

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

204760Orig1s000

PHARMACOLOGY REVIEW(S)

**ADDENDUM TO PHARMACOLOGY/TOXICOLOGY REVIEW OF
NDA 204,760 DATED 5/15/2014**

Reviewer: Yuk-Chow Ng, Ph.D.

Date: 9/4/2014

(b) (4)



Yuk-Chow Ng, Ph.D.
Pharmacologist
Division of Gastroenterology and Inborn Errors Products

David B. Joseph, Ph.D.
Lead Pharmacologist
Division of Gastroenterology and Inborn Errors Products

cc:
NDA 204,760
DGIEP
DGIEP/PM
DGIEP/D. Joseph
DGIEP/Y.-C. Ng
R/D Init.: D. Joseph 8/19/14

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/s/

YUK-CHOW NG
09/04/2014

DAVID B JOSEPH
09/04/2014

MEMORANDUM

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

FROM: David B. Joseph
Lead Pharmacologist

DATE: June 5, 2014

SUBJECT: NDA 204,760 (SD # 1 dated September 16, 2013)

Sponsor: AstraZeneca Pharmaceuticals LP

Drug Product: Movantik (naloxegol oxalate) tablets

Comments:

 (b) (4)
the clinical team recommends a waiver of clinical studies for the entire age range of pediatric patients. This recommendation will be presented to the Pediatric Review Committee for concurrence, which is required for granting of the waiver.

Recommendations:

There are no nonclinical issues which preclude the approval of Movantik. I concur with Dr. Ng's recommendation for approval and his recommended revisions in the label.

David B. Joseph, Ph.D. _____ Date _____
Lead Pharmacologist
Division of Gastroenterology and Inborn Errors Products

cc:
NDA (b) (4)
DGIEP
DGIEP/PM
DGIEP/D. Joseph
DGIEP/Y.-C. Ng
DGIEP/A. Rajpal
OND IO/A. Jacobs

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/s/

DAVID B JOSEPH
06/05/2014

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 204,760
Supporting document/s: 001
Applicant's letter date: 09/16/2013
CDER stamp date: 09/16/2013
Product: Movantik (naloxegol oxalate) tablets
Indication: Treatment of opioid-induced constipation in
adult patients with chronic non-cancer pain
Applicant: AstraZeneca
Review Division: Gastroenterology and Inborn Errors Products
Reviewer: Yuk-Chow Ng, Ph.D.
Supervisor/Team Leader: David B. Joseph, Ph.D.
Division Director: Donna Griebel, M.D.
Project Manager: Maureen D. Dewey

Disclaimer

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1 Executive Summary

1.1 Introduction

Naloxegol is a PEGylated derivative of naloxone. It acts as an antagonist at the μ - and δ -opioid receptors, and is a weak partial agonist at κ -opioid receptors, with the highest binding affinity at μ -opioid receptors. Naloxegol is a substrate of the P-glycoprotein (P-gp) transporter, which reduces its ability to cross the blood-brain barrier. Thus, naloxegol functions as a μ -opioid receptor antagonist in the gastrointestinal tract with reduced CNS effects. Opioid analgesics are the mainstay in the treatment of moderate-to-severe pain. However, their use is frequently associated with opioid-induced constipation (OIC). Naloxegol is an opioid receptor antagonist that has been developed as an oral treatment for opioid-induced constipation in adult patients with chronic non-cancer pain.

1.2 Brief Discussion of Nonclinical Findings

Pharmacokinetic studies demonstrated that distribution of naloxegol-related radioactivity into the rat brain and spinal cord was low compared to other tissues, suggesting relatively low CNS penetration of naloxegol in rats. A single time-point brain perfusion study in rats showed that the penetration of naloxegol was approximately 15 times slower than that of naloxone. However, in pharmacology studies, naloxegol produced a dose-dependent reduction in the centrally-mediated effects of morphine at plasma levels 15- to 112-times the human C_{max} at the Maximum Recommended Human Dose (MRHD). In addition, a comparison of the effects of naloxegol and naloxone on GI transit and analgesia showed that there was only a minimal separation between the dose-response curves for the peripheral and central antagonist effects for naloxegol; there was no separation between the central and peripheral antagonist effects for naloxone. Results from these nonclinical studies suggested that naloxegol has considerable CNS effects at high doses.

In the 2-year oral carcinogenicity study in rats, a dose-dependent increase in the incidence of benign interstitial (Leydig) cell adenoma of the testis was noted (statistically significant in trend test). A pair-wise comparison showed a statistically significant increase in the incidence of Leydig cell adenoma in the 400 mg/kg/day males. In addition, there was a significant increase in the incidence of Leydig cell hyperplasia in the 120 mg/kg/day males. The Sponsor examined the possibility that the naloxegol-induced increase in the incidence of benign Leydig cell tumors in rats was due to chronic exposure to elevated levels of plasma luteinizing hormone. It was demonstrated that plasma LH increased significantly after intravenous infusion of naloxegol. Plasma levels of testosterone also increased, but to a lesser extent. The results support the proposal that the observed drug-related increase in the incidence of benign Leydig cell adenoma and hyperplasia in rats was likely due to naloxegol-induced centrally mediated hormonal changes (i.e. elevated LH levels). Such a mechanism in tumor formation is common in rats. Given that the drug-induced increase in Leydig cell

adenoma incidence was statistically significant only at 400 mg/kg/day (818 times the human AUC at the MHRD), this effect is unlikely to be relevant to humans.

One of the metabolic pathways for naloxegol involves sequential shortening of the PEG chain moiety. As a result, ethylene glycol (EG) and diethylene glycol (DEG) are by-products of naloxegol metabolism. EG and DEG are known toxicants in animals and humans. Thus, potential exposure to EG and DEG in naloxegol-treated patients needs to be evaluated. In an Information Request dated Jan 16, 2014, the Clinical Pharmacology team stated: **“The proposed metabolism of naloxegol, a PEGylated product, is described as formation of partially shortened PEG chain products. Address the potential for the formation and systemic accumulation of ethylene glycol, diethylene glycol as well as their toxic metabolites as by-products of this metabolism.”** In their response, the Sponsor presented two assessments of the potential exposure to EG and DEG. In the first assessment, they assumed the entire 16.6 mg of PEG in a naloxegol oxalate tablet was fully metabolized to EG, DEG, or oxalic acid. The maximum theoretical doses for EG, DEG, and oxalic acid was 0.332, 0.244, and 0.483 mg/kg, respectively. The Sponsor indicated that these levels are significantly lower than the EPA Reference Doses of 2 and 1.6 mg/kg/day for EG and DEG, respectively, and are, therefore, considered safe. However, the cited Reference Doses are significantly higher than the Permitted Daily Exposure (PDE) of 6.2 mg per day for EG, as stated in ICH guidance Q3C. In the second assessment, potential exposure to EG and DEG was calculated based on metabolic profiles obtained from the clinical studies. Based on the relative abundance of the detected metabolites, which have varying PEG lengths, an administered naloxegol dose of 25 mg, or 38.3 μ moles, may potentially release 27.3 μ moles (1.7 mg) of EG or 10.8 μ moles (1.14 mg) of DEG. To account for the 12% of the metabolites in the excreta that could not be identified in the clinical studies, the Sponsor’s worst case scenario assumed that all 7 EG subunits in the PEG moiety were released as EG. This produces an additional 32.2 μ moles (2.0 mg) per day of EG for a possible total exposure of 3.7 mg EG per day. Under similar assumptions, a possible daily exposure of 2.6 mg DEG was estimated. These levels are less than the PDE of 6.2 mg per day for EG. It is noted that diethylene glycol is considered to have similar toxicity to that observed for ethylene glycol. This reviewer considers the estimation based on metabolic profiles to represent the most realistic assessment. Taken together, the Sponsor’s assessments provide a reasonable assurance of safety for the potential exposure to EG and DEG as metabolites of naloxegol.

1.3 Recommendations

1.3.1 Approvability

This application is recommended for approval.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

Established Pharmacologic Class (HIGHLIGHTS and section 11)

The Sponsor did not propose an EPC (established pharmacologic class) text phrase. Naloxegol is considered to be a peripherally-acting mu-opioid receptor antagonist with reduced CNS effects. Alvimopan and methylnaltrexone are also described in their respective labels as peripherally-acting mu-opioid receptor antagonists, and are proposed to exhibit a similar limitation as naloxegol in CNS activity. The existing EPC text phrase shown in the PRPLL for alvimopan and methylnaltrexone is “opioid antagonist”. This EPC text phrase is deemed as scientifically valid and clinically meaningful for naloxegol as well. Therefore, this reviewer recommends that, based on the mechanism of action, the EPC text phrase for naloxegol should be, “opioid antagonist”.

Sponsor’s Proposed Version:

8.1. Pregnancy

Pregnancy category (b) (4)



Evaluation:

This subsection should be revised to comply with the PLLR format. (b) (4)
no drug-related effects on embryo-fetal or post-natal development were demonstrated in these studies. However, we defer to the recommendation from the Maternal Health team for category C. The animal data from the embryo-fetal development studies in subsection 13.1 should be moved to this subsection. The recommended version shown below was developed in collaboration with the Maternal Health team (Carrie Ceresa and Jeanine Best).

For calculation of animal to human AUC ratios, the human AUC used was 330 ng·h/mL, a value that was measured in healthy volunteers administered 25 mg naloxegol. In the segment 2 embryo-fetal developmental study in rats, AUC in the 750 mg/kg/day females

was 479,000 ng·h/mL on GD 17. In the segment 2 embryo-fetal developmental study in rabbits, the AUC value in the 450 mg/kg/day females was 135,000 ng·h/mL on GD 20. The rat to human drug exposure comparison for the pre-/postnatal developmental study in rats is expressed based on body surface area, due to the absence of toxicokinetic data in this study (TK data for the same dose was not available in other rat studies).

Recommended Version:

Pregnancy Category C

Risk Summary

There are no adequate and well-controlled studies with Movantik in pregnant women. The use of Movantik during pregnancy may precipitate opioid withdrawal in a fetus due to the (b) (4) fetal blood brain barrier. No effects on embryo-fetal development were observed following administration of naloxegol in pregnant rats during the period of organogenesis at doses up to 1452 times the human AUC (area under the curve) at the maximum recommended human dose. No effects on embryo-fetal development were observed following administration of naloxegol in pregnant rabbits during the period of organogenesis at doses up to 409 times the human AUC at the maximum recommended human dose. Movantik should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Animal Data

Oral administration of up to 750 mg/kg/day naloxegol in rats (1452 times the human AUC at the maximum recommended human dose) and 450 mg/kg/day naloxegol in rabbits (409 times the human AUC at the maximum recommended human dose) during the period of organogenesis produced no adverse effects on embryo-fetal development. Oral administration of up to 500 mg/kg/day in rats (195 times the maximum recommended human dose based on body surface area) during the period of organogenesis through lactation produced no adverse effects on parturition or the offspring.

Sponsor's Proposed Version:

12.1 Mechanism of Action

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Evaluation:

Most of the information in the Sponsor's proposed version is excessive, and should be removed. The recommend version below was developed in collaboration with the Medical Team (Dr. Aisha Peterson and Dr. Anil Rajpal).

Recommended Version:**12.1 Mechanism of Action**

Naloxegol functions as a peripherally-acting mu-opioid receptor antagonist in the gastrointestinal tract, thereby decreasing the constipating effects of opioids. Administration of naloxegol at the recommended dose levels is expected to have minimal impact on opioid-mediated analgesia in the central nervous system. Due to poorer permeability and increased efflux of naloxegol across the blood-brain barrier, related to P-gp substrate properties, the CNS penetration of naloxegol is minimal.

Sponsor's Proposed Version:**12.2 Pharmacodynamics**

Use of opioids induces slowing of gastrointestinal motility and transit. Antagonism of gastrointestinal mu-opioid receptors by naloxegol inhibits opioid-induced delay of gastrointestinal transit time [REDACTED] (b) (4)

Evaluation:

The Sponsor's statement related to the pharmacologic activity (b) (4) (i.e. antagonism of opioid-induced delay of gastrointestinal transit time) is supported by one of the submitted pharmacology studies. Therefore, the proposed version is acceptable.

Sponsor's Proposed Version:

12.3 Pharmacokinetics

Absorption:

Following oral administration, naloxegol is absorbed rapidly, with peak concentrations (C_{max}) achieved at less than 2 hours. In a majority of subjects, a secondary plasma concentration peak of naloxegol was observed approximately 0.4 to 3 hours after the first peak. (b) (4)

(b) (4) Across the range of doses evaluated, peak plasma concentration and area under the plasma concentration-time curve (AUC) increase in a dose-proportional or almost dose-proportional manner.

Evaluation:

The Sponsor's statement regarding (b) (4) is supported by one of the submitted pharmacology studies. Therefore, the proposed version is acceptable.

Sponsor's Proposed Version:

13.1. Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Evaluation:

Statements regarding the

(b) (4)

The Sponsor provided a study which showed that infusion of naloxegol in male rats produced a transient elevation of plasma LH concentration, which suggests that the incidence of Leydig cell adenomas was hormonally mediated. However, the potential risk of Leydig cell tumor development in humans due to chronic elevation of LH levels is not understood. Nevertheless, it is reasonable for the label to include a statement indicating that the Leydig cell neoplasms in rats are unlikely to be relevant

(b) (4)

(b) (4)

For calculation of animal to human AUC ratios, the human AUC used was 330 ng·h/mL, a value that was measured in healthy volunteers administered 25 mg naloxegol. In the carcinogenicity study in mice, the AUC values in the 100 mg/kg/day males and 160 mg/kg/day females were 14,100 and 8,860 ng·h/mL, respectively, on week 52. In the 104-week carcinogenicity study in rats, the AUC values in the 120 and 400 mg/kg/day males were 81,100 and 270,000 ng·h/mL, respectively, and AUC in the 400 mg/kg/day females was 340,000 ng·h/mL on week 52. Toxicokinetics were not measured in the segment 1 fertility and early embryonic developmental study in rats. Therefore, AUC in the 1000 mg/kg/day group was estimated based on TK data from the 3-month oral toxicity study in rats, in which the AUC values in the 800 mg/kg/day males and females were 399,000 and 500,000 ng·h/mL, respectively, on day 29.

Information from the reproductive and developmental studies (b) (4)

Recommended Version:

Carcinogenesis

In a 104-week carcinogenicity study in CD-1 mice, naloxegol was not tumorigenic at oral doses up to 100 mg/kg/day in males and 160 mg/kg/day in females (43 and 27 times the human AUC at the maximum recommended human dose for male and female mice, respectively). In a carcinogenicity study in Sprague-Dawley rats, naloxegol was administered orally at doses of 40, 120, and 400 mg/kg/day for at least 93 weeks. Naloxegol did not cause an increase in tumors in female rats. In male rats, an increase in interstitial (Leydig) cell adenomas in testes was observed at 400 mg/kg/day (818 times the human AUC at the maximum recommended human dose). The no observed effect level for increased tumor incidence was 120 mg/kg/day in male and 400 mg/kg/day in female rats (246 and 1030 times the human AUC at the maximum recommended human dose for male and female rats, respectively). The Leydig cell neoplasms in rats are considered to be unlikely relevant to humans.

Mutagenesis

Naloxegol was not genotoxic in the *in vitro* bacterial reverse mutation (Ames) assay, mouse lymphoma TK^{+/-} mutation assay, or the *in vivo* mouse micronucleus assay.

Impairment of Fertility

Naloxegol was found to have no effect on fertility or reproductive performance in male and female rats at oral doses up to 1000 mg/kg/day (greater than 1000 times the human AUC at the maximum recommended human dose).

Sponsor's Proposed Version:

(b) (4)



Evaluation:

2 Drug Information

2.1 Drug

CAS Registry Number - 1354744-91-4

Generic Name – Naloxegol oxalate

Code Name - NKTR-118 oxalate; AZ13337019 oxalate; PEG-naloxol; NKTR-10018

Chemical Name -

(5 α ,6 α)-17-allyl-6-(2,5,8,11,14,17,20-heptaoxidocosan-22-yloxy)-4,5-epoxymorphinan-3,14-diol oxalate

Molecular Formula/Molecular Weight - C₃₄H₅₃NO₁₁•C₂H₂O₄ / 741.8

Structure or Biochemical Description

Table 1 Composition of naloxegol film-coated tablets 12.5 mg and 25 mg (as naloxegol oxalate 14.2 mg and 28.5 mg)

Components	mg per tablet (12.5 mg)	mg per tablet (25 mg)	Function	Standard
Tablet core				
Naloxegol oxalate	14.2 ^a	28.5 ^b	Drug substance	AstraZeneca
Mannitol	(b) (4)		(b) (4)	USP
Microcrystalline cellulose	(b) (4)		(b) (4)	NF
Croscarmellose sodium	(b) (4)		(b) (4)	NF
Propyl gallate	(b) (4)		(b) (4)	NF
Magnesium stearate	(b) (4)		(b) (4)	NF
Core tablet weight (mg)	(b) (4)		(b) (4)	(b) (4)
Tablet coating^{e, f}				
Hypromellose	(b) (4)		(b) (4)	USP
Titanium dioxide	(b) (4)		(b) (4)	USP
Polyethylene glycol (b) (4)	(b) (4)		(b) (4)	NF
Ferric oxide red	(b) (4)		(b) (4)	NF
Ferric oxide black	(b) (4)		(b) (4)	NF
(b) (4)	(b) (4)		(b) (4)	NF
(b) (4)	(b) (4)		(b) (4)	USP
Coated tablet weight (mg)	(b) (4)		(b) (4)	(b) (4)
^a	Corresponding to 12.5 mg naloxegol free base.			
^b	Corresponding to 25 mg naloxegol free base.			
^c	May be adjusted based on (b) (4)			
^d	Target amount corresponding to (b) (4) of core tablet weight. Amount may range from (b) (4) for the 12.5 mg tablet and (b) (4) for the 25 mg tablet (b) (4)			
^e	The film coating mixture (b) (4)			
^f	Coating constitutes (b) (4)			
^g	(b) (4)			

2.4 Comments on Novel Excipients

The excipients to be used in the naloxegol formulation appear to be safe. FDA Inactive Ingredients Database confirms that all the excipients listed in the table above are present in approved oral formulations at levels (e.g., mg/tablet) that exceed the estimated maximum daily dose (mg) in naloxegol tablets.

2.5 Comments on Impurities/Degradants of Concern

The Sponsor reported the following drug- and process-related impurities in the naloxegol batches used in the nonclinical studies and in naloxegol oxalate batches. The nonclinical studies were used to support the proposed specification limits (table taken from Sponsor's report).

Table 7 Levels of organic impurities found in naloxegol and naloxegol oxalate

Impurity (drug-related and process-related)	Acceptance Criteria	Batch analyses results	
		Naloxegol, preclinical studies ^a Range, n=17	Naloxegol oxalate, batches used to set the specification ^b Range, n=30
(b) (4)	NMT (b) (4)%	(b) (4)	(b) (4)
(b) (4)	NMT (b) (4)%	(b) (4)	(b) (4)
(b) (4)	NMT (b) (4)%	(b) (4)	(b) (4)
(b) (4)	NMT (b) (4)%	(b) (4)	(b) (4)
(b) (4)	NMT (b) (4)	(b) (4)	(b) (4)
Largest unspecified impurity ^h	NMT (b) (4)%	(b) (4)	(b) (4)

^a Naloxegol batches used in preclinical studies; 149002, 149003, 149005, 149006, 149007, 149008, 149010, 149011, 149012, 149013, 149014, 149015, 200237, 200278, 200279, 200388 and 1005.

^b Naloxegol oxalate batches manufactured at pilot or production scale; C548/1, C548/2, 1006, 1007, 1008, 1010, 1011, 1012, 1013, 1014, 1016, 1018, 1019, 1020, 1021, 1022, C564/1, C564/2, C564/3, RW1/C564/2, C564/5, C564/6, 1023, 1024, 1025, 1026, 1027, 1028, 1029 and 1030. The use of the batches are; technical, stability, clinical and intended commercial use.

^c For the (b) (4) (b) (4) was not individually specified in the specification valid at the time. Instead (b) (4) was included in the reporting of (b) (4) when applicable.

(b) (4)

(b) (4) are drug-related impurities that were present in the batches used in the nonclinical studies and in

naloxegol oxalate, the drug substance. The level of each impurity in the nonclinical batches was reported to be (b) (4) than that in the drug substance batches. The table below (taken from the Sponsor's report) shows the calculated levels of these impurities being dosed in the 3-month toxicity study in rats (LS-2007-028) and the calculated dose a patient would have received if (b) (4) were present at the acceptance criteria of (b) (4) (b) (4) respectively. In a response to an Information Request issued by the CMC team, the Sponsor verified the lot number for naloxegol used in the 3-month toxicity study in rats. The calculation shows that a sufficient safety margin can be established for each of the impurities. Because these impurities are (b) (4) (b) (4) there is very little safety concern regarding these impurities. Therefore, the acceptance criteria proposed by the Sponsor are deemed acceptable.

Table 3 Summary of qualification data obtained for (b) (4)

Impurity	Acceptance criteria (%)	Impurity level in qualification batch (%)	Impurity dose at specification limit ^a (mg/m ² /day)	Impurity dose at NOAEL ^b (mg/m ² /day)	Safety margin ^c
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(b) (4)

^a Impurity dose to humans at the specification limit (mg/m²/day)=(specification/ (b) (4))

^b impurity dose (mg/m²/day) at the NOAEL in 3 months oral rat toxicity study LS-2007-028 (b) (4)=(impurity concentration/ (b) (4)) Data are for males, the NOAEL for females was (b) (4)

^c Safety margin calculated as the ratio of the impurity dose at the NOAEL (rats) in mg/m²/day to the impurity dose in humans in mg/m²/day at the acceptance criteria.

(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

Adult patients with opioid-induced constipation with chronic non-cancer pain: 25 mg orally once daily; 12.5 mg once daily for patients who are taking moderate CYP3A4 inhibitors.

2.7 Regulatory Background

IND 78,781 was submitted by Nektar Therapeutics on 10/22/2007 for PEG-Naloxol (NKTR-118) for the treatment of opioid-induced constipation and other manifestations of opioid-induced bowel dysfunction in patients receiving opioid therapy for pain.

3 Studies Submitted

3.1 Studies Reviewed

Type of Study	Test system	Method of administration	Testing facility	Study number
1.1 Primary pharmacodynamics				
Binding characteristics of naloxegol and naloxone on cloned human μ -, κ - and δ -opioid receptor subtypes	CHO-K1 stable cell-lines expressing human opioid receptors	<i>In vitro</i>	NEKTAR Therapeutics Inc, USA	RD00001536.00
Binding characteristics of naloxegol and methylnaltrexone on cloned human μ -, δ - and cloned rat κ -opioid receptor subtypes	Membranes of CHO cell line expressing opioid receptors	<i>In vitro</i>	(b) (4)	OR Pharm 201302
Functional properties in a [³⁵ S]GTP γ S binding assay at cloned human μ -opioid receptors	CHO-K1 stable cell-lines expressing human μ -opioid receptors	<i>In vitro</i>	(b) (4)	OR Pharm 201302
Functional properties in a [³⁵ S]GTP γ S binding assay at cloned human μ -opioid receptors	Membranes of Human Embryonic Kidney 293 (HEK293) cell line expressing human μ -opioid receptors	<i>In vitro</i>	AZ R&D Montreal, Canada	hMOR GTP NKTR-118
Functional properties in a [³⁵ S]GTP γ S binding assay at cloned human δ and κ -opioid receptors	Membranes of CHO (δ) and HEK293 (κ) cell lines expressing opioid receptors	<i>In vitro</i>	(b) (4)	1022SY
Functional κ -opioid receptor agonism	Isolated rabbit <i>vas deference</i>	<i>Ex vivo</i>	(b) (4)	OR Pharm 201302
Pharmacology of naloxegol in rat models of analgesia and gastrointestinal transit	Sprague-Dawley rats	Oral gavage	NEKTAR Therapeutics Inc, USA	RD00001766.00
1.2 Secondary pharmacodynamics				
Receptors, ion channels, transporter and enzyme activity	Various recombinant cell lines	<i>In vitro</i>	(b) (4)	1012SY
1.3 Safety pharmacology				
Central Nervous System (Irwin Test)	Rat/Han Wistar	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2707
Central Nervous System (PTZ)	Rat/Han Wistar	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2801
Central Nervous System (Drug discrimination)	Rat/Han Wistar	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2736
Central Nervous System (Drug discrimination)	Rat/Han Wistar	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2819
Central Nervous System (Drug discrimination)	Rat/Han Wistar	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2924
Central Nervous System (Drug discrimination)	Rat/Han Wistar	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2966
Central Nervous System (Physical Dependence)	Rat/Han Wistar	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2746
Central Nervous System (Physical Dependence)	Rat/Han Wistar	oral	AZ R&D Södertälje, Sweden	SP-SPG-2747

Central Nervous System (Self-Administration)	Rat/Han Wistar	i.v.	AZ R&D Södertälje, Sweden	SP- D3820-SPG-2817
Central Nervous System (Self-Administration)	Rat/Han Wistar	i.v.	AZ R&D Södertälje, Sweden	SP-MethDev-SPG-2607
Central Nervous System (Analgesia)	Mouse/NMRI	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2959
Central Nervous System (Analgesia)	Mouse/NMRI	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2995
Central Nervous System (Analgesia)	Mouse/NMRI	subcutaneous	AZ R&D Södertälje, Sweden	SP-SPG-2958
Cardiovascular (hERG channel)	hERG-expressing HEK293 Cells	<i>In vitro</i>	(b) (4)	LS-2008-008
Cardiovascular (Cardiac ion channels)	Various recombinant cell lines	<i>In vitro</i>	AZ R&D Alderley Park, UK	1012SY
Cardiovascular (Myocyte contractility)	Dog ventricular myocyte	<i>Ex vivo</i>	AZ R&D Alderley Park, UK	2697SV
Cardiovascular (Isolated heart)	Rat isolated heart	<i>Ex vivo</i>	(b) (4)	3162SR
Cardiovascular (Telemetry)	Dog/Beagle	oral	AZ R&D Alderley Park, UK	1246ZD
Respiratory	Rat/Han Wistar	oral	AZ R&D Alderley Park, UK	3052SR
Gastrointestinal	Rat/Han Wistar	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2745
Gastrointestinal	Rat/Han Wistar	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2883
Renal	Rat/Han Wistar	oral	AZ R&D Södertälje, Sweden	SP-D3820-SPG-2854

Toxicology studies reviewed:

Type of study	Species and strain	Method of administration	Duration of dosing	Doses mg/kg ^a	GLP compliance	Testing facility	Study number
2.6.7.5 Single-dose toxicity	Rat (Sprague-Dawley)	po	Single Dose	500, 1000, 1500, 2000	Yes	AstraZeneca, Sweden	3143LR
	Rat (Sprague-Dawley)	iv	10 minutes	10, 30,100	No	AstraZeneca, UK	3504LR
	Dog (Beagle)	po, iv	Single Dose. Least 2 days between doses	iv 0.4 po 0.4, 2, 10, 20	No	(b) (4)	LS-2005-030
2.6.7.6/7 Repeat-dose toxicity	Mouse (CrI:CD1)	po	3 Months	0, 50, 400, 600, 800	Yes	(b) (4)	LS-2007-056
	Rat (Sprague-Dawley)	po	7 days	0, 100, 500, 1000	No	(b) (4)	LS-2007-005
	Rat (Sprague-Dawley)	po	1 month	0, 50, 150, 500	Yes	(b) (4)	LS-2007-011
	Rat (Sprague-Dawley)	po	3 months At least 94 Days	0, 50, 400, 600, 800 ^b	Yes	(b) (4)	LS-2007-028
	Rat (Sprague-Dawley)	po	6 months + 1 month recovery	0, 50, 200, 800 ^c	Yes	(b) (4)	LS-2007-040
	Dog (Beagle)	po	14 days	0, 25, 75, 200	Yes	(b) (4)	LS-2005-031
	Dog (Beagle)	po	14 days	0, 200, 500	Yes	(b) (4)	LS-2007-004
	Dog (Beagle)	po	1 month + 14-day recovery	0, 50, 150, 500	Yes	(b) (4)	LS-2007-012
2.6.7.8/9 Genotoxicity	Dog (Beagle)	po	9 months + 1-month recovery	0, 50, 200, 500	Yes	(b) (4)	LS-2007-041
	<i>Salmonella typhimurium</i> strains (TA1535, TA1537, TA98, TA100) and <i>Escherichia coli</i> strain WP2 <i>invA</i>	In vitro	Assay 1 (-/+ S9), plate incorporation for 48-72 hours. Assay 2 (-/+ S9), pre-incubation (30 minutes) followed by 48-72 hours plate incubation	50 - 5000 µg/plate	Yes	(b) (4)	LS-2007-006
	<i>Salmonella typhimurium</i> LT2 bacteria of strains TA1535, TA1537, TA98 and TA100, and <i>Escherichia coli</i> strain WP2 <i>invA</i> /pKM101	In vitro	All assays (-/+ S9), plate incorporation for 72 hours	49.1 - 5340 µg/plate	No	AstraZeneca, Sweden	2371BV

Type of study	Species and strain	Method of administration	Duration of dosing	Doses mg/kg ^a	GLP compliance	Testing facility	Study number	
	<i>Salmonella typhimurium</i> (TA1535, and TA100)	In vitro	Either: (-/+ S9), plate incorporation for 72 hours, OR (-/+ S9), pre-incubation (60 minutes) followed by 72 hours plate incubation	24 – 5210 µg/plate	No	AstraZeneca, Sweden	2449BV	
	<i>Salmonella typhimurium</i> strains (TA1535, TA1537, TA98, TA100) and <i>Escherichia coli</i> strain WP2 <i>uvrA</i> /pKM101	In vitro	(-/+S9), plate incorporation for 72 hours	5 – 5000 µg/plate	Yes	AstraZeneca, UK	2980BV	
	L5178Y TK+/- Mouse Lymphoma	In vitro	Assay 1, 4 hours in the presence and absence of S9. Assay 2, 24 hours in the absence of S9	0 – 1950 µg/ml	Yes	(b) (4)	LS-2007-007	
	Mouse NMRI Harlan	po	Single dose	500, 1000, 2000	Yes	(b) (4)	LS-2007-008	
2.6.7.10	Carcinogenicity	Mice Crl:CD1 (ICR)	po	2 year	M: 0, 25, 70/50, or 200/100 F: 0, 40, 120/80, 400/160	Yes	(b) (4)	LS-2008-006
		Rat (Sprague-Dawley)	po	2 year	0, 40, 120, 400 ^d	Yes	(b) (4)	LS-2008-007
2.6.7.11-14	Reproductive & development toxicity	Rat (Sprague-Dawley)	po	Males: at least 28 days prior to pairing & throughout mating phase Females: at least 14 days prior to pairing through to Day 7 postcoitum	0, 250, 500, 1000	Yes	(b) (4)	LS-2007-042
		Pregnant rat (Sprague-Dawley)	po	Day 6 to 17 postcoitum	0, 250, 500, 750, 1000	No	(b) (4)	LS-2007-043

Type of study	Species and strain	Method of administration	Duration of dosing	Doses mg/kg ^a	GLP compliance	Testing facility	Study number
2.6.7.17 Other toxicity studies	Pregnant rabbit (New Zealand White)	po	7 days (non-pregnant. Phase 1); D7 to 20 postcoitum (Phase 2) Day 0= day of mating	0, 30, 300, 600, 1000 (Phase 1); 0, 30, 150, 300, 450 (Phase 2)	No	(b) (4)	S-2009-004
	Pregnant rat (Sprague-Dawley)	po	Day 6 to Day 17 postcoitum	0, 250, <u>750</u> , 1000	Yes	(b) (4)	S-2007-044
	Pregnant rabbit (New Zealand White)	po	Day 7 to Day 20 postcoitum	0, 30, <u>150</u> , 450	Yes	(b) (4)	S-2009-005
	Pregnant rat (Sprague-Dawley)	po	Day 6 of gestation to Day 7 of lactation	0, 50, 250, <u>750</u> ^e	Yes	(b) (4)	ASU0139 (3270WR)
	Pregnant rat (Sprague-Dawley)	po	Day 6 of gestation to Day 20 of lactation	0, 50, 250, <u>500</u> ^f	Yes	(b) (4)	ASU0140 (3267WR)
	Rat (Sprague-Dawley)	po	14 days	0, 400, 800	Yes	AstraZeneca, UK	3368DR
	Rat (Sprague-Dawley)	iv	10 minutes	0, 80	No	AstraZeneca, USA	3533KR
	Mouse/CByB6F1 (Tg.rasH2 non-transgenic wild-type)	po	5 days	0, 100, 200, 400, 800, 1500	Yes	(b) (4)	10-2215 (0807DM)
	Mouse/CByB6F1 (Tg.rasH2 non-transgenic wild-type)	po	28 Days	0, <u>150</u> , 500, 1500	Yes	(b) (4)	10-2216 (0808DM)

^a Unless otherwise specified, for repeat-dose toxicity, the highest NOAEL (No Observed Adverse-Effect Level) is underlined.

^b 600 mg/kg NOAEL for males, 800 mg/kg NOAEL for females

^c 200 mg/kg NOAEL for males, 800 mg/kg NOAEL for females

^d 40 mg/kg NOAEL for males, 400 mg/kg NOAEL for females

^e 750mg/kg for maternal and pup toxicity

^f 500mg/kg for maternal and pup toxicity

M Males

F Females

3.2 Studies Not Reviewed

All studies related to drug discrimination, physical dependence, and self-administration were reviewed by the Controlled Substance Staff.

3.3 Previous Reviews Referenced

Pharmacology/Toxicology reviews of IND 78,781 by Niraj Mehta, Ph.D. dated 10/27/2009 and 5/19/2010.

4 Pharmacology

4.1 Primary Pharmacology

In vitro Pharmacology of NKTR-118 (Study No. RD00001536.00)

Objective: Receptor binding studies were performed to determine the effect of PEG (polyethylene glycol) conjugation of naloxone on the binding affinity at μ -opioid receptors, and to investigate whether PEGylation generated a significant change in the selectivity profile at μ -, κ - and δ -opioid receptors.

Methods and Materials: Competition binding studies were performed on cloned human μ -opioid receptors expressed in CHO cells using displacement of [3 H]naloxone by NKTR-118 or naloxone. Similar studies were performed using displacement of [3 H]naloxone at cloned human κ -opioid receptors or [3 H]DPDPE at the cloned human δ -opioid receptors. Competition binding assays also were performed on μ -, κ - and δ -opioid receptors using NKTR-118 and methylnaltrexone to compare the binding affinities of these compounds.

Results and Conclusions: As shown in the Sponsor's table below, NKTR-118 exhibited high affinity at the cloned human μ -opioid receptor with a K_i of 33.8 nM, but the binding affinity was 20-fold lower (as indicated by the increased K_i value) compared to naloxone. Reduction in binding affinity for NKTR-118, compared to naloxone, was also observed at the cloned human κ -opioid ($K_i=186.5$ nM) and δ -opioid ($K_i=53.5$ nM) receptors (47-fold lower and 5-fold lower for κ and δ -opioid receptors, respectively). The rank order of affinity for opioid receptors was $\mu > \delta > \kappa$ for NKTR-118, whereas that for naloxone was $\mu > \kappa > \delta$.

K_i values for in vitro binding of NKTR-118 and naloxone to opioid receptor subtypes (whole-cell preparations n=3)

Receptor	Naloxone K_i (nM)	NKTR-118 K_i (nM)	Fold decrease in affinity for NKTR-118
Mu	1.7 (\pm 0.8)	33.8 (\pm 22.2)	20.1 (\pm 6.5)
Kappa	4.0 (\pm 1.3)	186.5 (\pm 59.7)	46.6 (\pm 37.1)
Delta	10.3 (\pm 1.2)	53.5 (\pm 1.7)	5.2 (\pm 0.6)

NKTR-118 was also compared to methylnaltrexone in the whole cell preparations. The relative affinities at the different opioid receptors were derived from a single experiment, performed in triplicate samples. The Sponsor noted that variability of the affinities was high.

Receptor	Methyl naltrexone (MTNX) K_i (nM)	NKTR-118 K_i (nM)	Fold difference in affinity between MTNX and NKTR-118
Mu	8.46	77.3	↓ 9.1
Kappa	130	230	↓ 1.8
Delta	247	52.1	↑ 4.7

To obtain a more precise estimate of opioid receptor affinities for NKTR-118 and methylnaltrexone, a second study using membrane preparations of cells expressing cloned human μ - and δ - opioid receptors and cloned rat κ - opioid receptors was conducted. The study is described below.

Naloxegol: Opioid receptor pharmacology *in vitro* (Study No. OR Pharm 201302)

Naloxegol binding affinity at cloned mu, delta, and kappa opioid receptors

Objective: Receptor binding studies were performed to determine the effect of PEG conjugation of naloxone on the binding affinity at μ -opioid receptors, and to investigate whether PEGylation generated a significant change in the selectivity profile at μ -, κ - and δ -opioid receptors.

Methods and Materials: Competitive binding studies were performed on cloned human μ -opioid receptors expressed in membranes of CHO cells using displacement of [3 H]diprenorphine by NKTR-118 or other ligands; IC_{50} , K_i , and pK_i values were obtained. Similar studies were performed using displacement of [3 H]DADLE at cloned human δ -opioid receptors and [3 H]U69593 at cloned rat κ -opioid receptors.

Results and Conclusions:

At cloned human μ -opioid receptors labeled with the opioid receptor antagonist [3 H]diprenorphine, the pK_i values of naloxegol and methylnaltrexone were 8.13 ± 0.06 and 7.66 ± 0.08 respectively, corresponding to respective K_i values of 7.4 nM and 22.1 nM. Naloxegol was 3-fold more potent than methylnaltrexone at human μ -opioid receptors.

At cloned human δ -receptors labeled with the δ -opioid receptor agonist [3 H]DADLE, the pK_i values of naloxegol and methylnaltrexone were 6.69 ± 0.05 and 5.72 ± 0.35 respectively, corresponding to respective K_i values of 203 nM and 1.9 μ M. Naloxegol was 9.4-fold more potent than methylnaltrexone at human δ -receptors.

At cloned rat κ -opioid receptors labeled with the κ -opioid receptor agonist [3 H]U69693, the pK_i values of naloxegol and methylnaltrexone were 8.06 ± 0.05 and 7.96 ± 0.17 respectively, corresponding to respective K_i values of 8.65 nM and 10.9 nM. Naloxegol was equipotent with methylnaltrexone at rat κ -opioid receptors. The rank order of

affinity for opioid receptors is $\mu = \kappa > \delta$ for naloxegol, whereas that for methylnaltrexone is $\kappa > \mu > \delta$. The receptor binding data are summarized in the Sponsor's table below.

Table 1 **K_i values for binding of naloxegol and methylnaltrexone to opioid receptor subtypes *in vitro***

Receptor	Naloxegol K_i (nM)	methylnaltrexone K_i (nM)	K_i methylnaltrexone/ K_i naloxegol
Mu (human)	7.42	22.1	3.0
Delta (human)	203	1900	9.4
Kappa (rat)	8.65	10.9	1.25

Antagonism of morphine by naloxegol and methylnaltrexone at the cloned human mu opioid receptor

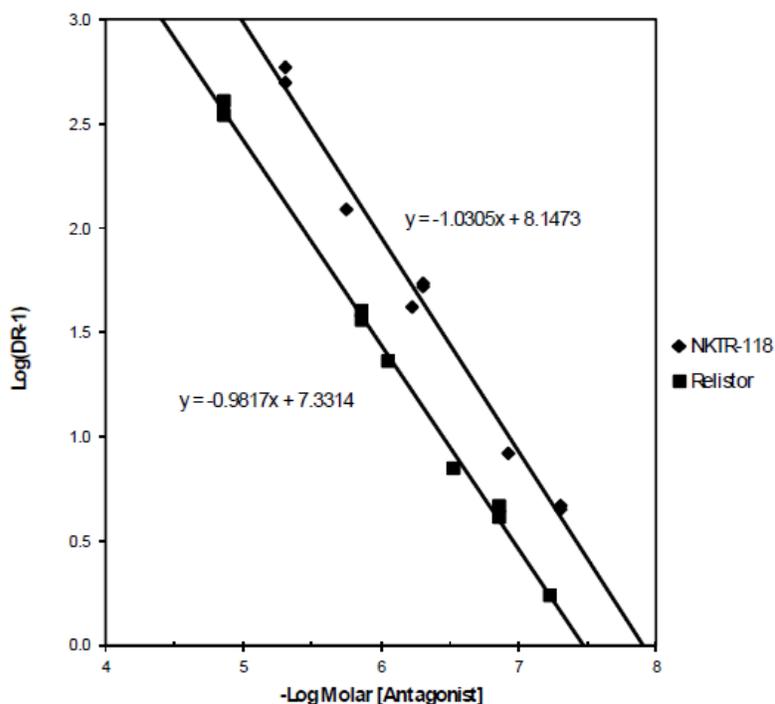
Objective: In order to assess whether naloxegol acts as a competitive antagonist, naloxegol was tested in Schild-type experiments for its ability to elicit right-shifts in the concentration-response curve of morphine in [³⁵S]GTP γ S binding experiments.

Methods and Materials: Human recombinant μ -opioid receptors stably expressed in CHO-K1 cells were used. Binding of [³⁵S]GTP γ S to GTP-binding proteins, as a function of agonist activity at the receptor, was measured.

Results and Conclusions: Naloxegol and methylnaltrexone elicited parallel right-shifts in the morphine dose-response curve with no reduction in E_{max} . The results for both compounds showed a very high degree of fit to the competitive antagonism model ($R_2 = 0.997$ to 0.998), with Schild slopes of -1.03 for naloxegol, and -0.982 for methylnaltrexone. Results of the Schild plot are shown in the figure below (taken from the Sponsor's report).

Schild Plot of Naloxegol and Methylnaltrexone Effects on Morphine Agonist Dose-response Curves

Figure 5 Schild plot of naloxegol and methylnaltrexone effects on morphine agonist dose-response curves at human mu receptors as measured by [³⁵S]GTPγS binding (pooled results of 3 independent experiments for each compound).



The pA₂ values of naloxegol and methylnaltrexone were determined to be 7.95 ± 0.11 and 7.43 ± 0.02 respectively, corresponding to K_B values of 11 nM and 37 nM, respectively. Naloxegol was 3.4-fold more potent than methylnaltrexone as an antagonist at human μ-opioid receptors (P=0.0012, pA₂ comparison). The difference in functional μ-opioid receptor antagonist potency was comparable to that seen in the μ-opioid receptor binding assay.

Assessment of kappa opioid receptor agonism by naloxegol in the isolated field-stimulated rabbit vas deferens assay

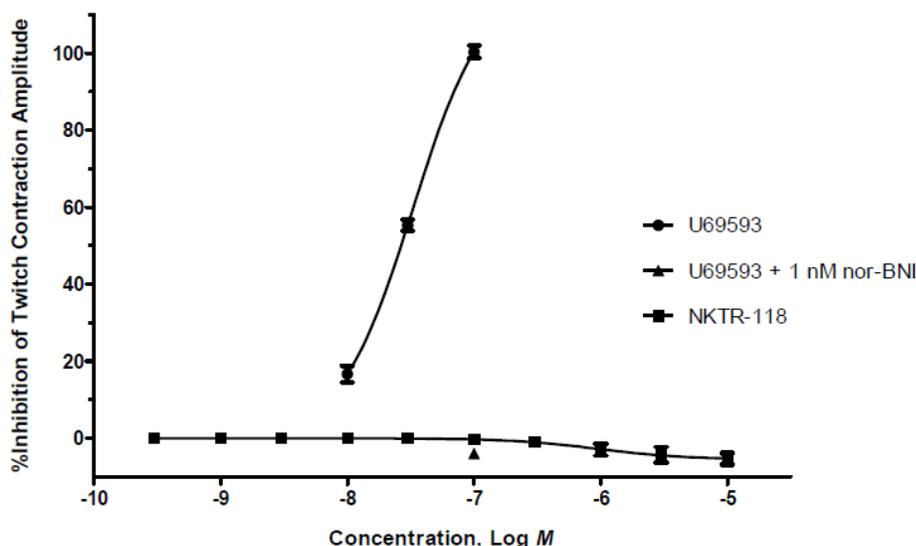
Objective: To assess the κ-opioid receptor agonism potential of naloxegol in an *ex vivo* functional assay. This study was conducted as a follow up to a study using an *in vitro* κ-opioid receptor activation assay, which demonstrated partial agonist activity of naloxegol based on stimulation of [³⁵S]GTPγS binding (see study no.1022SY).

Methods and Materials: Rabbit vas deferens was field-stimulated to induce twitch contraction. The ability of κ-opioid receptor agonist U69593 and naloxegol to inhibit the twitch contraction amplitude was assessed.

Results and Conclusions: In the field-stimulated rabbit vas deferens, the κ-opioid receptor agonist U69593 induced a concentration-dependent decrease in the twitch contraction amplitude (IC₅₀ = 33 nM), which was reversed by the kappa antagonist nor-BNI (1 nM). Naloxegol did not produce any decrease in the twitch contraction amplitude

at concentrations up to 10 μ M. A 100% inhibition was defined by the inhibitory effect of 100 nM U69593. The results show that naloxegol had no agonist activity at kappa opioid receptors in field-stimulated rabbit vas deferens assay.

Effect of Naloxegol on the Field-Stimulated Rabbit Vas Deferens Assay



NKTR-118: Opioid receptor functional assays <i>in vitro</i> (Study No. 1022SY)	
Materials and Methods	Results and Conclusions
The activity of NKTR-118 at δ - and κ -opioid receptors was assessed using [³⁵ S]GTP γ S functional assays to determine whether NKTR-118 is an agonist or antagonist at these two receptors. Binding of [³⁵ S]GTP γ S to GTP-binding proteins, as a function of agonist activity at the receptor, was measured. δ receptors were expressed in CHO cells and κ receptors were expressed in HEK-293 cells.	NKTR-118 alone had no effect on the binding of [³⁵ S]GTP γ S in the δ -opioid receptor functional assay, while NKTR-118 decreased the response to the reference agonist, DPDPE, in a concentration-dependent manner with an IC ₅₀ value of 0.866 μ M. NKTR-118 alone induced a concentration-dependent increase in the binding of [³⁵ S]GTP γ S in the κ -opioid receptor functional assay with an EC ₅₀ value of 0.047 μ M; however the maximum response induced was only 39% that of the reference agonist, U-69593. Therefore, NKTR-118 is an antagonist of the δ -opioid receptor, but exhibits partial agonist activity at the κ -opioid receptor in the [³⁵ S]GTP γ S binding assay.

<i>In Vitro</i> Functional Properties at the Cloned Human Mu Opioid Receptor (Study No. hMOR GTP NKTR-118)	
Materials and Methods	Results and Conclusions
NKTR-118 was tested in a	NKTR-118 and naloxone displayed no significant

<p>[³⁵S]GTPγS binding assay in order to determine its functional properties in membranes from HEK-293 cells transfected with the human μ-opioid receptor (hMOR). The opioid antagonist naloxone was also tested as a comparator. Two versions of the assay were designed to profile compounds for agonism and antagonism. The antagonist assay assessed NKTR-118 potency in reversing the effect of DAMGO ([D-Ala², NMe-Phe⁴, Gly-ol⁵]-enkephalin), a full agonist, or morphine, a partial agonist.</p>	<p>effect on hMOR [³⁵S]GTPγS at 30 μmol/L. When tested on hMOR in the antagonist mode of the assay, NKTR-118 inhibited both DAMGO and morphine activity with a mean %Relative I_{max} values of 85.7±2.7 and 85.9 ±8.9, respectively, and a mean pIC₅₀ values of 6.64±0.05 and 7.25±0.15, respectively. Naloxone inhibited both DAMGO and morphine activity with a mean %Relative I_{max} values of 85.6±4.3 and 87.9±3.3, respectively, and mean pIC₅₀ values of 7.30±0.08 and 7.95±0.08, respectively. Therefore, NKTR-118 displayed no significant agonist efficacy at the human μ-opioid receptor. When tested for antagonism, it was a full antagonist of human μ-opioid receptor.</p>
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Pharmacology of NKTR-118 in Rat Models of Analgesia and Gastrointestinal Transit (Study No. RD00001766.00)

Objective: The objective of this series of studies was to determine dose-response relationships for NKTR-118 in the morphine-induced effects on gastrointestinal transit (peripheral effects) and analgesia (central effects) using *in vivo* rat models.

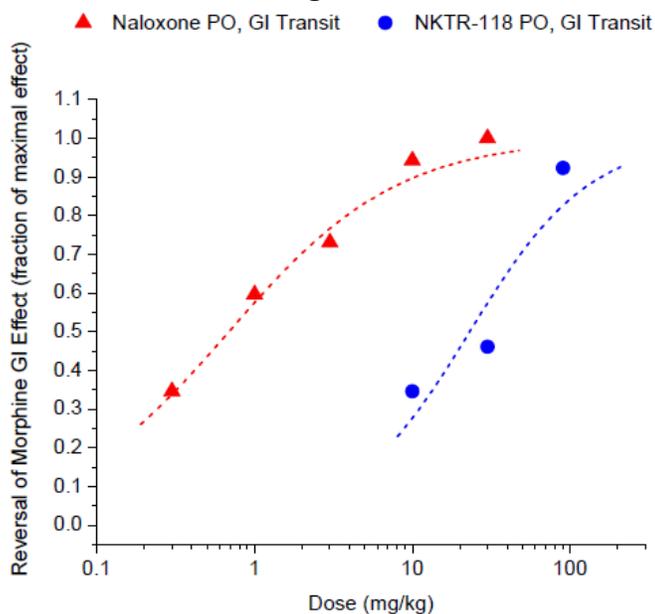
The Effect of NKTR-118 on Morphine-Induced Delay in Gastrointestinal Transit in Rats

Methods and Materials: The ability of NKTR-118 to antagonize the effects of morphine on gastrointestinal (GI) transit was investigated in a rat model in which the distance that a charcoal meal travels within a specified time interval is measured within the GI tract. Sprague-Dawley rats (7-8 animals/group) were administered morphine (10 mg/kg, IV) 5 minutes prior to oral administration of NKTR-118 (10, 30, or 90 mg/kg), naloxone (10 mg/kg), or saline. A 1 ml suspension of charcoal meal was given to the rats by gavage 25 minutes later. Animals were sacrificed 30 minutes after the meal and the distance that the charcoal meal travelled along the intestine from the pyloric sphincter and the total intestinal length were measure. Data was expressed as the percent of total intestinal length traveled.

Results and Conclusions: Morphine markedly reduced the distance travelled by the charcoal meal, and this distance was used as the measure of the full agonist effect. NKTR-118 treatment reversed, in a dose-dependent manner, the morphine-induced slowing of GI transit, but was less potent than naloxone. Naloxone at 10 mg/kg po produced a complete inhibition of the morphine effect on GI transit, whereas NKTR-118 produced the same inhibition at a dose of 90 mg/kg po (see figure below, taken from the study report). ED₅₀ values derived from the dose-response modeling of the antagonist

effect against morphine in the GI tract were 23.1 and 0.69 mg/kg for NKTR-118 and naloxone, respectively.

Dose-Response Relationship for Reversal of Morphine-induced Slowing of GI Transit in Rats

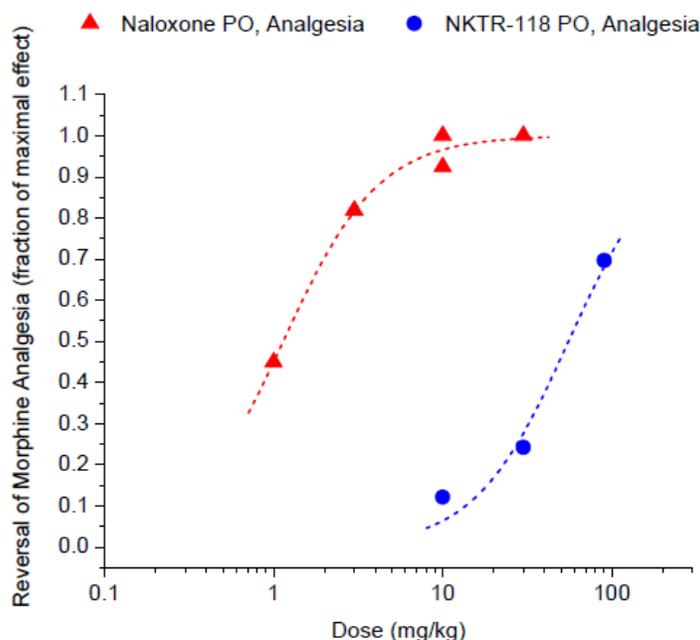


Effects of NKTR-118 on Morphine-Induced Analgesia in Rats

Materials and Methods: The ability of NKTR-118 to antagonize the analgesic effect of morphine, which is assumed to be a centrally mediated effect, was investigated using the hotplate test in rats. Male Sprague-Dawley rats (n=10) were administered morphine (5 mg/kg, IV) prior to oral administration of NKTR-118 (10, 30, or 90 mg/kg), naloxone (1, 3, 10, or 30 mg/kg), or saline. Following IV administration of 5 mg/kg morphine to provide analgesia, test articles were administered. Thirty minutes later, a thermal stimulus was applied to the hind paw. Latency for hind paw withdrawal in the presence of morphine plus saline was used as the measure of full analgesia. The antagonist effect of naloxegol or naloxone was evaluated by measuring the reversal of analgesia, evidenced by recovered sensitivity to the hot plate stimulus.

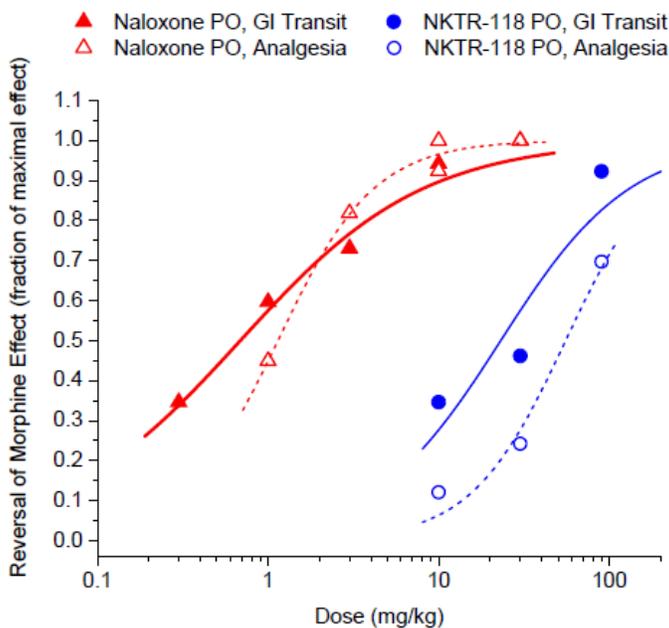
Results and Conclusions: NKTR-118 at doses of 10 and 30 mg/kg produced no significant reversal of analgesia. At 90 mg/kg, partial reversal of analgesia was observed. By contrast, naloxone produced a complete reversal of analgesia at 10 mg/kg, and partial reversal of analgesia was noted at doses starting at 1 mg/kg. NKTR-118 retains the antagonist properties of naloxone, but is significantly less potent than naloxone at reversing morphine analgesia. Overall, the results indicate that PEGylation of naloxone resulted in a significant reduction in antagonism of CNS opioid receptors. The results are shown in the figure below (taken from the Sponsor's study report).

Dose-Response Relationship for Reversal of Morphine Analgesia in Rats



The Sponsor combined the data from different rat studies assessing the central and peripheral pharmacological effects of NKTR-118 to determine the separation between the peripheral gastrointestinal effects and the analgesic central effects. The combined data are shown in the Sponsor’s figure below. It should be noted that the morphine dose was 10 mg/kg IV in the GI transit study, and 5 mg/kg IV in the analgesia study.

Dose-Response Relationships for Reversal of Morphine Effects in the GI Tract and CNS for Naloxone and NKTR-118



As shown in the figure above, comparison of the data from the GI transit and analgesia studies shows that the dose-response curves for naloxone overlap, indicating that there is no separation between the central and peripheral antagonist effects. When the dose-response curves are compared for NKTR-118 inhibition of morphine-induced GI effects and analgesia, there is only a limited separation of the desired antagonism of peripheral GI opioid receptors and the undesired antagonism of CNS opioid receptors in rats. The following table from the Sponsor shows the ED₅₀ values for analgesia and GI transit reversal (taken from the study report).

NKTR-118 and Naloxone Potency in Rats after Oral Administration

	ED ₅₀ for analgesia reversal (mg/kg)	ED ₅₀ for GI transit (mg/kg)	ED ₅₀ analgesia/ED ₅₀ GI
Naloxone	1.14	0.71	1.6
naloxegol	55.4	23.1	2.4

In conclusion, the results from the above studies show that, in the rat model, NKTR-118 antagonizes morphine-induced slowing of GI transit in a dose range that is slightly less than that which antagonizes morphine-induced analgesia. Thus, rats showed only a small dose separation for peripheral and central opioid antagonism effects with orally administered NKTR-118.

4.2 Secondary Pharmacology

NKTR-118: Selectivity Screening in Radioligand Binding, Enzyme and Electrophysiological Assays <i>in vitro</i> (Study No. 1012SY)	
Materials and Methods	Results and Conclusions
NKTR-118 was tested in a panel of 327 <i>in vitro</i> radioligand binding and enzyme activity assays, covering a diverse range of receptors, ion channels, transporters and enzymes. The study was performed at a single concentration of 10 µM NKTR-118 in duplicate to explore its pharmacological profile. NKTR-118 was also tested in a panel of seven cardiac ion channels (hCav1.2/β2/α2δ, hNav1.5, hKv4.3, hKChIP2.2, hKv7.1, hKCNE1, hCav3.2 hKv1.5, hHCN4) using <i>in vitro</i> electrophysiological assays over a range of concentrations. Recordings were made from human recombinant cell lines expressing each channel using automated, plate-based electrophysiology.	NKTR-118 at 10 µM inhibited 98%, 97%, and 90% of the radioligand binding at the opioid mu, kappa, and delta opioid receptors, respectively. NKTR-118 had no significant activity at the rest of the 324 radioligand binding and enzyme assays when tested at a concentration of 10 µM. NKTR-118 also had insignificant effects on the seven cardiac ion channels at up to 100 µM. Therefore, NKTR-118 appears to be specific for opioid receptors.

4.3 Safety Pharmacology

Central Nervous System

NKTR-118: Effects in the Irwin Screen after Single Oral Administration in Male Wistar Rats (Study No. SP-D3820-SPG-2707)	
Materials and Methods	Results and Conclusions
<p>The effects of NKTR-118 on gross behavioral and physiological parameters were assessed using a modified Irwin test in conscious male Han Wistar rats. Animals (6/group) received a single oral dose of NKTR-118 (free base) in sterile water at 0 (vehicle), 100, 300 or 1000 mg/kg. The animals were observed using a standard Irwin screen protocol at 15, 30, 60, 120, 240 and 1440 min after dosing.</p>	<p>There were no NKTR-118-related effects on behavioral scoring for up to 24 hours post-dose. There was a statistically significant decrease in bodyweight gain at 1000 mg/kg. The NOEL for behavioral effects was 1000 mg/kg (corresponding to a measured C_{max} value of 27,800 ng/mL). The effects of NKTR-118 on similar endpoints were also assessed using a functional observational battery (FOB) test during week 4 of dosing in the 28-day rat toxicology study (Study No. LS-2007-011). NKTR-118 had no significant effects at doses up to 500 mg/kg/day. Therefore, NKTR-118 had no significant effects in behavioral scoring. The NOEL for NKTR-118 was 500 mg/kg/day from the 28-day oral toxicity study in rats.</p>

NKTR-118: Effects on the Pentylenetetrazole Convulsion Threshold after Single Oral Administration in Male Wistar Rats (Study No. SP-D3820-SPG-2801)	
Materials and Methods	Results and Conclusions
<p>Potential proconvulsant effects of NKTR-118 were assessed by measuring its potential to decrease the threshold to convulsions induced by pentylenetetrazole (PTZ). Male Han Wistar rats (8 animals/group) were administered a single oral dose of NKTR-118 (free base) in sterile water at 0 (vehicle), 100, 300, or 1000 mg/kg. PTZ was administered via IV infusion at a rate of 13.8 mg/kg/min.</p>	<p>NKT-118 had no statistically significant effect on the threshold dose of PTZ required to induce convulsions at any test dose, indicating that NKTR-118 did not produce proconvulsant effects over the dose range. The NOEL was 1000 mg/kg (the highest dose tested). This corresponds to an estimated C_{max} value of 27,800 ng/mL.</p>

Morphine: Effects in the Mouse Grid Stimulation Analgesia Test After Single Subcutaneous Administration (Study No. SP-SPG-2958)	
Materials and Methods	Results and Conclusions
<p>The effects of single subcutaneous doses of morphine were assessed in the mouse grid stimulation analgesia test using latency to vocalization threshold as the analgesic endpoint. Morphine was administered at 0, 0.96, 3.2, 9.6, or</p>	<p>Morphine caused a dose dependent increase in the latency to vocalization, ranging from 5.3% at 0.96 mg/kg to 86.0% at 32.1 mg/kg. The ED_{50} for morphine-</p>

32.1 mg/kg. Analgesic effects were assessed at 30 min post-dose.	induced analgesia was 5.2 mg/kg.
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NKTR-118: Effects in the Mouse Grid Stimulation Analgesia Test 60 min After Single Oral Administration (Study No. SP-D3820-SPG-2959)	
Materials and Methods	Results and Conclusions
NKTR-118 was assessed for its potential to produce centrally mediated analgesic effects in male NMRI mice in a grid stimulation analgesia model using latency to vocalization threshold as the analgesic endpoint. Animals (8/group) were administered a single oral dose of NKTR-118 (free base) in saline at 0 (vehicle), 30, 100, 300, or 1000 mg/kg. Analgesic effects were assessed at 60 min post-dose.	Increases of less than 11% in latency to vocalization were noted at all NKTR-118 doses tested. Therefore, NKTR-118 at up to 1000 mg/kg, the highest dose tested, did not appear to produce significant analgesic effects.

NKTR-118: Effects in the Mouse Grid Stimulation Analgesia Test 120 min After Single Oral Administration (Study No. SP-D3820-SPG-2995)	
Materials and Methods	Results and Conclusions
NKTR-118 was assessed for its potential to produce centrally mediated analgesic effects in male NMRI mice in a grid stimulation analgesia model using latency to vocalization threshold as the analgesic endpoint. The animals (8/group) were administered a single oral dose of NKTR-118 at 0 (saline), 30, 100, 300, or 1000 mg/kg. Analgesic effects were assessed at 120 min post-dose.	Increases of less than 15% in latency to vocalization were noted at all NKTR-118 doses tested. Therefore, NKTR-118 at up to 1000 mg/kg, the highest dose tested, did not appear to produce significant analgesic effects. The NOEL was considered to 1000 mg/kg. This corresponds to an estimated C_{max} value of 23,125 ng/mL.

Cardiovascular

Effects of NKTR-118 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study No. LS-2008-008)	
Materials and Methods	Results and Conclusions
The <i>in vitro</i> effects of NKTR-118 on ionic currents in voltage-clamped human embryonic kidney cells (HEK293) that stably express the human ether-à-go-go-related gene (hERG) were assessed. Four concentrations of NKTR-118 (10, 30, 100 and 300 μ M) were tested.	NKTR-118 increased hERG potassium current by $16.0 \pm 1.7\%$, $25.9 \pm 0.6\%$, and $7.9 \pm 0.6\%$ at 10 μ M, 30 μ M, and 100 μ M, respectively, and inhibited hERG current by $13.3 \pm 0.4\%$ at 300 μ M versus $0.4 \pm 0.1\%$ in the control. Terfenadine (60 nM), the positive control, inhibited hERG potassium current by $77.0 \pm 2.6\%$. The IC_{50} for the inhibitory effect of NKTR-118 on hERG potassium current could not be calculated since only 13% inhibition occurred at the highest test dose. However, the Sponsor estimated

the IC ₅₀ to be >300 µM.

NKTR-118: The Effects on Cardiac Myocyte Contractility <i>In Vitro</i> (Study No. 2697SV)	
Materials and Methods	Results and Conclusions
The effects of NKTR-118 on contractility parameters (maximum contraction velocity and fraction sarcomere shortening) were assessed by measuring changes in sarcomere length in isolated canine ventricular myocytes. The effects of NKTR-118 were assessed at 0.1, 1, 10 and 100 µM.	Small but statistically significant increases in the maximum contraction velocity (109±3.0%) and fraction sarcomere shortening (108±2.1%) were noted at 0.1 and 10 µM NKTR-118, respectively. However, the changes are apparently not drug-related because they lacked dose-dependency. Therefore, NKTR-118 had no effect on contractility parameters in canine ventricular myocytes <i>in vitro</i> at doses up to 100 µM.

NKTR-118: Effects on Rat Isolated Heart (Study No. 3162SR)	
Materials and Methods	Results and Conclusions
The effects of NKTR-118 on cardiac function, including coronary flow, heart rate, and cardiac contractility were assessed using perfused isolated rat hearts (Langendorff preparation). Cumulative concentration-effect curves were constructed using nominal concentrations of 0.1, 1, 3, and 10 µM, and data were compared to that obtained in the presence of vehicle alone. Verapamil (1 µM) was then added as a positive control.	NKTR-118 at doses of 0.1, 1, 3, and 10 µM had no statistically significant effects on coronary flow or heart rate. There was also no inotropic effect, as there was no change in differential left ventricular pressure (LVP amplitude), mean LVP, dP/dt min, or dP/dt max. In each heart tested, 1 µM verapamil caused increases in coronary flow (up to +47.4%), and decreases in LVP amplitude (-73%), mean LVP (-51%), dP/dt min (-78%), and dP/dt max (-72%) parameters, demonstrating a negative inotropic effect. Therefore, NKTR-118 had no significant effects on cardiac function in isolated rat heart at concentrations up to 10 µM.

NKTR-118: Cardiovascular Effects in Conscious, Telemetered Beagle Dogs following Single Oral Administration (Study No. 1246ZD)

Objective: To evaluate the effects of NKTR-118 on arterial blood pressure, heart rate, left ventricular parameters, lead II electrocardiogram (ECG), body temperature, and clinical signs following oral administration in telemetered conscious beagle dogs.

Methods and Materials : Male beagle dogs (4/group), 15 to 28 months old for the first treatment phase and 18 to 31 months old for the second treatment phase, received on the first dosing occasion a single oral dose of vehicle (water). This was followed by single oral ascending doses of NKTR-118 at 25, 75, and 200 mg/kg at 2 to 5-day dosing intervals in the first dosing phase. In the second dosing phase, the animals received 0 and 5 mg/kg NKTR-118 at 2-day dosing intervals. The design of the study is summarized in the Sponsor's table below.

Design of the Telemetered Beagle Dog Study

Experimental day	Test item	Dose number	Dose levels
			mg/kg
1	Vehicle	1	0
6	NKTR-118	2	25
8	NKTR-118	3	75
13	NKTR-118	4	200
14	NKTR-118	5	200
97 (phase 2)	Vehicle	6	0
99 (phase 2)	NKTR-118	7	5

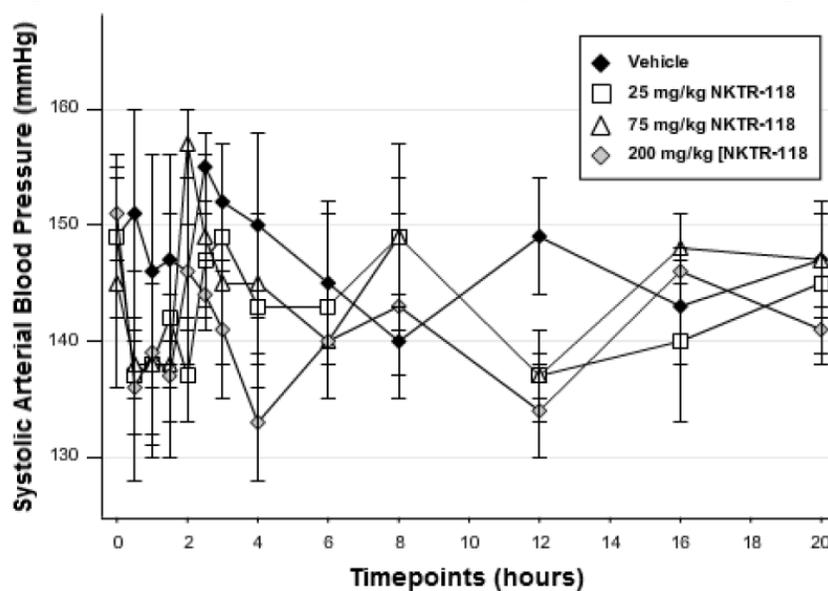
All dose levels and concentrations are nominal and are expressed in terms of the parent form of NKTR-118

Blood pressure, heart rate, left ventricular pressure, lead II ECG, and body temperature were monitored by telemetry for approximately 1 hour pre-dose and up to 20 hours post-dose (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, and 20 hr).

Results and Conclusions: NKTR-118 at 5 mg/kg had no effect on any of the cardiovascular parameters evaluated.

BP (Blood pressure): Decreases in both systolic (SBP) and diastolic blood pressure (DBP), compared to controls, were noted at ≥ 25 mg/kg NKTR-118. Maximum BP decreases in the drug-treated groups ranged from -9 to -12%, and were observed at 0.5 hr post-dose. Recovery occurred between 2 and 6 hr post-dose. In the 200 mg/kg group, further decreases in SBP and DBP (-11% and -15%, respectively), compared to controls, were observed sporadically during the 20 hour post-dose period with a maximum decrease observed at 4 hr. However, interpretation of the data is confounded by the observation that relative decreases in BP after 0.5 hr post-dose were associated with apparently random increases in SBP and DBP in the vehicle control group at 4 and 12 hr. Variability of the BP measurements is illustrated in the figure below (taken from the Sponsor's study report).

Figure 1 Effect of NKTR-118 on systolic arterial blood pressure



LV (left ventricular) Function: In animals receiving ≥ 25 mg/kg NKTR-118, LVSP (left ventricular systolic pressure) decreased by -7 to -13%, indices of cardiac myocardial contractility (LVdP/dt+) decreased by -19 to -22%, and indices of cardiac myocardial relaxation (LVdP/dt-) decreased by -15 to -19%, compared to controls, during the first 2 hours post-dose. There were other sporadic changes during the 20 hour post-dose period. However, these changes were not dose-related and/or occurred at a single time point, and are not considered as drug-related. Recovery generally was observed 4 to 8 hr post-dose. The changes in the left ventricular parameters are shown in the Sponsor's figures below.

Figure 14 Effect of NKTR-118 on left ventricular systolic pressure

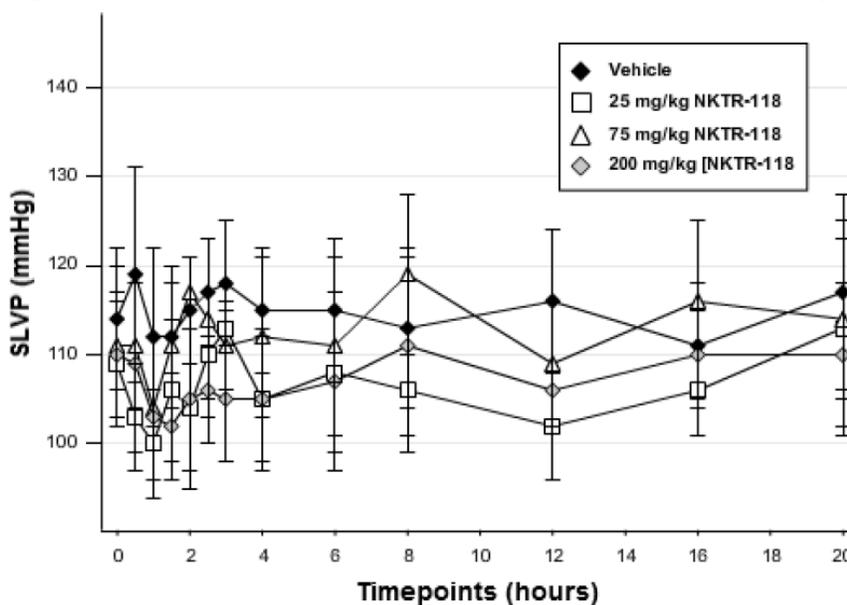
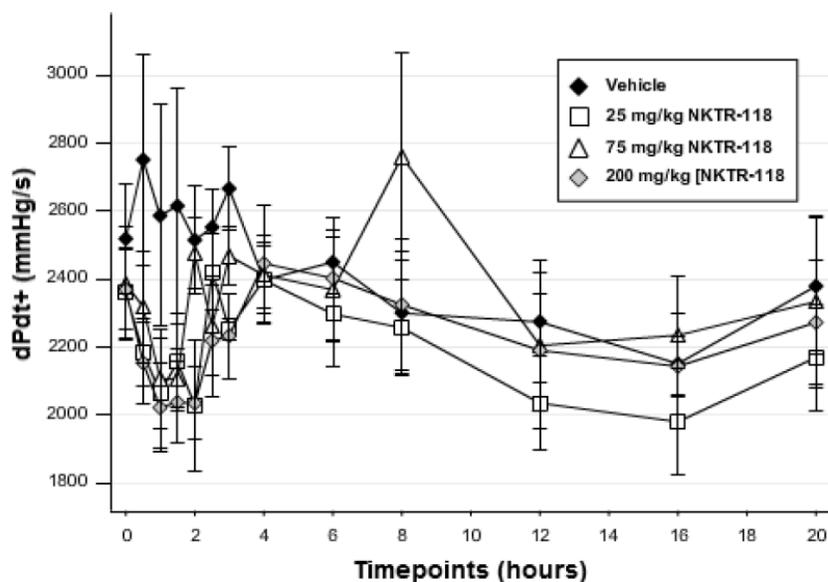
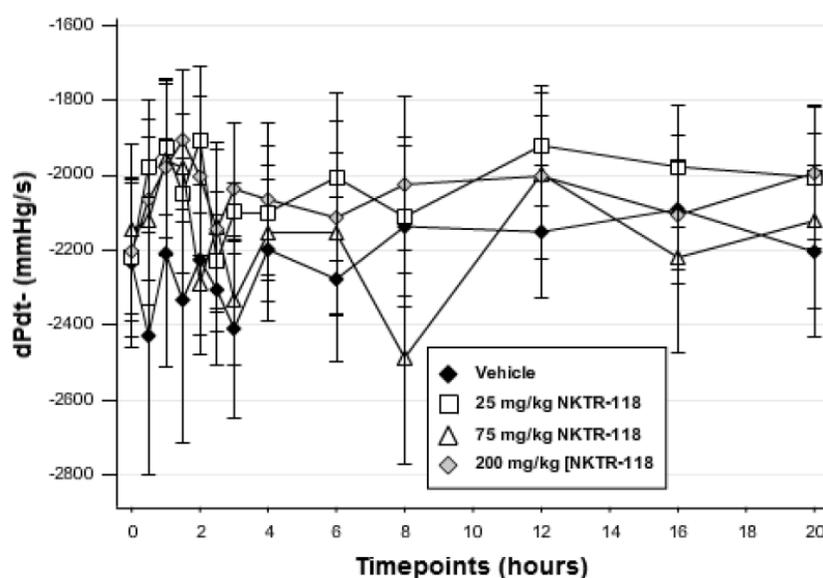
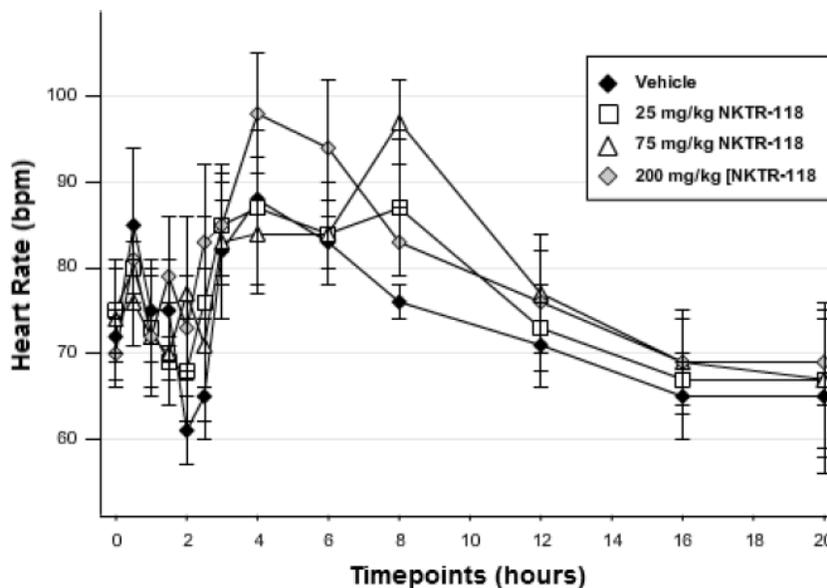


Figure 11 Effect of NKTR-118 on left ventricular dP/dt+**Figure 12** Effect of NKTR-118 on left ventricular dP/dt-

QA Interval: QA interval is the interval between the Q wave and the onset of the aortic blood pressure pulse. It is a measure of cardiac function; an increase in QA interval is correlated with a decrease in cardiac contractility. In the NKTR-118 treated groups, significant increases in QA interval were noted in the 75 and 200 mg/kg groups (up to 8%) between 0.5 and 1.5 hr. There were other sporadic changes during the 20 hour post-dose period. However, these changes were not dose-related and/or occurred at a single time point, and are not considered as drug-related.

HR (heart rate): In the NKTR-118 treated animals, significant increases in HR occurred in the 75 mg/kg group at the 2 and 8 hr time-points (+26% and +28%, respectively), and in the 200 mg/kg group at the 2 and 2.5 hr time-points (+20% and +28%, respectively). It was noted that the increase in HR occurred at time-points different from those when decreases in blood pressure or LV function occurred. Interpretation of the data is confounded by the high variations in HR in the vehicle control group. The changes in HR are shown in the Sponsor's figures below.

Figure 5 Effect of NKTR-118 on heart rate



ECG: In the 75 and 200 mg/kg NKTR-118 groups, increases in QT interval (+9% and +6%, respectively) were noted at 0.5 hr. Increases in QT_cR interval were noted in the 75 mg/kg group at 0.5 hr (+4%; 235±7 ms vs 226±6 ms in controls) and in the 200 mg/kg group at 3 hr (+3%; 240±6 ms vs 232±6 ms in controls), respectively. Other sporadic increases in QT and QT_cR were also noted. QT_cR was calculated as QT+β(HRM-HR) where β was the slope of QT versus HR, and HRM was the mean heart rate. However, these increases in QT_cR were not dose-dependent and/or occurred at a single timepoint, and the changes are not considered as drug-related. The Sponsor reported no other significant drug-related changes in ECG parameters. The changes in QT and QT_cR are shown in the Sponsor's figures below.

Figure 9 Effect of NKTR-118 on ECG QT interval

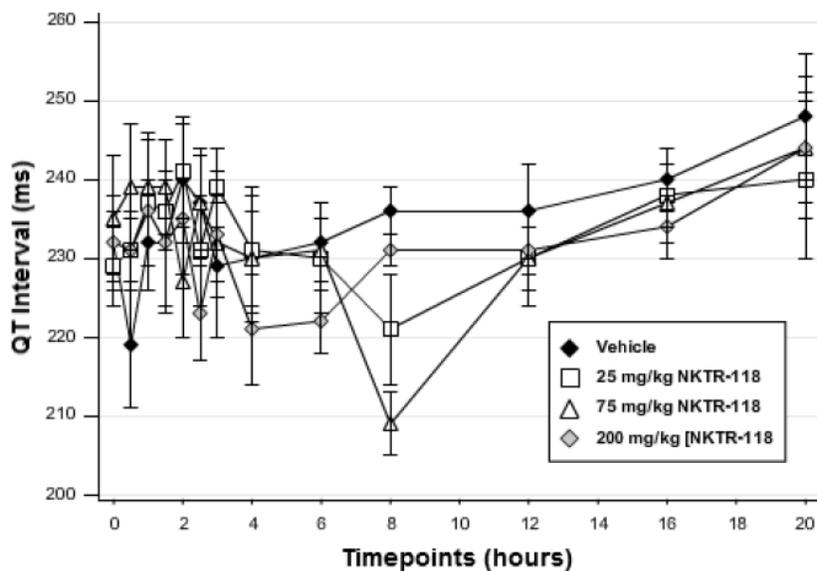
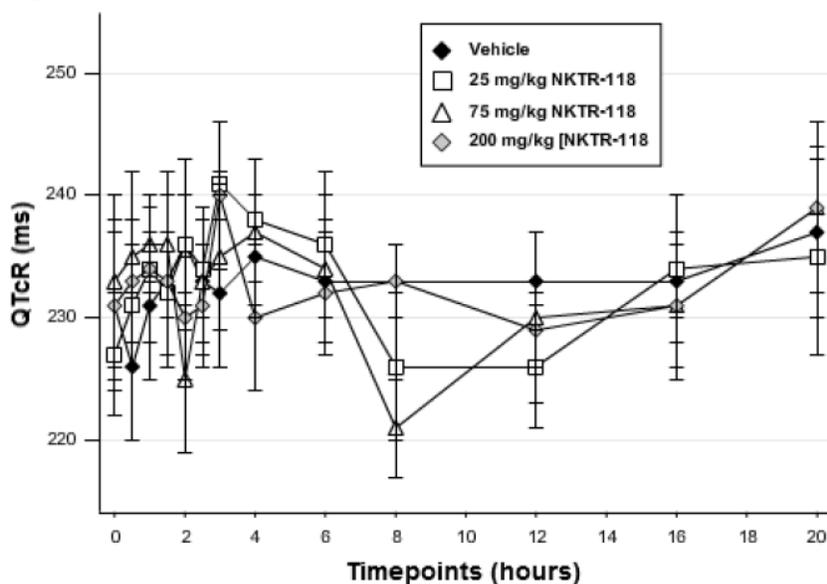


Figure 10 Effect of NKTR-118 on ECG QTcR



BT (Body temperature): There were no meaningful drug-related changes in body temperature.

There were no observable clinical effects, including no effect on food consumption or bodyweight, at any dose.

Plasma NKTR-118 levels are shown in the Sponsor's table below.

Table 4 Total plasma concentrations (mean ± standard deviation)

Dose	NKTR-118			
	5 mg/kg	25 mg/kg	75 mg/kg	200 mg/kg
C _{max} (µmol/L)	0.152 ± 0.219	0.829 ± 0.378	3.89 ± 1.98	11.4 ± 7.61
T _{max} (hours)	0.25	0.25	0.25	0.25

In summary, oral doses of NKTR-118 >5 mg/kg were associated with moderate decreases in arterial blood pressure, left ventricular systolic pressure, and indices of cardiac contractility and relaxation, compared to controls. The NOEL was considered to be 5 mg/kg. This dose corresponds to a measured C_{max} of 99 ng/ml (MW of NKTR-118 = 652), which is very close to the measured human C_{max} of 80 ng/ml at the proposed clinical dose of 25 mg per day.

Respiratory System

NKTR-118: Respiratory Effects in the Han Wistar Rat following Single Oral Administration (Study No. 3052SR)	
Materials and Methods	Results and Conclusions
The effects of NKTR-118 on respiratory parameters, including respiratory rate, tidal volume, minute volume, inspiration and expiration times, and peak inspiratory and expiratory flows were assessed in male Han Wistar rats using whole body plethysmography. Animals (8/group) were treated with a single oral dose of NKTR-118 (free base) in water, at 0 (vehicle), 100, 300, or 1000 mg/kg. Animals (8/group) in the positive control group were treated with 10 mg/kg theophylline. The animals were placed in whole body plethysmography chambers and ventilatory parameters were recorded pre-dose and for up to approximately 4 hours after dosing. Blood samples were taken at approximately 4 hours 20 minutes post-dose to confirm exposure levels of NKTR-118.	NKTR-118 at doses up to 1000 mg/kg had no significant effect on respiratory rate, tidal volume, minute volume, inspiration and expiration times, or peak inspiratory and expiratory flows. Theophylline (10 mg/kg) induced significant increases in respiration rate, minute volume, peak inspiratory and expiratory flows, and decreases in inspiration and expiration times, indicating that the recording conditions were optimal. The NOEL for respiratory function was considered to be 1000 mg/kg, the highest dose tested. This dose corresponds to an estimated C _{max} value of 27,800 ng/mL. Therefore, NKTR-118 at up to 1000 mg/kg had no significant effects on any of the respiratory parameters measured.

Gastrointestinal System

NKTR-118: Evaluation of Effects on Gastric Emptying and Intestinal Propulsion of a Semisolid Meal Following Single Oral Administration to Male Wistar Rats (Study No. SP-D3820-SPG-2745)

Objective: To assess the effects of NKTR-118 on gastric emptying and small intestinal propulsion of a semisolid charcoal meal in male Wistar rats.

Materials and Methods: Male Wistar rats (8/group) were treated orally with 0, 100, 300, or 1000 mg/kg NKTR-118. A meal containing charcoal as a non-absorbable marker was administered 60 minutes later. Fifteen minutes after administration of the charcoal meal, the rats were anesthetized and the stomach and small intestine were removed. The stomach with contents was weighed to estimate gastric emptying and the extension of the charcoal into the intestine was measured to obtain transport distance.

Results and Conclusions: NKTR-118 at ≥ 100 mg/kg produced significant increase in stomach weight, which ranged from 28 to 78% higher than the control stomach weight. NKTR-118 did not significantly affect intestinal transport distance at 100 or 300 mg/kg. Animals in the 1000 mg/kg group had 0% intestinal transport, and this group was excluded from statistical analysis. A NOEL could not be established for gastric emptying. The NOEL for intestinal transport was considered to be 300 mg/kg. The gastric emptying and intestinal transport data are shown in the Sponsor's table below.

Effects of NKTR-118 on Gastric Emptying and Intestinal Transport

NKTR-118 dose	Weight of stomach g \pmSEM	Intestinal transport distance % \pmSEM
Vehicle alone	3.55 \pm 0.1	54.1 \pm 2.2
100 mg/kg	4.54 \pm 0.2***	55.7 \pm 2.4
300 mg/kg	4.53 \pm 0.2***	53.8 \pm 2.7
1000 mg/kg	6.33 \pm 0.3***	0.0 \pm 0.0 ^a

Significance Level * P<0.05; ** P<0.01; *** P<0.001 compared to vehicle control.

^a Excluded from statistical analysis

NKTR-118: Evaluation of Effects on Gastric Emptying and Intestinal Propulsion of a Semisolid Charcoal Meal Following Single Oral Administration to Male Wistar Rats (Study No. SP-D3820-SPG-2883)

Objective: To assess the effects of NKTR-118 on gastric emptying and small intestinal propulsion of a semisolid charcoal meal in male Wistar rats.

Materials and Methods: Male Wistar rats (8/group) were treated orally with 0, 10, 30, or 100 mg/kg NKTR-118. A meal containing charcoal as a non-absorbable marker was administered 60 minutes later. Fifteen minutes after administration of the charcoal meal, the rats were anesthetized and the stomach and small intestine were removed. The stomach with contents was weighed to estimate gastric emptying and the extension of the charcoal into the intestine was measured to obtain intestinal propulsion distance.

Results and Conclusions: Stomach weight in the 100 mg/kg NKTR-118 group increased significantly by 15.3%. There were no effects on intestinal transit at any dose tested. The NOEL for gastric emptying was considered to be 30 mg/kg, and the NOEL for intestinal transport was considered to be >100 mg/kg. The gastric emptying and intestinal transport data are shown in the Sponsor's table below.

Effects of NKTR-118 on Gastric Emptying and Intestinal Transport

NKTR-118 dose	Weight of stomach g ±SEM	Intestinal transport distance % ±SEM
Vehicle alone	3.66±0.14	56.5±2.6
10 mg/kg	3.50 ±0.19	58.2 ±2.0
30 mg/kg	3.62 ±0.06	58.7 ±1.6
100 mg/kg	4.22 ±0.13*	57.5±1.3

Significance Level * P<0.05 compared to vehicle control.

Taken together, the above 2 studies show that the NOEL for decreased gastric emptying and decreased intestinal transport is 30 mg/kg and 300 mg/kg NKTR-118, respectively. These doses correspond to estimated C_{max} values of 1220 and 9000 ng/mL, respectively.

Renal System

NKTR-118: Effects on Water and Electrolyte Excretion after Single Oral Administration to Male Wistar Rats (SP-D3820-SPG-2854)

Objective: To investigate the effects of a single oral administration of NKTR-118 on water and electrolyte excretion in male Wistar rats.

Methods: Male Wistar rats (9/group) were administered orally a single dose of NKTR-118 at 0 (vehicle), 100, 300 or 1000 mg/kg. The animals were then placed individually in metabolic cages for 24 hr where they had access to tap water *ad libitum* but no food. Urine was collected and water consumption measured at 0 to 4 hr and 4 to 24 hr. Blood samples for clinical chemistry were taken pre- and post-test. Effects on renal excretion of water, electrolytes, creatinine, protein, and glucose were assessed at 0 to 4 and 4 to

24 hr post-dose, and plasma clinical chemistry analysis was performed. Positive control animals (9/group) received 30 mg/kg furosemide.

Results and Conclusions: NKTR-118 induced transient, dose-related increases in the urinary excretion of sodium (510%), potassium (190%), chloride (310%), and albumin (200%). At 1000 mg/kg, an increase in the excretion of phosphate (200%) and a delayed decrease in the excretion of calcium (-60%) was observed. The total (0 to 24 hr) absolute electrolyte excretions were not significantly affected. No clear drug-induced changes were seen in the plasma concentrations of solutes. The effects at doses up to 300 mg/kg were small, and not considered to be adverse. Therefore, the NOAEL is considered to be 300 mg/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Absorption

In Vitro Permeability of NKTR-118 (Study No. RD00001771.00)

Methods: The apparent permeability of NKTR-118 and naloxone was measured *in vitro* using a bi-directional permeability assay in Caco-2 cells. To determine whether active transport contributes to the permeability of NKTR-118, efflux ratios were calculated as the ratio of the basolateral to apical (B to A) versus the apical to basolateral (A to B) permeability. The efflux ratio reflects an asymmetry in the permeabilities in the A to B versus B to A direction. To evaluate the involvement of efflux transporters in altering permeability, additional experiments were conducted in the presence and absence of inhibitors of efflux transporters (cyclosporine A (10 μ M), verapamil (100 μ M), and elacridar (0.5 μ M)).

Results and Conclusions: NKTR-118 is a conjugate of naloxone and PEG (7 ethylene glycol units). The A to B and B to A permeability of NKTR-118 was 38- and 3-fold lower, respectively, than the corresponding permeability values for naloxone. The greater reduction in the A to B permeability compared to the B to A permeability of NKTR-118 suggested that factors in addition to the reduction in passive permeability may contribute to the reduced permeability of NKTR-118 compared to naloxone. In contrast, permeability of naloxone was equal in the A to B and B to A directions. The calculated efflux ratios for NKTR-118 and naloxone were 15.4 ± 5.5 and 0.91 ± 0.1 , respectively. An efflux ratio of greater than 2 indicates the involvement of an active efflux process. In the presence of the efflux transporter inhibitors cyclosporine, verapamil, and elacridar, efflux ratio for NKTR-118 was reduced from 15 to 1.0, 1.3, and 1.1, respectively. In contrast, efflux ratio for naloxone remained unchanged in the presence of the transporter inhibitors. The results suggested that NKTR-118 is a substrate for an apically directed efflux transporter(s). It was noted that in the presence of efflux inhibitors, the permeability of NKTR-118 was still approximately 14-fold lower

than that of naloxone. This suggested that the passive permeability of NKTR-118 was substantially reduced compared to that of naloxone. Taken together, the results suggested that, compared to naloxone, NKTR-118 has a reduced permeability in Caco-2 cells, resulting from a combination of reduced passive permeability and active efflux that is mediated by efflux transporters.

Permeability Evaluation of NKTR-118 (Study No. RD00002033.00)

Methods: The permeability of NKTR-118 was determined in rats using an *in situ* single-pass jejunal segment perfusion method. NKTR-118 was perfused at 0.02, 0.2, or 2.0 mg/ml, and metoprolol tartrate was used as a high permeability reference standard. Measurements of drug concentrations in the perfusate were used to calculate the effective permeability.

Results and Conclusions: The intestinal permeability of NKTR-118 in the *in situ* jejunal perfusion model in rats was approximately 40% of the permeability of metoprolol. Thus, under the study conditions, NKTR-118 exhibited moderate intestinal permeability. The results are summarized in the Sponsor's table below.

Table 4 Naloxegol intestinal permeability determined in an *in situ* jejunal perfusion model in the rat

Naloxegol perfusion concentration (mg/ml)	Naloxegol P_{eff} Mean \pm SD (10^{-4} cm/sec)	Metoprolol* P_{eff} Mean \pm SD (10^{-4} cm/sec)	Naloxegol/Metoprolol Permeability Ratio
2.0	0.143 \pm 0.312	0.232 \pm 0.323	0.420
0.2	0.105 \pm 0.180	0.303 \pm 0.120	0.319
0.02	0.065 \pm 0.089	0.166 \pm 0.076	0.471

* Metoprolol perfusion concentration 0.068 mg/ml

Pharmacokinetic Interpretation of Single IV and PO PEG7-Naloxol in Beagle Dogs (LS-2005-030); Pharmacokinetic Study of Intravenous and Orally Administered NKTR-118 (PEG-Naloxol) in Monkeys (Study No. LS-2007-037); The Disposition of [14 C]NKTR-118 in the Dog Following Oral Administration (Study No. 8259274); NKTR-118: Single Dose Intravenous Toxicity Study in the Rat (Study No. 3504LR)

Pharmacokinetic parameters for NKTR-118 were evaluated in a series of single dose pharmacokinetic studies (Study Nos. 3504LR, 8259274, LS-2005-30, LS-2007-37). The results are summarized in the Sponsor's table below.

Table 5 Mean pharmacokinetic parameters (SD) for naloxegol from single dose studies

Species, sex, strain,	Intravenous				Oral					
	Dose (mg/kg)	V _{ss} (L/kg)	Cl (L/h/kg)	t _{1/2} (h)	Dose (mg/kg)	C _{max} (ng/mL)	t _{max} (h)	AUC (µg.h/mL)	F (%)	t _{1/2} (h)
Rat, M Sprague Dawley	10, 30 & 100 ¹	3.14 (0.78)	2.65 (0.56)	2.92 (1.58)	-	-	-	-	-	-
Dog, M&F Beagle	0.4 ³	4.66* (2.08)	2.65 (0.65)	5.7 (0.8)	50 ²	1530 (218)	0.5 (0.2)	3740 (802)	20.6 [†]	9.58 (2.85)
Monkey, M Cynomolgus	1.0 ⁴	2.44 (0.7)	1.46 (0.23)	4.99 (2.19)	5 ⁴	41.6 (9.41)	0.69 (0.53)	78.1 (22.9)	2.26 (0.70)	2.77 (3.20)

1 Study 3504LR n=8 pooled across doses

2 Study 8259274 n=4 F dogs

3 Study LS-2005-30 n=6 M and F

4 Study LS-2007-37 n=3

* Recalculated from data within the study

† Cross-study value calculated for table, no SD available

The results showed that across species the volume of distribution ranges from 2.44 to 4.66 L/kg, consistent with extensive distribution of NKTR-118. The values for clearance ranged from 1.46 in monkey to 2.65 L/hr/kg in both rat and dog, resulting in half-lives of approximately 3 to 6 hours. Bioavailability ranged from 20% in dogs to 2% in monkeys after oral administration

5.1.2 Distribution

Plasma Protein Binding of NKTR-118 in Human, Rat, Mouse, Dog and Monkey Plasma (Study No. LS-2007-024)

Methods: The binding of NKTR-118 to plasma proteins from mouse, rat, dog, monkey, and human was studied *in vitro* by equilibrium dialysis and LC-MS/MS.

Results and Conclusions: In all species examined, the extent of binding was low and independent of concentration, except in dogs where the binding was reduced at higher concentrations. The Sponsor noted that the plasma protein binding of NKTR-118 was approximately 2-fold lower than that of naloxone in the same experiments. The results are summarized in the Sponsor's table below.

Table 8 Plasma protein binding of naloxexol in animals and human

Species	Concentrations ^a	% Bound ^b
Mouse (M CD1)	1.5, 15, 150 μ M	14.1
Rat (M Sprague Dawley)	1.5, 15, 150 μ M	20.8
Dog (M Beagle)	1.5, 15, 150 μ M	53.3, 29.5, 3.8 ^c
Monkey (M Cynomolgus)	1.5, 15, 150 μ M	9.7
Human (M&F Mixed)	1.5, 15, 150 μ M	4.2 ^d

^a concentrations equivalent to 978, 9780 and 97800 ng/mL

^b mean value from 1.5, 15 and 150 μ M

^c concentration dependent binding observed

^d mean value from 15 and 150 μ M

Comparison of Blood-Brain Permeability of Naloxone and Several PEGylated Conjugates using a Rat *in situ* Brain Perfusion Technique (Study No. RD00001811.00)

Methods: Uptake of naloxone and PEGylated analogues of naloxone into the brain was determined using a single time-point rat brain perfusion model. Test articles (20 μ M) were infused into the left carotid artery for 30 seconds and the brain was removed at 1 minute. The left central hemisphere was homogenized and analyzed for drug by LC-MS/MS. Atenolol and antipyrine were included in the infusion as markers of low and high permeation, respectively.

Results and Conclusions: Naloxone showed a high brain uptake of 60.2 ± 13.7 pmol/g brain/sec, which was twice as fast as antipyrine, the high permeation standard. NKTR-118 showed a much lower rate of uptake into the brain (4.1 ± 1.4 pmol/g brain/sec), as did an analogue with 5 PEG units; however, the analogue with 3 PEG units showed faster uptake (17.95 pmol/g brain/sec). The NKTR-118 uptake rate was comparable to that of atenolol, a marker of low permeability. Therefore, CNS penetration of NKTR-118 was approximately 15 times slower than that of naloxone in this *in situ* uptake model. The results are summarized in the table below (taken from the study report).

Table 2. Brain Uptake rates of Naloxone, NKTR-118, and Permeation Standards in Rats.

Drug	Mean Brain Uptake Rate (pmole/gm brain/sec)
Naloxone	60.2±13.7
NKTR-118	4.1±1.4
Antipyrine (high permeation standard)	28.2±14.3
Atenolol (low permeation standard)	5.2±2.2

NKTR-118: The Tissue Distribution of Total Radioactivity in the Rat Following Oral Administration of [¹⁴C]NKTR-118 (Quantitative Whole Body Autoradiography) (Study No. 192601)

Methods: The distribution of radioactivity into tissues following oral administration of [¹⁴C]NKTR-118 (50 mg/kg) was investigated by quantitative whole body autoradiography (QWBA) in 7 male pigmented Lister Hooded rats and 5 time-mated female Sprague Dawley rats (1 animal/time-point).

Results and Conclusions: The data are summarized in the Sponsor's tables below.

Table 3 Male pigmented rats, single oral administration of [¹⁴C]NKTR-118 (50 mg/kg): Tissue concentrations of total radioactivity

Sample	Expressed as µg equiv/g						
	001M (0.5 h)	002M (1 h)	003M (4 h)	004M (24 h)	005M (48 h)	006M (168 h)	007M (504 h)
Adrenal cortex	17.92	16.66	20.02	2.23	0.26	ND	ND
Adrenal medulla	13.93	10.89	10.08	1.11	0.27	ND	ND
Adrenal (whole)	17.28	15.42	18.36	1.72	0.28	ND	ND
Bladder wall	4.36	6.93	3.09	ND	ND	ND	ND
Blood	3.03	4.11	2.14	ND	ND	ND	ND
Bone marrow	11.11	10.72	5.36	0.28	ND	ND	ND
Brain	0.10 ^a	0.21	0.14	ND	ND	ND	ND
Brown fat	2.95	3.14	1.46	0.30	ND	ND	ND
Epididymis	2.26	5.91	15.41	0.80	ND	ND	ND
Eye	6.99	7.16	41.40	36.95	9.39	28.44	23.85
Harderian gland	12.15	15.16	8.34	ND	ND	ND	ND
Heart	3.62	5.18	2.45	ND	ND	ND	ND
Kidney cortex	32.51	27.29	28.60	1.66	1.14	0.26	ND
Kidney medulla	30.63	26.84	19.18	0.77	1.15	0.07	ND
Kidney (whole)	31.56	27.44	27.56	1.44	1.13	0.23	ND
Lachrymal gland	12.93	13.75	NP	ND	ND	ND	ND
Large intestine wall	8.83	14.09	12.09	ND	ND	ND	ND
Liver	43.40	66.49	58.74	4.61	7.74	1.03	ND
Lung	4.18	5.64	3.09	ND	ND	ND	ND
Lymph node	11.12	12.63	5.76	ND	ND	ND	ND
Pancreas	18.05	12.16	5.40	ND	ND	ND	ND
Pineal body	14.21	13.76	NP	ND	ND	ND	ND
Pituitary gland	18.38	18.94	13.85	0.71	ND	ND	ND
Preputial gland	12.29	28.34	25.32	3.03	ND	ND	ND
Prostate	4.89	6.56	4.49	ND	ND	ND	ND
Rectum wall	16.88	21.12	6.70	ND	ND	ND	ND
Salivary gland	28.59	16.18	9.51	0.30	ND	ND	ND

Table 3 Male pigmented rats, single oral administration of [¹⁴C]NKTR-118 (50 mg/kg): Tissue concentrations of total radioactivity

Sample	Expressed as µg equiv/g						
	001M (0.5 h)	002M (1 h)	003M (4 h)	004M (24 h)	005M (48 h)	006M (168 h)	007M (504 h)
Seminal vesicles	3.96	9.99	8.54	ND	ND	ND	ND
Skeletal muscle	3.87	4.24	2.12	ND	ND	ND	ND
Skin – albino	1.54	3.30	3.16	0.49	ND	ND	ND
Skin - pigmented	1.80	4.36	6.20	7.88	3.92	11.79	2.11
Small intestine wall	9.59	13.85	31.12	ND	ND	ND	ND
Spinal cord	0.06	0.15	0.02	ND	ND	ND	ND
Spleen	17.57	15.76	7.69	0.35	ND	ND	ND
Stomach wall	9.03	17.07	15.46	ND	0.48	ND	ND
Stomach wall - secretory	20.79	11.63	12.05	ND	ND	ND	ND
Testes	0.84	1.44	7.15	1.57	ND	ND	ND
Thymus	4.30	8.47	5.58	ND	ND	ND	ND
Thyroid gland	14.30	17.83	16.05	2.27	1.26	ND	ND
Uveal tract	44.53	46.94	156.57	101.82	47.44	70.10	43.05
White fat	0.39	0.25	0.29	ND	ND	ND	ND
Tissue	NA	NA	NA	0.14	0.10	0.04	0.02 ^a
Limit of reliable measurement:	0.16	0.06	0.09	0.06	0.09	0.16	0.09

NA Not applicable

ND Not distinguishable

NP Not present

^a Below the limit of reliable measurement

Table 4 Female time-mated rats, single oral administration of [¹⁴C]NKTR-118 (50 mg/kg): Tissue concentrations of total radioactivity

Sample	Expressed as µg equiv/g				
	008F (0.5 h)	009F (1 h)	010F (4 h)	011F (24 h)	012F (48 h)
Adrenal cortex	69.14	51.63	85.21	38.32	8.09
Adrenal medulla	56.34	36.41	57.24	NP	0.93
Adrenal (whole)	67.60	48.96	80.93	NP	7.50
Amniotic fluid	0.10a	0.21	0.24	ND	ND
Bladder wall	16.14	12.30	9.47	ND	ND
Blood	12.38	7.80	6.92	ND	ND
Bone marrow	38.74	29.84	24.40	ND	ND
Brain	0.34	0.15	0.28	ND	ND
Brown fat	5.48	4.71	9.02	ND	ND
Eye	2.46	6.02	4.17	0.77	0.23
Harderian gland	44.80	41.66	44.15	0.69	0.21
Heart	18.44	10.96	11.34	ND	ND
Kidney cortex	72.14	54.42	50.72	1.46	0.85
Kidney medulla	58.83	42.04	36.99	0.62	0.18
Kidney (whole)	66.96	52.29	49.34	1.13	0.66
Lachrymal gland	97.23	143.38	120.29	1.72	ND
Large intestine wall	23.61	25.20	37.45	ND	ND
Liver	68.67	56.01	58.78	4.42	2.68
Lung	16.36	11.01	10.60	ND	ND
Lymph node	34.10	27.50	30.28	ND	ND
Ovary	6.77	7.47	3.51	0.27	ND
Pancreas	36.56	26.07	20.84	ND	ND
Pineal body	73.72	36.52	NP	ND	ND
Pituitary gland	47.63	37.05	33.71	ND	ND
Placenta	21.09	12.89	14.78	0.16	0.06 ^a
Preputial gland	70.94	46.81	49.47	ND	ND
Rectum wall	31.29	18.76	19.20	ND	ND
Salivary gland	62.25	45.84	68.19	0.60	ND
Skeletal muscle	16.38	11.96	10.03	ND	ND
Skin	3.41	4.18	5.37	0.29	0.14

Table 4 Female time-mated rats, single oral administration of [¹⁴C]NKTR-118 (50 mg/kg): Tissue concentrations of total radioactivity

Sample	Expressed as µg equiv/g				
	008F (0.5 h)	009F (1 h)	010F (4 h)	011F (24 h)	012F (48 h)
Small intestine wall	42.26	28.98	35.71	ND	ND
Spinal cord	0.24	0.10	0.12	ND	ND
Spleen	95.80	63.00	56.16	0.85	0.25
Stomach wall	30.06	28.52	27.78	ND	4.01
Stomach wall - secretory	55.92	50.35	37.03	ND	0.38
Thymus	21.35	21.79	32.04	ND	ND
Thyroid gland.	52.89	29.65	45.39	ND	0.88
Uterus wall	31.19	82.21	185.03	19.04	4.00
Uveal tract	15.34	27.24	17.42	2.94	1.16
White fat	0.70	0.27	0.49	ND	ND
Foetus (whole)	3.10	1.99	2.23	0.17	0.06 ^a
Foetal brain	0.47	0.40	0.41	ND	ND
Foetal eye	3.23	2.17	3.92	ND	ND
Foetal liver	5.28	2.61	3.36	ND	ND
Foetal spinal cord	0.53	0.39	0.53	ND	ND
Foetal uveal	4.95	3.53	5.41	ND	ND
Tissue	NA	NA	NA	0.14	0.04 ^a
Limit of reliable measurement:	0.10	0.07	0.10	0.11	0.06

NA Not applicable

ND Not distinguishable

NP Not present

^a Below the limit of reliable measurement

In males, radioactivity was widely distributed to all tissues, with the high concentrations found in uveal tract, liver, kidney, and glandular tissues. However, the radioactivity was not quantifiable at 48 hours in most tissues. In pigmented tissues (skin, eye, and uveal tract), radioactivity concentrations persisted out to 504 hours. This indicated that NKTR-118-related radioactivity likely binds to melanin present in these tissues.

In albino females, radioactivity was widely distributed to all tissues including liver, kidney, small intestine wall, placenta and uterus. By 48 hours, the concentrations of radioactivity were low in the majority of tissues.

A sex difference was observed in the distribution of NKTR-118-related radioactivity; female rats showed an earlier maximum concentration (0.5 hr) in the majority of tissues and higher concentrations than the males. In both sexes, the elimination of radioactivity was rapid, with the levels of radioactivity below the limits of reliable measurement in most tissues by 24 hours post-dose.

In both males and females, distribution of radioactivity in the brain and spinal cord was low, indicating relatively low CNS penetration of NKTR-118 in rats. The data also demonstrated fetal exposure following a single oral dose of NKTR-118. Radioactivity levels in the fetal brain were lower than that in the liver or uveal tract. The radioactivity in fetal liver and fetal uvea was lower compared to that in the mother. These data demonstrated trans-placental transfer of NKTR-118-related radioactivity in rats.

Tissues with significant distribution of radioactivity are summarized in the Sponsor's table below.

Table 10 Concentrations of radioactivity in selected tissues after administration of [¹⁴C]-naloxegol (20mg/kg) to male and female rats

Tissues (M/F)	Concentration (µg equiv/g)					
	0.5h	1h	4h	24h	48h	168h
Blood (M)	3.03	4.11	2.14	ND	ND	ND
Pituitary gland (M)	18.38	18.94	13.85	0.71	ND	ND
Preputial gland (M)	12.29	28.34	25.32	3.03	ND	ND
Pigmented skin (M)	1.80	4.36	6.20	7.88	3.92	11.79
Uveal tract (M)	44.53	46.94	156.57	101.82	47.44	70.10
Blood (F)	12.38	7.80	6.92	ND	ND	NS
Brain (F)	0.34	0.15	0.28	ND	ND	NS
Kidney (F)	66.96	52.29	49.34	1.13	0.66	NS
Liver (F)	68.67	56.01	58.78	4.42	2.68	NS
Placenta (F)	21.09	12.89	14.78	0.16	BLQ	NS
Small intestine wall (F)	42.26	28.96	35.71	ND	ND	NS
Uterus (F)	31.19	82.21	185.03	19.04	4.00	NS
Fetus (whole) (F)	3.10	1.99	2.23	0.17	BLQ	NS

BLQ Below the limit of reliable quantitation
 ND Not distinguishable
 NS No sample at this time

5.1.3 Metabolism

Cross-Species Comparison of Metabolic Profiles of NKTR-118 in Human, Dog, Monkey, Rat, and Mouse Hepatocytes (Study No. RD00001767.00)

Methods: NKTR-118 or naloxone (10 or 50 μ M) was incubated with human, monkey, dog, rat, or mouse hepatocytes for up to 2 hours. Aliquots of hepatocyte media were removed at 0.5, 1, or 2 hours after the beginning of incubation and subjected to analysis of metabolites.

Results and Conclusions: Comparison of the estimated hepatic intrinsic clearance values showed that hepatic metabolism of NKTR-118 in humans is 6- to 12-fold slower than in the studied animal species. Eleven metabolites were identified and their abundance relative to the remaining NKTR-118 was determined. These results showed the similarity of the metabolic pathways across species, with all metabolites detected in human hepatocyte incubations also present in all animal species. The metabolites identified were designated as M1, M2, M4, M6, M7, M13, M25, M26, a component which is possibly M24, and two other metabolites. The results are summarized in the Sponsor's table below.

Metabolite Profile comparison

Metabolite	Relative abundance of metabolite as % naloxegol peak by MS				
	Mouse	Rat	Dog	Monkey	Human
M1	15.07	31.25	3.91	27.29	21.83
M6	17.32	11.81	1.83	13.52	17.37
M7	8.95	6.46	3.11	5.76	16.43
(M+16) possibly M24*	5.43	3.37	0.91	2.94	11.17
M13	5.66	3.62	0.19	3.35	13.49
M50	1.5	1.92	0.83	3.71	8.27
M4	5.28	2.91	0.76	23.80	BLD
M49	11.94	7.12	3.63	19.18	10.47
M2	28.86	31.53	70.77	1.52	0.97
M26	BLD	BLD	2.49	BLD	BLD
M25	BLD	BLD	11.55	BLD	BLD

BLD = Below the limit of detection

* position of hydroxylation not determined in this study nor in later AZ metabolism profiling

In mouse and rat hepatocytes, NKTR-118 was mainly metabolized by glucuronidation, N-dealkylation, and PEG-chain shortening by one ethylene glycol sub-unit. In dog hepatocytes, NKTR-118 was mainly metabolized by glucuronidation. In monkey hepatocytes, NKTR-118 was mainly metabolized by N-dealkylation, demethylation, and oxidation of the PEG-chain. In human hepatocytes, the major metabolites were the result of shortening of the PEG-chain with the removal of one, two, or three ethylene glycol sub-units, as well as N-dealkylated and oxidized metabolites. Naloxol and naloxone were not observed in the hepatocyte incubations. Two metabolites detected

in the hepatocyte incubations (an N-glucuronide and a glutathione adduct) were not observed in subsequent metabolism studies, and were not included in the metabolite maps presented. Thus, NKTR-118 metabolic pathways are similar across mouse, rat, dog, monkey, and human hepatocytes. All metabolites that were observed in human hepatocytes were also observed in all animal species studied.

The major metabolic processes observed are summarized in the Sponsor's schematic below.

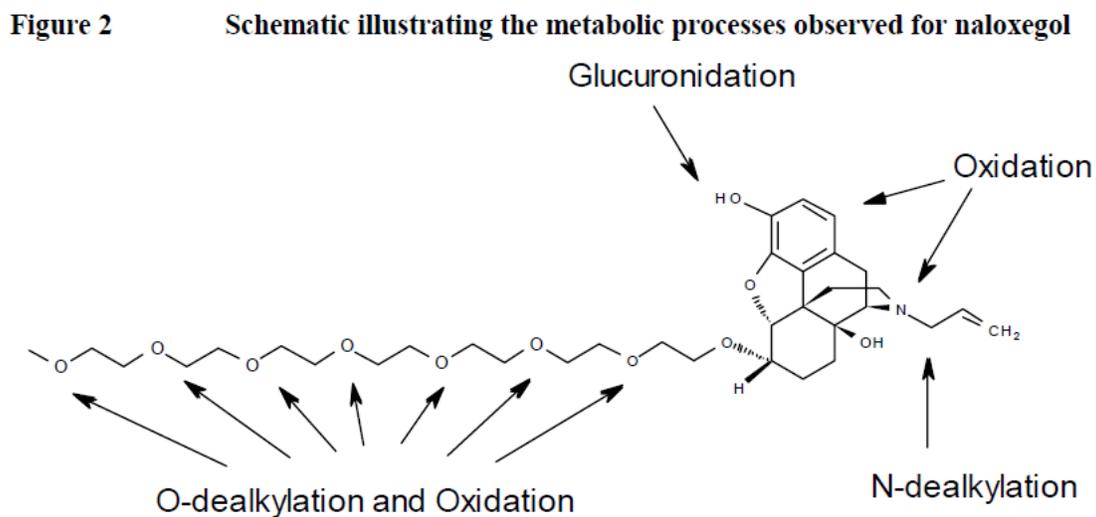
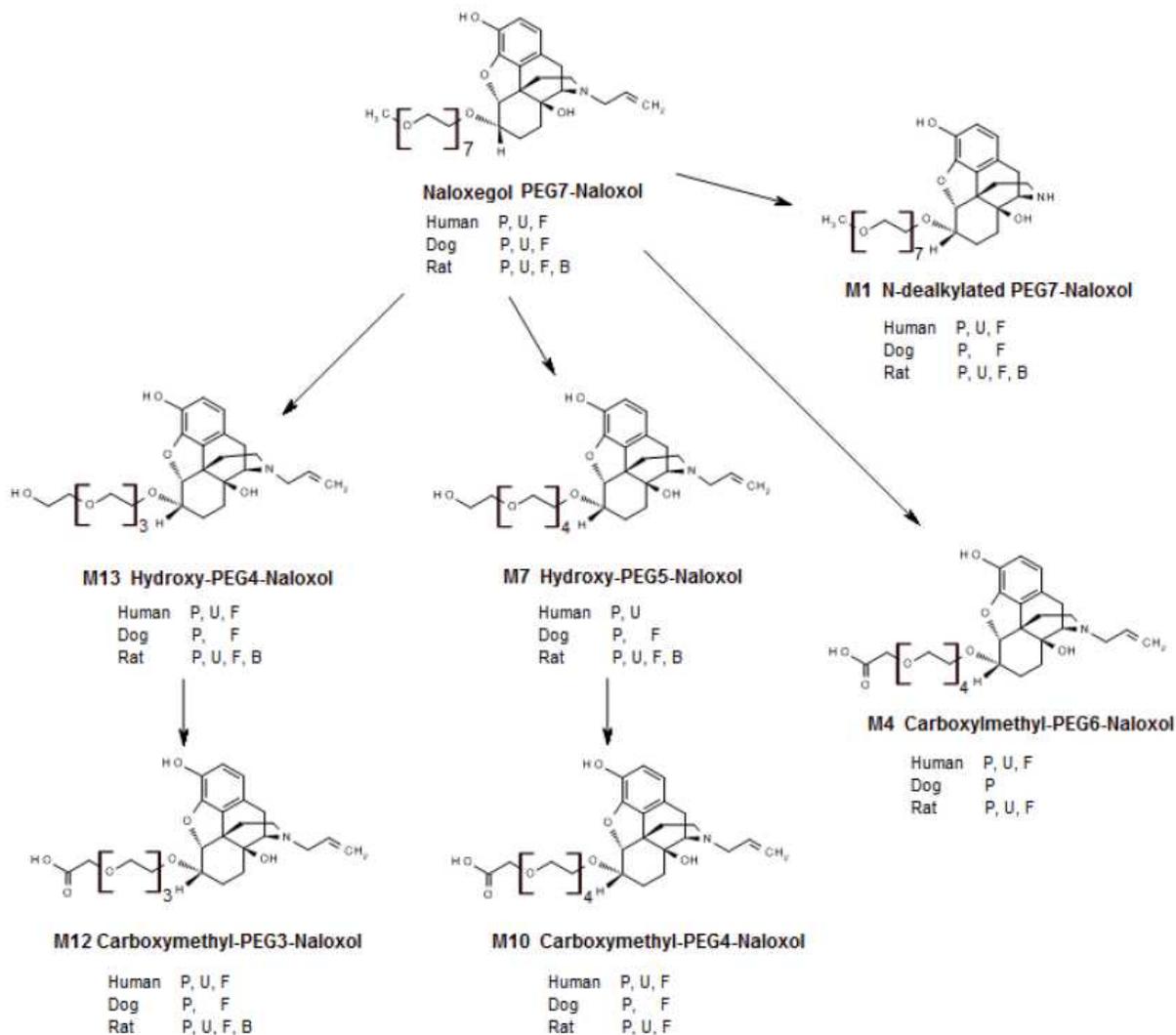


Figure 3 Identified human metabolites and their detection in rat, dog and human
(P = Plasma, U =Urine, F = Feces and B = Bile)



Cross-Species Comparison of Metabolic Profiles of NKTR-118 in Human, Dog, Rat, and Mouse Plasma (Study No. LS-2008-607)

Methods: The metabolites of NKTR-118 in plasma from mouse, rat, dog, and human after a single oral dose of NKTR-118 at 5 mg/kg, 100 mg/kg, 25 mg/kg, or 250 mg, respectively, were compared using LC-MS.

Results and Conclusions: Seventeen metabolites were identified in human plasma. The most abundant component was NKTR-118 at approximately 80% of the mass chromatogram, with no other component representing >10% of the mass chromatogram. All of the human metabolites were present in plasma from mice, rats, and dogs. The metabolites were designated as M1, M2, M4, M6, M7, M8, M9, M10,

M12, M13, M16, M17, and M41. Four other components were also identified: a glutathione adduct, a component that could be M11, and two components showing the addition of oxygen (one of which may be M24). The metabolites in the plasma are summarized in the Sponsor's table below.

Metabolite Number	Metabolite Description	MW	Mouse	Rat	Dog	Human
			Abundance (% total) based on MS response			
M16	Carboxymethyl-PEG2-naloxol	475.03	0.42	0.80	0.16	1.32
M12	Carboxymethyl-PEG3-naloxol	519.04	0.48	0.40	0.49	2.19
M2	PEG7-Naloxol-glucuronide	827.00	19.2	14.2	31.8	0.01
M10	Carboxymethyl-PEG4-naloxol	563.07	1.35	0.20	0.63	2.43
M49	PEG7-naloxol-gluathione	956.00	-	0.16	0.55	-
M41	Carboxymethyl-PEG5-naloxol	607.12	1.11	0.35	0.45	0.71
M4	Carboxymethyl-PEG6-naloxol	651.13	0.92	0.33	0.49	0.57
Metabolite Number	Metabolite Description	MW	Mouse	Rat	Dog	Human
			Abundance (% total) based on MS response			
M48	HO-PEG2-naloxol	417.07	-	0.75	-	1.07
M17	HO-PEG3-naloxol	461.08	3.49	0.56	0.10	2.04
M8	PEG7-naloxol-cysteine	770.24	-	0.24	1.54	0.38
M13	HO-PEG4-naloxol	505.08	-	0.62	0.29	5.08
Possibly M11	N-dealkylated 2hydroxyl mPEG7 naloxol	627.00	-	1.04	0.34	-
M7	HO-PEG5-naloxol	549.13	0.70	0.88	2.98	2.34
M6	HO-PEG6-naloxol	593.13	1.07	1.27	2.54	1.29
M1	N-dealkylated mPEG7-naloxol	611.11	0.72	10.1	0.48	2.57
M9	Demethylated PEG7-naloxol	637.20	2.38	0.43	1.88	0.23
Possibly M24	2-hydroxyl mPEG7-naloxol	667.18	0.24	1.10	3.57	1.92
NKTR-118	Naloxegol	651.32	68.0	66.6	51.7	75.9

Cross-Species Comparison of Metabolic Profiles of NKTR-118 in Human, Rat, and Mouse Urine (Study No. LS-2008-606)

Methods: The metabolites of NKTR-118 in urine from mouse, rat, and human after a single oral dose of NKTR-118 at 5 mg/kg, 100 mg/kg, and 250 mg, respectively, were compared using LC-MS.

Results and Conclusions: Sixteen metabolites were identified in human urine. The most abundant component was NKTR-118 at approximately 80% of the mass chromatogram, with no other component representing >10% of the mass chromatogram. All of the human metabolites were present in urine from mice and rats. The metabolites were designated M1, M2, M4, M6, M7, M8, M9, M10, M13, M16, M29, M38, M41, and M46. There were two additional components which could be M11 and M24. The comparisons of the components in plasma and urine of animals and humans showed that all human metabolites were present in the animal species examined. The metabolites in urine are summarized in the Sponsor's table below.

Metabolite Number	Metabolite	MW	Mouse	Rat	Human
			Abundance (% total) based on MS response		
M16	Carboxymethyl-PEG2- naloxol	475.03	0.11	0.89	0.80
M12	Carboxymethyl-PEG3- naloxol	519.04	0.08	0.02	1.16
M2	PEG7-Naloxol-glucuronide	827.00	22.2	33.3	0.27
M10	Carboxymethyl-PEG4- naloxol	563.07	0.27	2.01	1.78
M49	PEG7-naloxol-glutathione	956.00	-	-	-
M41	Carboxymethyl-PEG5- naloxol	607.12	0.36	0.04	0.49
M4	Carboxymethyl-PEG6- naloxol	651.13	-	0.17	0.68
M48	HO-PEG2-naloxol	417.07	0.16	0.03	0.50
M17	HO-PEG3-naloxol	461.08	1.85	0.08	1.34
M8	PEG7-naloxol-cysteine	770.24	2.32	0.26	0.92
M13	HO-PEG4-naloxol	505.08	0.5	0.72	4.73
Possibly M11	N-dealkylated 2hydroxyl mPEG7 naloxol	627.00	0.33	0.13	0.16
M7	HO-PEG5-naloxol	549.13	1.82	1.21	5.52
M6	HO-PEG6-naloxol	593.13	3.13	3.58	2.03
M1	N-dealkylated mPEG7-naloxol	611.11	3.13	3.86	2.33
M9	Demethylated PEG7-naloxol	637.20	1.83	0.33	0.18
Possibly M24	2-hydroxyl mPEG7-naloxol	667.18	0.26	3.26	1.38
NKTR-118	naloxegol	651.32	61.7	50.1	75.8

***In Vivo* Metabolism of [¹⁴C]NKTR-118 Based on Metabolites Observed in Plasma, Urine, Bile, and Feces from the Rat (Study No. DMR3); Study of Metabolism in the Dog following Oral Administration of [¹⁴C]NKTR-118 (Study No. AZS120109-01); Metabolism of NKTR-118 in Healthy Male Volunteers Following Oral Administration of [¹⁴C]NKTR-118 (Study No. SP-D3820-SPE-0530)**

Methods: *In vivo* metabolism of NKTR-118 was studied using samples from rats, dogs, and humans that were administered [¹⁴C]NKTR-118 at 50, 50, and 25 mg, respectively.

Results and Conclusions: The Sponsor's table below summarizes the results from the 3 individual studies.

Table 13 Summary of the amounts of metabolites present in plasma and excreta quantified by radioactivity measurement in rat, dog and human

Sample (time)	Male rat				Female Dog			Male Human		
	Plasma (1-24h)	Bile (0-72)	Urine (0-72)	Faeces (0-96)	Plasma (1)	Urine (0-72)	Faeces (0-96)	Plasma (4)	Urine (0-24)	Feces (0-96)
Total No. Metabolites ^a	6	15	13	19	3	4	7	4	3	6
% of chromatogram (plasma) % dose (excreta) represented by component										
P	15.4	1.5	7.4	13.9	8.3	2.5	26	64	9.9	16.2
M1		10.1	0.3	15.3			7.5	5.5		13.7
M2	5	7.7	2.4	0.8	62	9.3				
M3			0.5							
M4				2.8						3.8
M7							6.5	8.2	0.7	6.5
M10							13	12	1.5	10.9
M12										9.1
M13								10	1.1	4.5
M26					12	2.8				
M47					18	2.5				

^a Present at >1% of the radioactivity

Note: adding these components doesn't give study total recoveries as limited pools are used for profiling and other samples (cage wash etc.) are not included

The data showed that about 20%, 29%, and 25% of the administered dose in rats, dogs, and humans, respectively, were recovered unchanged in urine and feces. Although M7, M10, M12, and M13 were not found in these studies in rats and dogs, they were identified in repeated-dose studies in these animal species.

Metabolites in Plasma from Repeated Dose Studies: Comparison of Exposures between Human, Rat, and Dog (Study No. SP-D3820-SPE-0535)

Methods: The circulating metabolites of NKTR-118 were compared using pooled plasma samples from repeated-dose studies in rats treated with 50, 200, or 800 mg/kg/day NKTR-118 (Study No. LS-2007-040), dogs treated with 50, 200, or 500 mg/kg/day NKTR-118 (Study No. LS-2007-041), and humans treated 12.5, 25, or 50 mg /day NKTR-118 (Study No. D3820C00020).

Results and Conclusions: Four significant metabolites in human plasma also were present in rats and dogs. All four metabolites were present in animals at substantially

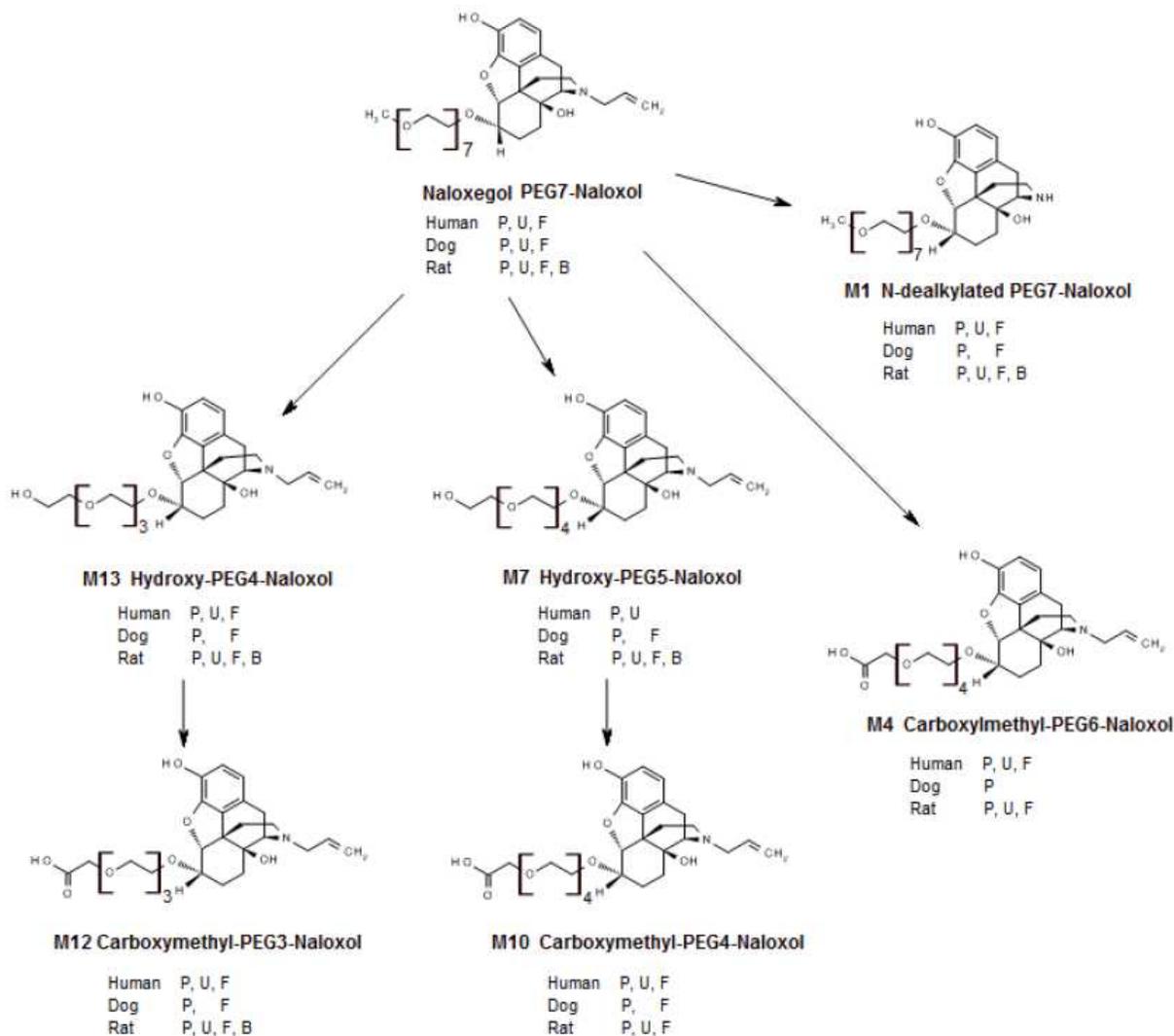
higher concentrations than those observed in humans. The animal exposures are shown in the Sponsor's table below as a multiple of the human exposure after a 25 mg dose (330 ng•hr/ml).

Table 11 Comparative exposure of humans and animals to circulating metabolites based on data from the toxicology studies

Circulating metabolites	Exposure presented as a multiple of the human exposure after a 25 mg dose of naloxegol			
	Rat		Dog	
Species	M	F	M	F
Sex	M	F	M	F
NOEL Dose (mg/kg)	200	800	200	200
M1	36	38	8	9
M7	93	122	149	112
M10	19	33	205	182
M13	25	52	7	6

The Sponsor's figure below shows the metabolic pathway based on the identified metabolites.

Figure 3 Identified human metabolites and their detection in rat, dog and human
(P = Plasma, U =Urine, F = Feces and B = Bile)



5.1.4 Excretion

The Disposition of [¹⁴C]NKTR-118 in the Rat Following Oral Administration (Study No. 192617) and The Disposition of [¹⁴C]NKTR-118 in the Dog Following Oral Administration (Study No. 8259274)

Methods: Rats and dogs were administrated a single oral dose of [¹⁴C]NKTR-118 at 50 mg/kg. The rat study included one group of bile duct-cannulated males. Radioactivity was measured in collected samples of excreta and bile (rats only).

Results and Conclusions: Results from the 2 studies are summarized in the Sponsor's table below.

Table 14 Recovery of naloxegol related material in excretion balance studies

Species, sex	Collection interval (h)	Dose route	Dose (mg/kg)	Radioactivity (% of dose)						
				Faeces	Urine	Bile	Carcass	CO ₂	Cage wash	Total
Rat M(n=3)	0-168	PO	50	78.9	18.8		0.3	0.0	0.7	98.7
Rat M (n=3)	0-72	PO	50	15.0	13.2	66.0	0.6		0.7	95.5
Rat F (n=3)	0-168	PO	50	66.4	30.2		0.2	0.0	1.4	98.2
Dog F (n=4)	0-168	PO	50	63.4	25.2				4.5	93.1

Over 93% of the radioactivity was recovered in the excreta in both rats and dogs. The excretion was mainly fecal; however, urinary excretion in female rats and dogs (25 to 30%) was higher than that in male rats (18.8%). The Sponsor noted that in humans, the mean total recovery was 84.2%, with 67.7% recovered in feces and 16.0% in urine.

The excretion of radioactivity was rapid in rats, with 94.8% and 84.8% of the dose (radioactivity) recovered over the first 24 hours in male and female rats, respectively. Excretion was slower in dogs, where 32.2% and 70.7% of the dose was recovered at 24 and 48 hours post-dose, respectively. The radioactivity in blood and plasma from rats was highest at the first sampling time (1h post-dose) and fell to very low levels at 24 hours. The plasma radioactivity between 1 and 8 hours post-dose in female animals was approximately double that in males. In dogs, the plasma radioactivity was highest at 0.5 hr and was measurable out to 24 hr and beyond in some animals.

In bile-duct cannulated male rats, the total recovery of radioactivity was 95.5% of the dose, of which 79.2% was recovered in bile and urine; this confirms absorption of about 80% of the oral dose. The majority of the radioactivity (92.6% of the dose) was recovered by 24 hr post-dose. In male rats, the excretion was predominantly biliary.

5.1.5. Pharmacokinetic Drug Interactions

Investigation of the Potential of NKTR-118 to Inhibit Human Cytochrome P450 Isoforms (Study No. LS-2007-064)

The potential of NKTR-118 to inhibit CYP isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) was investigated in human liver microsomes using standard substrates. The results showed that NKTR-118 was a weak inhibitor of CYP2D6 with an IC₅₀ of 84.7µM. NKTR-118 showed some inhibition of CYP2C19 and CYP3A4-mediated midazolam metabolism, but the IC₅₀ values were greater than 100 µM, which was the highest concentration tested. These concentrations are quite high compared to the estimated clinical C_{max} of 0.12 µM. The results are summarized in the Sponsor's table below.

Table 15 Summary of the effects of naloxegol on the activity of CYP isoforms

	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4 Midazolam	CYP3A4 Testosterone
IC ₅₀ (μM)	NC	NC	NC	84.7	NC	NC
Inhibition at 100μM (%)	-	-	38.2	-	41.4	-

NC not calculated (>100 μM)
1μM is equivalent to 652 ng/mL

Assessment of the Potential of AZ13337019 to Produce Time Dependent Inhibition of Cytochrome P450 Isoforms in Human Liver Microsomes (Study No. ADME-AZS-Wave3-130226)

Potential inhibition of P450 isoforms, CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5, by NKTR-118 was investigated in human liver microsomes. There was no time-dependent inhibition at 10 μM NKTR-118, and only a small inhibition of CYP3A4/5 at 50 μM. The inhibition was not considered clinically important.

Study to Investigate the Cytochrome P450 Induction Potential of NKTR-118 in Fresh Human Hepatocytes from Three Individual Donors (Study No. LS-2007-073)

The potential of NKTR-118 to induce cytochrome P450 isoforms in humans was studied using hepatocytes from three individual human donors. NKTR-118 produced no induction of CYP1A2 or CYP3A4 at concentrations up to 10 μM. In fact, there was evidence of a reduction in the activity of CYP3A4 in cells from 2 donors at 10 μM. Therefore, NKTR-118 was not considered to be an inducer of CYP1A2 or CYP3A4 isoforms in humans.

Evaluation of Induction Potential of CYP1A2, CYP2B6 and CYP3A4 by Naloxegol in Human Hepatocytes (Study No. 440003796)

The potential of naloxegol to induce cytochrome P450 isoforms was investigated using hepatocytes from three different human donors. There was no induction of CYP1A2, CYP2B6, or CYP3A4 at concentrations up to 16 μM. However, there was evidence of a concentration related reduction (19 to 40%) in the activity of CYP3A4 from all donors at 16 μM naloxegol. Therefore, naloxegol was not considered to be an inducer of CYP1A2, CYP2B6, or CYP3A4 in humans.

Assessment of NKTR-118 as Substrate of Human BCRP, OATP1B1 and PATP1B3 (Study No. OPT-2010-113)

The potential for NKTR-118 to be a substrate of the OATP1B1 and OATP1B3 transporters was assessed in transfected MDCK cells by measuring the uptake of [¹⁴C]NKTR-118. There was only a very small difference in the uptake of [¹⁴C]NKTR-118 between the transporter-expressing cells and control cells, when compared to the reference substrates. Therefore, NKTR-118 was not considered to be a substrate of OATP1B1 or OATP1B3.

The potential of NKTR-118 to be a substrate of BCRP was assessed in Caco2 cells expressing native BCRP. There was reduction in the efflux ratio of [¹⁴C]NKTR-118 in the presence of the chrysin, a BCRP inhibitor. However, this reduction was largely due to the modest change in the A to B flux of [¹⁴C]NKTR-118. The net B to A flux of [¹⁴C]NKTR-118 in Caco-2 cells was reduced by about 20% in the presence of 100 µM chrysin, a concentration that was expected to abolish BCRP transport. Therefore, the efflux of [¹⁴C]NKTR-118 did not appear to be substantially mediated by BCRP.

Assessment of NKTR-118 as Inhibitor of Human Pgp, OAT1, OAT3, OC2, BCRP, OATP1B3 Mediated Transport (Study NO. OPT-2010-114)

The potential for NKTR-118 to inhibit the transport of substrates of human Pgp, OAT1, OAT3, OC2, BCRP, and OATP1B3 was studied in MDCK and Caco-2 cells using standard substrates. The IC₅₀ values for NKTR-118 against all these transporters were greater than 100 µM, the highest concentration tested. Therefore, NKTR-118 had minimal effects in the inhibition of the transporters.

Assessment of the Metabolism of NKTR-118 by Six Human Cytochrome P450 Isoforms (Study No. LS-2007-063)

The objective of this study was to assess the metabolic stability of NKTR-118 and naloxone in the presence of human cytochrome P450 isoforms (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP2C8) expressed in Bactosomes™ using a validated assay system. The results showed that NKTR-118 is metabolized exclusively by CYP3A4 Bactosomes™. NKTR-118 was metabolically stable in the presence of CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP2C8 Bactosomes™. In contrast, multiple P450 isoforms including CYP2C19, CYP3A4, CYP2C9, CYP2D6, and CYP2C8 are involved to varying degrees in the metabolism of naloxone. The results are summarized in the Sponsor's tables below.

Table 2: Cytochrome P450 Isoform Identification Results for NKTR-118 and Naloxone; CYP1A2, CYP2C9 and CYP2C19.

Isoform	Compound ID	Replicate	t _{1/2} (min)	% Parent Remaining Following Incubation For:						Comments
				0min	5min	15min	30min	45min	45min Control	
CYP1A2	NKTR-118	1	ND	100	92.9	91.9	102	91.5	96.0	
		2	ND	100	99.2	106	111	96.9	107	
		3	ND	100	108	100	99.0	94.2	107	
		Average (±SD)	ND	100	100 (7.59)	99.4 (7.11)	104 (6.23)	94.2 (2.71)	103 (6.13)	
	Naloxone	ND	100	99.8	101	107	94.6	108		
CYP2C9	NKTR-118	1	ND	100	105	94.1	103	97.0	105	
		2	ND	100	110	106	104	102	108	
		3	ND	100	104	99.6	92.7	96.9	117	
		Average (±SD)	ND	100	106 (3.03)	99.7 (5.73)	100 (6.44)	98.5 (2.69)	110 (6.39)	
	Naloxone	145	100	97.7	86.8	81.3	82.0	103		
CYP2C19	NKTR-118	1	ND	100	96.4	92.4	96.2	94.5	96.8	
		2	ND	100	102	103	97.4	96.5	98.1	
		3	ND	100	104	101	102	108	103	
		Average (±SD)	ND	100	101 (3.79)	98.8 (5.63)	98.7 (3.32)	99.6 (7.29)	99.3 (3.61)	
	Naloxone	12.5	100	78.0	41.1	19.3	17.0*	105	45 min time point excluded.	

*Excluded from the profile due to non-linear parent disappearance

ND: t_{1/2} not determined due to less than 10% parent disappearance following 45 min incubation

Table 3: Cytochrome P450 Isoform Identification Results for NKTR-118 and Naloxone; CYP2D6, CYP3A4 and CYP2C8.

Isoform	Compound ID	Replicate	t _{1/2} (min)	% Parent Remaining Following Incubation For:						Comments
				0min	5min	15min	30min	45min	45min Control	
CYP2D6	NKTR-118	1	ND	100	104	99.6	98.2	93.5	94.9	
		2	ND	100	101	91.7	97.4	104	113	
		3	ND	100	103	94.6	96.8	104	99.6	
		Average (±SD)	ND	100	103 (1.60)	95.3 (3.97)	97.4 (0.711)	101 (6.16)	103 (9.42)	
	Naloxone	229	100	96.0	90.0	90.4	85.7	96.8		
CYP3A4	NKTR-118	1	5.71	100	53.4	10.4	2.77	1.82*	94.0	45 min time point excluded.
		2	6.02	100	55.1	12.1	3.32	1.85*	107	45 min time point excluded.
		3	6.08	100	60.9	13.4	3.53	2.22*	108	45 min time point excluded.
		Average (±SD)	5.94(0.199)	100	56.5 (3.91)	12.0 (1.49)	3.21 (0.392)	1.96 (0.223)*	103 (7.95)	45 min time point excluded.
	Naloxone	53.9	100	92.6	80.5	69.5	55.0	102		
CYP2C8	NKTR-118	1	ND	100	99.4	111	101	101	109	
		2	ND	100	94.6	95.9	92.0	92.7	91.9	
		3	ND	100	109	99.1	104	110	112	
		Average (±SD)	ND	100	101 (7.31)	102 (7.67)	99.1 (6.41)	101 (8.40)	104 (10.9)	
	Naloxone	120	100	96.3	81.9	80.0	77.1	93.7		

*Excluded from the profile due to non-linear parent disappearance

ND: t_{1/2} not determined due to less than 10% parent disappearance following 45 min incubation

In Vitro Identification of Cytochrome P450 and FMO Isoforms Responsible for the Metabolism of NKTR-118 (Study No. NKTR118DMX3)

Methods: The objective of this study was to identify the NADPH-dependent cytochrome P450 (CYP) and flavin monooxygenases (FMO) enzymes that are

responsible for the metabolism of NKTR-118. The mass spectrometer responses for NKTR-118 and seven metabolites were analyzed following incubations of NKTR-118 (0.5 to 100 μM) with human liver microsomes (HLM, 0.4 mg/mL) to establish apparent Michaelis Constant (K_m) values for metabolite formation. In addition, incubations with individually expressed human CYP and FMO enzymes were conducted to determine their potential contribution to NKTR-118 metabolism. Due to the biotransformation of NKTR-118 in HLM to many minor metabolites, and the lack of authentic metabolite standards, no experiments were conducted with the specific chemical inhibitors of the CYP or FMO enzymes in the presence of HLM.

Results and Conclusions: Michaelis Constant (K_m) values for the formation of *N*-despropylene NKTR-118 (M1), hydroxyl PEG6 naloxol (M6), hydroxyl PEG5 naloxol (M7), *O*-desmethyl NKTR-118 (M9), hydroxyl PEG4 naloxol (M13), hydroxyl PEG3 naloxol (M17), and hydroxyl PEG2 naloxol (M44) were determined to be 59 ± 7.8 , 36 ± 4.5 , 22 ± 4.2 , 35 ± 5.1 , 35 ± 8.7 , 43 ± 5.7 , and 43 ± 5.2 μM , respectively. CYP3A4 and 3A5 appear to be the major isoforms for the metabolism of NKTR-118, while CYP2D6 had minor contributions to the formation of M9. FMO enzymes appeared to have no contribution to the metabolism of NKTR-118. The experiments in this study provided *in vitro* evidence that NKTR-118 is primarily metabolized by CYP 3A4 and 3A5.

The Determination of the Log(P) and pK_a Values for NKTR-118 (Study No. LS-2009-604)

The objective of this study was to determine the pK_a (negative logarithm of the acid-base dissociation constant) and LogP (partition coefficient) values of NKTR-118. NKTR-118 had two pK_a values (8.45 ± 0.01 and 9.48 ± 0.06), consistent with the predicted ionizable groups on the molecule. The octanol:water partition coefficient measured for the neutral form of NKTR-118 was 1.43 ± 0.03 , averaged over 3 replicates.

Figure 1 This is an example. Replace figure caption here to match inserted figure

5.2 Toxicokinetics

N/A

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose toxicity studies were conducted in mice (oral), rats (oral, IV), and dogs (oral, IV). Full study reports were provided within the NDA. The results are summarized below.

In the acute oral tolerability study in mice (Study No. LS-2007-008), as part of the mouse micronucleus study, NMRI mice (5/sex/group) were treated with a single dose of 500, 1000, or 2000 mg/kg NKTR-118 followed by 24 hours of observation. An additional group was treated with 2000 mg/kg on two consecutive days. Clinical signs, including decreased motor activity, hunched posture, and pilo-erection, were noted in the 2000 mg/kg animals. No clinical signs were observed in the 500 or 1000 mg/kg animals.

In the oral tolerability study in Sprague Dawley rats (Study No. 3143LR), animals (3/sex/group) were administered 0 (vehicle), 500, 1000, 1500, or 2000 mg/kg NKTR-118 free base, or 500 or 1000 mg/kg NKTR-118 oxalate by oral gavage. The dose levels for the oxalate salt groups are expressed as free base equivalent. Bodyweights and clinical signs were recorded for approximately 14 days after dosing, and the animals were terminated for gross necropsy examination. Toxicokinetic parameters were analyzed. Notable clinical signs included decreased motor activity, half-shut eyes, pilo-erection, and respiration changes in the 2000 mg/kg NKTR-118 free base groups, and half-shut eyes and pilo-erection in male rats at 1000 mg/kg NKTR-118 oxalate. There were no drug-related gross necropsy findings. Exposure to NKTR-118 was generally similar after dosing with 500 or 1000 mg/kg of NKTR-118 free base or NKTR-118 oxalate. The results are summarized in the Sponsor's table below.

Table 6 Comparison of toxicokinetic exposure parameters (mean \pm SD; n=3) after oral administration of naloxegol doses of free base and oxalate salt solutions at doses of 500 and 1000 mg/kg to rats

Dose (mg/kg)	Drug material	C _{max} (ng/ml)		AUC (ng.h/ml)	
		Male	Female	Male	Female
500	Free base	15700 \pm 3580	48600 \pm 13200	155000 \pm 3830	352000 \pm 14200
	Oxalate salt	15000 \pm 3710	24300 \pm 5800	175000 \pm 15500	342000 \pm 26200
1000	Free base	33500 \pm 61304	42400 \pm 2760	423000 \pm 166000	634000*
	Oxalate salt	19200 \pm 4540	43200 \pm 208	312000 \pm 67800	743000 \pm 103000

* No SD calculated (n=2)

In the intravenous pharmacokinetic study in Sprague Dawley rats (Study No. 3504LR), animals (3/sex/group) were treated with 10, 30, or 100 mg/kg/day NKTR-118. Notable clinical signs included soft feces and associated uro-genital staining in the 30 and 100 mg/kg groups.

In the pharmacokinetic study in Beagle dogs (Study No. LS-2005-030), animals (3/sex/group) were treated with 0.4, 2, 10, or 20 mg/kg NKTR-118 free base by oral gavage, or intravenously with 0.4 mg/kg NKTR-118 free base. Clinical signs were limited to sporadic soft feces

6.2 Repeat-Dose Toxicity

Short- and medium-term repeated-dose toxicity studies were conducted in rats and dogs. These included a 7-day, a 1-month, and a 3-month oral toxicity study in rats, two 14-day oral studies in dogs, and a 1-month oral toxicity study in dogs. The Sponsor provided full study reports for these studies, which are summarized below.

In the 7-day oral toxicity study in Sprague-Dawley rats (Study No. LS-2007-005, non-GLP), animals (5/sex/group) were administered 0 (vehicle), 100, 500, or 1000 mg/kg/day NKTR-118 by oral gavage. Animals in the toxicokinetic group (5/sex/group) were administered 100 or 1000 mg/kg/day NKTR-118. There were no deaths or drug-related clinical signs. Small decreases in bodyweight and bodyweight gain in the 1000 mg/kg/day group on days 4 and 7 were associated with lower food consumption. Decreases in blood urea nitrogen levels were noted in the 500 and 1000 mg/kg/day females, and increases in cholesterol levels were noted in the 500 and 1000 mg/kg/day males and females, compared to controls. Increased adrenal weights in the 1000 mg/kg/day group correlated with histopathological findings of vacuolation of the adrenal cortical cells (also observed in the 500 mg/kg/day males). Increased liver weights in the 500 and 1000 mg/kg/day group (both sexes) were associated with diffuse hepatocellular hypertrophy (females only). The NOEL (no observed effect level) was considered to be 100 mg/kg/day, which corresponds to AUC_{0-24hr} of 13,100 and 53,400 ng·h/mL, and C_{max} of 2,370 and 5,080 ng/ml in males and females, respectively.

In the 1-month oral toxicity study in Sprague-Dawley rats (Study No. LS-2007-011, GLP), main-study animals (10/sex/group) were administered 0 (vehicle), 50, 150, or 500 mg/kg/day NKTR-118 by oral gavage for 28 days. Recovery animals (5/sex/group) were administered 0 or 500 mg/kg/day NKTR-118 for 28 days, followed by a 14-day recovery period. Toxicokinetic animals (2/sex in controls and 9/sex in drug-treated groups) received 0 (vehicle), 50, 150, or 500 mg/kg/day NKTR-118 for 28-days. There were no deaths, and there were no drug-related clinical signs, changes in bodyweight or food consumption, neurological effects, or ophthalmology findings. Apparent drug-related increases in cholesterol levels were noted in the 150 mg/kg/day males and 500 mg/kg/day males and females, and higher triglyceride levels were noted in all drug-treated groups. The changes in cholesterol and triglyceride levels were no longer present at the end of the 14-day recovery period. There were no drug-related changes in organ weights, or gross or histopathological findings. The NOAEL (no observed adverse effect level) was considered to be 500 mg/kg/day NKTR-118, which corresponds to AUC_{0-24hr} of 262,660 and 372,020 ng·h/mL, and C_{max} of 23,444 and 36,341 ng/ml NKTR-118 in males and females, respectively, on day 28. Exposure to NKTR-118 was less than dose-proportional on day 1, but greater than dose-proportional in males and approximately linear in females on day 28. Exposure was generally higher in females.

In the 14-day oral toxicity study in Beagle dogs (Study No. LS-2005-031, GLP), animals (3/sex/group) were administered 0 (vehicle), 25, 75, or 200 mg/kg/day NKTR-118 by oral gavage. There were no deaths. Drug-related clinical signs were limited to soft stool and diarrhea in all drug-treated groups. There were no drug-related changes in

bodyweight, food consumption, ECG, blood pressure, clinical pathology parameters, or organ weights, and there were no drug-related macroscopic or microscopic findings. The NOAEL was considered to be >200 mg/kg/day, corresponding to an AUC_{0-24hr} of 24,421 and 19,297 ng·h/mL and C_{max} of 20,144 and 16,600 ng/mL, in male and female dogs, respectively.

In another 14-day oral toxicity study in Beagle dogs (Study No. LS-2007-004, GLP), animals (3/sex/group) were administered 0 (vehicle), 200, or 500 mg/kg/day NKTR-118 by oral gavage. One 200 mg/kg/day female was sacrificed on day 1 and one 500 mg/kg/day female was found dead on day 9. The Sponsor attributed the deaths to incorrect dosing based on macroscopic findings in the lung, and the deaths were not considered to be drug-related. Drug-related clinical signs were limited to abnormal (loose) feces in the 200 and 500 mg/kg/day males and females and excess salivation in the 500 mg/kg/day males and females. Minimal decreases in bodyweight, compared to controls, in the 500 mg/kg/day males were correlated with significant decrease in food consumption. There were no drug-related changes in clinical pathology parameters or organ weights, and there were no drug-related macroscopic or microscopic findings. The NOAEL was considered to be 500 mg/kg/day, the highest dose tested. This dose corresponds to an AUC_{0-24hr} of 90,743 and 65,696 ng·h/mL and C_{max} of 103,949 and 55,797 ng/mL in male and female dogs, respectively.

In the 1-month oral toxicity study in Beagle dogs (Study No. LS-2007-012, GLP), animals (4/sex/group) were administered 0 (vehicle), 50, 150, or 500 mg/kg/day NKTR-118 by oral gavage. Reversibility of the treatment effects was evaluated over a 14-day recovery period (2/sex for the control and high-dose groups). There were no deaths. Clinical signs, including excessive salivation and emesis, were noted in the 150 and 500 mg/kg/day males and females. Slight decreases in bodyweight gain (6 to 8%), compared to controls, were noted in the 500 mg/kg/day males and females. Bodyweights in the 500 mg/kg/day group remained decreased during the recovery period. There were no changes in food consumption, or in any of the clinical pathology, ophthalmology, respiratory, or ECG parameters, and there were no macroscopic findings. Increased incidence of mild lymphocyte depletion was noted in the thymus of the 500 mg/kg/day group. However, depletion of cortical lymphocytes in the thymus was also noted in controls. The Sponsor considered the finding to be stress-related. The NOAEL was considered to be 150 mg/kg/day, based on excessive salivation, emesis, and decreased bodyweight gain at 500 mg/kg/day. This dose corresponds to an AUC_{0-24hr} of 14,657 and 11,548 ng·h/mL and C_{max} of 12,492 and 14,061 ng/mL in male and female dogs, respectively. In general, the plasma exposure to NKTR-118 was comparable between males and females. There was greater than dose-proportional increase in AUC_{0-24hr} in males and females on day 1 and 28. There was no drug accumulation upon repeated dosing.

A 3-month oral toxicity study in rats (Study No. LS-2007-028) was reviewed under IND 78,781 by Dr. Niraj Mehta (Pharmacology/Toxicology review dated 5/19/2010). His review is included verbatim below.

3-Month Oral Gavage Toxicity and Toxicokinetic Study with NKTR-118 in Rats

Key Study Findings: Based on the body weight decreases $\geq 10\%$ male rats, the MTD (maximum tolerated dose) in males was 600 mg/kg/day. A MTD could not be defined in females, since there was no observed test article-related toxicity in female rats.

Study #: Covance No. 7985-101 (LS-2007-028)

EDR 4.2.3.2 pgs. 1-1122 (Serial No. 0022)

Conducting Laboratory and Location:

(b) (4)

Date of Study Initiation: August 14, 2007 (report dated September 4, 2008)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: Lot# 149006; 98% pure

Methods: The test and control/vehicle articles were administered once daily by oral gavage for at least 94 days as described in the sponsor's table below. The animals were euthanized upon completion of the treatment periods.

Group	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration (mg/mL) ^a
	Male	Female		
Toxicity Animals				
1 (Control) ^b	10	10	0	0
2 (Low)	10	10	50	10
3 (Mid)	10	10	400	40
4 (Mid-High)	10	10	600	60
5 (High)	10	10	800	80
Toxicokinetic Animals^c				
6 (Control) ^b	5	5	0	0
7 (Low)	8	8	50	10
8 (Mid)	8	8	400	40
9 (Mid-High)	8	8	600	60
10 (High)	8	8	800	80

a For Groups 2 and 7, the dose volume was 5 mL/kg. For Groups 1, 3, 4, 5, 6, 8, 9, and 10, the dose volume was 10 mL/kg.

b Groups 1 and 6 received control article only.

c Toxicokinetic animals were included solely for the purpose of blood sample collections. Two animals/sex/group served as replacement animals. One Group 8 male, one Group 8 female, and one Group 9 female were used as replacements during the study.

Doses: 0, 50, 400, 600, 800 mg/kg/day

Species/strain: CrI:CD(SD) rats

Number/sex/group: See table above.

Route, formulation, volume, and infusion rate: The test and control articles were prepared weekly based on dose concentrations of the test article as supplied. The vehicle control article was reverse osmosis water stored under ambient conditions. The dose volume is outlined in the table above.

Age: 7-8 weeks

Weight: Males; 204 to 311 g, Females; 163 to 241 g

Sampling Times: Blood samples were collected (via jugular vein) from unfasted rats on days 1, 29, and 89 of the dosing phase. Samples were collected from three animals/sex in the control group at approximately 3 hours postdose. Blood was collected from the first three animals/sex in the drug-treated groups at predose and approximately 3 and 12 hours postdose, and the second set of three animals/sex in the drug-treated groups at approximately 0.5, 6, and 24 hours postdose. For hematology, coagulation, and clinical pathology, blood samples were collected from all surviving animals (animals fasted overnight) on days 29 and 95 of the dosing phase. Beginning on day 78 of the dosing phase, blood for clinical pathology was collected (if possible) from toxicity (Groups 1-5) and toxicokinetic (Groups 6-10) animals sacrificed at an unscheduled interval.

Observations and Times:

Mortality: Mortality checks were performed twice daily (a.m. and p.m.).

Clinical Signs: Cage-side observations were performed once daily. Detailed observations were conducted on all animals once during the predose phase, on toxicity animals before dosing on day 1 of the dosing phase and weekly thereafter, and on the day of scheduled sacrifice.

Bodyweight: Body weights were recorded once during the predose phase, and weekly thereafter.

Food Consumption: Food consumption was measured weekly.

Hematology: The following parameters were measured on blood samples prior to scheduled necropsy.

red blood cell (erythrocyte) count	platelet count
hemoglobin	white blood cell (leukocyte) count
hematocrit	differential blood cell count
mean corpuscular volume	blood smear
mean corpuscular hemoglobin	reticulocyte count
mean corpuscular hemoglobin concentration	

Coagulation

prothrombin time	activated partial thromboplastin time
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Clinical Chemistry: The following parameters were measured on blood samples prior to scheduled necropsy.

glucose	alanine aminotransferase
urea nitrogen	alkaline phosphatase
creatinine	gamma glutamyltransferase
total protein	aspartate aminotransferase
albumin	calcium
globulin	inorganic phosphorus
albumin/globulin ratio	sodium
cholesterol	potassium
total bilirubin	chloride

Urinalysis: Samples were taken for urinalysis from all surviving animals on days 29 and 95 of the dosing phase. The following parameters were measured.

appearance (clarity and color)	pH
bilirubin	microscopic examination of sediment
blood	protein
glucose	volume
ketones	specific gravity
urobilinogen	

Necropsy/Gross Pathology: For all toxicity group rats, regardless of timing or circumstances of death, examination consisted of the external features of the carcass; external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues.

Organ weights: At scheduled sacrifices, the following organs (when present) will be weighed; paired organs will be weighed together (toxicity animals group)

adrenal (2)	prostate
brain	salivary gland [mandibular (2)]
epididymis (2)	seminal vesicle
heart	spleen
kidney (2)	testis (2)
liver with gallbladder (drained)	thymus
lung	thyroid (2 lobes) with parathyroid
ovary (2)	uterus
pituitary gland	

Histopathology: The following organs/tissues were examined in all toxicity group animals:

adrenal (2)	ovary (2)
aorta	pancreas
brain	prostate
cecum	rectum
colon	salivary gland [mandibular (2)]
duodenum	sciatic nerve
epididymis (2) ^a	seminal vesicle
esophagus	skeletal muscle (thigh)
eye (2) ^a	skin/subcutis
optic nerve (2) ^a	spinal cord (cervical, thoracic, and lumbar)
femur with bone marrow (articular surface of the distal end)	spleen
gallbladder	sternum with bone marrow
Harderian gland ^a	stomach
heart	testis (2) ^a
ileum	thymus
jejunum	thyroid (2 lobes) with parathyroid
kidney (2)	tongue
lesions	trachea
liver	urinary bladder
lung with large bronchi	uterus
lymph node (mesenteric)	vagina
mammary gland	
pituitary gland	

a Preserved in modified Davidson's fixative.

Toxicokinetics: Blood samples were collected from each control animal at approximately 3 hours postdose and in the test article treated groups at predose and approximately 0.5, 3, 6, 12, and 24 hours postdose on days 1, 29, and 89 of the dosing phase. The TK data for NKTR-118 and its rapidly metabolized analog, NKTR-118-Glucuronide, were analyzed.

RESULTS:

Mortality: A total of five toxicity and toxicokinetic animals, one 50 mg/kg/day male, one 800 mg/kg/day male, one toxicokinetic male and female given 400 mg/kg/day, and one toxicokinetic

female given 600 mg/kg/day of test article, were found dead or sacrificed at unscheduled intervals.

The 50 mg/kg/day male was found in moribund condition on day 78 of the dosing phase with a red discharge in the pan paper and limited use of his left hind leg, which was swollen. Bilaterally large kidneys with dark red fluid in the renal pelvis, and a distended urinary bladder were at necropsy. Microscopically, multiple renal cysts and a distended renal pelvis and minimal lymphocytic infiltrate of the epididymis were observed.

The 800 mg/kg/day male was observed with clinical signs that included general debilitation, head tilt and ataxia, hunched posture, and sensitivity to touch on day 60 of the dosing phase. No macroscopic changes were noted in this animal. Upon microscopic examination, inflammation was noted in the meninges surrounding the brain and spinal cord, macrophage infiltrate in the lungs, atrophy of the thymus, inflammation/cell infiltrate of the kidney, and chronic inflammation of the prostate.

In the TK group, a 400 mg/kg/day male was found in moribund condition on day 19 of the dosing phase. Adverse clinical signs included hunched posture, red haircoat and nose, and red discharge in the cage pan. No macroscopic observations were noted in this animal. One 400 mg/kg/day female was found in moribund condition on day 78 of the dosing phase. Clinical signs prior to sacrifice included head tilt and hyperactive and small optic nerves were noted at necropsy. One 600 mg/kg/day female was found in moribund condition on day 61 with clinical signs that included thin appearance, hunched posture, rough/yellow haircoat, hypoactivity, and sensitivity to touch, and a distended bladder was noted at necropsy.

Clinical Signs: Prevalent test article-related clinical signs over the course of the study included a clear oral discharge, which was noted in 2/10, 5/10, and 6/10 males dosed with 400, 600, and 800 mg/kg/day NKTR-118 respectively, and 8/10, 8/10, and 9/10 females, dosed with 400, 600, and 800 mg/kg/day NKTR-118, respectively. In most instances, the clear oral discharge occurred on random days through the course of the dosing period and lasted for 1-12 days. Additionally, yellow staining of the perineal area was noted in 1/10, 4/10, and 5/10 males dosed with 400, 600, and 800 mg/kg/day NKTR-118 respectively, and 3/10, 7/10, and 6/10 females, dosed with 400, 600, and 800 mg/kg/day NKTR-118, respectively. In most instances, the yellow staining of the perineal area occurred on random days through the course of the dosing period and lasted for 1-3 weeks. Neither of these clinical signs were noted in any of the control animals. Rough hair coat was noted in 5/10 males and 2/10 females in the 800 mg/kg/day groups compared to 0/20 animals in the control group.

One male given 800 mg/kg/day, sacrificed due to general debilitation, was observed to have head tilt and ataxia, hunched posture, and sensitive to touch on day 60 of the dosing phase.

Two females given 400 and 800 mg/kg/day NKTR-118 respectively, were observed with palpable masses starting on days 92 and 77 of the dosing phase. These masses were verified and collected at necropsy. These masses were diagnosed as neoplasms based on microscopic examination and classified as a mammary carcinoma in the 400 mg/kg/day female and a myxoma of the skin in the 800 mg/kg/day female.

Other random clinical signs observed in test article-treated animals included red haircoat, labored/audible breathing, and few feces.

Bodyweight: The effects on bodyweight gain are shown in the table below. The weight gain values were calculated from the starting and final mean bodyweights.

Dose (mg/kg/day)	Initial Bodyweight (g)	Final Bodyweight (g)	Bodyweight Gain (g)	%Control Weight Gain
Males				
0	254 ± 19.2	570 ± 46.8	316	100%
50	255 ± 19.9	568 ± 52.0	313	99%
400	256 ± 25.3	547 ± 43.4	291	92%
600	251 ± 25.0	539 ± 55.1	288	91%
800	257 ± 25.5	536 ± 54.0	279	88%
Females				
0	198 ± 17.6	317 ± 31.7	119	100%
50	201 ± 19.6	344 ± 39.0	143	120%
400	197 ± 17.1	323 ± 25.8	126	106%
600	199 ± 18.3	331 ± 38.4	132	111%
800	197 ± 15.9	333 ± 29.7	136	114%

Values are the mean ± S.D., except for bodyweight gain.

na: not applicable

There was a dose-dependent reduction of weight gain in males of 1%, 8%, 9% and 12% in the 50, 400, 600 and 800 mg/kg/day groups, respectively. An increase in weight gain of 20%, 6%, 11% and 14% occurred in the 50, 400, 600 and 800 mg/kg/day females, respectively. Weight gain during Week 6 was significantly ($p \leq 0.05$) decreased in the 600 and 800 mg/kg/day males (16 ± 7.9 g and 17 ± 5.1 g, respectively, compared to 24 ± 6.3 g in the control males).

Food Consumption: The initial mean (\pm S.D.) food consumption values (dosing phase days 1-7) for Group 1 (control) male and female rats were 201 ± 21.6 and 142 ± 11.7 g/animal respectively, and the food consumption values during the last week of the dosing phase (days 85-91) in male and female rats were 216 ± 23.0 and 152 ± 13.0 g/animal, respectively. On days 64-70 (Week 10) during the dosing phase, female rats in the 800 mg/kg/day had a significant increase ($p \leq 0.05$) of 17% in mean food consumption values. There were no other significant changes in food consumption.

Hematology: The results are shown in the tables below.

Parameter	Dose (mg/kg/day)	Change on Day 29 compared to control
Reticulocyte counts	600 (male) 50, 400, 600, 800 (female)	↑ 17% ↓ 18%, ↑ 12%, ↑ 12%, *↑ 26%
Platelet counts	400 (male)	↑ 15%
White blood cells	50, 600, 800 (female)	↑ 16%, ↓ 12%, ↓ 12%
Neutrophils	50, 400 (female)	↑ 15%, ↑ 26%
Lymphocytes	50, 600, 800 (female)	↑ 16%, ↓ 13%, ↓ 16%
Monocytes	400 (male)	↑ 20%
Coagulation parameters		
Prothrombin time	800 (male) 800 (female)	*↓ 5% *↓ 5%

Parameter	Dose (mg/kg/day)	Change on Day 95 compared to control
Reticulocyte counts	400, 600, 800 (male) 600, 800 (female)	↑ 20% , ↑ 12%, ↑ 26% ↑ 16%, ↑ 13%
Platelet counts	400, 800 (male)	*↑ 15%, ↑ 11%
White blood cells	600,800 (male) 50 (female)	↓ 12%, ↓ 16%, ↑ 12%,
Neutrophils	400, 800 (female)	↑ 23%, ↑ 58%,
Lymphocytes	600, 800 (male) 50 (female)	↓ 17%, ↓ 19% ↑ 12%

*- Statistically significant; $p \leq 0.05$

On day 29, there were a few small, but statistically significant changes in specific hematologic parameters when compared to control animals. These changes included an increase of reticulocyte counts in 800 mg/kg/day females, and decreases in prothrombin time in 800 mg/kg/day males and females. On day 95, there was a statistically significant increase of platelet counts in 400 mg/kg/day males. The percent changes for some of the parameters presented (i.e. neutrophils) in the table above were skewed due to intergroup variability.

Clinical Chemistry: The results are shown in the tables below.

Parameter	Dose (mg/kg/day)	Change on Day 29 compared to control
Cholesterol	400, 600, 800 (male) 400, 600, 800 (female)	*↑ 51%, *↑ 54%, *↑ 88% *↑ 53%, *↑ 84%, *↑ 77%
Total bilirubin	400, 600, 800 (female)	↓ 50%, ↓ 50%, ↓ 50%
Aspartate aminotransferase	50, 400, 600, 800 (male)	↓ 13%, ↓ 11%, ↓ 13%, ↓ 11%
Alanine aminotransferase	800 (male) 600, 800 (female)	↑ 29% *↑ 45%, ↑ 28%
Alkaline phosphatase	400, 600, 800 (male)	↓ 18%, ↓ 17%, ↓ 18%
Gamma	800 (female)	*↑ 50%

glutamyltransferase		
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Parameter	Dose (mg/kg/day)	Change on Day 95 compared to control
Urea nitrogen	800 (female)	↑ 19%
Cholesterol	400, 600, 800 (male) 400, 600, 800 (female)	*↑ 49%, *↑ 50%, *↑ 82% *↑ 51%, *↑ 67%, *↑ 65%
Aspartate aminotransferase	50, 400, 800 (male) 50, 400, 800 (female)	↓ 16%, ↓ 18%, ↑ 18% ↓ 16%, ↑ 13%, ↓ 13%
Alanine aminotransferase	600, 800 (male) 400, 600, 800 (female)	↑ 23%, *↑ 129% ↑ 35%, ↑ 24%, ↑ 16%
Alkaline phosphatase	50, 600 (male) 400, 600, 800 (female)	↓ 17%, *↓ 18% ↑ 23%, ↑ 20%, ↑ 23%
Phosphate	800 (female)	↑ 16%

There were significant, robust increases of cholesterol levels in male and female rats dosed with 400, 600, and 800 mg/kg/day of NKTR-118, respectively. Although there were two groups of animals with statistically significant changes in alanine aminotransferase levels, there were concomitant levels of intergroup variability associated with these statistically significant changes (3 animals had levels 2 to 5-fold greater than other animals in the same group). On day 95, there was a significant decrease of alkaline phosphatase levels in the 600 mg/kg/day males. In addition, absolute levels of alkaline phosphatase were reduced on day 95 when compared to day 29 for both control and test article-treated males and females.

Urinalysis: The results are shown in the tables below.

Parameter	Dose (mg/kg/day)	Change on Day 29 compared to control
Urine volume	50, 400, 600, 800 (male) 400, 600, 800 (female)	↑ 27%, ↑ 43%, ↑ 45%, ↑ 32% ↑ 36%, ↑ 34%, *↑ 85%
Urine pH	800 (male)	*↑ 4%.

Parameter	Dose (mg/kg/day)	Change on Day 95 compared to control
Urine volume	50, 800 (male) 50, 800 (female)	↓ 26%, ↓ 31% ↓ 23%, ↑ 41%

On day 29, there were small, but statistically significant increase of urine volume in 600 mg/kg/day females and an increase of urine pH in 800 mg/kg/day males.

Organ Weights: Absolute weight, organ/body weight ratio, and organ/brain weight ratio were reported. The organ weight changes are summarized below and '*' denotes values that are statistically different from the control value; $p \leq 0.05$.

Adrenals: Organ/body weight ratio was increased by 18% in 800 mg/kg/day males.

Absolute weight was increased by 14%, 22%, 22%, and *26%, and organ/brain weight ratio was increased by 14%, 22%, 23%, and *32% in 50, 400, 600, and 800 mg/kg/day females, respectively. Organ/body weight ratio was increased by *21%, 19%, and *24% in 400, 600, and 800 mg/kg/day females, respectively.

Kidneys: Organ/body weight ratio was increased by *13% in 800 mg/kg/day males.

Absolute weight was increased by 12%, *16%, and *13%, organ/body weight ratio was increased by *11%, *14%, and *10%, and organ/brain weight ratio was increased by 12%, *16%, and *18% in 400, 600, and 800 mg/kg/day females, respectively.

Liver: Absolute weight was increased by 12%, 17%, and *27%, and organ/body weight ratio was increased by *18%, *27%, and *36% in 400, 600, and 800 mg/kg/day males, respectively. Organ/brain weight ratio was increased by 18% and *30% in 600 and 800 mg/kg/day males, respectively.

Absolute weight was increased by *19%, *36%, and *36%, organ/body weight ratio was increased by *18%, *32%, and *34%, and organ/brain weight ratio was increased by *14%, *36%, and *43% in 400, 600, and 800 mg/kg/day females, respectively.

Ovary: Absolute weight and organ/brain weight ratio were both increased by 16% in 50 mg/kg/day females.

Pituitary: Absolute weight was increased by 11%, 19%, and 14%, and organ/body weight ratio was increased by 10%, 17%, and 12% in 400, 600, and 800 mg/kg/day females, respectively. Organ/brain weight ratio was increased by 11%, 11%, 20%, and 19% in 50, 400, 600, and 800 mg/kg/day females, respectively.

Prostate: Organ/body weight ratio was decreased by 13% and 13%, and organ/ brain weight ratio was decreased by 13% and 10% in 400 and 800 mg/kg/day males, respectively.

Salivary gland: Absolute weight, organ/body weight ratio, and organ/brain weight ratio were increased by 12%, 10%, and 12% respectively, in the 600 mg/kg/day females.

Testes: Organ/body weight ratio was decreased by *13% and *16% in 600 and 800 mg/kg/day males, respectively.

Thymus: Absolute weight, organ/body weight ratio, and organ/brain weight ratio were decreased by 24%, *26%, and 21% respectively, in the 800 mg/kg/day females.

Thyroid/Parathyroid: Absolute weight, organ/body weight ratio, and organ/ brain weight ratio were increased by 14%, 20%, and 15% respectively, in 600 mg/kg/day males.

Absolute weight, organ/body weight ratio, and organ/ brain weight ratio were increased by 23%, 15%, and 22% respectively, in 50 mg/kg/day females.

Uterus: Absolute weight was increased by 24% and 17%, organ/body weight ratio was increased by 22%, and 17%, and organ/brain weight ratio was increased by 23% and 17% in 400 and 600 mg/kg/day females, respectively. Absolute weight and organ/body weight ratio were decreased by 12% and 14% respectively, in 800 mg/kg/day females.

Necropsy/Gross Pathology: Macroscopic finding of discolored stomach was seen in 2/6 males and 2/7 females a dosed with 800 mg/kg/day of test-article compared to 1/10 male and 0/10 female control animals. There were no other test article-related effects observed.

Histopathology: There were no test article-related effects observed.

Toxicokinetics: NKTR-118 was readily absorbed and reached peak plasma concentrations at approximately 0.5 hr following oral administration. The C_{max} on day 1 of dosing was 1.19, 11.8, 17.6, and 22.2 $\mu\text{g/mL}$ in males and 5.61, 31.1, 39.4, and 42.5 $\mu\text{g/mL}$ in females, following administration of 50, 400, 600, and 800 mg/kg/day NKTR-118, respectively. In the high dose treatment group (800 mg/kg/day), the mean plasma NKTR-118 concentrations were 1.22 $\mu\text{g/mL}$ in males and 18.2 $\mu\text{g/mL}$ in females at 24 hour postdose on day 1. The mean $AUC_{(0-24\text{hr})}$ on day 1 dosing was 3.81, 53.7, 121, and 161 $\text{hr}\cdot\mu\text{g/mL}$ in males and 120.7, 245, 387, and 656 $\text{hr}\cdot\mu\text{g/mL}$ in females following 50, 400, 600, and 800 mg/kg/day NKTR-118 treatments, respectively.

The increase in peak plasma NKTR-118 concentrations with dose level was approximately dose-proportional in males and less than dose-proportional in females. Total plasma NKTR-118 exposure, as assessed by $AUC_{(0-24\text{hr})}$, showed greater than dose-proportional increase in males on all TK collection days and in females on days 1 and 29 of the dosing phase. Total plasma NKTR-118 exposure for females on day 89 was approximately dose proportional.

Mean plasma NKTR-118 C_{max} on day 1 in females was approximately 2 to 5-fold higher than that of males; these differences were more prominent at the low dose. On days 29 and 89, the differences in plasma NKTR-118 C_{max} between males and females were 20 to 40% except at the low dose, where the C_{max} was approximately 2-fold higher in females. Similar trends of higher plasma exposure to NKTR-118 was observed in females when compared to males based on $AUC_{(0-24\text{hr})}$.

Accumulation of NKTR-118 was assessed as the plasma NKTR-118 AUC ratios for days 29 and 89 with reference to day 1. Male rats had a 2.1 to 4.7-fold higher exposure on day 89 compared to day 1, with the majority of the accumulation occurring during days 1-29. In general, minimal accumulation was observed in female rats during days 1-89 (most accumulation observed occurred days 29-89). The TK data is summarized in the sponsor's table below.

Mean Plasma Toxicokinetic Data Summary of NKTR-118

Parameter	Administered Dose (mg/kg)							
	50		400		600		800	
Sex	M	F	M	F	M	F	M	F
Day 1								
AUC ₍₀₋₂₄₎ (hr*µg/mL)	3.81	20.7	53.7	245	121	387	161	656
C _{max} (µg/mL)	1.19	5.61	11.8	31.1	17.6	39.4	22.2	42.5
Day 29								
AUC ₍₀₋₂₄₎ (hr*µg/mL)	9.65	24	194	273	257	364	399	500
C _{max} (µg/mL)	2.08	4.39	22.2	26.3	24.8	37.7	48.2	45.9
Day 89								
AUC ₍₀₋₂₄₎ (hr*µg/mL)	11.3	36.4	252	367	337	474	478	580
C _{max} (µg/mL)	3.1	7.26	31.3	31.9	36.2	46.7	54.6	56.6

Abbreviations: M = male, F = female, AUC₍₀₋₂₄₎ = area under the plasma concentration versus time curve from 0 to 24 hours, C_{max} = maximum plasma concentration.

NKTR-118 was rapidly metabolized to NKTR-118-Glucuronide, which reached peak plasma concentrations typically within 0.5 hr following oral administration of NKTR-118. The mean plasma NKTR-118-Glucuronide C_{max} on day 1 was 1.99, 13.8, 14.9, and 14.5 µg/mL in males and 2.56, 13.5, 13.4, and 20.1 µg/mL in females, following 50, 400, 600, and 800 mg/kg/day NKTR-118 treatments, respectively. In the high-dose treatment group (800 mg/kg/day), the mean plasma NKTR-118-Glucuronide concentrations remained above 2.01 µg/mL in males and 13.8 µg/mL in females at 24 hours postdose on day 1. The mean AUC_(0-24hr) on day 1 was 4.71, 49.3, 87.3, and 100 hr*µg/mL in males and 6.25, 79.9, 112, and 250 hr*µg/mL in females following 50, 400, 600, and 800 mg/kg/day NKTR-118 treatments, respectively.

Conclusions: The 13-week oral toxicity study with NKTR-118 in rats resulted in five total deaths: one 50 mg/kg/day male, one 800 mg/kg/day male, one 400 mg/kg/day male and female, and one 600 mg/kg/day female. The 50 mg/kg/day male was found in moribund condition with a red discharge in the pan and limited use of his left hind leg. Macroscopic examination showed enlarged kidneys with dark red fluid in the renal pelvis, and a distended urinary bladder. Microscopically, the animal had multiple renal cysts and a distended renal pelvis. The 400 mg/kg/day male was found in moribund condition with clinical symptoms that included hunched posture, red haircoat and nose, and red discharge in the cage pan. The 400 mg/kg/day female was found in moribund condition with clinical signs that included head tilt and hyperactivity. The 600 mg/kg/day female was found in moribund condition with clinical signs that included thin appearance, hunched posture, rough/yellow haircoat, hypoactivity, and sensitivity to touch. The 600 mg/kg/day female found a distended bladder. Lastly, the 800 mg/kg/day male was observed with clinical signs that included general debilitation, head tilt and ataxia, hunched posture, and sensitivity to touch. No macroscopic changes were noted. Microscopic findings included inflammation in the meninges surrounding the brain and spinal cord, macrophage infiltrate in the lungs, atrophy of the thymus, and inflammation/cell infiltrate of the kidney. The deaths in this study are not considered as drug-related, given the clear lack of dose-dependence.

Common test article-related adverse clinical signs included a clear oral discharge and yellow staining of the perineal area. Rough haircoat was observed in 5/10 males and 2/10 females in the 800 mg/kg/day dose group. Two females given 400 and 800 mg/kg/day were observed with

palpable masses starting on days 92 and 77 of the dosing phase. These masses were diagnosed as neoplasms based on microscopic examination and classified as a mammary carcinoma in the 400 mg/kg/day female and a myxoma of the skin in the 800 mg/kg/day female.

There was a dose-dependent reduction in body weight gain in males (-8%, -9%, and -12% decrease relative to controls at 400, 600, and 800 mg/kg/day, respectively) without a significant change in food consumption. Females gained weight relative to controls with minor increases in food consumption on certain weeks throughout the dosing phase. In addition, clinical chemistry results showed significant increases in cholesterol levels in males and females dosed with ≥ 400 mg/kg/day of NKTR-118. There were no changes in gross pathology. Significant, dose-dependent increases in liver weights were observed in both males and females, however there were no concurrent histopathological changes. Toxicokinetic analyses revealed that the C_{max} increased dose-proportionally in males and less than dose proportionally in females. Females had greater exposures than males at all doses. Male rats had a 2.1 to 4.7-fold higher AUC on day 89 compared to day 1, whereas female rats had minimal drug accumulation.

Based on the decrease in bodyweight gain (12%) in the 800 mg/kg/day males, the NOAEL (no observed adverse effect level) and the MTD (maximum tolerated dose) in males was 600 mg/kg/day, which corresponded to $AUC_{(0-24hr)}$ and C_{max} values of 337 hr $\cdot\mu$ g/mL and 36.2 μ g/mL, respectively, measured at day 89. Since no test article-related adverse effects were observed in females, the NOAEL for females in the 13-week oral toxicity study was 800 mg/kg/day, which corresponded to $AUC_{(0-24hr)}$ and C_{max} values of 580 hr $\cdot\mu$ g/mL and 56.6 μ g/mL, respectively, measured at day 89. A MTD could not be defined in females, since there was no observed test article-related toxicity in female rats.

A 3-month oral toxicity study in mice (Study No. 7985-109) was reviewed under IND 78,781 by Dr. Niraj Mehta (Pharmacology/Toxicology review dated 10/27/2009). His review is included verbatim below.

3-Month Oral Gavage Toxicity and Toxicokinetic Study with NKTR-118 in Mice

Key Study Findings: Based on toxicity, mortality, and body weight gain decreases ($\geq 10\%$) observed in the 13-week oral toxicity study in mice, the MTD in males appears to be greater than 50, but less than 400 mg/kg/day, and in females the MTD appears to be < 600 mg/kg/day.

Study #: (b) (4) No. 7985-109 (LS-2007-056)

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: August 28, 2007 (report dated August 28, 2008)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: Lot# 149005; 98% pure

Methods: The test and control/vehicle articles were administered once daily by oral gavage for at least 90 days as described in the sponsor's table below. The animals were euthanized upon completion of the treatment periods.

Group	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration (mg/mL) ^a
	Male	Female		
Toxicity Animals				
1 (Vehicle Control) ^b	10	10	0	0
2 (Low)	10	10	50	10
3 (Mid)	10	10	400	40
4 (Mid-High)	10	10	600	60
5 (High)	10	10	800	80
Toxicokinetic Animals^c				
6 (Vehicle Control) ^b	21	21	0	0
7 (Low)	57	57	50	10
8 (Mid)	57	57	400	40
9 (Mid-High)	57	57	600	60
10 (High)	57	57	800	80

a For Groups 2 and 7, the dose volume was 5 mL/kg. For Groups 1, 3, 4, 5, 6, 8, 9, and 10, the dose volume was 10 mL/kg.

b Groups 1 and 6 received vehicle control article only.

c Toxicokinetic animals were included solely for the purpose of blood sample collections. Three animals/sex/group served as replacement animals. Due to mortalities, one Group 7 female, three Group 8 males, three Group 8 females, three Group 9 males, two Group 9 females, three Group 10 males, and three Group 10 females were used as replacement animals during the study.

Doses: 0, 50, 400, 600, 800 mg/kg/day

Species/strain: Crl:CD1(ICR) mice

Number/sex/group: See table above.

Route, formulation, volume, and infusion rate: The test and control articles were prepared weekly based on dose concentrations of the test article as supplied. The vehicle control article was reverse osmosis water stored under ambient conditions. The dose volume is outlined in the table above.

Age: 6.6 to 7.4 weeks

Weight: Males; 26.0-41.5 g, Females; 18.7-32.4 g

Sampling Times: Blood samples were collected (via cardiac puncture) from unconscious (via carbon dioxide inhalation) mice on days 1, 30, and 91 of the dosing phase. Samples were collected from three animals/sex in the control group at approximately 3 hours postdose and from three animals/sex in the test article treated groups at predose and approximately 0.5, 3, 6, 12, and 24 hours postdose. For hematology and clinical pathology, blood samples were collected from all toxicity animals (Groups 1-5) prior to scheduled sacrifice and samples were collected from

toxicity animals that died or were sacrificed at an unscheduled interval¹. Animals were not fasted prior to sample collection for toxicokinetic analysis or clinical pathology.

¹ Beginning on day 8 of the dosing phase blood (Clinical Chemistry only) was collected from toxicokinetic animals euthanized at an unscheduled interval. toxicokinetic animals euthanized at an unscheduled interval.

Observations and Times:

Mortality: Mortality checks were performed twice daily (a.m. and p.m.).

Clinical Signs: Cage-side observations were performed once daily. Detailed observations were conducted on all animals once during the predose phase, on toxicity animals before dosing on day 1 of the dosing phase and weekly thereafter, and on the day of scheduled sacrifice.

Bodyweight: Body weights were recorded once during the predose phase, and weekly thereafter.

Food Consumption: Food consumption was measured weekly.

Hematology: The following parameters were measured on blood samples prior to scheduled necropsy.

red blood cell (erythrocyte) count	platelet count
hemoglobin	white blood cell (leukocyte) count
hematocrit	differential blood cell count
mean corpuscular volume	blood smear
mean corpuscular hemoglobin	reticulocyte count
mean corpuscular hemoglobin concentration	

Clinical Chemistry: The following parameters were measured on blood samples prior to scheduled necropsy.

glucose	alanine aminotransferase
urea nitrogen	alkaline phosphatase
creatinine	gamma glutamyltransferase
total protein	aspartate aminotransferase
albumin	calcium
globulin	inorganic phosphorus
albumin/globulin ratio	sodium
cholesterol	potassium
total bilirubin	chloride

Gross Pathology: All animals in the main phase of the study were examined for external features of the carcass; external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues.

Organ weights: At scheduled sacrifices, the following organs (when present) will be weighed; paired organs will be weighed together (main phase animals).

adrenal (2)	prostate
brain	salivary gland [mandibular (2)]
epididymis (2)	seminal vesicle
heart	spleen
kidney (2)	testis (2)
liver with gallbladder (drained)	thymus
lung	thyroid (2 lobes) with parathyroid
ovary (2)	uterus
pituitary gland	

Histopathology: The following organs/tissues were examined in all main phase animals:

adrenal (2)	ovary (2)
aorta	pancreas
brain	prostate
cecum	rectum
colon	salivary gland [mandibular (2)]
duodenum	sciatic nerve
epididymis (2) ^a	seminal vesicle
esophagus	skeletal muscle (thigh)
eye (2) ^a	skin/subcutis
optic nerve (2) ^a	spinal cord (cervical, thoracic, and lumbar)
femur with bone marrow (articular surface of the distal end)	spleen
gallbladder	sternum with bone marrow
Harderian gland ^a	stomach
heart	testis (2) ^a
ileum	thymus
jejunum	thyroid (2 lobes) with parathyroid
kidney (2)	tongue
lesions	trachea
liver	urinary bladder
lung with large bronchi	uterus
lymph node (mesenteric)	vagina
mammary gland	
pituitary gland	

a Preserved in modified Davidson's fixative.

Toxicokinetics: Blood samples were collected from each control animal at approximately 3 hours postdose and in the test article treated groups at predose and approximately 0.5, 3, 6, 12, and 24 hours postdose on days 1, 30, and 91 of the dosing phase. The TK data for NKTR-118 and metabolite, NKTR-118-Glucuronide, were analyzed.

RESULTS:

Mortality: A total of nine main phase animals were found dead or sacrificed prematurely; two males and two females given 800 mg/kg/day of test article, and four males and one female given 600 mg/kg/day of test article. The condition, day of death, clinical symptoms, gross pathology,

and histopathology results from these animals are summarized in the table below.

Dose	Sex	Day of Death	Clinical Symptoms	Gross Pathology (GP:)/ Histopathology (HP:)
600	M	Moribund Day 46	Hypoactive, cold to touch, swollen abdomen, few feces, hunched posture, & general debilitation	<u>GP:</u> Distended stomach, duodenum, ileum, colon, cecum, & jejunum (contains gas). <u>HP:</u> Mixed infiltrate of liver and inflammation and necrosis of liver, lymphocytic depletion and necrosis of thymus, dilation of duodenum, ileum, colon, cecum, & jejunum, & myeloid hyperplasia of marrow (femur and sternum).
600	M	Moribund Day 52	Swollen abdomen, audible breathing, & general debilitation	<u>GP:</u> Distended duodenum, ileum, colon, cecum, & jejunum (contains gas), & discolored (red/dark red) stomach mucosa. <u>HP:</u> Lymphocytic depletion and necrosis of thymus, mononuclear infiltrate of kidneys, & dilation of duodenum, ileum, colon, cecum, & jejunum.
600	M	Found dead Day 60	None	<u>GP:</u> None <u>HP:</u> Lymphocytic/macrophage infiltrate of liver, lymphocytic/macrophage infiltrate (alveolus) of lungs, & myeloid hyperplasia of marrow (femur and sternum).
600	M	Day 8 -death (b) (4)	None	<u>GP:</u> Perforated trachea <u>HP:</u> Lymphocytic infiltrate (peribronchial/perivascular) of lungs, & myeloid hyperplasia of marrow (femur & sternum).
600	F	Moribund Day 91	Few feces, thin appearance, rough haircoat, hunched posture, & general debilitation	<u>GP:</u> Large bilateral lymph node & discolored lobe (red/dark red) and raised area of liver. <u>HP:</u> Neutrophil infiltrate of trachea, extramedullary hematopoiesis of liver, extramedullary hematopoiesis of spleen, lymphocytic depletion/necrosis of spleen, lung inflammation, mandibular salivary gland atrophy, uterine atrophy, & myeloid hyperplasia of marrow (femur & sternum), & plasma cell hyperplasia and neutrophil infiltrate of lymph node (mandibular)
800	M	Moribund Day 85	Few feces, thin appearance, rough haircoat, & general debilitation	<u>GP:</u> Large bilateral lymph node. <u>HP:</u> Extramedullary hematopoiesis of liver, necrosis and inflammation of liver, extramedullary hematopoiesis of spleen, macrophage infiltrate (alveolus) of lungs, chronic progressive nephropathy of kidneys, lymphocytic/macrophage infiltrate of prostate, myeloid hyperplasia of marrow (femur & sternum), & plasma cell hyperplasia of lymph node (mandibular).
800	M	Moribund Day 85	Rough haircoat, yellow haircoat, hunched posture, few feces, & general debilitation	<u>GP:</u> Large bilateral lymph node. <u>HP:</u> Extramedullary hematopoiesis of spleen, macrophage infiltrate (alveolus) of lungs, lymphocytic depletion and necrosis of thymus, salivary gland atrophy chronic progressive nephropathy of kidneys, myeloid hyperplasia of marrow (femur & sternum), & plasma cell hyperplasia of lymph node (mandibular).
800	F	Moribund Day 58	None	<u>GP:</u> None. <u>HP:</u> Lymphocytic/macrophage infiltrate of liver, lymphocytic infiltrate (peribronchial/perivascular) of lungs, uterine atrophy, & myeloid hyperplasia of marrow (femur & sternum).

800	F	Moribund Day 83 (death gavage related)	Hypoactive, limited use of right front paw, swollen cranial head & right shoulder	<u>GP</u> : Perforated esophagus (cranial), & mammary (multiple, clear, gelatinous regions) <u>HP</u> : Fibrosis/fibroplasia of esophagus, degeneration/necrosis of heart (myocardium), depletion of lymphocytes/necrosis of spleen, & lymphocytic infiltrate (peribronchial/perivascular) of lungs, thymic necrosis, interstitial infiltrate of kidney, atrophy of mandibular salivary gland, moderate skin inflammation, & periesophagus necrosis.
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In the TK animal groups (Groups 6-10), 13 males (four 400 mg/kg/day, four 600 mg/kg/day, and five 800 mg/kg/day) and 15 females (one 50 mg/kg/day, five 400 mg/kg/day, one 600 mg/kg/day, and eight 800 mg/kg/day) were found dead or sacrificed at unscheduled intervals. Three males and two females given 400 mg/kg/day and one male and one female given 800 mg/kg/day had macroscopic observations of distended intestines containing gas.

Clinical Signs: There were minor treatment-related clinical signs in mice given 50 mg/kg/day of NKTR-118, which included one male with rough haircoat starting on day 15 and continuing until the end of the study. This animal also had yellow staining noted on days 57, 64, and 71. In females administered 50 mg/kg/day, adverse clinical signs included one female with few feces (days 64 and 71), a second female with dorsal abdomen alopecia (days 71-93), and a third female with alopecia and a sore/scab on the left lateral abdomen.

Test article-related clinical signs of audible or labored respiration was noted in 6/20, 4/20, and 5/20 animals in the 400, 600, and 800 mg/kg/day groups, respectively. Incidences of few feces were noted in 1/20, 5/20, 4/20, and 8/20 animals in the 50, 400, 600, and 800 mg/kg/day groups, respectively. Incidences of few feces were more predominant in males than females. Other signs of general distress included rough haircoat, observed in two males given 400 mg/kg/day, one male given 600 mg/kg/day, and eight males given 800 mg/kg/day. Rough haircoat was noted less frequently in females; two females given 600 mg/kg/day and one female given 800 mg/kg/day. Yellow haircoat was observed in two males given 800 mg/kg/day and one male given 50 mg/kg/day.

Bodyweight: The effects on body weight gain are shown in the table below. The weight gain values were calculated from the starting and final mean body weights.

Dose (mg/kg/day)	Initial Body weight (g)	Final Body weight (g)	Body weight Gain (g)	%Control Weight Gain
Males				
0	33.2 ± 2.99	38.6 ± 2.92	5.4	100%
50	32.9 ± 2.80	39.7 ± 3.21	6.8	130%
400	32.9 ± 3.05	37.6 ± 2.92	4.7	87%
600	32.8 ± 2.86	36.7 ± 4.52	3.9	72%
800	32.9 ± 1.85	31.6 ± 4.55	-1.3	*na
Females				
0	26.0 ± 1.46	31.8 ± 1.94	5.8	100%
50	25.8 ± 1.49	33.1 ± 1.98	7.3	126%
400	25.9 ± 1.87	33.1 ± 3.14	7.2	124%

600	25.9 ± 2.56	33.4 ± 2.91	7.5	129%
800	25.0 ± 1.93	30.4 ± 2.68	5.4	93%

Values are the mean ± S.D., except for bodyweight gain. na: not applicable

* $p \leq 0.05$

The final bodyweight of male mice dosed with 800 mg/kg/day of NKTR-118 was lower than the initial body weight at the start of the dosing period. The 800 mg/kg/day male mice had a decrease in body weight of 2.8 ± 3.10 g and 5.9 ± 3.05 g during weeks 12 and 13 of the dosing period, respectively. The overall difference in weight between initial and final body weight values, and the decrease in weight gain observed on week 13 in 800 mg/kg/day male mice were statistically significant when compared to control male mice. As noted in male mice, 800 mg/kg/day female mice had a small decrease in bodyweight of 1.9 ± 2.99 g during week 13. However, at the conclusion of the dosing period, female mice in the 50, 400, and 600 mg/kg/day groups exhibited an increase in weight gain of 26%, 24%, and 29% respectively, when compared to control females.

Food Consumption: The initial mean (\pm S.D.) food consumption values (Days 1-7) for Group 1 (control) male and female mice were 35.9 ± 2.21 and 32.5 ± 2.32 g/animal respectively, and the food consumption values during the last week of the dosing phase (days 85-91) in male and female mice were 36.9 ± 2.84 and 38.1 ± 4.10 g/animal, respectively. Male and female mice in the 800 mg/kg/day group had a significant decrease ($p \leq 0.05$) of 34% and 16% respectively, in mean food consumption values during the last week of the dosing phase.

Hematology: The results are shown in the table below.

Parameter	Dose (mg/kg/day)	Change compared to control
Reticulocyte counts	800 (male)	*↓ 61%
	800 (female)	↓ 32%
Platelet counts	800 (male)	↑ 45%
	50, 400, 600, 800 (female)	↑ 31%, ↓ 11%, ↑ 29%, ↑ 23%
White blood cells	50, 600, 800 (male)	↑ 17%, ↑ 69%, ↑ 32%
	50, 400, 600, 800 (female)	↑ 34%, ↑ 40%, ↑ 69%, *↑ 138%
Neutrophils	600, 800 (male)	↑ 290%, ↑ 262%
	50, 400, 600, 800 (female)	↑ 24%, ↑ 76%, *↑ 191%, *↑ 612%
Lymphocytes	50, 800 (male)	↑ 24%, ↓ 24%
	50, 400, 600, 800 (female)	↑ 39%, ↑ 31%, ↑ 43%, ↑ 43%
Eosinophils	400, 600 (male)	↑ 53%, ↑ 60%
	400, 600, 800 (female)	↑ 57%, ↑ 100%, ↑ 57%
Large unstained cells	400 (female)	↑ 50%

*- Statistically significant; $p \leq 0.05$

Significant reductions in reticulocyte counts were observed in 800 mg/kg/day males, and significant increases in white blood cells were observed in 800 mg/kg/day females. The percent

changes for some of the parameters presented in the table above were skewed because of intergroup variability and/or a decrease in the total number of animals tested, especially in higher dose males (≥ 600 mg/kg/day). Specifically, although males and females in groups dosed with ≥ 600 mg/kg/day of test article showed a robust increase in neutrophils, individual animals within each group had 5 to 12-fold differences in neutrophil counts. However, there was a consistent trend of increased neutrophils in animals dosed at ≥ 600 mg/kg/day. There decreases in animals tested were a result of animal deaths/premature sacrifices, or an insufficient quantity of test material (blood).

Clinical Chemistry: The results are shown in the table below.

Parameter	Dose (mg/kg/day)	Change compared to control
Glucose	400, 600, 800 (male) 50, 400, 600, 800 (female)	*↓ 20%, *↓ 36%, *↓ 44% ↓ 16%, ↓ 21%, *↓ 29%, *↓ 36%
Urea nitrogen	600, 800 (female)	↑ 17%, ↑ 22%
Total protein	600,800 (male) 800 (female)	*↑ 10%, *↑ 23% *↑ 17%
Albumin	800 (female)	*↑ 12%,
Globulin	800 (male) 800 (female)	↑ 47% ↑ 27%
Cholesterol	400, 600, 800 (male) 50, 400, 600, 800 (female)	*↑ 32%, *↑ 47%, ↑ 29% ↑ 18%, ↑ 27%, *↑ 56%, *↑ 71%
Aspartate aminotransferase	400, 600, 800 (male) 400, 800 (female)	↓ 29%, ↓ 13%, ↓ 25% ↑ 81%, ↑ 53%
Alanine aminotransferase	600 (male) 600 (female)	↑ 23% ↑ 59%
Alkaline phosphatase	600 (male) 400, 600, 800 (female)	↓ 20% *↓ 29%, *↓ 43%, *↓ 48%
Sodium	800 (male)	*↑ 2%
Potassium	600 (female)	↓ 17%

*- Statistically significant; $p \leq 0.05$

The following statistically significant changes were observed: dose-dependent decrease in glucose level was observed in male and female mice; dose-dependent decrease in alkaline phosphatase levels was observed in female mice at doses ≥ 400 mg/kg/day; and a dose-dependent increase in cholesterol level was observed in female mice (only statistically significant at doses ≥ 600 mg/kg/day). There was a statistically significant increase of total protein levels in 600 and 800 mg/kg/day males and 800 mg/kg/day females, respectively. 800 mg/kg/day females had a small, but statistically significant increase of albumin levels, and 400, 600 and 800 mg/kg/day males had significantly higher cholesterol levels. Intergroup variability within both the control group and test article-treated groups resulted in random increases in levels of aspartate aminotransferase and alanine aminotransferase. As noted previously, the percent changes for some of the parameters presented (i.e. aspartate aminotransferase and alanine aminotransferase) in the table above were skewed because of intergroup variability and/or a decrease in the total number of animals tested, especially in higher

dose males (≥ 600 mg/kg/day). The decreases in animals tested were a result of animal deaths/premature sacrifices, or an insufficient quantity of test material (blood).

Organ Weights: Absolute weight, organ/body weight ratio, and organ/brain weight ratio were reported. The organ weight changes are summarized below, and ‘*’ denotes values that are statistically different from the control value; $p \leq 0.05$.

Adrenals: Absolute weight was increased by 25% and 30%, organ/body weight ratio was increased by 26% and 55%, and organ/brain weight ratio was increased by 22% and 31% in 400 and 800 mg/kg/day males, respectively.

Brain: Organ/body weight ratio was decreased by *17% in 800 mg/kg/day males.

Epididymis: Absolute weight and organ/brain weight ratio were decreased by 18% and 17%, respectively.

Heart: Organ/body weight ratio was decreased by 12% and *14% in 50 and 400 mg/kg/day females, respectively.

Kidneys: Absolute weight was decreased by 12% and *20%, and organ/brain weight ratio was decreased by 12% and *18% in 600 and 800 mg/kg/day males, respectively.

Organ/body weight ratio was decreased by *13% in 400 mg/kg/day females.

Liver/gall bladder: Absolute weight decreased by *15% in 400 mg/kg/day males. Organ/body weight ratio was decreased by *14% and increased by *12% in 400 and 800 mg/kg/day males. Organ/brain weight ratio was decreased by *16% in 400 mg/kg/day males.

Absolute weight was increased by *21% and 14%, organ/body weight ratio was increased by *16% and *21%, and organ/brain weight ratio was increased by *23% and *20% in 600 and 800 mg/kg/day females, respectively

Lung: Organ/body weight ratio was decreased by 15% in 50 mg/kg/day animals and increased by 26% in 800 mg/kg/day males.

Organ/body weight ratio was decreased by *13% and 13% in 50 and 400 mg/kg/day, respectively.

Ovaries: Absolute weight and organ/brain weight ratio were decreased by 22% and 18% respectively, in 800 mg/kg/day females. Organ/body weight ratio was decreased by 15%, 14%, and 18% in 400, 600, and 800 mg/kg/day females, respectively.

Pituitary: Absolute weight was decreased by 16%, 13%, 13%, and 16% in 50, 400, 600, and 800 mg/kg/day males, respectively. Organ/body weight ratio was decreased by 22% and 12% in 400 and 800 mg/kg/day males, respectively. Organ/brain weight ratio was decreased by 12% in 800 mg/kg/day males.

Absolute weight was increased by 17% and 17%, and organ/body weight ratio was increased by 12% and 12% in 50 and 600 mg/kg/day males, respectively. Absolute weight and organ/body weight ratio was decreased by 17% and 14% respectively, in 800 mg/kg/day males. Organ/brain weight ratio was increased by 21%, 12%, and 21% in 50, 400, and 600 mg/kg/day males respectively, and decreased by 12% in 800 mg/kg/day females.

Prostate: Absolute weight and organ/brain weight were both decreased by 27%, and organ/body weight ratio was decreased by 15% in 800 mg/kg/day males.

Salivary gland (mandibular): Absolute weight was decreased by 15% and *33%, and organ/body weight ratio was decreased by 13% and 22% in 600 and 800 mg/kg/day males, respectively. Organ/brain weight ratio was decreased by *32% in 800 mg/kg/day males.

Absolute weight and organ/brain weight ratio were decreased by 21% and 17% respectively, in 800 mg/kg/day females. Organ/body weight ratio was decreased by 13% and 17% in 600 and 800 mg/kg/day females, respectively.

Seminal vesicles: Absolute weight was decreased by 12% and *36% in 400 and 800 mg/kg/day males, respectively. Organ/body weight and organ/brain weight ratios were decreased by 25% and *34% respectively, in 800 mg/kg/day males.

Spleen: Absolute weight was decreased by 23% and increased by 45% in 400 and 800 mg/kg/day males, respectively. Organ/body weight ratio was decreased by 23% and increased by 77% in 400 and 800 mg/kg/day males, respectively. Organ/brain weight ratio was decreased by 24% in 400 mg/kg/day males.

Absolute weight was increased by 24% in 600 mg/kg/day females. Organ/body weight ratio was increased by 24% and 18%, and organ/brain weight ratio was increased by 29% and 18% in 600 and 800 mg/kg/day females, respectively.

Testis: Organ/body weight ratio was decreased by *14% and 12% in 50 and 800 mg/kg/day males, respectively.

Thymus: Absolute weight was decreased by 20%, 14%, and *58%, and organ/body weight ratio was decreased by 19%, 12% and *52% in 400, 600, and 800 mg/kg/day males, respectively. Organ/brain weight ratio was decreased by 30% and *58% in 400 and 800 mg/kg/day males, respectively.

Absolute weight was decreased by 20%, and organ/body weight and organ/brain weight ratios were both decreased by 16% in 800 mg/kg/day females.

Thyroid/parathyroid: Absolute weight and organ/body weight ratio were increased by 23% and 24% respectively, in 400 mg/kg/day males. Organ/brain weight ratio was increased by 13% and 20% in 50 and 400 mg/kg/day males, respectively.

Absolute weight was increased by 25% and 16%, organ/body weight ratio was increased by 19%

and 22%, and organ/brain weight ratio was increased by 24% and 23% in 600 and 800 mg/kg/day females, respectively

Uterus: Absolute weight was decreased by 34%, 21%, and *46%, and organ/brain weight ratio was decreased by *32%, 20%, and 43% in 400, 600, and 800 mg/kg/day females, respectively. Organ/body weight ratio was decreased by 16%, *37%, 26% and *46%, in 50, 400, 600, and 800 mg/kg/day females, respectively.

Gross Pathology: Macroscopic finding of enlargement of the mandibular lymph node was seen in 4/8 males and 2/8 females dosed with 800 mg/kg/day of test article compared to 0/10 male and 0/10 female control animals.

Histopathology:

Liver: Minimal to slight necrosis was noted in 3/8 males in the 800 mg/kg/day group, 1/9 females in the 600 mg/kg/day group, and 3/10 females in the 400 mg/kg/day group respectively (one female had moderate necrosis), compared to 1/10 and 0/10 control males and females, respectively. Minimal to slight, extramedullary hematopoiesis occurred in 3/6 and 4/8 males in the 600 and 800 mg/kg/day groups respectively, compared to 0/10 control male mice. Minimal to slight, inflammation was observed in 4/8 males in the 800 mg/kg/day group, and 3/10 females in the 400 mg/kg/day group, compared to 0/20 control animals.

Lymph Node: Plasma cell hyperplasia was noted in 4/4 males and 2/2 females in the 800 mg/kg/day group. No control animals were examined and the severity of incidences was not provided.

Bone Marrow (femur): Mild to marked, myeloid hyperplasia was observed in 2/10, 5/6, and 8/8 males in the 400, 600, and 800 mg/kg/day groups respectively, and 7/9 and 7/8 females in the 600 and 800 mg/kg/day groups, respectively. There were no incidences in control animals (0/20). The most severe incidences (marked myeloid hyperplasia) were noted in 6/8 males and 3/7 females in the 800 mg/kg/day group.

Bone Marrow (sternum): Mild to marked, myeloid hyperplasia was observed in 7/10, 5/6, and 6/7 males in the 400, 600, and 800 mg/kg/day groups respectively, and 6/10, 9/9, and 7/8 females in the 400, 600, and 800 mg/kg/day groups, respectively. There were no incidences in control animals (0/20). The most severe incidences (marked myeloid hyperplasia) were noted in 5/7 males and 3/7 females in the 800 mg/kg/day group.

Salivary gland: Atrophy was observed in 5/8 males of the 800 mg/kg/day group (severity of incidences were not provided).

Spleen: Minimal to slight, necrosis and depletion of lymphocytes was observed in 5/8 males in the 800 mg/kg/day group compared to 0/10 control male animals. Minimal to moderate, extramedullary hematopoiesis was examined in 2/6 and 7/8 males in the 600 and 800 mg/kg/day groups respectively, and 5/9 and 6/8 females dosed with 600 and 800 mg/kg/day respectively, compared to 0/20 control mice. The most severe incidences (moderate extramedullary hematopoiesis) were noted in 4/7 males dosed with 800 mg/kg/day of the test article.

Thymus: Necrosis and depletion of lymphocytes was observed in 5/6 males and 3/8 females respectively, in the 800 mg/kg/day group compared to 0/10 and 1/10 control male and female animals, respectively (severity of incidences were not provided).

Uterus: Mild to moderate, atrophy was observed in 1/10, 4/10, 7/10, 6/9, and 7/8 females in the control, 50, 400, 600, and 800 mg/kg/day groups, respectively. The most severe incidences (moderate atrophy) were observed in 3/7 of the 800 mg/kg/day females.

Toxicokinetics: NKTR-118 was readily absorbed and reached peak plasma concentrations at approximately 0.5 hr following oral administration. C_{max} values increased in proportion to dose on day 1 in males and females, however no clear C_{max} versus dose relationship was observed on days 30 and 91. $AUC_{(0-24)}$ values appeared to be greater than dose-proportional at higher doses in males and females on day 1, and only in females on day 91; no clear $AUC_{(0-24)}$ versus dose relationship was observed at other time points. There was a high prevalence of inter-animal variability, thus making it difficult to accurately evaluate sex-based differences. However, there were a few sex-based differences in exposure, which included an increase in drug exposure on days 1 and 30 in females administered 400 mg/kg/day NKTR-118 and day 91 in females administered 800 mg/kg/day. In males, there was an increase in drug exposure on days 30 and 91 in the 800 and 600 mg/kg/day respectively, when compared to females. Accumulation of NKTR-118 was assessed as the plasma NKTR-118 AUC ratios for days 30 and 91 with reference to day 1. Male and female mice had a 0.8 to 3.3-fold higher exposure on day 91 compared to day 1, with the majority of that accumulation occurring during the day 1 to day 30 period. The TK data is summarized in the sponsor's table below.

Mean Plasma Toxicokinetic Data Summary of NKTR-118

Parameter	Administered Dose (mg/kg/day)								
	Sex	50		400		600		800	
Day 1									
$AUC_{(0-24)}$ (hr* μ g/mL)		1.60	1.39	21.4	13.5	25.1	29.0	46.5	33.5
C_{max} (μ g/mL)		0.849	0.802	9.54	4.74	11.5	14.1	18.5	15.9
Day 30									
$AUC_{(0-24)}$ (hr* μ g/mL)		3.29	2.72	24.7	43.1	46.3	44.1	66.1	44.5
C_{max} (μ g/mL)		1.93	1.74	13.3	25.6	23.2	25.7	22.8	19.2
Day 91									
$AUC_{(0-24)}$ (hr* μ g/mL)		1.73	1.84	16.3	16.0	60.2 ^a	44.6	38.2	79.5
C_{max} (μ g/mL)		0.945	0.701	6.26	7.80	28.5	21.1	16.0	31.4

Abbreviations: M = male, F = female, $AUC_{(0-24)}$ = area under the plasma concentration versus time curve from 0 to 24 hours, C_{max} = maximum plasma concentration.

a This value was calculated after removing an outlier datum from one animal at the 12-hr timepoint.

NKTR-118 is known to be rapidly metabolized to a glucuronide conjugate. Peak plasma NKTR-118-Glucuronide concentrations occurred typically within 0.5 hr following oral administration of NKTR-118 except in females that received the highest dose on day 91 (T_{max} : 3.19 hr). Plasma NKTR-118-Glucuronide concentrations generally increased with dose in an apparent linear fashion. The mean plasma NKTR-118-Glucuronide C_{max} on day 1 of dosing was 7.49, 38.1, 32.1, and 53.3 μ g/mL in males and 3.22, 20.4, 27.7, and 43.7 μ g/mL in females, following administration of 50, 400, 600, and 800 mg/kg/day NKTR-118, respectively. In the high dose treatment group (800 mg/kg/day), the mean plasma NKTR-118-Glucuronide concentrations were 0.128 μ g/mL in males and 0.0957 μ g/mL in females at 24 hr postdose on day 1. The mean $AUC_{(0-24)}$ on day 1 was 11.8, 93.0, 83.5, and 147 hr* μ g/mL in males and 5.38, 53.7, 69.5, and 91.2 hr* μ g/mL in females following administration of 50, 400, 600, and 800 mg/kg/day NKTR-

118, respectively.

Conclusions: In the 3-month oral toxicity study in mice, a total of 9 main study animals were found dead or sacrificed prematurely; four males and one female given 600 mg/kg/day, and two males and two females given 800 mg/kg/day. In the TK groups, a total of 28 (13 males and 15 females) animals were found dead or sacrificed prematurely; 1/57 females given 50 mg/kg/day, 4/57 males and 4/57 females given 400 mg/kg/day, 4/57 and 1/57 females given 600 mg/kg/day, and 5/57 males and 8/57 females given 800 mg/kg/day.

Other predominant clinical signs included few feces (more prevalent in males) in 1/20, 5/20, 4/20, and 8/20 animals in the 50, 400, 600, and 800 mg/kg/day groups respectively. Rough haircoat occurred in two 400 mg/kg/day males, one 600 mg/kg/day male, and eight 800 mg/kg/day males.

In addition, there was a decrease of body weight between initial body weight measurements (32.9 ± 1.93 g) and final body weight measurements (31.6 ± 4.55 g) in male mice administered 800 mg/kg/day of NKTR-118. A significant decrease (19%) in total body weight of the 800 mg/kg/day males occurred during the last two weeks of the dosing period. Subsequently, there was a 13% and 28% reduction in body weight gain in 400 and 600 mg/kg/day males, respectively. Females gained weight relative to controls in doses ≤ 600 mg/kg/day and there was no significant decrease of weight gain in 800 mg/kg/day females. Also, 800 mg/kg/day male and female mice had a significant decrease of 34% and 16% respectively, in mean food consumption values during the last week of the dosing phase.

Hematology parameters showed increased neutrophil counts in males and females dosed with ≥ 600 mg/kg/day of NKTR-118. Clinical chemistry parameters included a dose-dependent increase of glucose levels in both males and females, an increase of cholesterol levels in males and females dosed at ≥ 400 mg/kg/day, and dose-dependent decreases of alkaline phosphatase levels in females.

Enlarged lymph nodes were observed in males dosed with 800 mg/kg/day. There was a significant decrease in weight of the thymus and spleen in 800 mg/kg/day males, and a decrease of uterine weight in 800 mg/kg/day females. These were consistent with microscopic changes noted in the animals (discussed below). Significant histopathology findings included mild to marked, myeloid hyperplasia of bone marrow (femur) in males dosed with 400, 600, and 800 mg/kg/day, and females dosed with 600 and 800 mg/kg/day. The most severe incidences (marked myeloid hyperplasia) were noted in males and females in the 800 mg/kg/day group. In addition, mild to marked, myeloid hyperplasia of bone marrow (sternum) was observed in males and females dosed with 400, 600, and 800 mg/kg/day, respectively. The most severe incidences (marked myeloid hyperplasia) were noted in males and females in the 800 mg/kg/day group. Other microscopic findings included minimal to slight necrosis of the liver in 800 mg/kg/day males and 400 mg/kg/day females, and plasma cell hyperplasia of the lymph nodes in 800 mg/kg/day males and females. Minimal to moderate extramedullary hematopoiesis of the spleen was noted in males and females dosed with ≥ 600 mg/kg/day and necrosis of the spleen was noted in 800 mg/kg/day males. Thymic necrosis was noted in 800 mg/kg/day males and

females. Mild to moderate, uterine atrophy was observed in females dosed with 50, 400, 600, and 800 mg/kg/day groups, respectively.

Based on the toxicity, mortality, and body weight gain decreases ($\geq 10\%$) observed in the 13-week oral toxicity study in mice, the NOAEL (no observed adverse effect level) and MTD in males appears to be greater than 50, but less than 400 mg/kg/day. Specifically, morbidity and mortality were observed in males at doses ≥ 600 mg/kg/day, and a decrease in body weight gain was observed in males dosed at ≥ 400 mg/kg/day. Microscopic examinations in male mice dosed with 800 mg/kg/day showed necrosis of the spleen, liver, and thymus. Based on toxicity and mortality, the NOAEL and MTD in females appear to be < 600 mg/kg/day. Test article-related morbidity was noted in females dosed at ≥ 600 mg/kg/day. There were no significant changes in body weight gain in female mice. Lastly, microscopic examinations showed necrosis of the thymus in 800 mg/kg/day females.

Study title: 26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with NKTR-118 in Rats with a 4-Week Recovery Period

Study no.:	7985-104
Study report location:	N/A
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	11-09-2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NKTR-118; 149005, 149010, 149011, 149012, 149013; 98%

Key Study Findings

Animals (15/sex/group) were treated orally with 0 (vehicle), 50, 200, or 800 mg/kg/day NKTR-118 for 26 weeks. Recovery animals (10/sex/group) were treated orally with 0 or 800 mg/kg/day NKTR-118 for 26 weeks, followed by a 4-week recovery period. There were 8 unscheduled deaths; however, none the deaths appear to be drug-related. Drug-related clinical signs included colored and rough hair coats, minor skin lesions, and hunched appearance in the 800 mg/kg/day group, excessive salivation in the 200 and 800 mg/kg/day groups, and abnormal respiration in the 200 and 800 mg/kg/day males; these clinical signs were reversible after the recovery period. Bodyweight and bodyweight gains at the end of the dosing phase was reduced by 9.4% and 14.5%, respectively, in the 800 mg/kg/day males. The bodyweight changes were not correlated with any decrease in food consumption. Increases in cholesterol levels, up to 75% compared to controls, were noted in the 200 and 800 mg/kg/day group. Increases in liver weight in the 200 and 800 mg/kg/day females correlated with dose-dependent hepatocellular hypertrophy. The increase in liver weight in the females was partially reversed after the recovery period. Other microscopic findings included pancreatic atrophy in the 800 mg/kg/day group, and dilatation of the uterus in the 800 mg/kg/day females; the changes were partially reversed after the recovery period. Carcinoma of

the mammary gland was noted in one female in each of the 200 and 800 mg/kg/day group. A metastatic carcinoma of unknown origin was noted in the mandibular lymph node of one main study 800 mg/kg/day female, and a tumor (astrocytoma) was noted in the brain of one main study 800 mg/kg/day female. Pituitary adenoma of the pars distalis was noted in the 800 mg/kg/day group (1 male and 1 female). The NOAEL is considered to be 50 mg/kg/day based on observed changes in clinical signs, cholesterol levels, and pancreatic atrophy in males and females, and microscopic liver findings in females at doses \geq 200 mg/kg/day.

Methods

Doses:	0 (vehicle), 50, 200, 800 mg/kg/day
Frequency of dosing:	once daily
Route of administration:	oral (gavage)
Dose volume:	10 ml/kg
Formulation/Vehicle:	solution / reverse osmosis water
Species/Strain:	Rats / CrI:CD(SD)
Number/Sex/Group:	Main study: 15/sex/group; Recovery group: 10/sex/group
Age:	6 to 7 weeks old
Weight:	Males: 190 to 284 g F: 165 to 205 g
Satellite groups:	Toxicokinetic group: control (3/sex), drug-treatment group (9/sex)
Unique study design:	N/A
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

Study Design of the 26-Week NKTR-118 Oral Toxicity Study in Rats

Group ^a	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration ^c (mg/mL)
	Male	Female		
Toxicity Animals				
1 (Control) ^b	25	25	0	0
2 (Low)	15	15	50	5
3 (Mid)	15	15	200	20
4 (High) ^b	25	25	800	80
Toxicokinetic Animals				
5 (Control)	3	3	0	0
6 (Low)	9	9	50	5
7 (Mid)	9	9	200	20
8 (High)	9	9	800	80

Observations and Results

Mortality

All animals were checked twice daily for morbidity and mortality.

There were 8 unscheduled deaths during the course of the study: **1)** Two control females, one 200 mg/kg/day female, and one 800 mg/kg/day female were found dead on days 23, 86, 23, and 23 of the dosing phase, respectively. There was no definitive cause of death based on macroscopic or microscopic examination. The Sponsor considered these deaths to be accidental and unrelated to drug administration; **2)** One control female was found dead on day 127 of the dosing phase. The cause of death was most likely secondary to a kidney mass/tumor observed macroscopically and diagnosed microscopically as a nephroblastoma; **3)** One 800 mg/kg/day male was found dead on day 46 of the dosing phase. The cause of death was not evident from macroscopic or microscopic examination. This animal was on a rack that was disconnected from the water source for an undetermined length of time on day 26 of the dosing phase, and displayed clinical signs consistent with dehydration which included rough haircoat, yellow haircoat, low food consumption, and bodyweight loss. Yellow haircoat persisted until the animal was found dead. No remarkable findings were noted for this animal at necropsy; **4)** One control male was found dead on day 4 of the recovery phase. This animal was found to be hypoactive with labored respiration, few feces, cold to the touch, pale ears, and dilated pupils, and died prior to being sacrificed. The cause of death in this animal was attributed to infection/inflammation of the prostate gland; **5)** One toxicokinetic 50 mg/kg/day female was sent to necropsy following the last scheduled blood collection on day 182 of the dosing phase with a mammary gland mass subsequently diagnosed microscopically as a mammary carcinoma. None of the deaths appear to be drug-related.

Clinical Signs

Animals were checked twice daily for abnormalities, and signs of pain or distress. Detailed observations were conducted on main study animals once during the pre-dose phase, before dosing on day 1 and weekly thereafter, and on the day of scheduled sacrifice. Daily cageside observations were conducted on main study animals during the dosing and recovery phases, except on days when detailed observations were conducted.

On day 26 of the dosing phase, it was noted that the waterline to the rack housing the last 15 males given 800 mg/kg/day and all 25 of the females given 800 mg/kg/day was disconnected from the water supply. The Sponsor reported that the animals had clinical signs consistent with dehydration, which included decreased food consumption, body weight loss, hunched appearance, yellow haircoat in the perineal area, rough haircoat, and red haircoat. Food consumption and bodyweight increased more rapidly than the control animals during the interval following the return of water to these animals. By day 30 of the dosing phase, the water-deprived animals were described by the Sponsor as normal/no remarkable observations, with the exception of a few animals with yellow haircoat,

Main Study:

The following notable clinical signs were observed.

Summary of Notable Clinical Signs

Clinical Signs	Sex	Main Study			
		Dose (mg/kg/day) (# of animals)			
		0 (25)	50 (15)	200 (15)	800 (25)
Appearance <i>Hunched</i>	M	0	0	0	4
	F	0	0	0	2
Discharge <i>Excessive salivation</i>	M	0	0	1	14
	F	0	0	4	18
Respiratory <i>Audible</i>	M	0	0	1	2
	M	0	0	0	1
Skin and Pelage <i>Alopecia, front paws</i>	F	0	0	0	1
	M	0	0	0	1
<i>Alopecia, front leg</i>	M	0	0	0	1
<i>Red haircoat, cranial head</i>	F	0	0	0	2
<i>Red haircoat, front legs</i>	M	0	0	0	1
	F	0	0	0	5
<i>Red haircoat, mouth</i>	M	0	0	0	2
	F	0	0	0	6
<i>Red haircoat, perineal area</i>	M	0	0	0	2
<i>Red skin, mouth</i>	M	0	0	0	1
<i>Rough haircoat,</i>	M	0	0	0	8
	F	1	0	0	3
<i>Rough haircoat, mouth</i>	M	0	0	0	1
<i>Rough haircoat, perineal area</i>	M	0	0	0	1
<i>Sore/Scab, distal tail</i>	F	0	0	0	1
<i>Sore/Scab, nose</i>	F	0	0	0	1
<i>Sore/Scab, right front leg</i>	M	0	0	0	1
<i>Yellow Haircoat, perineal area</i>	M	0	0	0	10
	F	0	0	0	18

Drug-related clinical signs included colored and rough hair coats, minor skin lesions, and hunched appearance in the 800 mg/kg/day group, excessive salivation in the 200 and 800 mg/kg/day groups, and abnormal respiration in the 200 and 800 mg/kg/day males.

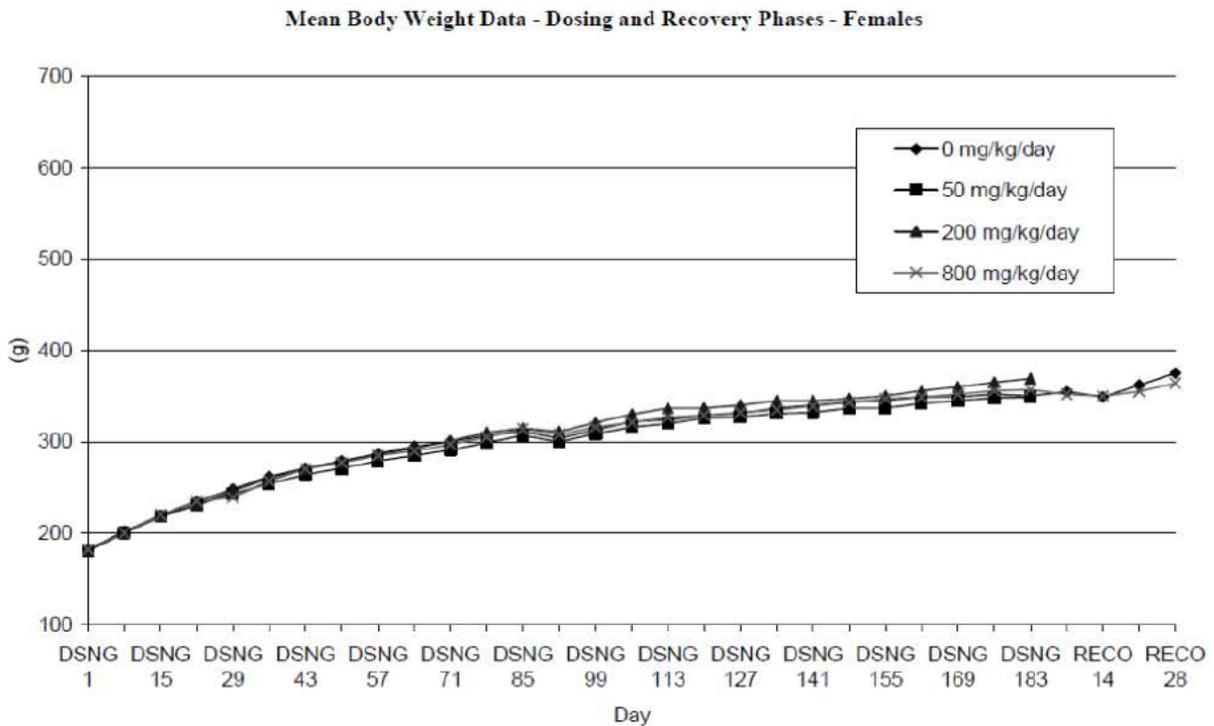
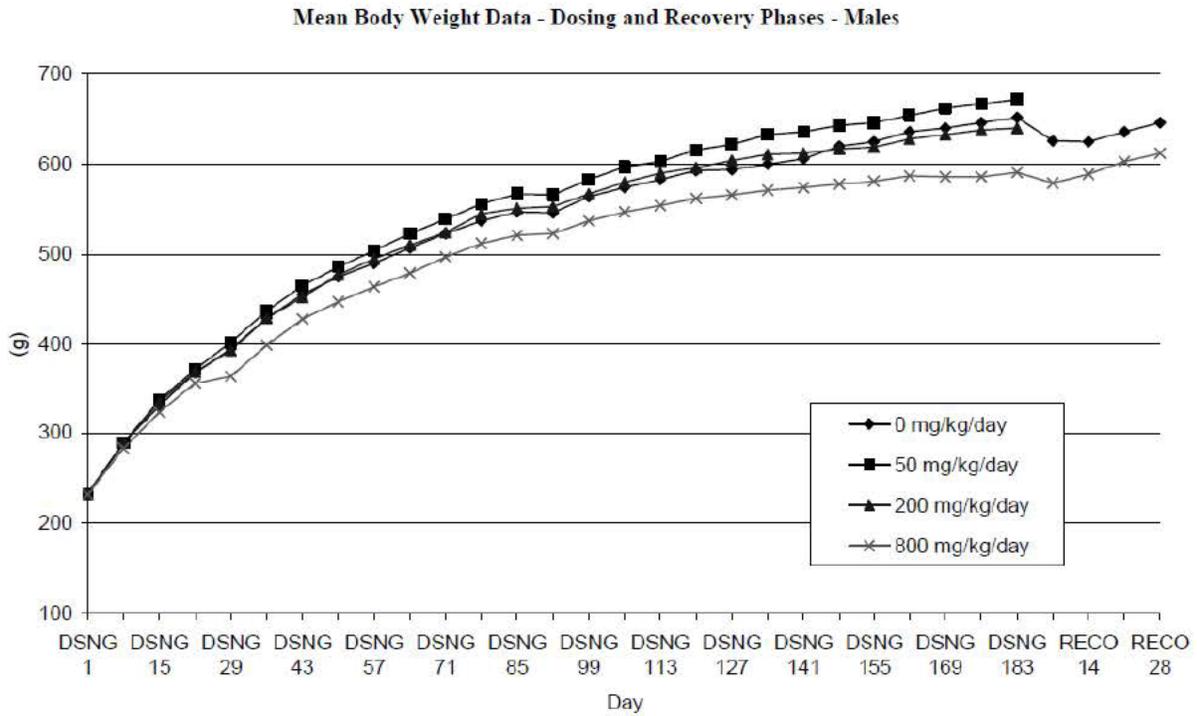
Recovery Phase:

There were no drug-related clinical signs.

Bodyweights

Animals were weighed during the pre-dose phase, prior to dosing on day 1 of the dosing phase, and once weekly thereafter.

Bodyweight and bodyweight gains at the end of the dosing phase were significantly decreased by 9.4% and 14.5%, respectively, in the 800 mg/kg/day males, compared to controls. These changes are considered to be drug-related. There were no drug-related changes in bodyweight or bodyweight gain in females at the end of the dosing phase. Decreases in bodyweight on days 29 and 36 in the 800 mg/kg/day males (-7.8% and -7.0%, respectively) were likely due to water deprivation resulting from disconnection of the waterline from the animal rack housing the 15 males given 800 mg/kg/day. Decreases in bodyweight gains in the 800 mg/kg/day males (-70.4%) and females (-66.7%), during days 22 through 29 also were likely related to the absence of water availability. At the end of the recovery period, bodyweight gain in the 800 mg/kg/day males increased by 104.5% compared to controls. Other sporadic changes in bodyweight and bodyweight gains were not considered drug-related. The bodyweight data are summarized in the figures below (taken from the Sponsor's study report).

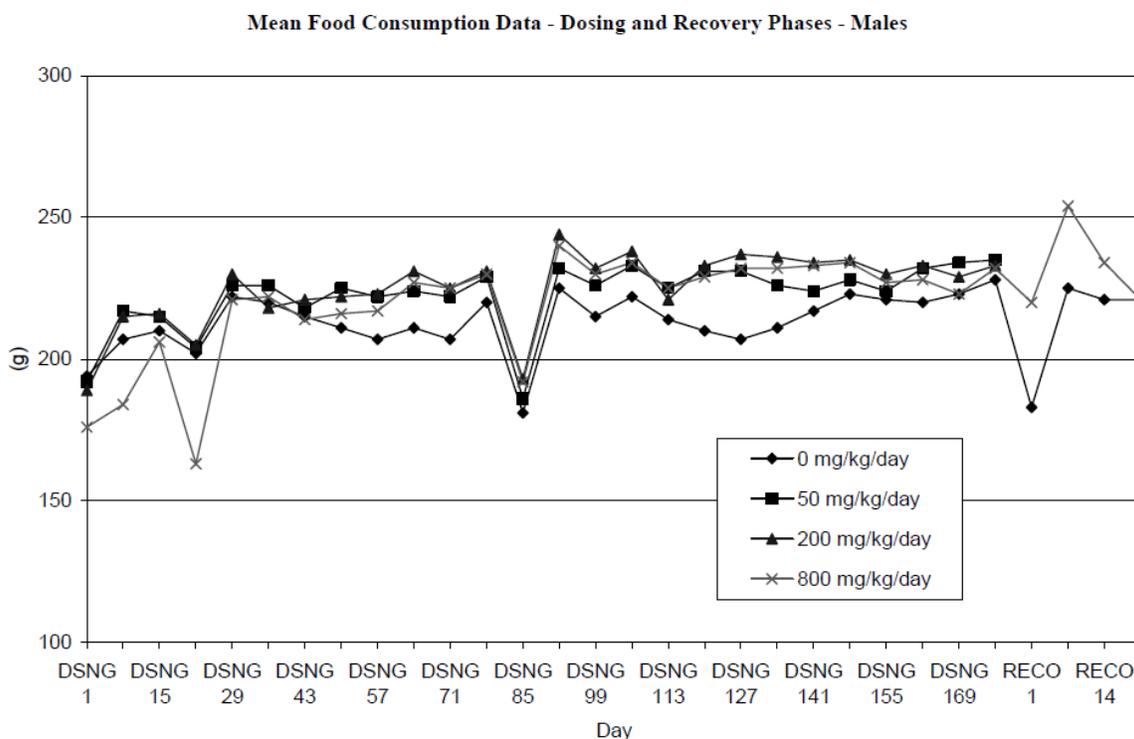


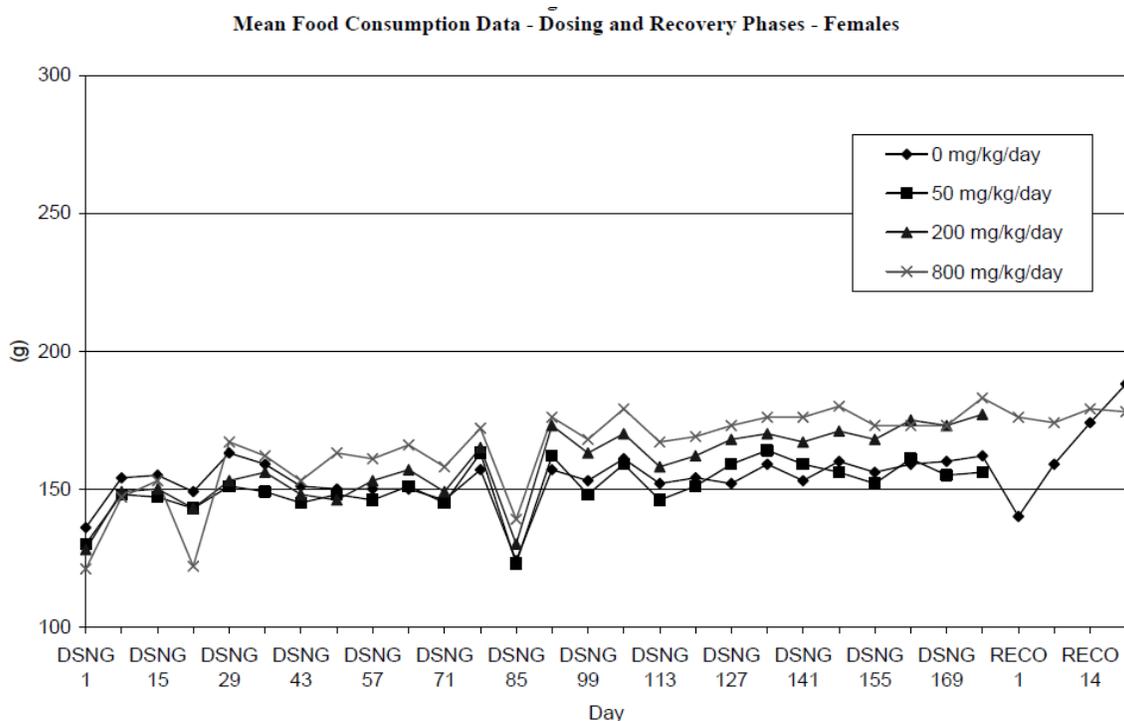
Feed Consumption

Food consumption was measured weekly for main study animals during the dosing and recovery phases.

Food consumption in the 800 mg/kg/day males was generally higher compared to controls starting on day 50, after being slightly reduced on days 1 through 50. However, statistically significant increases were noted only during a small number of intervals. In the 200 and 800 mg/kg/day females, increased food consumption was noted during most intervals. The increase in food consumption was considered to be drug-related. However, increased food consumption was not correlated with bodyweight increases, and was not considered toxicologically important. Decreased food consumption noted for days 22 through 29 and days 85 through 92 was due to fasting of the animals for collection of clinical pathology samples on days 23 and 86 of the dosing phase, respectively, as well as the lack of water availability noted on day 26 of the dosing phase.

During the first two weeks of the recovery phase, food consumption in the 800 mg/kg/day males and females increased compared to controls (up to 20.2% and 25.7%, respectively). However, part of the reasons for the relative increase was due to an unexplained decrease in food consumption in the controls. The results are shown in the figures below (taken from the Sponsor's study report).





Ophthalmoscopy

Examinations were performed during the pre-dose phase, the final week of the dosing phase, and the final week of the recovery phase. There were no drug-related ophthalmoscopic findings.

ECG

Not performed.

Hematology

Blood samples were collected for hematology from fasted animals via a jugular vein during weeks 4 and 13, and at scheduled sacrifices in the main study and recovery phase. Blood was also collected, when possible, from animals sacrificed at unscheduled intervals. The following parameters were examined (table taken from Sponsor’s study report).

- | | |
|---|------------------------------------|
| red blood cell (erythrocyte) count | platelet count |
| hemoglobin | white blood cell (leukocyte) count |
| hematocrit | differential blood cell count |
| mean corpuscular volume | blood smear |
| mean corpuscular hemoglobin | reticulocyte count |
| mean corpuscular hemoglobin concentration | |

Notable changes are summarized in the table below.

Changes in Hematology Parameters at the End of the Treatment Period

Parameter	Days	Sex	% change		
			50 mg/kg/day	200 mg/kg/day	800 mg/kg/day
Reticulocytes	23	M			+17.9*
		F			+15.5*
	86	M			+17.1*
	184	M			+17.4*
	Recovery	M			-17.2*
		F			-23.7*
Percent Reticulocytes	23	M			+18.5*
		F			+19.1*
	86	M		+27.8*	+22.2*
	184	M			+21.1*
	Recovery	M			-18.2*
		F			-21.1*
Lymphocytes	23	M			-15.6
	86	M			-13.5
	184	M		-16.4*	-22.7*
	Recovery	M			-2.1
	Percent Lymphocytes	184	M		-11.6*
	Recovery	M			+4.7
Percent Eosinophils	23	M			+25.0
	86	M			+45.5
	184	M			+35.7
	Recovery	M			+71.4
	Neutrophils	23	F		
86		F			-14.1
184		F			+45.5*
Recovery		F			+2.0
Percent neutrophils		23	M		
	86	M			+7.2
	184	M		+45.3*	+39.5*
	Recovery	M			-16.0
	Monocytes	184	F		+47.1*
Recovery		F			+0
Percent Monocytes	184	F			+27.3*
	Recovery	F			+9.1
Platelets	23	F			+18.9*
	86	F			+14.2*
	184	F			+16.5*
	Recovery	F			+6.1

There were significant increases in reticulocytes, eosinophils, neutrophils, monocytes, and platelets, and significant decreases in lymphocytes in the 200 and 800 mg/kg/day males. However, the changes occurred either early during the drug treatment period or in a single gender, and their clinical significance is unclear. All the changes were reversible after the recovery period.

There were other changes that were not dose-dependent and/or small in magnitude (less than 10%). These changes are considered sporadic and not drug-related.

Clinical Chemistry

Blood samples were collected for clinical chemistry from fasted animals via the jugular vein during weeks 4 and 13, and at scheduled sacrifices. Blood was also collected (when possible) from animals sacrificed at unscheduled intervals. The following parameters were examined (table taken from Sponsor's study report).

glucose	alkaline phosphatase
urea nitrogen	gamma glutamyltransferase
creatinine	aspartate aminotransferase
total protein	calcium
albumin	inorganic phosphorus
globulin	sodium
albumin/globulin ratio	potassium
cholesterol	chloride
total bilirubin	triglycerides
alanine aminotransferase	

Summary of Notable Changes in Clinical Chemistry Parameters

Parameter	Days	Sex	% change		
			50 mg/kg/day	200 mg/kg/day	800 mg/kg/day
Cholesterol	23	M		+8.9	+55.2*
		F		+44.3*	+54.0
	86	M		+19.8	+53.5*
		F		+48.8*	+53.7*
	184	M		+16.2	+44.8*
		F		+75.2*	+54.3*
	Recovery	M			-3.8
		F			+28.6
Alkaline Phosphatase	23	M		-25.9*	-23.1*
		F		-17.2*	-18.7*
	86	M		-21.6*	-13.6
		F			+4.1
	184	M		-15.7	-7.1
		F			+29.0
	Recovery	M			-17.1
		F			-11.2

There were dose-dependent increases in cholesterol levels in the 200 and 800 mg/kg/day group. This change is considered drug-related. Transient significant decreases in alkaline phosphatase levels were noted in the 200 and 800 mg/kg/day group on day 23.

There were other sporadic changes that were not dose-dependent, small in magnitude (less than 10%), and/or occurred only in one gender. Those changes were not considered as drug-related.

Urinalysis

Urine samples were collected from fasted animals. Samples were collected during weeks 4 and 13 of the dosing phase and at scheduled sacrifice in the main study and recovery phase. The following parameters were examined (table taken from the Sponsor's study report).

appearance (clarity and color)	bilirubin
blood	glucose
ketones	protein
microscopic examination of sediment	specific gravity
pH	volume
urobilinogen	

There were no meaningful drug-related changes in the parameters examined. Small but statistically significant changes in specific gravity (-1.2%) in the 800 mg/kg/day males, and urine pH in the 800 mg/kg/day males.

Gross Pathology

Observations were made at scheduled sacrifice (day 184 in the main study and day 29 in the recovery phase).

Main Study:

Stomach/GI: Depressed area was observed in the 800 mg/kg/day group (1/15 F); discolored area was observed in the 200 mg/kg/day (2/15 M) and 800 mg/kg/day groups (1/15 M and 5/15 F).

Skin: Subcutis abrasion was observed in the 800 mg/kg/day group (1/15 M).

Ovary: Cyst was observed in the 50, 200, and 800 mg/kg/day groups (1/15 F each).

Uterus: Distended uterus was observed in the 800 mg/kg/day group (1/15 F).

Lymph node: Large lymph node was observed in the 800 mg/kg/day group (1/15 F).

Recovery Phase:

Lung: Discolored lung was observed in the control group (1/9 M and 1/7 F) and the 800 mg/kg/day group (3/9 M and 1/9 F).

Lymph node/other: Mass was observed in the 800 mg/kg/day group (1/9 F).

Organ Weights

Organs were collected on the day of sacrifice (day 184 in the main study and day 29 in the recovery phase). The following organs were collected and their weights were measured (table taken from the Sponsor's study report).

adrenal (2)	prostate
brain	salivary gland [mandibular (2)]
epididymis (2)	seminal vesicle
heart	spleen
kidney (2)	testis (2)
liver	thymus
lung	thyroid (2 lobes) with parathyroid
ovary (2)	uterus
pituitary gland	

Main Study:

Adrenal: Organ weight/body weight ratio increased by 34.7% in the 800 mg/kg/day males.

Brain: Organ/brain weight ratio increased by 12.6% in the 800 mg/kg/day males.

Kidney: Organ weight/body weight ratio increased by 19.3% and 21.2% in the 200 and 800 mg/kg/day males, respectively.

Liver: Absolute weight, organ weight/body weight ratio, and organ weight/brain weight ratio increased by 21.6%, 32.1%, and 20.8%, respectively, in the 800 mg/kg/day males. Absolute weight, organ weight/body weight ratio, and organ weight/brain weight ratio increased by 17.2%, 13.1%, and 14.9%, respectively, in the 200 mg/kg/day females, and by 31.7%, 30.3% and 30.5%, respectively, in the 800 mg/kg/day females.

Pituitary: Organ weight/body weight ratio increased by 13.6% in the 800 mg/kg/day males.

Recovery Phase:

Epididymis: Absolute weight and organ weight/body weight ratio increased by 9.2% and 15.7%, respectively, in the 800 mg/kg/day males.

Liver: Organ weight/body weight ratio increased by 15.9% in 800 mg/kg/day females.

Lung: Absolute weight, organ weight/body weight ratio, and organ weight/brain weight ratio increased by 20.3%, 28.0%, and 19.6%, respectively, in the 800 mg/kg/day males.

Testis: Organ weight/body weight ratio increased by 10.0% in the 800 mg/kg/day males.

There were other sporadic organ weight changes; however, those changes lacked dose-dependency and/or were small in magnitude, and were not considered as drug-related.

Histopathology

Tissues and organs were collected on the day of sacrifice (day 183 in the main study and day 29 in the recovery phase). The organs/tissues that were examined are listed in the table below (taken from Sponsor's study report). All tissues from main study animals in the control and high-dose groups, animals that died or were sacrificed at an unscheduled interval, and the mammary mass collected from one female were processed and examined microscopically. The kidney, pituitary gland, liver, as well as macroscopic lesions in the low- and mid-dose groups from main study animals were also processed and examined microscopically.

adrenal (2)	optic nerve (2) ^a
aorta	ovary (2)
brain	pancreas
cecum	pituitary gland
cervix	prostate
colon	rectum
duodenum	salivary gland [mandibular (2)]
epididymis (2) ^a	sciatic nerve
esophagus	seminal vesicle
eye (2) ^a	skeletal muscle (thigh)
femur with bone marrow (articular surface of the distal end)	skin/subcutis
Harderian gland	spinal cord (cervical, thoracic, and lumbar)
heart	spleen
ileum	sternum with bone marrow
jejunum	stomach
kidney (2)	testis (2) ^a
lesions	thymus
liver	thyroid (2 lobes) with parathyroid
lung with large bronchi	tongue
lymph node (mandibular)	trachea
lymph node (mesenteric)	urinary bladder
mammary gland (females)	uterus
	vagina

Adequate Battery - Yes

Peer Review - Yes

Histological Findings

Organ/Tissue	Sex	Main Study				Recovery	
		Dose (mg/kg/day)				Dose (mg/kg/day)	
		0	50	200	800	0	800
Adrenal glands Increased vacuolation	M	0/15	--	--	2/15	0/9	0/9
Brain B-Astrocytoma	M	0/15	--	--	0/15	0/9	0/9
	F	0/15	--	--	1/15	0/7	0/9
Heart Fibrosis	M	0/15	--	--	1/15	0/9	0/9
Kidneys Chronic progressive nephropathy	M	12/15	15/15	14/15	15/15	8/9	8/9
	F	7/15	5/15	10/14	10/15	4/7	7/9
Liver Hypertrophy, Hepatocellular Increased hepatocytes Karyomegaly	F	0/15	0/14	2/14	6/15	0/7	0/9
	M	0/15	0/15	0/15	0/15	0/9	0/9
	F	0/15	0/15	0/15	0/15	0/7	2/9
Mammary Lobuloalveolar hyperplasia M-carcinoma	F	0/15	1/1	1/1	1/15	2/7	1/9
	F	0/14	0/1	1/1	0/15	0/7	1/9
Lung Alveolus macrophage infiltrate Lymphocytes/macrophages infiltrate Chronic inflammation Pigment	M	3/15	0/1	0/1	7/15	6/9	7/9
	F	3/15	--	0/1	1/15	5/7	5/9
	M	1/15	0/1	0/1	0/15	0/9	0/9
	F	0/15	--	0/1	3/15	4/7	5/9
	M	2/15	1/1	1/1	1/15	8/9	6/9
	F	2/15	--	0/1	8/15	1/7	0/9
	M	0/15	0/1	0/1	1/15	0/9	1/9
Lymph Node (mandibular) Primary unknown metastatic carcinoma	F	0/15	--	--	1/15	0/7	0/9
Pancreas Lymphocytes/macrophages infiltrate Pigment Atrophy Chronic inflammation Hemorrhage	M	8/15	--	--	5/15	3/9	6/9
	F	4/15	--	--	7/15	0/7	3/9
	M	5/15	--	--	9/15	5/9	5/9
	F	2/15	--	--	0/15	0/7	1/9
	M	1/15	--	--	2/15	0/9	1/9
	F	0/15	--	--	2/15	0/7	0/9
	M	0/15	--	--	1/15	0/9	0/9
	M	0/15	--	--	1/15	0/9	1/9

Pituitary B-adenoma	M	0/15	0/15	0/15	1/15	0/9	0/9
	F	0/15	0/15	0/14	1/15	0/7	0/9
Hyperplasia/Hypertrophy	M	0/15	1/15	0/15	3/15	1/9	1/9
Prostate gland Chronic/Chronic active inflammation	M	2/15	-- --	--	4/15	3/9	5/9
Rectum Eosinophilic inflammation	M	0/15	--	--	1/15	0/9	0/9
Skin/Subcutis Erosion/Ulcer	M	0/15	--	--	1/15	0/9	0/9
	M	0/15	--	--	1/15	0/9	0/9
Spleen Increased pigment	F	3/15	-	--	7/15	2/7	1/9
Stomach Subacute inflammation	M	0/15	--	0/2	1/15	0/9	0/9
Uterus Dilatation	F	0/15	--	--	2/15	0/7	1/9

M: malignant

B: benign

Dose-dependent hepatocellular hypertrophy was noted in the 200 and 800 mg/kg/day females. The hypertrophy was described as centrilobular to midzonal hepatocellular hypertrophy, characterized by minimally enlarged hepatocytes with increased cytoplasm, sometimes accompanied by enlarged nuclei. Hepatocellular hypertrophy was no longer present after the recovery period, suggesting this change was reversible. This hypertrophy correlated with increased liver weights in these dose groups. Pancreatic atrophy was noted in the 800 mg/kg/day males and females, and the change was partially reversible after the recovery period. Dilatation of the uterus was noted in the 800 mg/kg/day females, and this change was partially reversed after the recovery period.

Carcinoma of the mammary gland was noted in one recovery phase 800 mg/kg/day female and one main study 200 mg/kg/day female. Because there was no clear dose relationship, the neoplasms were not considered to be drug-related. A metastatic carcinoma of unknown origin was diagnosed in the mandibular lymph node of one main study 800 mg/kg/day female, and a tumor (astrocytoma) was diagnosed in the brain of one main study 800 mg/kg/day female. These two neoplasms were considered most likely spontaneous and not related to NKTR-118 administration. Pituitary adenoma of the pars distalis was noted in two main study 800 mg/kg/day animals (1 male and 1 female). The Sponsor indicated that pituitary adenomas are common, spontaneously occurring neoplasms in aging rats and are among the most common early occurring tumors in rats. Because proliferative pituitary changes (adenomas and/or hyperplasia/hypertrophy) occurred with low incidence in several groups in this study, including controls, they were not considered as drug-related.

The other microscopic findings were not dose-dependent, present in control and treatment groups, and/or were low in incidence. Therefore, these changes were not considered to be drug-related.

Special Evaluation

None

Toxicokinetics

Maximum plasma levels of NKTR-118 were achieved within the first collection time (0.25 hour) after oral administration. Plasma exposure to NKTR-118 (AUC_{0-24hr}) was greater than dose proportional in males, and approximately dose proportional in females. Females had higher (1.2- to 4.3-fold) plasma NKTR-118 exposure than males. After repeated dosing, there was moderate accumulation of NKTR-118 in males (1.2- to 2.5-fold) and females (0.87- to 1.5-fold). The average C_{max} and AUC_{0-24hr} values for both sexes at 200 mg/kg/day (the lowest observed adverse effect level) were 14.1 $\mu\text{g/mL}$ and 104.5 $\mu\text{g}\cdot\text{h/mL}$, respectively, on week 26. The data are summarized in the Sponsor's table below.

Plasma NKTR-118 Toxicokinetic Parameters

Day/Week	Dose (mg/kg/day)	C_{max} ($\mu\text{g/mL}$)	$AUC(0-24hr)$ ($\text{hr}\cdot\mu\text{g/mL}$)	
			Male	Female
Day 1	50	1.21	4.48	3.85
	200	9.19	32.7	16.4
	800	17.7	169	35.0
Week 4	50	1.32	5.41	5.43
	200	7.77	56.7	15.9
	800	29.3	314	28.7
Week 13	50	1.26	6.96	4.33
	200	10.8	72.7	18.0
	800	36.0	389	40.3
Week 26	50	2.82	9.17	6.14
	200	13.0	82.0	15.1
	800	36.9	389	52.4

NKTR-118 was metabolized quickly to NKTR-118–glucuronide; peak plasma levels of NKTR-118–glucuronide were achieved within 0.25 hour after oral administration of NKTR-118. After repeated dosing, there was accumulation of NKTR-118–glucuronide in males (2.0- to 5.7-fold) and females (1.8- to 4.2-fold) on dosing week 26.

Dosing Solution Analysis

Mean dose concentrations were between 98.6 and 109% of the theoretical concentrations. The low- and high-dose formulations used on day 1 of the dosing phase were 113 and 111% of theoretical concentrations, respectively. There was no evidence of formulation issues, instrument failure, or dilution errors; however, due to the initial results being greater than 10% of the theoretical yield, the backup samples were analyzed. The mean concentrations of the backup samples were 109 and 105% of theoretical concentrations for the low- and high-dose formulations, respectively. The high-dose samples from week 13 were not diluted within the range of the standard concentrations, and the results were calculated using responses not within the established linearity range of the analytical method. The Sponsor suggested that this was acceptable for the analysis of these samples and acceptable results were obtained. Formulations were solutions at all concentrations and, therefore, homogeneity was not analyzed. All dose formulations were considered acceptable for use on study.

Study title: 39-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with NKTR-118 in Dogs with a 4-Week Recovery Period

Study no.:	7985-105
Study report location:	N/A
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	10-31-2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NKTR-118; 149013, 149012, 149014, 149011, 149010, 149007, 149008; 98%

Key Study Findings

Animals (6/sex/group) were treated orally with 0 (vehicle), 20, 200, or 500 mg/kg/day NKTR-118 for 39 weeks. Recovery animals (4/sex/group) were treated orally with 0 or 500 mg/kg/day NKTR-118 for 39 weeks, followed by a 4-week recovery period. NKTR-118 was generally well tolerated. There were no deaths. Drug-related clinical signs included tremors, ataxia, hypoactive behavior, and skin lesions in the 500 mg/kg/day group, retching and increased incidence of emesis in the 200 and 500 mg/kg/day groups, and dose-dependent excessive salivation. These clinical signs were no longer present after the recovery period. Sinus tachycardia was observed in the control, 200, and 500 mg/kg/day groups, and was not considered drug-related. Increases in cholesterol levels (up to 51%), were observed in the 200 mg/kg/day males and females and the 500 mg/kg/day males. There were no meaningful macroscopic or microscopic findings. The NOAEL is considered to be 200 mg/kg/day, based on tremors, ataxia, and hypoactive behavior in the 500 mg/kg/day animals.

Methods

Doses: 0 (vehicle), 50, 200, or 500 mg/kg/day
 Frequency of dosing: once daily
 Route of administration: oral (gavage)
 Dose volume: 10 ml/kg
 Formulation/Vehicle: solution / reverse osmosis water
 Species/Strain: dogs/beagle
 Number/Sex/Group: Main study: 6/sex/group
 Recovery: 4/sex/group
 Age: 4.5 to 5.5 months old
 Weight: Males: 5.2 to 7.9 kg
 Females: 4.7 to 8.2 kg
 Satellite groups: N/A
 Unique study design: N/A
 Deviation from study protocol: There were minor deviations that did not affect the quality or integrity of the study.

Study Design of the 39-Week Oral Toxicity Study in Dogs

Group ^a	No. of Animals ^b		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
	Male	Female		
1 (Control)	10	10	0	0
2 (Low)	6	6	50	5
3 (Mid)	6	6	200	20
4 (High)	10	10	500	50

a Group 1 received control article only.

b Animals designated for recovery sacrifice (four/sex in Groups 1 and 4) underwent 4 weeks of recovery following dose administration.

Observations and Results

All animals were observed twice daily for morbidity and mortality. There were no deaths.

Clinical Signs

Animals were observed twice daily for abnormalities, and signs of pain or distress. During the dosing phase, cageside observations were made at approximately 1 hour postdose based on the last animal dosed. Detailed observations were performed once during the pre-dose phase, before dosing on day 1, weekly thereafter, and on the day of terminal sacrifice.

Main Study:

The following notable clinical signs were observed.

Summary of Notable Clinical Signs

Clinical Signs	Sex	Main Study			
		Dose (mg/kg/day) (# of animals)			
		0 (10)	50 (6)	200 (6)	500 (10)
Appearance					
<i>Tremors</i>	F	0	0	0	2
<i>Tremors, entire body</i>	M	0	0	0	3
	F	0	0	0	2
<i>Tremors, hind legs</i>	M	0	0	0	3
	F	0	0	0	3
<i>Tremors, left hind leg</i>	F	0	0	0	1
<i>Tremors, cranial head</i>	F	0	0	0	2
Behavior					
<i>Ataxic</i>	M	0	0	0	1
	F	0	0	0	5
<i>Hyperactive</i>	F	0	0	0	1
<i>Hypoactive</i>	M	0	0	0	7
	F	0	0	0	1
<i>Retching</i>	M	0	0	3	6
	F	0	0	1	6
<i>Difficulty during dosing</i>	M	0	0	3	2
	F	0	0	1	2
Discharge					
<i>Excessive salivation</i>	M	0	2	6	10
	F	0	1	3	10
<i>Vomitus, clear</i>	M	0	1	3	7
	F	1	0	4	8
<i>Vomitus, cloudy</i>	M	0	0	3	1
	F	0	0	2	1
<i>Vomitus, containing food</i>	M	4	2	5	10
	F	4	2	6	10
<i>Vomitus, foamy</i>	F	3	3	5	8
<i>Vomitus, white in color</i>	M	2	2	5	6
	F	0	2	3	6
Skin and Pelage					
<i>Broken skin, ears</i>	F	0	0	0	1

<i>Broken skin, left ear</i>	F	0	0	0	1
<i>Broken skin, right ear, behind</i>	F	0	0	0	1
<i>Broken skin, left hind leg</i>	M	0	0	0	1
<i>Red skin, ears</i>	F	0	0	0	1
<i>Red skin, ears, inside</i>	F	0	0	0	1
<i>Scab, ear</i>	F	0	0	0	1
<i>Scab, left ear</i>	F	0	0	0	1
<i>Scab, left hind leg</i>	M	0	0	0	1

Drug-related clinical signs included tremors, ataxia, hypoactive behavior, and skin lesions in the 500 mg/kg/day animals, retching and higher incidence of emesis in the 200 and 500 mg/kg/day animals, and dose-dependent excessive salivation. There were other sporadic clinical signs that were not dose-dependent, and they are not considered as drug-related.

Recovery Phase:

There were no drug-related clinical signs.

Bodyweights

Animals were weighed during the pre-dose phase, prior to dosing on day 1, and once weekly thereafter.

There were no meaningful drug-related effects.

Feed Consumption

Food consumption was measured daily for weeks 1 through 5, and weekly thereafter through the end of the recovery phase.

There were no significant drug-related effects.

Ophthalmoscopy

Examinations were performed during the pre-dose phase, on day 272 of the dosing phase, and on day 27 of the recovery phase.

There were no drug-related ophthalmoscopic findings.

ECG

ECG was recorded using 10 leads once during the pre-dose phase, on weeks 4, 13, 26, and 40 of the main study, and within 3 days of the recovery phase sacrifice. A heart rate correction for the QT interval (QT_c) was calculated (QT_c (Seconds) = $(QT / ((60/HR)^{(1/3)}))$)

Sinus bradycardia (<70/minute) was observed in 2 control males (week 40) and one 500 mg/kg/day female (recovery phase). Sinus tachycardia (>170/minute) was observed at week 4 in one male in each of the 50 and 500 mg/kg/day groups, at week 13 in two 50 mg/kg/day males, at week 40 in a 50 mg/kg/day male, at weeks 4 and 26 in a 200 mg/kg/day male, and at weeks 4, 26, and 40 in a 500 mg/kg/day female. The data are summarized in the table below.

Summary of Sinus Tachycardia Incidences

Week	Dose (mg/kg/day)			
	Control	50	200	500
4		1M	1M	1M, 1F
13		2M		
26			1M	1F
40		1M		1F

The Sponsor noted that bradycardia can be observed sporadically in young dogs, and that sinus tachycardia is a frequent arrhythmia in dogs and is usually associated with nervousness and excitement. The incidence of sinus tachycardia did not appear to be dose-dependent. Therefore, the sporadic heart rate findings are not considered as drug-related. The Sponsor reported that all other electrocardiogram parameters were within expected limits at all intervals examined.

Hematology

Blood samples were collected twice during the pre-dose phase, at weeks 4, 13, and 26, and before scheduled sacrifices in the main study and recovery phase. The following hematologic parameters were evaluated (table taken from the Sponsor's study report).

red blood cell (erythrocyte) count	platelet count
hemoglobin	white blood cell (leukocyte) count
hematocrit	differential blood cell count
mean corpuscular volume	blood smear
mean corpuscular hemoglobin	reticulocyte count
mean corpuscular hemoglobin concentration	

Hematocrit in the 500 mg/kg/day males was decreased by 6.1% on dosing day 275. There were other changes that were not dose-dependent. There were no drug-related effects in the recovery phase. Therefore, there were no meaningful drug-related effects on hematology parameters.

Clinical Chemistry

Blood samples were collected twice during the pre-dose phase, at weeks 4, 13, and 26, and before scheduled sacrifices in the main study and recovery phase. The following parameters were evaluated (table taken from Sponsor's study report).

glucose	alkaline phosphatase
urea nitrogen	gamma glutamyltransferase
creatinine	aspartate aminotransferase
total protein	calcium
albumin	inorganic phosphorus
globulin	sodium
albumin/globulin ratio	potassium
cholesterol	chloride
total bilirubin	triglycerides
alanine aminotransferase	

Increases in cholesterol levels were observed on dosing days 24, 87, 178 and 275 in the 200 and 500 mg/kg/day males (up to +31.6% and +46.3%, respectively), and on dosing day 275 in the 200 mg/kg/day females (+50.6%). The increase in cholesterol levels was small in magnitude and it lacked a microscopic correlate. The cholesterol levels in the 500 mg/kg/day group decreased by recovery day 29. There were other sporadic changes, but they lacked dose-dependency and were small in magnitude.

Urinalysis

Urine samples were collected once during the pre-dose phase, at weeks 4, 13, and 26, and before scheduled sacrifices in the main study and recovery phase. The following parameters were evaluated (table taken from the Sponsor's study report).

appearance (clarity and color)	ketones
volume	bilirubin
specific gravity	urobilinogen
pH	blood
protein	microscopic examination of sediment
glucose	

There were no drug-related effects.

Gross Pathology

Observations were made at scheduled sacrifices (day 275 in the main study and day 29 in the recovery phase).

Main Study

Subcutis skin: Abrasion was observed in the 500 mg/kg/day group (1/6 M and 1/6 F).

Mammary gland: Thickened female mammary gland was observed in the control (1/6), 50 mg/kg/day (3/6), 200 mg/kg/day (4/6), and 500 mg/kg/day (3/6) females.

Discoloration was observed in the duodenum (1/6 M and 1/6 F), colon (1/6 M), ileum (1/6 M), jejunum (1/6 M), cecum (1/6 M), and rectum (1/6 M) in the 500 mg/kg/day groups.

The Sponsor attributed the thickening, abnormal shape, and/or enlargement of the mammary gland to normal sexual maturation. The macroscopic findings are not considered as adverse.

Recovery Phase

The macroscopic findings were no longer apparent at the end of the recovery period.

Organ Weights

Organs were collected on the day of sacrifice (day 275 in the main study and day 29 in the recovery phase). The following organs were collected and their weights were measured (table taken from the Sponsor's study report).

adrenal (2)	prostate
brain	salivary gland [mandibular (2)]
epididymis (2)	spleen
heart	testis (2)
kidney (2)	thymus
lung	thyroid (2 lobes) with parathyroid
ovary (2)	uterus
pituitary gland	liver with gallbladder (drained)

Heart: Heart weight/brain weight ratio decreased by 17.5% in the 500 mg/kg/day males compared to controls.

Kidney: Absolute weight and organ weight/brain weight ratio decreased by 24.0% and 26.6%, respectively, in the 500 mg/kg/day males compared to controls.

Liver/gall bladder: Absolute weight increased by 10.5% and 27.2% in the 200 and 500 mg/kg/day males, respectively, and organ weight/brain weight ratio increased by 23.1% in the 500 mg/kg/day males.

Pituitary: Absolute weight and organ weight/brain weight ratio increased by 47.7% and 45.3%, respectively, in the 500 mg/kg/day males.

The significant changes were observed only in males, lacked macroscopic and microscopic correlates, and were reversible at the end of the recovery period. Therefore, the findings were not considered as adverse.

Histopathology

Tissues and organs were collected on the day of sacrifice (day 275 in the main study and day 29 in the recovery phase). The organs/tissues that were examined are listed in the table below (taken from Sponsor's study report).

adrenal (2)	optic nerve (2) ^a
aorta	ovary (2)
brain	pancreas
cecum	pituitary gland
cervix	prostate
colon	rectum
duodenum	salivary gland [mandibular (2)]
epididymis (2) ^a	sciatic nerve
esophagus	skeletal muscle (thigh)
eye (2) ^a	skin/subcutis
femur with bone marrow (articular surface of the distal end)	spinal cord (cervical, thoracic, and lumbar)
gallbladder	spleen
heart	sternum with bone marrow
ileum	stomach
jejunum	testis (2) ^a
kidney (2)	thymus
lacrimal gland	thyroid (2 lobes) with parathyroid
lesions	tongue
liver	trachea
lung with large bronchi	urinary bladder
lymph node (mandibular)	uterus
lymph node (mesenteric)	vagina
mammary gland (females)	

Adequate Battery - Yes

Peer Review - No

Histological Findings

Sporadic microscopic findings were observed. However, the findings lacked dose-dependency and were low in frequency. There were no meaningful drug-related microscopic effects.

Special Evaluation

N/A

Toxicokinetics

Blood samples were collected twice during the pre-dose phase, at weeks 4, 13, and 26, before scheduled sacrifices, and at a single time-point once weekly during the recovery phase. The TK data for the parent drug and the glucuronide conjugate are shown in the Sponsor's tables below.

Plasma NKTR-118 Toxicokinetic Parameters

Day/Week	Sex	Dose (mg/kg/day)	TK Parameter Value (Mean ± SD) †			
			C _{max} µg/mL	C _{24hr} µg/mL	AUC(0-24hr) hr•µg/mL	T _{max} hr
Day 1	M	50	3.42 ± 1.27	0.00 ± 0.00	5.77 ± 1.53	0.250
		200	28.8 ± 20.0	0.0497 ± 0.0792	36.0 ± 21.4	0.250
		500	58.7 ± 18.9	0.0115 ± 0.0243	86.1 ± 14.2	0.250
	F	50	4.93 ± 1.76	0.00 ± 0.00	6.93 ± 1.01	0.250
		200	16.9 ± 2.18	0.00 ± 0.00	30.2 ± 4.04	0.250
		500	69.1 ± 51.5	0.0431 ± 0.0571	88.0 ± 37.6	0.250
Week 4	M	50	5.09 ± 1.77	0.00 ± 0.00	8.97 ± 2.34	0.250
		200	29.8 ± 14.6	0.0375 ± 0.0919	43.3 ± 19.3	0.250
		500	58.2 ± 36.7	0.097 ± 0.114	94.7 ± 31.9	0.250
	F	50	4.34 ± 2.08	0.0075 ± 0.0184	6.78 ± 1.79	0.250
		200	23.9 ± 7.25	0.0332 ± 0.043	35.5 ± 8.05	0.250
		500	59.8 ± 20.7	0.109 ± 0.0864	102 ± 23.9	0.250
Week 13	M	50	5.47 ± 2.37	0.00817 ± 0.020	9.34 ± 1.85	0.250
		200	29.9 ± 8.23	0.0237 ± 0.0373	48.2 ± 8.67	0.270
		500	62.5 ± 22.8	0.129 ± 0.120	119 ± 29.4	0.250
	F	50	4.18 ± 1.51	0.0118 ± 0.0186	9.36 ± 1.72	0.250
		200	22.5 ± 4.36	0.0747 ± 0.101	57.5 ± 6.20	0.250
		500	53.7 ± 39.9	0.258 ± 0.185	116 ± 55.5	0.250
Week 26	M	50	6.01 ± 3.24	0.00 ± 0.00	11.1 ± 2.82	0.250
		200	32.7 ± 17.5	0.0237 ± 0.028	48.5 ± 19.7	0.250
		500	93.3 ± 45.2	0.0933 ± 0.119	149 ± 54.2	0.625
	F	50	3.19 ± 1.36	0.00 ± 0.00	6.45 ± 2.57	0.250
		200	16.8 ± 6.67	0.0168 ± 0.0274	33.8 ± 6.29	0.250
		500	58.3 ± 22.2	0.218 ± 0.267	116 ± 32.6	1.00
Week 39	M	50	6.60 ± 2.45	0.0175 ± 0.0273	13.3 ± 3.32	0.250
		200	38.3 ± 15.6	0.030 ± 0.0241	54.4 ± 17.3	0.250
		500	138 ± 83.5	0.251 ± 0.147	194 ± 62.8	0.250
	F	50	6.12 ± 1.86	0.0325 ± 0.0396	11.7 ± 3.54	0.250
		200	38.8 ± 24.5	0.110 ± 0.0842	57.4 ± 25.9	0.250
		500	106 ± 49.6	0.351 ± 0.276	205 ± 46.9	0.250

Plasma NKTR-118-glucuronide Toxicokinetic Parameters

Day/Week	Sex	Dose (mg/kg/day)	TK Parameter Value (Mean ± SD) [‡]			
			C _{max} μg/mL	C _{24hr} μg/mL	AUC(0-24hr) hr•μg/mL	T _{max} hr
Day 1	M	50	8.08 ± 3.18	0.0113 ± 0.0278	20.0 ± 7.26	0.260
		200	103 ± 50.1	0.061 ± 0.149	185 ± 85.4	1.00
		500	213 ± 42.3	0.0894 ± 0.133	472 ± 80.2	1.00
	F	50	11.5 ± 5.17	0.011 ± 0.0269	21.9 ± 9.49	0.250
		200	98.1 ± 29.9	0.00833 ± 0.0204	172 ± 53.5	1.00
		500	167 ± 77.7	0.167 ± 0.146	352 ± 167	1.00
Week 4	M	50	13 ± 4.86	0.0308 ± 0.0422	29.6 ± 10.4	0.250
		200	140 ± 86.1	0.162 ± 0.242	282 ± 131	1.00
		500	347 ± 89.4	0.701 ± 0.931	719 ± 187	1.00
	F	50	26.9 ± 13	0.0957 ± 0.119	44.6 ± 18.8	0.25
		200	155 ± 49.9	0.278 ± 0.211	296 ± 87.7	1.00
		500	382 ± 118	0.695 ± 0.519	776 ± 248	1.00
Week 13	M	50	16.4 ± 11.7	0.0695 ± 0.0766	31.7 ± 15.9	0.250
		200	146 ± 69.0	0.169 ± 0.143	292 ± 107	1.00
		500	455 ± 136	0.613 ± 0.303	986 ± 297	1.00
	F	50	13.1 ± 4.55	0.0658 ± 0.0794	27.1 ± 7.27	0.250
		200	171 ± 51.9	0.431 ± 0.471	344 ± 99.0	1.00
		500	377 ± 121	1.27 ± 0.885	843 ± 351	1.00
Week 26	M	50	21.7 ± 12.1	0.0673 ± 0.0386	42.1 ± 20.4	0.250
		200	209 ± 56.8	0.185 ± 0.122	415 ± 80.3	1.00
		500	510 ± 207	0.702 ± 0.684	1120 ± 406	1.00
	F	50	18.6 ± 8.98	0.0505 ± 0.0545	32.0 ± 8.48	0.25
		200	164 ± 65.7	0.195 ± 0.136	318 ± 106	1.00
		500	443 ± 166	1.55 ± 1.50	1110 ± 443	1.00
Week 39	M	50	20.1 ± 7.56	0.139 ± 0.137	42.8 ± 19.7	0.250
		200	204 ± 57.7	0.283 ± 0.201	407 ± 93.1	1.00
		500	485 ± 123	1.95 ± 1.45	1120 ± 335	1.00
	F	50	35.1 ± 30.9	0.156 ± 0.152	52.7 ± 24.3	0.250
		200	272 ± 130	0.808 ± 0.701	515 ± 288	1.00
		500	487 ± 94.2	1.33 ± 0.885	1270 ± 409	1.00

[‡] T_{max} value is reported as median.

Maximum plasma levels of NKTR-118 were achieved between 0.25 to 1 hour after oral administration. Plasma exposure of NKTR-118 (AUC_{0-24hr}) was slightly more than dose proportional in the 200 and 500 mg/kg/day males and females. Males and females showed similar plasma NKTR-118 exposure, except on week 26 when females showed

lower exposure. After repeated dosing, there was moderate accumulation of NKTR-118 in males (1.5- to 2.3-fold) and females (0.6- to 2.3-fold) in all dosing groups on week 39. In general, the accumulation increased with time in all treatment groups. The average C_{max} and AUC_{0-24hr} values for both sexes in the 200 mg/kg/day group (NOAEL) on dosing day 275 were 38.6 $\mu\text{g/mL}$ and 55.9 $\mu\text{g}\cdot\text{h/mL}$, respectively.

NKTR-118 was metabolized quickly to NKTR-118-glucuronide; peak plasma levels of NKTR-118-glucuronide were achieved at 0.25 to 1 hour after oral administration of NKTR-118. Both AUC_{0-24hr} and C_{max} for NKTR-118-glucuronide exceeded that of the parent compound by at least 2-fold at all doses. After repeated dosing, there was moderate accumulation of NKTR-118-glucuronide in males (2.1- to 2.4-fold) and females (2.4- to 3.6-fold) on dosing week 39.

Dosing Solution Analysis

The dosing formulations with nominal concentrations of 5 and 50 mg/mL from weeks 1, 4, 13, 26 and 39 were within $\pm 10\%$ of the nominal concentration (90 to 110% of the mean target). All dose formulations with the nominal concentration of 20 mg/mL were within $\pm 10\%$ of the nominal concentration, except for week 39 (87.2% of the mean target). The backup samples were analyzed and they were within $\pm 10\%$ of the nominal concentration. Therefore, the dose formulations were acceptable for use in the study and interpretation of study results.

7 Genetic Toxicology

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

Study title: NKT 10018 Bacterial Mutation Test	
Study no.:	LS-2007-006
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	02/13/2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NKT-10018, 149002, 96%

Key Study Findings: NKT-10018 (same drug substance as NKTR-118) was positive in the Ames test.

Methods	
Strains:	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535 and TA1537, and <i>Escherichia coli</i> WP2 <i>uvrA</i>
Concentrations in definitive study:	1.58, 5.0, 15.8, 50, 158, 500, 1581, and 5000 µg per plate
Basis of concentration selection:	No precipitate or background lawn toxicity was observed at the maximum dose tested (5000 µg/plate)
Negative control:	Sterile water
Positive control:	Plate incorporation method in the absence of metabolic activation: sodium azide (NaAz, 0.5 µg/plate) for TA1535 and TA100, 2-nitrofluorene (2NF, 1.0 µg/plate) for TA98, 9-aminoacridine (9AC, 50 µg/plate) for TA1537, and 4-nitroquinoline N-oxide (NQO, 0.5 µg/plate) for WP2 <i>uvrA</i> . Plate incorporation method in the presence of metabolic activation: 2-aminoanthracene (2AA, 5.0 µg/plate) for TA1535, 15 µg/plate AA for WP2 <i>uvrA</i> , and Benzo[a]pyrene (BaP, 5 µg/plate) for TA98, TA1537, TA100. The only difference in positive control compounds used for the pre-incubation method was the 9AC dose (10 µg/plate) for TA1537 in the absence of metabolic activation.
Formulation/Vehicle:	NKT-10018 dissolved in sterile water
Incubation & sampling time:	Both the plate incorporation method and the pre-incubation method were used in the study. Phenobarbital/5,6-benzoflavone-induced rat liver S9 (10%) was used as the metabolic activation system. The plates (triplicate in the confirmation assay) were incubated for approximately 48 to 72 hours at 37°C. The condition of the bacterial background lawn was evaluated for evidence of test article toxicity, and precipitate was evaluated after the incubation period by visual examination. Revertant colonies for a given tester strain and activation condition were counted by automated colony counter. When the plates exhibited precipitation, the colonies were counted visually at the discretion of the study director.

Study Validity: Selection of bacterial tester strains was appropriate. The positive control compounds produced the expected responses. Dose selection was adequate based upon use of the limit dose (i.e., 5000 µg/plate). Neither precipitate nor background lawn toxicity was observed at the maximum dose of 5000 µg/plate. The S9 concentration (10%) was within acceptable limits. The criteria for a positive mutagenic response was a dose-related increase in revertant colony numbers at least twice the concurrent vehicle control value with any bacterial strain (1.5 times vehicle control for strain TA100) either in the presence or absence of S9 mix. Provided mean value(s) lay outside the historical control range, this was considered to be indicative of mutagenic activity. The criteria for a negative mutagenic response was the absence of a dose-related increase of at least 2 times (1.5 times for TA100) the concurrent vehicle control value. If the results failed to satisfy the criteria for a clear positive or negative response, the results were considered equivocal. The Sponsor considered it acceptable to conclude an equivocal response if no clear conclusion can be made. Therefore, the study is deemed valid.

Results: There was no visible thinning of the background lawn of non-revertant bacteria following exposure to NKT-10018, indicating that NKT-10018 was non-toxic to the bacteria at the levels tested. No precipitation was observed. The mean revertant colony counts for the vehicle controls were close to or within the laboratory historical control range. Appropriate positive controls (with S9 mix where required) induced increases in revertant colony numbers to at least twice the concurrent vehicle control value with the appropriate bacterial strain (1.5 times for strain TA100), confirming sensitivity of the test system and activity of the S9 mix. There were significant increases in revertant colony numbers, compared to vehicle controls, with strain TA1535 following exposure to 1581 or 5000 µg/plate of NKT-10018 in the absence of S9 (2.2 and 4.1 times, respectively) and 5000 µg/plate of NKT-10018 in presence of S9 mix (4.0 times) in the plate incorporation assay. In the pre-incubation assay, there were significant increases in revertant colony numbers with strains TA1535 and TA100 following exposure to 5000 µg/plate of NKT-10018 (2.4 and 1.6 times the vehicle control, respectively) in the absence of S9 mix. The results are summarized in the Sponsor's tables below.

Table 1 NKT-10018 - Plate Incorporation Assay in the Absence of S9 Mix

Strain	Conc. (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †
			x_1	x_2	x_3	mean	SD	x_1	x_2	x_3	
TA1535	Water	0	20	22	19	20	2				1.0
	50	0	17	16	18	17	1				0.8
	158	0	31	34	24	30	5				1.5
	500	0	32	27	33	31	3				1.5
	1581	0	35	44	53	44	9				2.2 +
	5000	0	84	76	90	83	7				4.1 +
TA1537	Water	0	18	12	13	14	3				1.0
	50	0	20	22	14	19	4				1.3
	158	0	19	14	13	15	3				1.1
	500	0	13	25	15	18	6				1.2
	1581	0	13	13	15	14	1				1.0
	5000	0	15	22	12	16	5				1.1
TA98	Water	0	33	23	41	32	9				1.0
	50	0	34	22	29	28	6				0.9
	158	0	25	33	26	28	4				0.9
	500	0	33	26	32	30	4				0.9
	1581	0	29	22	37	29	8				0.9
	5000	0	32	25	15	24	9				0.7
TA100	Water	0	155	164	171	163	8				1.0
	50	0	142	159	145	149	9				0.9
	158	0	144	162	136	147	13				0.9
	500	0	160	171	166	166	6				1.0
	1581	0	155	189	173	172	17				1.1
	5000	0	202	212	214	209	6				1.3
WP2 <i>uvrA</i>	Water	0	50	53	51	51	2				1.0
	50	0	60	62	60	61	1				1.2
	158	0	42	57	61	53	10				1.0
	500	0	54	64	57	58	5				1.1
	1581	0	51	48	57	52	5				1.0
	5000	0	64	55	56	58	5				1.1

* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation

+ Substantial dose-related increase in revertant colony counts (fold response ≥ 2 for TA1535)

Table 2 NKT-10018 - Plate Incorporation Assay in the Presence of S9 Mix

Strain	Conc. (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †
			x_1	x_2	x_3	mean	SD	x_1	x_2	x_3	
TA1535	Water	+	31	24	19	25	6				1.0
	50	+	23	18	23	21	3				0.9
	158	+	30	18	30	26	7				1.1
	500	+	29	28	29	29	1				1.2
	1581	+	42	53	51	49	6				1.97
	5000	+	108	100	90	99	9				4.0 +
TA1537	Water	+	18	11	15	15	4				1.0
	50	+	18	18	25	20	4				1.4
	158	+	20	18	25	21	4				1.4
	500	+	12	27	17	19	8				1.3
	1581	+	15	9	17	14	4				0.9
	5000	+	15	14	17	15	2				1.0
TA98	Water	+	42	36	37	38	3				1.0
	50	+	52	45	58	52	7				1.3
	158	+	28	41	32	34	7				0.9
	500	+	51	46	42	46	5				1.2
	1581	+	54	33	53	47	12				1.2
	5000	+	53	50	54	52	2				1.4
TA100	Water	+	142	178	181	167	22				1.0
	50	+	184	165	159	169	13				1.0
	158	+	156	158	179	164	13				1.0
	500	+	175	188	197	187	11				1.1
	1581	+	205	208	199	204	5				1.2
	5000	+	245	215	210	223	19				1.3
WP2 <i>uvrA</i>	Water	+	54	65	51	57	7				1.0
	50	+	62	69	73	68	6				1.2
	158	+	70	86	73	76	9				1.3
	500	+	65	78	67	70	7				1.2
	1581	+	63	53	69	62	8				1.1
	5000	+	79	72	62	71	9				1.3

* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation

+ Substantial dose-related increase in revertant colony counts (fold response ≥ 2 for TA1535)

Positive Controls for the Plate Incorporation Assay

Strain	Treatment	Conc. ($\mu\text{g}/\text{plate}$)	S9	Number of revertants			Fold		response †
				x_1	x_2	x_3	mean	SD	
TA1535	NaAz	0.5	0	341	301	348	330	25	16
TA1537	9AC	50	0	245	178	162	195	44	14
TA98	2NF	1	0	188	171	173	177	9	5.5
TA100	NaAz	0.5	0	553	572	598	574	23	3.5
WP2 <i>uvrA</i>	NQO	0.5	0	188	211	185	195	14	3.8
TA1535	2AA	5	+	472	449	453	458	12	19
TA1537	BaP	5	+	121	131	122	125	6	8.5
TA98	BaP	5	+	461	471	461	464	6	12
TA100	BaP	5	+	1548	1516	1534	1533	16	9.2
WP2 <i>uvrA</i>	2AA	15	+	374	350	477	400	67	7.1

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation

Table 4 NKT-10018 - Pre-incubation Assay in the Absence of S9 Mix

Strain	Conc. (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †
			x_1	x_2	x_3	mean	SD	x_1	x_2	x_3	
TA1535	Water	0	24	27	25	25	2				1.0
	50	0	29	17	19	22	6				0.9
	158	0	23	24	24	24	1				0.9
	500	0	21	22	17	20	3				0.8
	1581	0	37	28	32	32	5				1.3
	5000	0	58	60	61	60	2				2.4 +
TA1537	Water	0	14	17	18	16	2				1.0
	50	0	19	23	13	18	5				1.1
	158	0	11	12	8	10	2				0.6
	500	0	11	15	16	14	3				0.9
	1581	0	17	13	12	14	3				0.9
	5000	0	15	19	16	17	2				1.0
TA98	Water	0	34	25	23	27	6				1.0
	50	0	40	35	38	38	3				1.4
	158	0	16	28	44	29	14				1.1
	500	0	31	36	26	31	5				1.1
	1581	0	30	28	37	32	5				1.2
	5000	0	30	37	41	36	6				1.3
TA100	Water	0	125	130	111	122	10				1.0
	50	0	175	153	159	162	11				1.3
	158	0	153	163	179	165	13				1.4
	500	0	142	146	136	141	5				1.2
	1581	0	139	126	114	126	13				1.0
	5000	0	179	206	189	191	14				1.6 +
WP2 <i>uvrA</i>	Water	0	45	55	46	49	6				1.0
	50	0	39	66	47	51	14				1.0
	158	0	46	54	57	52	6				1.1
	500	0	54	56	55	55	1				1.1
	1581	0	57	44	61	54	9				1.1
	5000	0	35	46	35	39	6				0.8

* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation

+ Substantial dose-related increase in revertant colony counts (fold response ≥ 2 for TA1535 or ≥ 1.5 for TA100)

Table 5 NKT-10018 - Pre-incubation Assay in the Presence of S9 Mix

Strain	Conc. ($\mu\text{g}/\text{plate}$)	S9	Number of revertants					Plate observations *			Fold response †
			x_1	x_2	x_3	mean	SD	x_1	x_2	x_3	
TA1535	Water	+	18	28	28	25	6				1.0
	50	+	15	19	21	18	3				0.7
	158	+	13	11	14	13	2				0.5 A
	500	+	20	21	7	16	8				0.6
	1581	+	27	27	18	24	5				1.0
	5000	+	40	25	28	31	8				1.3
TA1537	Water	+	17	13	13	14	2				1.0
	50	+	16	19	12	16	4				1.1
	158	+	14	17	20	17	3				1.2
	500	+	12	12	11	12	1				0.8
	1581	+	11	18	23	17	6				1.2
	5000	+	10	17	11	13	4				0.9
TA98	Water	+	33	44	38	38	6				1.0
	50	+	51	42	41	45	6				1.2
	158	+	49	35	56	47	11				1.2
	500	+	39	51	49	46	6				1.2
	1581	+	42	46	32	40	7				1.0
	5000	+	42	50	53	48	6				1.3
TA100	Water	+	137	185	175	166	25				1.0
	50	+	165	199	175	180	17				1.1
	158	+	145	190	156	164	23				1.0
	500	+	178	166	145	163	17				1.0
	1581	+	210	178	175	188	19				1.1
	5000	+	165	171	180	172	8				1.0
WP2 <i>uvrA</i>	Water	+	67	61	63	64	3				1.0
	50	+	85	83	64	77	12				1.2
	158	+	64	63	63	63	1				1.0
	500	+	52	65	55	57	7				0.9
	1581	+	60	69	62	64	5				1.0
	5000	+	59	53	58	57	3				0.9

* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation

A Apparent decrease in colony counts considered to be due to normal variation rather than toxicity since not dose-related and counts are within historical control range

Table 6 Positive Controls for the Pre-incubation Assay

Strain	Treatment	Conc. (µg/plate)	S9	Number of revertants					SD	Fold response †
				x_1	x_2	x_3	mean			
TA1535	NaAz	0.5	0	330	299	315	315	16	12	
TA1537	9AC	10	0	946	720	766	811	119	50	
TA98	2NF	1	0	131	116	144	130	14	4.8	
TA100	NaAz	0.5	0	597	541	546	561	31	4.6	
WP2 <i>uvrA</i>	NQO	0.5	0	1285	1258	1270	1271	14	26	
TA1535	2AA	5	+	398	394	392	395	3	16	
TA1537	BaP	5	+	160	145	119	141	21	9.9	
TA98	BaP	5	+	339	416	411	389	43	10	
TA100	BaP	5	+	1179	1277	1415	1290	119	7.8	
WP2 <i>uvrA</i>	2AA	15	+	535	572	501	536	36	8.4	

† Fold response in mean revertants compared to concurrent vehicle control

SD Sample standard deviation

In summary, NKT-10018 (NKTR-118) was positive in the Ames test.

Study Title: AZ13337019 also known as NKTR-118: Genetic Toxicity Evaluation using a Limited Bacterial Reverse Mutation Test (Study No. 2371BV)

Objective: In this non-GLP study, the Sponsor investigated the mutagenic potential of different batches of AZ13337019 (NKTR-118) and (b) (4)

Methods and Materials: NKTR-118 batch 200278 was tested for mutagenic activity using the *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100, and *E. Coli* strain WP2 *uvrA*/pKM101. NKTR-118 batches DRJ-2325-05 and DRJ-2325-03, and (b) (4) were tested using TA100 and TA1535. The assays were conducted using the plate incorporation method in the presence and absence of S9 mix. The positive controls were 2-aminoanthracene (all strains) in the presence of S9 mix, and sodium azide (TA1535 and TA100), 2-nitrofluorene (TA98), 9-aminoacridine HCl (TA1537), and potassium dichromate (*E.coli uvrA*/pKM101) in the absence of S9 mix.

A test article was considered to be mutagenic if 1) The number of revertant colonies in any strain was increased in the presence of one or more doses of the test compound, with or without metabolic activation; 2) There was a dose-related increase in the number of revertant colonies; and 3) Unclear increases were shown to be reproducible.

Results and Conclusions: NKTR-118 batch 200278 was tested twice and was found to be mutagenic in strains TA1535 and TA100, both in the presence and absence of metabolic activation. The maximum increase seen in strain TA1535 was 44 to 59 times and 33 to 47 times the solvent control in the presence and absence of S9 mix,

respectively. The maximum increase seen in strain TA100 was 5.3-6.0 times and 6.6-7.2 times the solvent control in the presence and absence of S9 mix, respectively.

NKTR-118 batch DRJ-2325-05, purified through chromatography from batch 200278, was mutagenic to strain TA1535 both in the presence and absence of S9 mix. The maximum increase seen was 3.3 and 4.2 times the solvent control in the presence and absence of S9 mix, respectively.

(b) (4) was mutagenic to TA1535 in the presence of S9 mix. The maximum increase seen was 9.8 times the solvent control value.

NKTR-118 batch DRJ-2325-03, the oxalate salt from batch 200278, was not mutagenic in strains TA1535 or TA100 in the presence or absence of metabolic activation.

In summary, NKTR-118 batches 200278 and DRJ-2325-05 showed mutagenic activity, both in the presence and absence of metabolic activation. However the mutagenic activity of batch DRJ-2325-05 (purified through chromatography) was much lower than that for batch 200278. NKTR-118 batch DRJ-2325-03, the oxalate salt, was not positive in the Ames test. It appears that a degradant is responsible for the mutagenic activity of NKTR-118 free base. It was also concluded that naloxone-HCl dihydrate was mutagenic in the presence of metabolic activation.

Study Title: Further evaluation of AZ13337019, also known as NKTR-118 and MPEG7-Naloxol: Genetic Toxicity Evaluation using a Limited Bacterial Reverse Mutation Test (Study No. 2449BV)

Objective: In this non-GLP study, the Sponsor investigated the impurities or degradation products that may account for the strong mutagenic activity in some batches of NKTR-118.

Methods and Materials: The study was performed using *Salmonella typhimurium* strains TA1535, TA100, TA98, TA1537, and *E.coli uvrA/pKM101*. The assays were performed using the plate incorporation method, in the presence and absence of S9 mix. The positive controls were 2-aminoanthracene (all strains) in the presence of S9 mix, and sodium azide (TA1535 and TA100), 2-nitrofluorene (TA98), 9-aminoacridine HCl (TA1537), and potassium dichromate (*E.coli uvrA/pKM101*) in the absence of S9 mix. Various batches of NKTR-118, NKTR-118 that was stored under different conditions, re-processed NKTR-118 batches, impurities ((b) (4)) and liquid residues generated from batch re-purification were tested in this study.

Results and Conclusions: Some batches of NKTR-118 showed strong mutagenic activity in the Ames test, while others did not. The results are summarized in the Sponsors table below.

Table 1 Summary of results presented as fold increases compared to solvent controls

Compound/Batch	Table No.	TA1535 – S9 mix	TA1535 + S9 mix	TA100 – S9 mix	TA100 + S9 mix	Conclusion
mPEG ₇ -naloxol batch 1005	Table 4 and Table 5	1.5	3.2	1.0	1.1	Mutagenic
mPEG ₇ -naloxol batch 149015	Table 6 and Table 7	21.4	40.5	4.8	4.9	Mutagenic
mPEG ₇ -naloxol batch 200237	Table 8 and Table 9	10.3	18.4	2.6	2.8	Mutagenic
mPEG ₇ -naloxol batch 200388	Table 10 and Table 11	7.9	16.1	2.2	2.1	Mutagenic
mPEG ₇ -naloxol batch 149003	Table 12 and Table 13	2.5	3.2	1.6	1.1	Mutagenic
mPEG ₇ -naloxol batch 149006	Table 14 and Table 15	9.4	14.0	2.4	2.2	Mutagenic
mPEG ₇ -naloxol batch 200279	Table 16 and Table 17	28.2	43.6	5.5	4.9	Mutagenic
(b) (4) or (b) (4) from batch 200278)	Table 18 and Table 19	(b) (4)	■	■	■	Mutagenic
(b) (4) (b) (4) from batch 200278)	Table 20 and Table 21	■	■	■	(b) (4)	Not mutagenic
(b) (4) batch 200278)	Table 22 and Table 23	(b) (4)	■	■	■	Mutagenic

(b) (4)	Table 24 and Table 25	(b) (4)	■	■	■	Not mutagenic
(b) (4) (b) (4) from batch 200278)	Table 26 and Table 27	(b) (4)	■	■	■	Not mutagenic
mPEG ₇ -naloxol oxalate batch 01-kzwd-582-59	Table 28 and Table 29	1.7	1.5	1.1	1.1	Not mutagenic
mPEG ₇ -naloxol batch 01-kzwd582-60	Table 30 and Table 31	4.4	3.2	1.3	1.2	Mutagenic
mPEG ₇ -naloxol oxalate batch 06-kpfw-577-04 (b) (4) solution of NKTR-118 batch 200279 (b) (4)	Table 32 and Table 33	(b) (4)	■	■	■	Not mutagenic
mPEG ₇ -naloxol stability test at 25°C 60 % RH of batch 149003	Table 34 and Table 35	3.0	3.0	2.2	1.3	Mutagenic
mPEG ₇ -naloxol stability test at 5°C of batch 149003	Table 36 and Table 37	8.2	9.3	2.9	2.3	Mutagenic
mPEG ₇ -naloxol batch 200279 bulk	Table 38 and Table 39	2.3	5.6	1.8	1.6	Mutagenic
mPEG ₇ -naloxol batch 200279 bulk, vacuum evaporated (01-kxrg550-22)	Table 40 and Table 41	2.0	4.9	1.6	1.4	Mutagenic
1:1 mixture of NKTR-118 (batch 149015) (b) (4) (b) (4)	Table 42 and Table 43	(b) (4)	■	■	■	Mutagenic

NKTR-118 batch 200279 a (storage: light, air, open)	Table 44 and Table 45	31.5	39.7	2.9	3.9	Mutagenic
NKTR-118 batch 200279 b (storage: closed, air, 5% water)	Table 46 and Table 47	7.6	9.7	1.5	1.9	Mutagenic
NKTR-118 batch 200279 c (storage: oxygen, 50°C, closed)	Table 48 and Table 49	70.3	85.0	6.3	7.8	Mutagenic
NKTR-118 ba 200279 d (storage: 50°C, 0.5 mL water, 2w)	Table 50 and Table 51	2.8	3.6	0.9	1.4	Mutagenic
(b) (4)	Table 52 and Table 53	(b) (4)				Mutagenic
NKTR-118 Inert API dissolved in water; DRJ-2325-15	Table 54 and Table 55	8.9	11.7	2.0	1.9	Mutagenic
NKTR-118 Inert API dissolved in DMSO; DRJ-2325-15	Table 56 and Table 57	11.9	13.6	2.2	2.3	Mutagenic
NKTR-118 vacuum pumped 68h batch 149006a	Table 58 and Table 59	18.4	20.4	3.1	2.6	Mutagenic
NKTR-118 vacuum reference batch 149006b	Table 60 and Table 61	15.1	16.1	2.7	2.1	Mutagenic
NKTR-118 phosphate batch 03-KVKM327-25	Table 62 and Table 63	4.4	4.7	1.6	1.3	Mutagenic
NKTR118 Batch: 149015 prep.chromatography fraction 1 to 9	Table 64	Fraction 6: 29.6 Fraction 7: 8.5	Fraction 6: 28.7 Fraction 7: 8.7	Not tested	Not tested	Mutagenic

(b) (4)	Table 65	(b) (4)	Not tested	(b) (4)	Not tested	Mutagenic
(b) (4)	Table 66	(b) (4)	Not tested	(b) (4)	Not tested	Mutagenic
Ms-PEG naloxol oxalate batch DRJ-2325-19 purified	Table 69 and Table 70	29.7	11.5	2.7	2.1	Mutagenic
(b) (4)	Table 71 and Table 72	(b) (4)				Mutagenic
MPEG ₇ Naloxol N-oxide batch 03-kdhf771-41	Table 73 and Table 74	1.3	4.0	1.3	1.1	Mutagenic
(b) (4)	Table 75 and Table 76	(b) (4)				Mutagenic
(b) (4)	Table 77 and Table 78	(b) (4)				Mutagenic
(b) (4)	Table 79 and Table 80	(b) (4)				Not mutagenic

Table 2 (b) (4) Summary of fold increase, compared to solvent controls, plate incorporation test

Compound/Batch	Table No.	TA1535 -S9 mix ^a	TA1535 +S9 mix ^a	TA100 -S9 mix ^a	TA100 +S9 mix ^a	E.coli -S9 mix ^b	E.coli +S9 mix ^b	TA98 -S9 mix ^a	TA98 +S9 mix ^a	TA1537 -S9 mix ^a	TA1537 +S9 mix ^a
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(b) (4)	Table 67 and Table 68	(b) (4)									
batch 03-KDHF771-39 plate incorporation test											

(b) (4)

The results showed that when stored under appropriate conditions, NKTR-118 was not mutagenic. [REDACTED] (b) (4), a degradation product of NKTR-118, showed strong mutagenic activity. When NKTR-118 free base was converted to NKTR-118 oxalate, no mutagenic activity was observed. It was concluded that NKTR-118 itself was not mutagenic in the Ames test. The mutagenic activity observed with NKTR-118 can be attributed to [REDACTED] (b) (4), a NKTR-118 degradation product. The degradation did not occur when NKTR-118 was converted to its oxalate salt.

Study title: NKTR-118 oxalate, also known as naloxegol oxalate and AZ13337019 oxalate: Genetic Toxicity Evaluation using a Bacterial Reverse Mutation Test	
Study no.:	2980BV
Study report location:	N/A
Conducting laboratory and location:	Safety Assessment UK, AstraZenca R&D Alderley, Macclesfield, England
Date of study initiation:	11/01/2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NKTR-10018 oxalate (NKTR-118 oxalate), C564/1, 98.7%

Key Study Findings: NKTR-118 oxalate was negative in the Ames test.

Methods	
Strains:	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA1535 and TA1537, and <i>Escherichia coli</i> WP2 <i>uvrA</i> /pKM101
Concentrations in definitive study:	5, 16, 50, 160, 500, 1600, or 5000 µg per plate
Basis of concentration selection:	Neither precipitate nor background lawn toxicity was observed at the maximum dose tested (5000 µg/plate).
Negative control:	Sterile water
Positive control:	Absence of metabolic activation: sodium azide (NaAz, 0.5 µg/plate) for TA1535 and TA100, 2-nitrofluorene (2NF, 0.5 µg/plate) for TA98, 9-aminoacridine (9AC, 50 µg/plate) for TA1537, and potassium dichromate (25 µg/plate) for WP2 <i>uvrA</i> /pKM101).

	Presence of metabolic activation: 2-aminoanthracene (2AA) 2.0 µg/plate for TA1535, TA100, TA98, TA1537, and 20 µg/plate for WP2 <i>uvrA</i> /pKM101
Formulation/Vehicle:	NKTR-118 oxalate dissolved in sterile water
Incubation & sampling time:	The plate incorporation method was used in the study. Aroclor 1254-induced rat liver S9 (10%) was used as the metabolic activation system. The plates (triplicate in the confirmation assay) were incubated for 72 hours at 37°C. The condition of the bacterial background lawn was evaluated for evidence of test article toxicity, and precipitate was evaluated after the incubation period by visual examination. Revertant colonies for a given tester strain and activation condition were counted by automated colony counter. In cases where the mean incidence of revertant colonies on the solvent control plates was 20 or less, or if cytotoxicity or precipitate interfered with image analysis, the plate(s) was scored and manually entered into an analysis program.

Study Validity: The study is deemed valid for the following reasons. Selection of bacterial tester strains was appropriate. The positive control compounds produced the expected responses. Dose selection for the plate incorporation method was adequate based upon use of the limit dose (i.e., 5000 µg/plate). Neither precipitate nor background lawn toxicity was observed at the maximum dose of 5000 µg/plate. The S9 concentration (10%) was within acceptable limits.

Study Criteria: A test article was considered to be mutagenic if 1) For TA1535 or TA1537, the mean number of revertant colonies of one or more doses of the test item, with or without metabolic activation, is equal to or greater than 2 times the concurrent solvent control mean value and the relevant historical mean value; for any other strain, the mean number of revertant colonies is equal to or greater than 2 times the concurrent solvent control mean value in the presence of one or more doses of the test item, with or without metabolic activation; 2) There was a dose-related increase in the number of revertant colonies; and 3) Any increase was reproducible. These criteria are acceptable.

Results: There was no visible thinning of the background lawn of non-revertant bacteria following exposure to NKTR-118 oxalate, indicating that the test article was non-toxic to the bacteria at the levels tested. No precipitation was observed. The mean revertant colony counts for the vehicle controls were close to or within the laboratory historical control range. There was no consistent increase in numbers of revertant colonies in

any of the five tester strains, either in the presence or absence of S9 mix. The results are summarized in the Sponsor's tables below.

NKTR-118 oxalate without metabolic activation

(Dose/plate) µg µmol	TA1535		TA1537		TA98		TA100		E.coli WP2 uvrA/ pKM101	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Solvent control 1	17	1	12	1	32	6	133	5	167	16
Solvent control 2	16	1	13	1	35	6	111	12	177	14
5 0.00674	17	2	12	3	35	6	122	3	161	12
16 0.0216	16	2	12	2	27	3	117	16	180	14
50 0.0674	18	1	11	1	28	1	124	13	186	9
160 0.216	16	2	12	2	32	6	124	4	171	7
500 0.674	18	1	12	2	25	5	119	3	170	26
1600 2.16	17	2	13	2	31	10	120	19	167	32
5000 6.74	21	5	12	2	33	6	117	9	162	23
Positive control 1	422	13	499	135	181	24	559	54	1170	23

NKTR-118 oxalate with metabolic activation

(Dose/plate) µg µmol	TA1535		TA1537		TA98		TA100		E.coli WP2 uvrA/ pKM101	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Solvent control 1	17	3	14	1	36	8	136	37	178	6
Solvent control 2	17	2	14	1	34	6	132	2	170	8
5 0.00674	16	1	15	1	30	3	128	16	183	15
16 0.0216	19	3	15	2	39	8	122	4	170	19
50 0.0674	17	4	12	2	37	6	146	5	188	13
160 0.216	16	2	13	2	36	13	132	5	162	28
500 0.674	16	2	13	1	32	4	137	4	178	16
1600 2.16	18	1	13	2	39	8	132	5	175	8
5000 6.74	16	1	14	2	39	3	146	6	171	18
Positive control 1	164	28	251	22	1238	352	1868	42	2317	176

Conclusions: NKTR-118 oxalate was negative in the Ames test under the conditions used in the assay.

7.2 In Vitro Assays in Mammalian Cells

Study title: Cell Mutation Assay at the Thymidine Kinase Locus (TK^{+/-}) in Mouse Lymphoma L5178Y Cells with NKT-10018	
Study no.:	LS-2007-007
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	02/13/2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NKT-10018, 149002, 96%

Key Study Findings: NKT-10018 (NKTR-118) was not mutagenic in the *in vitro* L5178Y TK^{+/-} mouse lymphoma mammalian cell gene mutation assay.

Methods	
Cell line:	L5178Y/TK ^{+/-} mouse lymphoma
Concentrations in definitive study:	Expt. I (4 hr treatment): 162.5, 325, 650, 1300, 1950, and 2600 µg/ml Expt. II (24 hr treatment): 360.6, 544.2, 829.8, 1212.5, 1763.2, 2070.1, and 2756.9 µg/ml
Basis of concentration selection:	The doses of the definitive studies were selected based on results obtained in a dose-ranging study, performed in the presence (4 hr treatment) and absence (4 hr and 24 hr treatments) of metabolic activation. NKTR-118 concentrations between 40.6 and 5200 µg/mL were used. RSG (relative suspension growth) values below 50% were observed at ≥1300 µg/mL in the absence and at ≥2600 µg/mL in the presence of metabolic activation. In addition, precipitation was observed at 2600 and 5200 µg/mL, the 2 highest concentrations tested, both in the presence and absence of metabolic activation.
Negative control:	Cell culture medium
Positive control:	Without S9: Methylmethane sulfonate (MMS) 19.5 and 13.0 µg/ml in Expt. I and II, respectively With S9: Cyclophosphamide (CPA) 3.0 and 4.5 µg/ml in Expt. I and II, respectively
Formulation/Vehicle:	NKTR-118 dissolved in deionized water
Incubation & sampling time:	Phenobarbital/beta-naphthoflavone-induced rat liver S9 (10%) was used as the metabolic activation system. Cell cultures (2/concentration) in suspension were exposed to solvent, vehicle control, or NKTR-118 in the presence or absence of S-9 for 4 or 24 hours. Following treatment, cells were washed and cultured in suspension for a 48-hour expression period. Following the expression period, cells were plated (2 plates/concentration) in soft agar medium with and without the selective

	agent, trifluorothymidine (TFT). After appropriate period of time the colonies were counted manually. The colony size distribution was determined in the controls and at all concentrations of the test article.
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Study Validity:

The study is deemed valid for the following reasons. The Sponsor's criteria for an acceptable assay are: 1) All plates, from either the cloning efficiency period 2 (i.e. cloning efficiency determined after the expression period to measure viability of the cells without selective agent) or the TFT resistance-testing portion of the experiment are analyzable; 2) The absolute cloning efficiency period 2 at the time of mutant selection (CE) of the negative/vehicle controls is 65 to 120%; 3) The total suspension growth of the negative/vehicle control calculated by the day 1 fold-increase in cell number multiplied by the day 2 fold-increase in cell number is 8 to 32; 4) The range of the negative/vehicle control MF (mutant frequency) is in the range of 50 to 170 x 10⁻⁶ cells; 5) The positive controls (MMS and CPA) should yield an absolute increase in total MF (i.e. an increase above spontaneous background MF, an induced MF (IMF)) of at least 300 x 10⁻⁶ cells. At least 40% of the IMF should be reflected in the small colony MF. Alternatively, the positive controls should induce at least 150 small colonies; 6) The upper limit of cytotoxicity observed in the positive control culture should be the same as for the experimental cultures (i.e. the relative total growth (RTG) should be greater than 10% of the concurrent selective control group); and 7) The highest concentration of the test item should be 10 mM or 5000 µg/mL, unless limited by toxicity or solubility of the test item. If toxicity occurred, the highest concentration should lower the cloning efficiency period 1 or the relative total growth to 10 to 20% of survival. If precipitation is noted, the highest analyzed concentration should be the lowest concentration where precipitation is observed by the unaided eye. These criteria are acceptable.

Study Criteria:

The Sponsor's criteria for positive results are: 1) If the induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10⁶ cells above the corresponding solvent control or negative control; and 2) A relevant increase of the mutation frequency should be dose-dependent. Results of test groups are rejected if the relative total growth and the viability of the cells in suspension immediately after treatment (cloning efficiency) is less than 10% of the vehicle control. These criteria are acceptable.

Results:

Results of the study are summarized in the Sponsor's table below.

Table 1: Summary of results of experiment I and II

	conc. µg per mL	S9 mix	relative cloning efficiency 1	relative total growth	mutant colonies/ 10 ⁶ cells	threshold	relative cloning efficiency 1	relative total growth	mutant colonies/ 10 ⁶ cells	threshold
Column	1	2	3	4	5	6	7	8	9	10
Experiment I / 4 h treatment			culture I				culture II			
Neg. control with medium		-	100.0	100.0	61		100.0	100.0	73	
Solv. control with water		-	100.0	100.0	75	201	100.0	100.0	91	217
Pos. control with MMS	19.5	-	76.6	27.4	421	201	108.3	28.9	423	217
Test item	162.5	-	123.2	129.3	44	201	82.7	103.9	95	217
Test item	325.0	-	148.5	102.4	73	201	68.3	107.3	130	217
Test item	650.0	-	103.1	44.5	54	201	14.5	62.7	111	217
Test item	1300.0	-	60.4	19.6	57	201	38.9	32.2	109	217
Test item	1950.0	-	39.6	10.5	60	201	38.3	15.4	107	217
Test item	2600.0 (p)	-	12.8	culture was not continued [#]			10.7	culture was not continued [#]		
Neg. control with medium		+	100.0	100.0	45		100.0	100.0	73	
Solv. control with water		+	100.0	100.0	57	183	100.0	100.0	47	173
Pos. control with CPA	4.5	+	14.6	11.9	377	183	11.9	32.3	84	173
Pos. control with CPA	6.0	+	14.0	8.7	377	183	8.1	10.0	445	173
Test item	162.5	+	109.8	111.9	43	183	92.7	88.0	87	173
Test item	325.0	+	101.8	124.0	61	183	96.2	123.6	123	173
Test item	650.0	+	101.8	117.4	43	183	92.7	79.4	104	173
Test item	1300.0	+	36.2	23.5	108	183	57.9	36.6	116	173
Test item	1950.0	+	10.8	5.1	139	183	26.0	13.5	32	173
Test item	2600.0 (p)	+	7.8	culture was not continued [#]			18.8	culture was not continued [#]		
Experiment II / 24 h treatment			culture I				culture II			
Neg. control with medium		-	100.0	100.0	75		100.0	100.0	61	
Solv. control with water		-	100.0	100.0	69	195	100.0	100.0	151	277
Pos. control with MMS	13.0	-	58.7	36.4	305	195	66.9	29.1	543	277
Test item	360.6	-	132.6	126.0	85	195	111.7	136.4	117	277
Test item	544.2	-	95.4	67.1	79	195	167.1	148.4	106	277
Test item	829.8	-	89.7	76.2	62	195	82.5	101.0	87	277
Test item	1212.5	-	36.1	12.0	85	195	53.0	18.2	111	277
Test item	1763.2	-	14.9	0.5	25	195	0.0	0.1	50	277
Test item	2070.1	-	2.4	culture was not continued [#]			0.0	culture was not continued [#]		
Test item	2756.9 (p)	-	4.1	culture was not continued [#]			0.0	culture was not continued [#]		

threshold = number of mutant colonies per 10⁶ cells of each solvent control plus 126

not determined, culture not continued due to exceedingly strong toxic effects

The values printed in bold are judged as invalid, since the acceptance criteria (page 23) are not met.

(p) precipitation visible to the unaided eye

The range of the negative and solvent controls was from 45 to 151 mutant colonies per 10⁶ cells; the range of the groups treated with the NKTR-118 was from 32 to 139 mutant colonies per 10⁶ cells. The lowest negative and solvent controls (45 and 47 colonies) fell just short of the lowest limit of the acceptance criteria. Since the mean values of both parallel cultures (45 and 73 equal to a mean of 59, 47 and 57 equal to a mean of 52) remained within the acceptable range, the data were judged as acceptable by the Sponsor. The positive controls, MMS (19.5 µg/mL in experiment I and 13.0 µg/mL in experiment II) and CPA (4.5 and 6.0 µg/mL in experiment I), showed significant increases in induced total mutant colonies and in the relative quantity of small versus large induced colonies in at least one of the concentrations

(the latter data not shown in the above table).

There were no significant or reproducible dose-dependent increases in mutant colony numbers up to the maximum NKT-10018 concentration, with or without metabolic activation. All individual mutation frequencies remained within the historical range of negative and solvent controls. The threshold of 126 above the mutation frequency of the corresponding solvent control was not reached at any of the NKT-10018 doses tested.

Conclusions: NKT-10018 (NKTR-118) was not mutagenic in mouse lymphoma L5178Y/TK^{+/−} cells in the absence or presence of metabolic activation.

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

Study title: Micronucleus Assay in Bone Marrow Cells of the Mouse with NKT-10018

Study no:	LS-2007-008
Study report location:	N/A
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	2-12-2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NKT-10018; 149002; 96%

Key Study Findings:

NKT-10018 (NKTR-118) did not increase the mean incidence of micronucleated PCE at any of the doses tested. NKT-10018 is considered to be non-clastogenic in the *in vivo* mouse micronucleus assay.

Methods

Doses in definitive study:	0 (vehicle), 500, 1000, or 2000 mg/kg
Frequency of dosing:	single administration
Route of administration:	oral (gavage)
Dose volume:	10 mL/kg bodyweight
Formulation/Vehicle:	dissolved in deionized water
Species/Strain:	mouse/NMRI
Number/Sex/Group:	6/sex/group/sacrifice time-point; sacrifices occurred at 24 and 48 hr
Satellite groups:	N/A
Basis of dose selection:	The dose selection was based on a pilot study in mice (2M +2F per group), where mice were given single oral (gavage) doses of 1000 mg/kg or 2000 mg/kg NKT-10018. Animals were monitored for clinical signs, mortality, and body weight change for 48 hours post-dosing. The drug did not produce any mortalities or clinical signs at 1000 mg/kg; however, at 2000 mg/kg clinical signs including reduction in spontaneous activity, abdominal position, eyelid closure, and ruffled fur were observed.
Negative control:	Deionized water
Positive control:	Cyclophosphamide (40 mg/kg)

Bone marrow cells were collected for micronuclei analysis at 24 hours (all doses) and 48 hours (high dose group only) after a single administration of NKT-10018. Ten animals (5 males, 5 females) per test group were evaluated for the occurrence of micronuclei. At least 2000 PCEs per animal were scored for micronuclei. To determine the cytotoxic effect due to the treatment with NKT-10018, the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample, and reported as the number of PCEs per 2000 erythrocytes.

Study Validity

The Sponsor considered the study as valid if 1) the negative controls are in the range of their historical control data; 2) the positive controls are in the range of their historical control data; 3) at least 4 animals per group and sex can be evaluated; and 4) the ratio of PCEs (polychromatic erythrocytes) to erythrocytes is not less than 20% of the negative control value.

Study Criteria

A test item was classified as clastogenic by the Sponsor if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group. The criteria are adequate, and the study is deemed valid.

Results

Clinical signs, including reduction in spontaneous activity, abdominal position, and ruffled fur were noted in the 2000 mg/kg animals. The data are summarized in the Sponsor's table below.

Clinical Signs at 2000 mg/kg in the Main Experiment

Toxic Reactions	hours post-treatment male / female				
	1 h	2-4 h	6 h	24 h	48 h*
reduction of spontaneous activity	11/12	6/9	6/8	0/0	0/0
abdominal position	7/9	0/0	0/0	0/0	0/0
ruffled fur	12/12	12/12	12/12	0/0	0/0

*: data only from 6 animals per sex.

Results of the micronucleus assay are summarized in the Sponsor's table below.

Summary of Micronucleus Assay Results

test group	dose mg/kg b.w.	sampling time (h)	PCEs with micronuclei (%)	range	PCE per 2000 erythrocytes
vehicle	0	24	0.055	0 - 3	1104
test item	500	24	0.075	0 - 2	1060
test item	1000	24	0.070	0 - 3	1206
test item	2000	24	0.070	0 - 3	1097
positive control	40	24	2.290	29 - 59	1204
test item	2000	48	0.060	0 - 5	1025

Data were combined for male and female mice.

NKT-10018 had no effect on the incidence of micronuclei. The mean number of PCEs was not decreased after treatment with NKT-10018 compared to the mean value of PCEs in the vehicle control, indicating that NKT-10018 did not have cytotoxic effects in bone marrow. Cyclophosphamide (40 mg/kg), the positive control, induced a significant increase in micronucleated PCEs in male and female mice, compared to vehicle control (2.290% vs 0.055% in controls, $p < 0.0001$).

In summary, NKT-10018 did not increase the mean incidence of micronucleated PCE in any of the doses tested. NKT-10018 is not considered to be clastogenic in the *in vivo* mouse micronucleus assay.

7.4 Other Genetic Toxicity Studies

Study Titles: (b) (4): **Genetic Toxicity Evaluation using a Limited Bacterial Reverse Mutation Test (Study No. 2370BV);** (b) (4) **Genetic Toxicity Evaluation using the Mouse Lymphoma Cell Thymidine Kinase Locus Assay (Study No. 2372MV);** (b) (4): **Genetic Toxicity Evaluation using a Bacterial Reverse Mutation Test (Study No. 3071BV);** and (b) (4) (b) (4): **Genetic Toxicity Evaluation using a Bacterial Reverse Mutation Test (Study No. 3098BV)**

Genotoxicity of three potential impurities, (b) (4) was evaluated. (b) (4) (also known as (b) (4)), is an impurity that can be found in the manufacturing of naloxegol. The Sponsor's study, using the plate incorporation method, showed that (b) (4) mutagenic in the Ames assay both in the presence or absence of S9 metabolic activation. Also, (b) (4) was found to be mutagenic in the mouse lymphoma L5178Y/TK⁺ cells in the absence of S9 metabolic activation.

(b) (4) also known as (b) (4) is a synthetic impurity reported by the Sponsor. It has been detected in late development and production scale batches of naloxegol oxalate. The Sponsor's study, using the plate incorporation method, showed that, (b) (4) is mutagenic in the Ames assay in the presence of S9 metabolic activation.

(b) (4), is an impurity that can be found in the manufacturing of naloxegol. The Sponsor's study, using the liquid pre-incubation method, showed that (b) (4) is mutagenic in the Ames assay both in the presence and absence of S9 metabolic activation

8 Carcinogenicity

Study title: 2-Year Oral Gavage Carcinogenicity and Toxicokinetic Study with NKTR-118 in Rats

Study no.:	7985-111
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11-25-2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NKTR-118; 149015 (98%), 200237 (98%), 200388 (98%), 09-0581 (97%), 200279 (98%)
CAC concurrence:	Yes (meeting minutes dated 11/26/2008)

Key Study Findings

- Male and female rats (60/sex/group) received 0 (vehicle), 40, 120, or 400 mg/kg/day NKTR-118 (naloxegol) by oral gavage.
- Due to low survival in the control groups (20 or less in in males and females), all surviving animals were sacrificed on week 93 (males) or week 94 (females) based on the Executive CAC recommendations.
- There was a drug-related negative dose-response relationship in mortality rates in male rats. Pair-wise comparison showed a statistically significant decrease in mortality rates in the 400 mg/kg/day males and 120 mg/kg/day females, compared to their respective controls.
- Drug-related clinical signs included clear oral discharge, brown and yellow haircoat in the perineal area, and red and sore/scab skin in the hind paws.
- Body weight and bodyweight gains were significantly reduced (87% and 83%, respectively) in the 400 mg/kg/day males compared to controls at terminal sacrifice. There were no meaningful drug-related changes in food consumption during the treatment period.
- Drug-related lesions of the hind foot, including abrasion and crusted area, were noted when scheduled sacrifice and premature death animals were both considered. However, the relative incidence of these findings in animals at the terminal sacrifice was comparable between the controls and 400 mg/kg/day group.

- There was a dose-dependent statistically significant increase in the incidence of benign interstitial (Leydig) cell adenoma of the testis (0%, 0%, 6.7%, and 11.7% in the 0, 40, 120, and 400 mg/kg/day males, respectively), based on the significance criteria for either rare or common tumors. Pair-wise comparison showed a statistically significant increase in the incidence of benign interstitial cell adenoma in the 400 mg/kg/day males compared to controls, based on the significance criteria for rare tumors.
- The Sponsor reported a dose-dependent statistically significant decrease in the incidence of pituitary adenoma (M: 65%, 55%, 61.7%, and 51.7% in the 0, 40, 120, and 400 mg/kg/day groups, respectively; F: 88.3%, 81.7%, 76.7%, and 75% in the 0, 40, 120, and 400 mg/kg/day groups, respectively). Also, there was a dose-dependent significant decrease in the incidence of mammary carcinoma (31.7%, 16.7%, 20%, and 15% in the 0, 40, 120, and 400 mg/kg/day females, respectively).
- Non-neoplastic findings included interstitial (Leydig) cell hyperplasia (5%, 8.3%, 20%, and 13.3% in the 0, 40, 120, and 400 mg/kg/day males, respectively); pododermatitis, renal pelvis dilatation, tubule cell vacuolation, and pituitary hyperplasia were noted in the 120 or 400 mg/kg/day males and females.
- Plasma NKTR-118 AUC increased with dose in a greater than dose proportional manner in both males and females, except during week 52 in the females, where apparent dose-proportionality was observed. Females showed higher plasma NKTR-118 exposure (approximately 1.3 to 4.8-fold) when compared to corresponding males. The sex-related differences in plasma NKTR-118 reduced with time. Accumulation of the metabolite, NKTR-118-glucuronide, in plasma was greater than that of NKTR-118, and male rats exhibited greater accumulation than female rats.

Adequacy of Carcinogenicity Study

This study used doses (0 (vehicle), 40, 120, or 400 mg/kg/day) that were recommended by the Executive CAC. The study length was acceptable since all surviving animals were sacrificed on week 93 (males) or week 94 (females) based on recommendations by the Executive CAC, due to low survival in the control groups. Analysis of mortality showed no drug-related increase in mortality rate; rather, there was a significant negative dose-response relationship in mortality rates in male rats. The carcinogenicity study was conducted appropriately.

Appropriateness of Test Models

The Crl:CD(SD) strain is an appropriate model because this strain is known to be responsive to known carcinogens and historical control data has been established in the conducting laboratory. The test model used by the Sponsor was appropriate.

Evaluation of Tumor Findings

Testicular benign interstitial cell adenoma was noted in 0/60, 0/60, 4/60, and 7/60 male rats treated with 0 (vehicle), 40, 120, and 400 mg/kg/day NKTR-118, respectively. The increase in the incidence of adenoma of the testis was dose-dependent ($p=0.0016$ significant based on either the rare or common tumor criteria), and pair-wise comparison showed a statistically significant increase in the 400 mg/kg/day males (11.7%) compared to controls (0%) ($p=0.0115$, significant based on the rare tumor criteria). The incidence rate at the high dose was slightly higher than the Charles River 2-year control group historical incidence range of 1.11 to 9.33%. Therefore, the increased incidence of benign interstitial cell adenoma in the testis of NKTR-118 treated rats is considered to be drug-related. Also, increased interstitial cell hyperplasia was noted in the treatment groups (3/60, 5/60, 12/60, and 8/60 males in the 0, 40, 120, and 400 mg/kg/day groups, respectively).

Methods

Doses:	0 (vehicle), 40, 120, or 400 mg/kg/day
Frequency of dosing:	once daily
Dose volume:	10 ml/kg
Route of administration:	oral gavage
Formulation/Vehicle:	solution/reverse osmosis water
Basis of dose selection:	Executive Carcinogenicity Assessment Committee recommendations; the Committee recommended doses of 40, 120, and 400 mg/kg/day by oral gavage, based on the rodent to human plasma AUC ratio exceeding 25-fold.
Species/Strain:	Rats/Crl:CD(SD)
Number/Sex/Group:	60
Age:	6 to 7 weeks old
Animal housing:	Individually in stainless steel or polycarbonate cages with bedding
Paradigm for dietary restriction:	 (b) (4)
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	Toxicokinetics: control: 7 rats/sex treatment: 13 rats/sex/group
Deviation from study protocol:	Due to low survival in the control groups, all surviving animals were sacrificed on week 93 (males) or week 94 (females), based on the Executive CAC recommendations conveyed on August 2, 2010. There were other minor deviations that did not affect the quality or integrity of the study.

The design of the study is shown in the table below (taken from the Sponsor's report).

Study Design of 104-week Carcinogenicity Study in Rats

Group ^a	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
	Male	Female		
Carcinogenicity Animals				
1 (Control)	60	60	0	0
2 (Low)	60	60	40	4
3 (Mid)	60	60	120	12
4 (High)	60	60	400	40
Toxicokinetic Animals^b				
5 (Control)	7	7	0	0
6 (Low)	13	13	40	4
7 (Mid)	13	13	120	12
8 (High)	13	13	400	40

a Groups 1 and 5 received reverse osmosis water only.

b Toxicokinetic animals included solely for the purpose of blood sample collections. Cohorts of three sex/group each were bled twice at each sample-collection interval (for test article groups, nine animals/sex/group designated for sample collections). The control group consisted of three animals/sex bled at two intervals. An additional four animals/sex/group were included in the study design as replacements to compensate for possible mortality.

Observations and Results

Mortality

All animals were observed twice daily for morbidity. Due to low survival in the control groups, all surviving animals were sacrificed on week 93 (males) or week 94 (females), based on the Executive CAC recommendations conveyed on August 2, 2010.

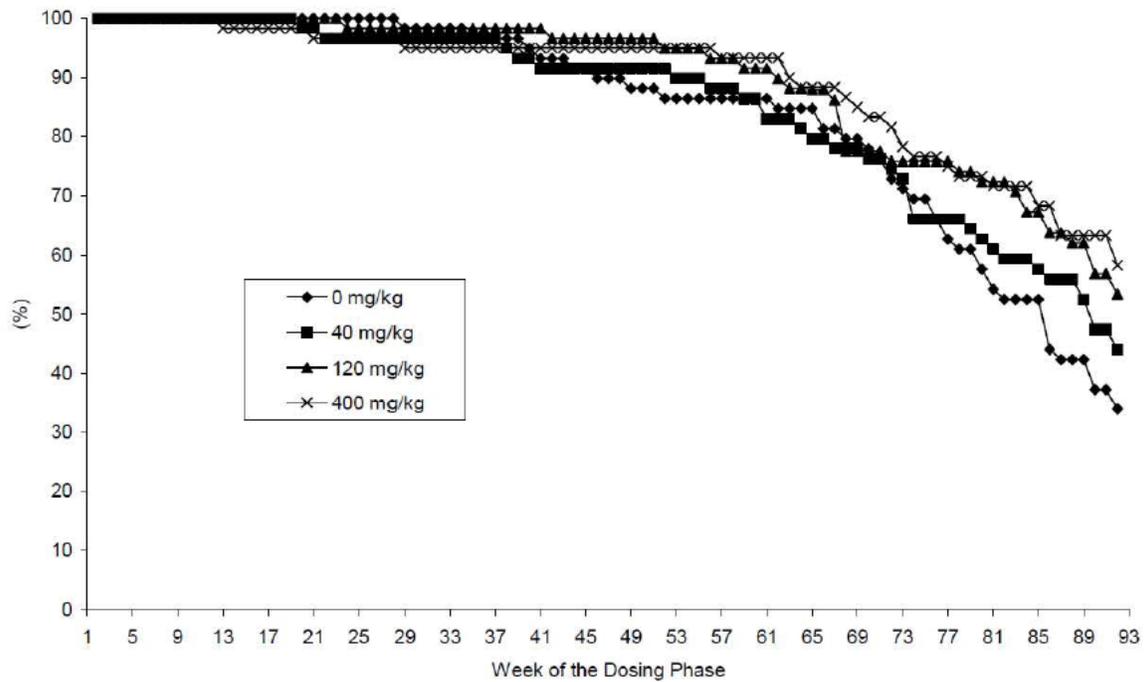
The Sponsor reported that in males, a significantly negative (decreased) trend occurred in mortality rate ($p=0.0028$, Cox-Tarone test), along with a significantly lower mortality rates in the 120 mg/kg/day (46%, $p=0.0216$) and 400 mg/kg/day (42%, $p=0.0069$) males, compared to controls (66%). In females, a significantly negative (decreased) trend occurred in mortality rate ($p=0.0155$) was also observed, and the 120 mg/kg/day females showed a significantly lower mortality rate (45%, $p=0.0122$) compared to controls (68%). In the 400 mg/kg/day females, the mortality rate (53%) was lower compared to controls (68%), but the difference was not statistically significant. The survival rates are summarized in the table and figures below taken from the Sponsor's study report.

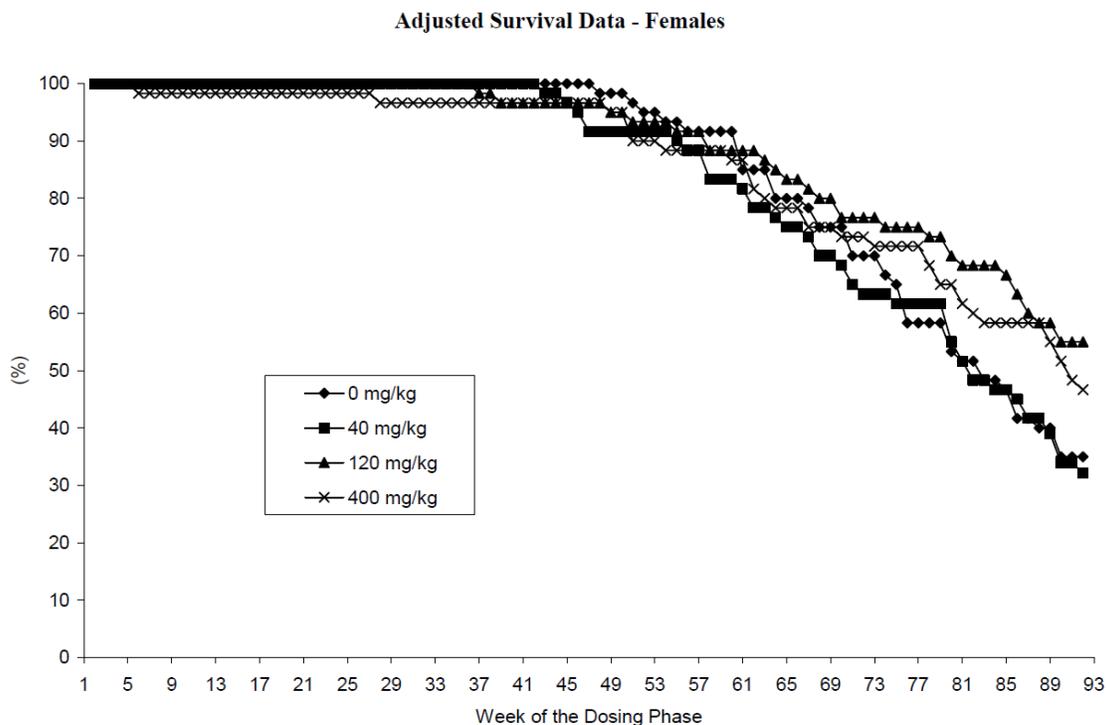
Survival Rates of the NKTR-118 Treated Rats

Adjusted Survival Rates (% alive at the end of each week)									
Dose (mg NKTR-118/kg/day)	Sex	Male				Female			
		0	40	120	400	0	40	120	400
Week 26		100	97	98	97	100	100	100	98
Week 52		86	92	95	95	95	92	93	90
Week 78		61	66	74	73	58	62	73	68
Week 90		37	47	57	63	35	34	55	52
Week 92 or 93 ^a		34	44	53	58	32	31	55	47

a Sacrifice occurred at Week 93 for males and Week 94 for females.

Adjusted Survival Data - Males





According to the FDA statistical review by Dr. Mohammad Atiar Rahman, dated 2/11/14, there was a statistically significant negative dose-response relationship ($p=0.0109$) in mortality rates in male rats. Pairwise comparisons showed a statistically significant decrease in mortality in the 400 mg/kg/day males and 120 mg/kg/day females, compared to their respective controls.

The Sponsor reported no specific drug-related cause of death in the unscheduled sacrifices or deaths. Where a probable cause of death was determined, the most common cause of death, regardless of sex, was pituitary adenoma.

Clinical Signs

Animals were observed twice daily for abnormalities, and signs of pain or distress. Detailed observations were performed once during the predose phase, before dosing on day 1, weekly thereafter, and on the day of terminal sacrifice. The following notable clinical signs were observed.

Summary of Notable Clinical Signs

Clinical signs	Sex	Dose (mg/kg/day)			
		0	40	120	400
Number/sex/group		60	60	60	60
Appearance					
<i>Malocclusion</i>	M	2	5	4	10
Discharges					
<i>Clear oral</i>	M	1	1	5	13
	F	1	5	6	19

Mass	M	17	15	20	27
	F	31	26	30	29
Skin and Pelage					
<i>Alopecia, perineal area</i>	F	0	1	3	7
<i>Brown haircoat, perineal area</i>	M	1	3	3	10
	F	3	5	6	10
<i>Hind paws red skin</i>	M	10	15	20	26
	F	10	10	12	20
<i>Red skin, left hind paw</i>	F	7	6	7	11
<i>Red skin, right hind paw</i>	F	3	6	6	10
<i>Hind paws scaly skin</i>	M	20	19	29	30
<i>Hind paws sore/scab</i>	M	6	13	12	20
	F	9	8	12	26
<i>Left hind paws sore/scab</i>	M	10	15	18	28
	F	8	8	9	16
<i>Right hind paws sore/scab</i>	M	12	14	17	26
	F	4	8	6	10
<i>Yellow haircoat</i>	M	3	3	5	27
	F	5	12	20	44

Drug-related clinical signs included clear oral discharge, brown and yellow haircoat in the perineal area, and red and sore/scab skin in the hind paws. The Sponsor indicated that clear oral discharge was likely related to the taste of the test article formulation, and haircoat staining and hind foot lesions were likely related to decreased grooming. These clinical signs are not considered adverse. All other clinical signs were present in the control groups, showed no relationship to dose, or are common of aged rats; therefore, these were not considered to be drug-related.

There were no meaningful drug-related effects on the incidence of masses. The table below summarizes the noteworthy masses. The incidences were generally low and occurred only in one sex.

Summary of Masses in the 2-Year Carcinogenicity Study in Rats

Masses	Sex	Dose (mg/kg/day)			
		0	40	120	400
Number/sex/group		60	60	60	60
Dorsal abdomen/midline	M	0	1	0	3
Ventral abdomen/midline	M	7	5	8	11
Inguinal Area/right	M	1	2	3	6
Ventral abdomen/right	M	1	2	3	5
Ventral abdomen/left	F	3	7	10	6

Ventral Throat/right	F	0	1	0	2
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Body Weights

Animals were weighed during the predose phase, prior to dosing on day 1 of the dosing phase, once weekly during weeks 2 through 14, and once every 4 weeks thereafter. As shown below in the reviewer's table, and the figures and table from the Sponsor's report, NKTR-118 significantly reduced body weight and bodyweight gain in the 400 mg/kg/day males compared to controls by week 4. There were only minimal changes in body weights in drug-treated females compared to controls.

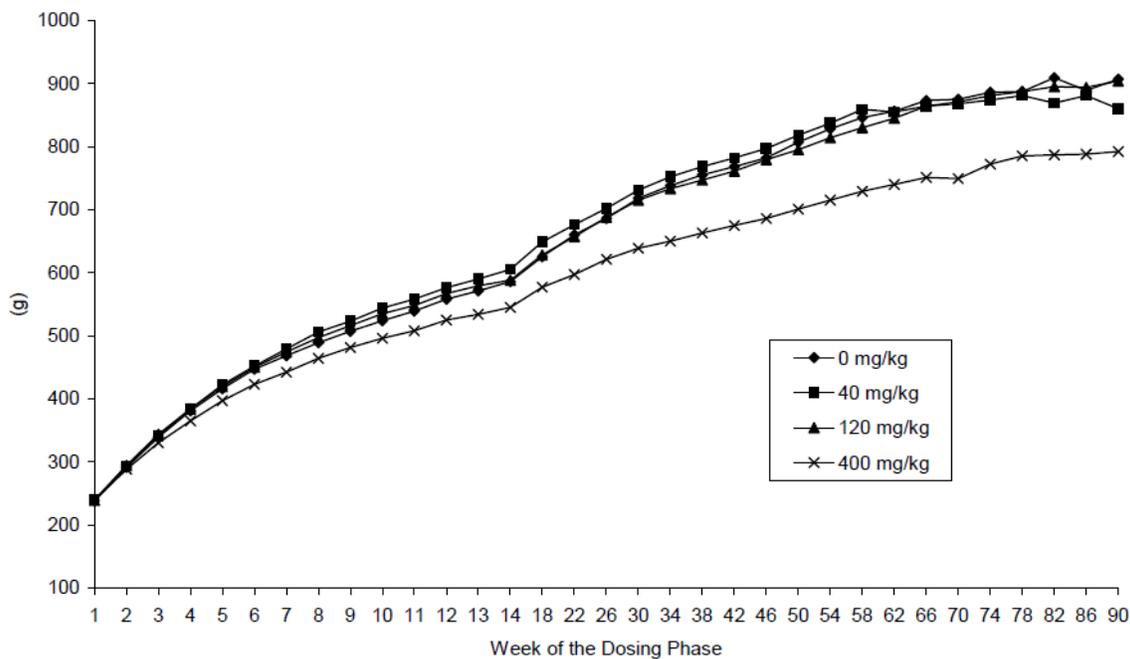
Changes in Body Weight in the 2-Year Carcinogenicity Study in Rats

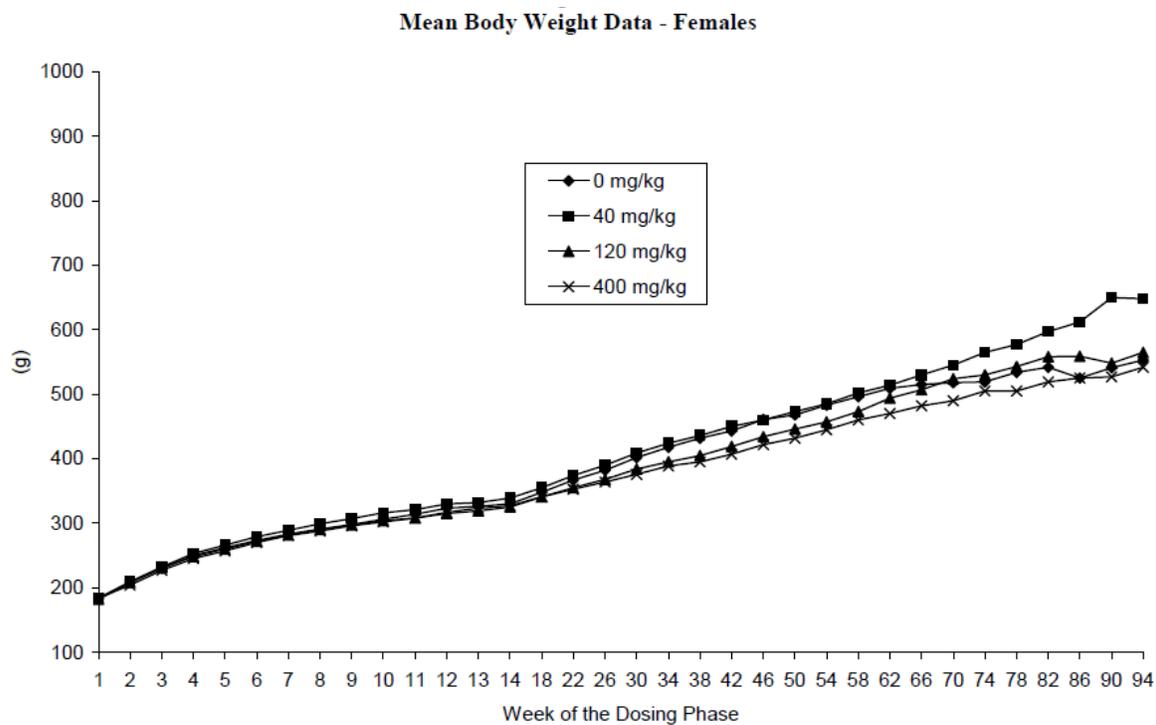
MALES	Dose (mg/kg/day)			
	0	40	120	400
Group	1	2	3	4
Week 0 Weight (g)	238	239	239	239
Week 26 Weight (g)	686	702	688	621*
% of Control, Wk 26	100	102.3	100.3	90.5
Δ Wk26-Wk0 (g)	448	463	449	382
BW Gain, % of Control	100	103.4	100.2	85.3
Week 54 Weight (g)	828	837	814	715*
% of Control, Wk 54	100	101.1	98.3	86.4
Δ Wk54-Wk0 (g)	590	598	575	476
BW Gain, % of Control	100	101.4	97.5	80.7
Week 78 Weight (g)	887	881	887	785*
% of Control, Wk 78	100	99.3	100	88.5
Δ Wk74-Wk0 (g)	649	642	648	546
BW Gain, % of Control	100	98.9	99.9	84.1
Week 90 Weight (g)	907	860	904	792*
% of Control, Wk 90	100	94.8	99.7	87.3
Δ Wk90-Wk0 (g)	669	621	665	553
BW Gain, % of Control	100	92.8	99.4	82.7

FEMALES	Dose (mg/kg/day)			
	0	40	120	400
Group	1	2	3	4
Week 0 Weight (g)	183	184	182	184
Week 26 Weight (g)	382	390	368	364

% of Control, Wk 26	100	102.1	96.3	95.3
ΔWk26-Wk0 (g)	199	206	186	180
BW Gain, % of Control	100	103.5	93.5	90.5
Week 54 Weight (g)				
	483	485	457	445*
% of Control, Wk 54	100	100.4	94.6	92.1
ΔWk54-Wk0 (g)	300	301	275	261
BW Gain, % of Control	100	100.3	91.7	87.0
Week 78 Weight (g)				
	534	577	543	505
% of Control, Wk 78	100	108.1	101.7	94.6
ΔWk74-Wk0 (g)	351	393	361	321
BW Gain, % of Control	100	112.0	102.8	91.5
Week 94 Weight (g)				
	553	648*	565	542
% of Control, Wk 90	100	117.2	102.2	98.0
ΔWk90-Wk0 (g)	370	464	383	358
BW Gain, % of Control	100	125.4	103.5	96.8

Mean Body Weight Data - Males

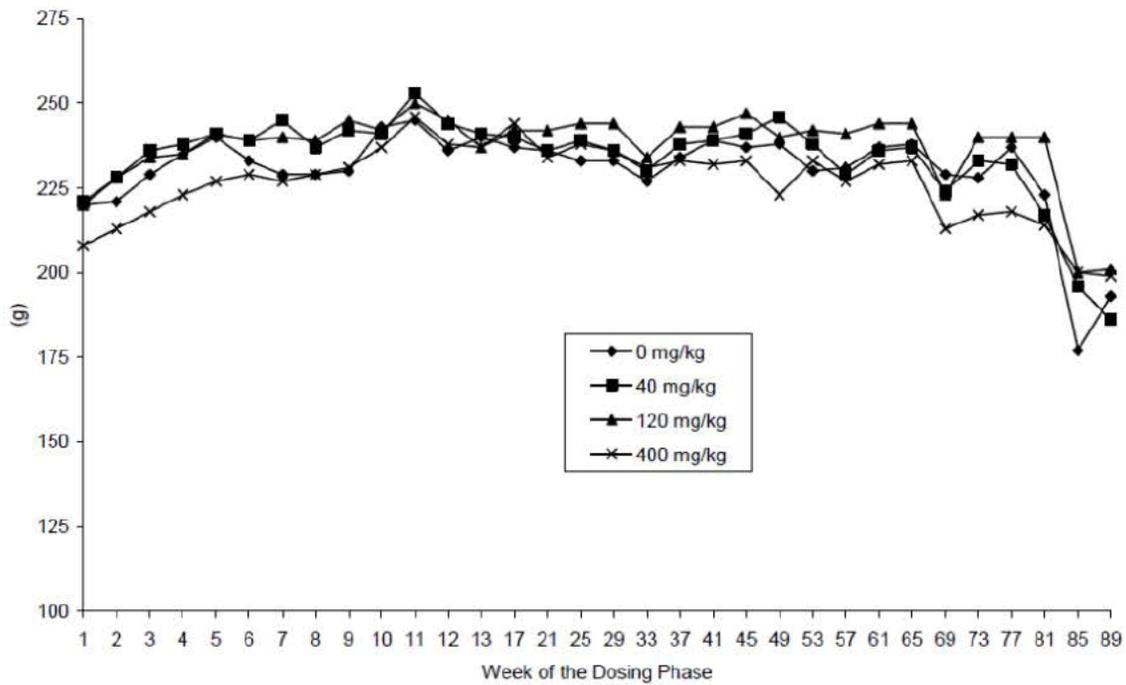




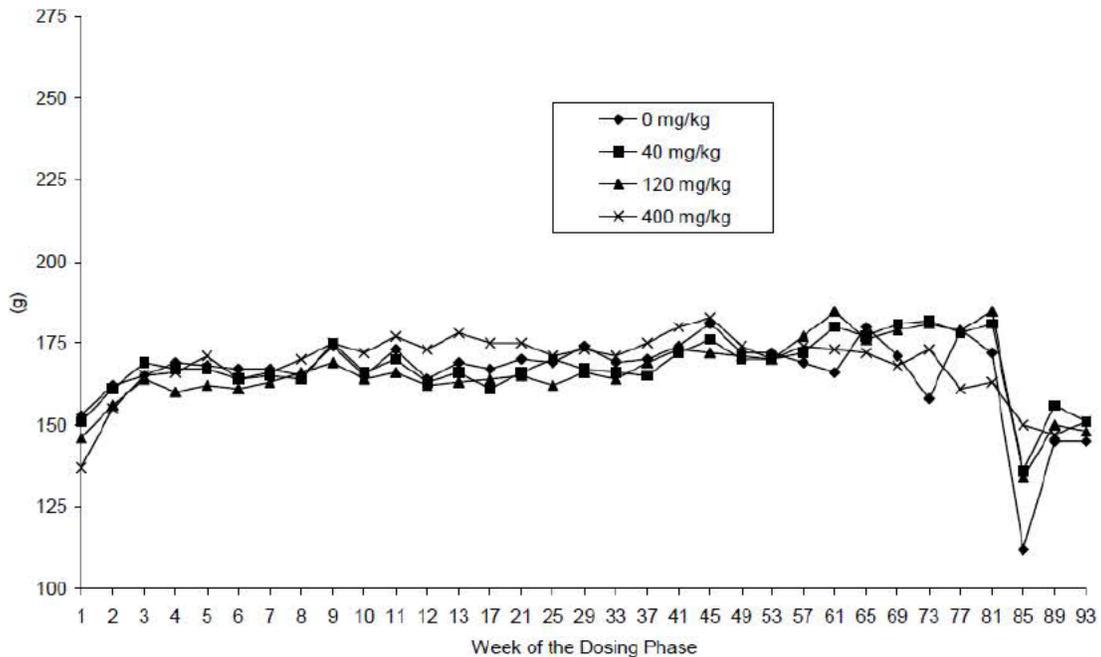
Feed Consumption

Food consumption was recorded weekly at weeks 1 through 13, every 4 weeks thereafter, and at weeks 89 and 93 of the dosing phase for males and females, respectively. As shown in the figures below (taken from the Sponsor's report), there were no meaningful drug-related changes in food consumption during the treatment period; only sporadic changes were noted.

Mean Food Consumption Data - Males



Mean Food Consumption Data - Females



Gross Pathology

Observations were made at sacrifice or immediately after premature death. Necropsies were performed on all carcinogenicity animals during scheduled sacrifice, animals found dead, and animals sacrificed prior to study termination.

The major findings are shown in the table below.

Macroscopic Findings in the 2-Year Carcinogenicity Study in Rats

Organ/Tissue	Sex	Doses (mg/kg/day)			
		Control	40	120	400
Number / group / sex examined		60	60	60	60
Foot/Foot Pad					
<i>Abrasion</i>	M	1	1	0	2
	F	0	0	0	2
<i>Crusted</i>	M	11	13	11	22
	F	8	7	10	18
Lung					
<i>Discolored</i>	M	1	6	4	3
	F	2	0	4	2
Pituitary					
<i>Discolored</i>	M	2	1	4	4
	F	4	5	8	6
Seminal vesicle					
<i>Large</i>	M	0	1	1	2
Stomach (GI)					
<i>Discolored</i>	M	2	5	7	7
	F	4	4	5	7

Drug-related lesions of the hind foot, including abrasion and crusted area, were observed when scheduled sacrifice and premature death animals were both considered. However, the relative incidence of these findings in animals at the scheduled sacrifice was comparable in the controls and the 400 mg/kg/day groups (55% and 49% in the 0 and 400 mg/kg/day males, respectively, and 32% and 43% in the 0 and 400 mg/kg/day females, respectively). The Sponsor indicated that pododermatitis in rats is a common finding in carcinogenicity studies, and the macroscopic findings in the foot/foot pad were not considered toxicologically significant. The other macroscopic findings are not considered adverse due to the lack of dose-dependency and/or corresponding microscopic findings.

Histopathology

All main study animals, including those euthanized in moribund condition or found dead before the end of the dosing period, were submitted to full necropsy. The animals were examined for palpable masses, and abnormalities were identified and correlated with postmortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities.

The following tissues and organs from all carcinogenicity animals were collected for microscopic examination during scheduled and unscheduled sacrifice, and from animals found dead during the study (table taken from Sponsor's study report).

Tissues and Organs Collected

adrenal (2)	optic nerve (2) ^a
aorta	ovary (2)
brain	pancreas
cecum	pituitary gland
cervix	prostate
colon	rectum
duodenum	salivary gland [mandibular (2)]
epididymis (2)	sciatic nerve
esophagus	seminal vesicle
eye (2) ^a	skeletal muscle (thigh)
femur with bone marrow (articular surface of the distal end)	skin/subcutis
Harderian gland	spinal cord (cervical, thoracic, and lumbar)
heart	spleen
ileum	sternum with bone marrow
jejunum	stomach
kidney (2)	testis (2) ^a
lesions	thymus
liver	thyroid (2) with parathyroid
lung with large bronchi	tongue
lymph node (mandibular)	trachea
lymph node (mesenteric)	urinary bladder
mammary gland	uterus
	vagina

Peer Review - Yes

A peer review was performed on selected tissues at [REDACTED] (b) (4). All testes, all recorded neoplasms, and 10% of animals (all tissues) were examined microscopically.

Neoplastic

In the Sponsor's analysis, neoplastic lesions were chosen for statistical analyses if the incidence in at least one of the treated groups was increased or decreased by at least two occurrences compared to the control group. The incidental tumors (i.e., tumors not assigned as the cause of death by the study pathologist) were analyzed by linear logistic regression of tumor prevalence. Fatal and palpable (superficial) tumors were analyzed by the Cox-Tarone binary regression method using the death time or the first palpation time (as applicable) as a surrogate for the tumor onset time. One-sided positive trends in common (background incidence rate > 1%) and rare (background incidence < 1%) tumors (if applicable) defined by the study pathologist were evaluated

at the 0.005 and 0.025 significance levels, respectively. High-dose group comparisons in common and rare tumors were evaluated at the 0.01 and 0.05 significance levels, respectively. Other intermediate, pair-wise, and one-sided group comparisons were evaluated at the 5.0% significance level. Benign and malignant neoplastic incidences were evaluated separately and combined, where appropriate.

In the FDA statistical review, the tumor data were analyzed for dose response relationships and pair-wise comparisons of control groups with each of the treated groups; the analyses were performed using the Poly-K method. To adjust for multiple testing, the dose response relationship was tested at levels of $\alpha=0.005$ for common tumors and $\alpha=0.025$ for rare tumors. This method is appropriate for the submission of experiments in two species, in order to keep the false-positive rate at the nominal level of approximately 10%. A rare tumor is defined as one in which the published spontaneous tumor rate is less than 1%. For multiple pair-wise comparisons of treated groups with the control, test levels of $\alpha=0.01$ for common tumors and $\alpha=0.05$ for rare tumors were used, in order to keep the false-positive rate at the nominal level of approximately 10% for the experiments in both species.

Noteworthy neoplastic tumor findings are summarized in the table below.

Summary of Neoplastic Changes in the 2-Year Carcinogenicity Study in Rats

Organ/Tissue	Sex	Doses (mg/kg/day)			
		Control	40	120	400
Number/group/sex examined		60	60	60	60
Adrenal Cortex <i>Hematopoietic neoplasm</i>	M	0	2	0	0
	F	0	0	0	0
Adrenal Medulla <i>Pheochromocytoma</i>	M	3	4	3	1
	F	0	0	2	0
Body (whole/cavity) <i>Histiocytic sarcoma</i>	M	1	1	3	1
	F	1	0	1	0
<i>Lymphosarcoma</i>	M	0	2	1	2
	F	0	1	0	0
<i>Hemangiosarcoma</i>	M	2	1	1	1
	F	0	1	0	0
Bone Marrow (Femur) <i>Hematopoietic neoplasm</i>	M	0	2	2	1
	F	1	1	0	0
Bone Marrow (Sternum) <i>Hematopoietic neoplasm</i>	M	0	2	2	1
	F	1	1	0	0
Brain <i>Granular Cell tumor</i>	M	2	0	1	0
	F	1	0	0	1
Liver <i>Hepatocellular adenoma</i>	M	0	0	2	1

	F	2	1	1	1
<i>Hematopoietic Neoplasm</i>	M	0	2	1	1
	F	1	1	1	0
Mammary					
<i>Fibroadenoma</i>	F	15	13	19	18
<i>Carcinoma</i>	F	19	10	12	9
Pancreas					
<i>Acinar cell adenoma</i>	M	0	1	2	0
	F	0	0	0	0
<i>Islet cell adenoma</i>	M	3	3	2	0
	F	0	1	3	3
<i>Islet cell carcinoma</i>	M	0	1	0	2
	F	1	3	0	1
Parathyroid					
<i>Adenoma</i>	M	2	0	1	1
	F	1	0	0	0
Pituitary					
<i>Adenoma</i>	M	39	33	37	31
	F	53	49	46	45
Skin					
<i>Keratoacanthoma</i>	M	2	1	3	3
	F	0	2	0	0
<i>Squamous cell papilloma</i>	M	1	2	0	0
	F	0	0	0	0
<i>Pilomatricoma</i>	M	0	2	3	1
	F	0	0	0	0
<i>Basal cell tumor</i>	M	0	1	0	3
	F	0	0	0	0
<i>Fibroma</i>	M	1	1	2	0
	F	0	0	0	1
<i>Lipoma</i>	M	0	0	3	0
	F	1	0	0	1
<i>Fibrosarcoma</i>	M	1	1	2	0
	F	0	1	0	0
Spleen					
<i>Hematopoietic Neoplasm</i>	M	0	3	1	1
	F	0	0	0	0
Testis					
<i>Benign Interstitial cell adenoma</i>	M	0	0	4	7
Thyroid					
<i>Follicular Cell adenoma</i>	M	0	2	2	0
	F	2	0	0	0
<i>C-cell adenoma</i>	M	7	7	10	5
	F	5	4	8	7

<i>Follicular Cell carcinoma</i>	M	2	2	1	1
	F	0	1	0	0
<i>C-cell carcinoma</i>	M	1	0	1	2
	F	0	0	0	0
Uterus					
<i>Endometrial stromal polyp</i>	F	3	1	2	4
Vagina					
<i>Granular cell tumor</i>	F	0	0	2	2

There was a dose-dependent increase in the incidence of benign interstitial (Leydig) cell tumors of the testis. The incidence was 0/60, 0/60, 4/60 (6.7%), and 7/60 (11.7%) in the 0, 40, 120, and 400 mg/kg/day males, respectively. The Sponsor reported that benign interstitial (Leydig) cell adenomas showed a significant positive trend ($p=0.0013$) with a borderline increased incidence (not statistically significant) at 400 mg/kg/day compared to controls ($p=0.0101$), when analyzed as a common tumor. The Charles River 2-year control group historical incidence ranges from 1.11 to 9.33%. According to the FDA statistical review by Dr. Mohammad Atiar Rahman dated 2/11/2014, based on the criteria of adjustment for multiple testing, there was a statistically significant positive trend in the incidence of benign interstitial cell adenoma in the testis of male rats ($p=0.0016$). Pair-wise comparison showed a statistically significant increase in the 400 mg/kg/day males ($p=0.0115$, significant based on the rare tumor criteria), compared to controls.

A request was made by this reviewer to Dr. Rahman for a combined statistical analysis of pancreas islet cell adenoma and carcinoma, thyroid follicular cell adenoma and carcinoma, and thyroid C-cell adenoma and carcinoma. No statistically significant changes were found in any of these combinations.

There was a dose-dependent decrease in the incidence of pituitary adenoma in the males (65%, 55%, 61.7%, and 51.7% in the 0, 40, 120, and 400 mg/kg/day males, respectively) and females (88.3%, 81.7%, 76.7%, and 75% in the 0, 40, 120, and 400 mg/kg/day females, respectively). The Sponsor reported a significant negative trend in the incidence of pituitary tumor in both males ($p=0.0094$) and females ($p=0.0088$), with significantly lower rates in the 120 mg/kg/day females ($p=0.0098$) and in the 400 mg/kg/day males ($p=0.0112$) and females ($p=0.0194$), compared to controls.

There was also a dose-related decrease in the incidence of mammary carcinoma in the females (31.7%, 16.7%, 20%, and 15% in the 0, 40, 120, and 400 mg/kg/day females, respectively). The Sponsor reported a significant negative trend ($p=0.0082$) along with significantly lower incidence of mammary carcinoma in the 120 mg/kg/day ($p=0.0335$) and 400 mg/kg/day ($p=0.0110$) females, compared to controls.

The FDA statistical review by Dr. Mohammad Atiar Rahman, dated 2/11/2014, did not evaluate the apparent drug-related decreases in tumor incidence.

Non-neoplastic

The following noteworthy non-neoplastic microscopic findings were observed.

Non-Neoplastic Microscopic Findings in the 2-Year Carcinogenicity Study in Rats

Organ/Tissue	Sex	Doses (mg/kg/day)			
		0	40	120	400
Number / group / sex		60	60	60	60
Foot <i>Pododermatitis</i>	M	16	17	13	25
	F	10	8	11	21
Kidney <i>Pelvis dilatation</i>	M	37	44	47	49
	F	15	26	23	33
<i>Tubule cell vacuolation</i>	M	0	0	0	1
	F	0	3	1	7
Lung <i>Hemorrhage</i>	M	19	28	27	23
	F	14	17	13	24
Pituitary <i>Hyperplasia</i>	M	6	4	8	10
	F	3	3	4	6
Stomach <i>Hyperkeratosis</i>	M	1	2	1	1
	F	2	3	1	6
Testes <i>Interstitial cell hyperplasia</i>	M	3	5	12	8

An increase in the incidence of interstitial (Leydig) cell hyperplasia in the males was noted (5%, 8.3%, 20%, and 13.3% in the 0, 40, 120, and 400 mg/kg/day males, respectively). The Sponsor reported that the incidence of interstitial (Leydig) cell hyperplasia showed a positive trend ($p=0.1301$) that was not statistically significant, with a significantly increased incidence in the 120 mg/kg/day males, compared to controls ($p=0.0343$). Other apparent drug-related non-neoplastic findings, including pododermatitis, renal pelvis dilatation and tubule cell vacuolation, and pituitary hyperplasia, were noted in the 400 mg/kg/day males and females.

Toxicokinetics

Blood samples (3 animals/sex/ timepoint) at predose and postdose (0.5, 3, 6, 12, and 24 hr) were collected on day 1, and on weeks 26 and 52. Plasma samples were analyzed using a validated LC-MS/MS methods for quantitation of NKTR-118 and its metabolite, NKTR-118-Glucuronide.

As shown in the tables below (taken from the Sponsor's report), plasma NKTR-118 C_{max} increased with dose in a dose proportional manner at all time-points for TK measurements in both males and females. However, the increase in plasma NKTR-118 AUC_{0-24hr} was greater than dose proportional. Females generally had higher plasma NKTR-118 exposure compared to males. There was some accumulation of NKTR-118 in males and minimal or no accumulation in females after 52 weeks of NKTR-118 administration. Plasma NKTR-118-Glucuronide exposure (C_{max} and AUC_{0-24hr}) generally increased more than dose proportionally in males and females. There was significant accumulation of NKTR-118-Glucuronide in males and females after 52 weeks of NKTR-118 administration.

Toxicokinetics in the 2-Year Carcinogenicity Study in Rats

Day/ Week	Sex	Dose (mg/kg/day)	NKTR-118			NKTR-118-glucuronide		
			T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-24hr} (hr•ng/mL)	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-24hr} (hr•ng/mL)
Day 1	Male	40	0.500	1700	6660	0.500	1860	5870
		120	0.500	4200	17600	0.500	5720	16800
		400	0.507	19300	112000	0.507	12500	61800
	Female	40	0.513	3570	19500	0.513	1410	5090
		120	2.97	12500	83800	0.507	3760	18800
		400	3.01	41200	403000	0.520	8630	88100
Week 26	Male	40	0.513	4480	14100	0.513	6260	14800
		120	0.523	8410	46700	0.523	18700	77900
		400	3.00	33600	255000	0.563	54700	361000
	Female	40	0.500	6220	28500	0.500	5580	16100
		120	0.500	15200	97600	0.500	13800	71400
		400	2.99	41600	385000	0.500	52600	416000
Week 52	Male	40	0.500	3850	16800	0.500	6790	21600
		120	0.520	15000	81100	0.520	29600	112000
		400	0.520	36200	270000	0.520	75600	437000
	Female	40	0.493	6370	35700	0.493	5120	18600
		120	3.06	13700	106000	0.500	13900	81100
		400	0.500	38100	340000	0.500	76600	473000

Dosing Solution Analysis

Stock assay solutions were tested appropriately for stability and drug concentration. Mean concentrations of the 4 mg/ml, 12 mg/ml, and 40 mg/ml dosing solutions ranged from 78.8% to 115.1% of nominal concentrations. Various individual samples were outside the $\pm 10\%$ nominal range but deviations were infrequent and did not affect the integrity of the study over the 2-year dosing period. Dose formulations are solutions at the concentrations used and, therefore, no homogeneity analysis was performed.

Study title: 2-Year Oral Carcinogenicity and Toxicokinetic Study with NKTR-118 in Mice

Study no.: 7985-110
Study report location: N/A
Conducting laboratory and location: [REDACTED] (b) (4)
Date of study initiation: 11-25-2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: NKTR-118; 149015 (98%), 200278 (98%), 200388 (98%), 09-0581 (97%), 200279 (98%)
CAC concurrence: Yes (meeting minutes dated 11/26/2008)

Key Study Findings

- Male mice were treated with 0 (vehicle), 25, 70, or 200 mg/kg/day NKTR-118 and female mice were treated with 0 (vehicle), 40, 120, or 400 mg/kg/day NKTR-118 by oral gavage. Due to increased mortality relative to controls in the 200 mg/kg/day males and 400 mg/kg/day females, dose levels for animals in the mid- and high dose groups were reduced. Starting on day 118 of the dosing phase, the middle and high dose males received doses of 50 and 100 mg/kg/day, respectively; starting on day 117 of the dosing phase, the middle and high dose females received doses of 80 and 160 mg/kg/day, respectively.
- There were no significant differences in mortality rates at study termination among treatment groups in either sex.
- Apparent drug-related clinical signs included convulsions, abdominal swelling, hypoactivity, sensitive to touch, squinted eyes, labored and/or irregular respiration, and cold to touch. Yellow haircoat occurred before dose reduction in males and females on days 118 and 117, respectively. There were no drug-related clinical signs after dose reduction.
- There were no drug-related changes in body weight or bodyweight gain.
- There were no statistically significant dose-response relationships in tumor incidence in either sex. Pair-wise comparisons also did not show a statistically significant increase in tumor incidence in any drug-treated group compared to their respective controls in either sex.
- There were no meaningful drug-related non-neoplastic findings.
- Plasma NKTR-118 C_{max} and AUC_{0-24hr} increased with dose in a greater than dose proportional manner on day 1 in both males and females. After reducing the middle and high doses on week 17, plasma C_{max} and AUC values increased

dose proportionally on weeks 26 and 52 in males and females. Females had lower plasma NKTR-118 exposure (approximately 40 to 70%) compared to males, based on a mg/kg/day comparison, on day 1 and on weeks 26 and 52. There was minimal or no accumulation of NKTR-118 in males and females after 52 weeks of NKTR-118 administration. Plasma NKTR-118-Glucuronide exposure (C_{max} and AUC) generally increased with dose in greater than dose proportional manner in males, but dose proportionally in females. Females consistently had lower NKTR-118-Glucuronide exposure than males in the middle and high dose groups. There was minimal or no accumulation of NKTR-118-Glucuronide in males or females after 52 weeks of NKTR-118 administration. Exposure to NKTR-118-glucuronide was consistently greater than that of NKTR-118.

Adequacy of Carcinogenicity Study

The mouse carcinogenicity study used the doses (0, 25, 70, and 200 mg/kg/day for males and 0, 40, 120, and 400 mg/kg/day for females) that were recommended by the Executive CAC. Due to increased mortality relative to controls in males and females, dose levels for the mid- and high dose groups were reduced, according to the Executive CAC recommendations. The study length was acceptable since the mice were treated for up to 104 weeks. There was no drug-related increase in mortality rate at study termination. The carcinogenicity study was conducted appropriately.

Appropriateness of Test Models

The CrI:CD1(ICR) strain is an appropriate model because this strain is known to be responsive to known carcinogens, and historical control data has been established in the conducting laboratory. The test model used by the Sponsor was appropriate.

Evaluation of Tumor Findings

A borderline significant increase ($p=0.0497$) in the incidence of liver hepatocellular carcinoma in the 25 mg/kg/day males (10/60, 16.7%) compared to controls (4/60, 6.7%) was reported by the Sponsor. However, there were no significant increases at higher doses, and there was no significant trend ($p=0.3256$). According to the FDA statistical review by Dr. Mohammad Atiar Rahman dated 2/11/2014, none of the tumor incidences showed a statistically significant dose-response relationship in either males or females. Pair-wise comparisons also did not show a statistically significant increase in tumor incidence in any drug-treated group compared to their respective controls in either sex.

A significant decrease ($p=0.0416$) in the incidence of bronchiolar-alveolar adenoma of the lungs was noted in the 70/50 mg/kg/day males (6/60, 10%) compared to controls (15/60, 25%), but there was no significant trend ($p=0.0932$, negative direction). In females, the whole body/cavity histiocytic sarcoma incidence showed a significant decrease ($p=0.0199$) in the 120/80 mg/kg/day group (0/60, 0%) compared to controls (5/60, 8.3%), but there was no significant trend ($p=0.0699$, negative direction). The

FDA statistical review by Dr. Mohammad Atiar Rahman dated 2/11/2014, did not evaluate the apparent drug-related decreases in tumor incidence.

Therefore, there were no significant drug-related neoplastic findings in male or female mice treated with NKTR-118 in the 2-year oral carcinogenicity study in mice.

Methods

Doses: Males: 0 (vehicle), 25, 70/50, and 200/100 mg/kg/day
 Females: 0 (vehicle), 40, 120/80, and 400/160 mg/kg/day

Frequency of dosing: Once daily
 Dose volume: 10 ml/kg

Route of administration: Oral gavage
 Formulation/Vehicle: Solution/reverse osmosis water

Basis of dose selection: Executive Carcinogenicity Assessment Committee recommendations. The Committee recommended doses of 0, 25, 70, and 200 mg/kg/day by oral gavage for males, based on mortality, and 0, 40, 120, and 400 mg/kg/day by oral gavage, based on adverse histopathology observations.

Species/Strain: Mice/Crl:CD1(ICR)
 Number/Sex/Group: 60
 Age: 6 to 7 weeks old
 Animal housing: Individually in stainless steel cages with bedding

Paradigm for dietary restriction: Certified Rodent Diet #2014C (Harlan Laboratories, Inc.)

Dual control employed: No
 Interim sacrifice: No
 Satellite groups: Toxicokinetic groups: Control - 22/sex; Treatment - 58/sex/group

Deviation from study protocol: Due to increased mortality in the 400 mg/kg/day females and 200 mg/kg/day males, dose levels for animals in the mid- and high dose groups were reduced, according to FDA Executive CAC recommendations made on 4/09/2009.

The design of the study is shown in the table below (taken from the Sponsor's report).

Study Design of the 104-week Carcinogenicity Study in Mice

Group ^a	No. of Animals		Dose Level (Male) (mg/kg/day)	Dose Level (Female) (mg/kg/day)	Dose Concentration (Male) (mg/mL)	Dose Concentration (Female) (mg/mL)
	Male	Female				
Carcinogenicity Animals						
1 (Control)	60	60	0	0	0	0
2 (Low)	60	60	25	40	2.5	4
3 (Mid) ^c	60	60	70/50	120/80	7/5	12/8
4 (High) ^c	60	60	200/100	400/160	20/10	40/16
Toxicokinetic Animals^b						
5 (Control)	22	22	0	0	0	0
6 (Low)	58	58	25	40	2.5	4
7 (Mid) ^c	58	58	70/50	120/80	7/5	12/8
8 (High) ^c	58	58	200/100	400/160	20/10	40/16

a Groups 1 and 5 received vehicle control article only.

b Toxicokinetic animals included solely for the purpose of blood sample collections. Four animals/sex/group served as replacement animals to compensate for possible mortality.

c Starting on Day 118 of the dosing phase for males, the mid- and high-dose groups received a dose level of 50 and 100 mg/kg/day, respectively, and a dose concentration of 5 and 10 mg/mL, respectively. Starting on Day 117 of the dosing phase for females, the mid- and high-dose groups received a dose level 80 and 160 mg/kg/day, respectively, and a dose concentration of 8 and 16 mg/mL, respectively.

Due to increased mortality relative to controls in the 400 mg/kg/day females and to a lesser extent in the 200 mg/kg/day males, dose levels for animals in the mid- and high dose groups were reduced, according to Executive CAC recommendations made on 4/9/2009. Starting on day 118 of the dosing phase, the middle and high dose males received doses of 50 and 100 mg/kg/day, respectively; starting on day 117 of the dosing phase, the middle and high dose females received doses of 80 and 160 mg/kg/day, respectively.

Observations and Results

Mortality

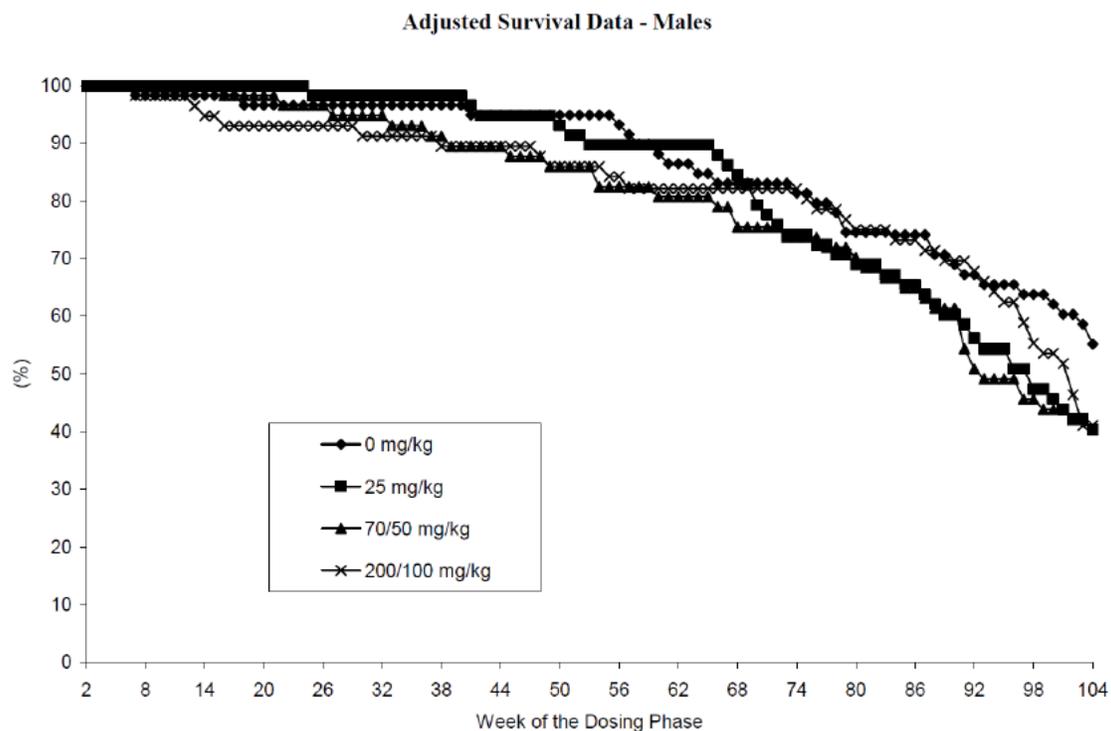
All animals were observed twice daily for morbidity and mortality. Death occurred in the 200 mg/kg/day males (7 animals) and 400 mg/kg/day females (16 animals) through day 117 of dosing. These animals were found dead or sacrificed in moribund condition with respiratory distress (labored or irregular) and abdominal swelling.

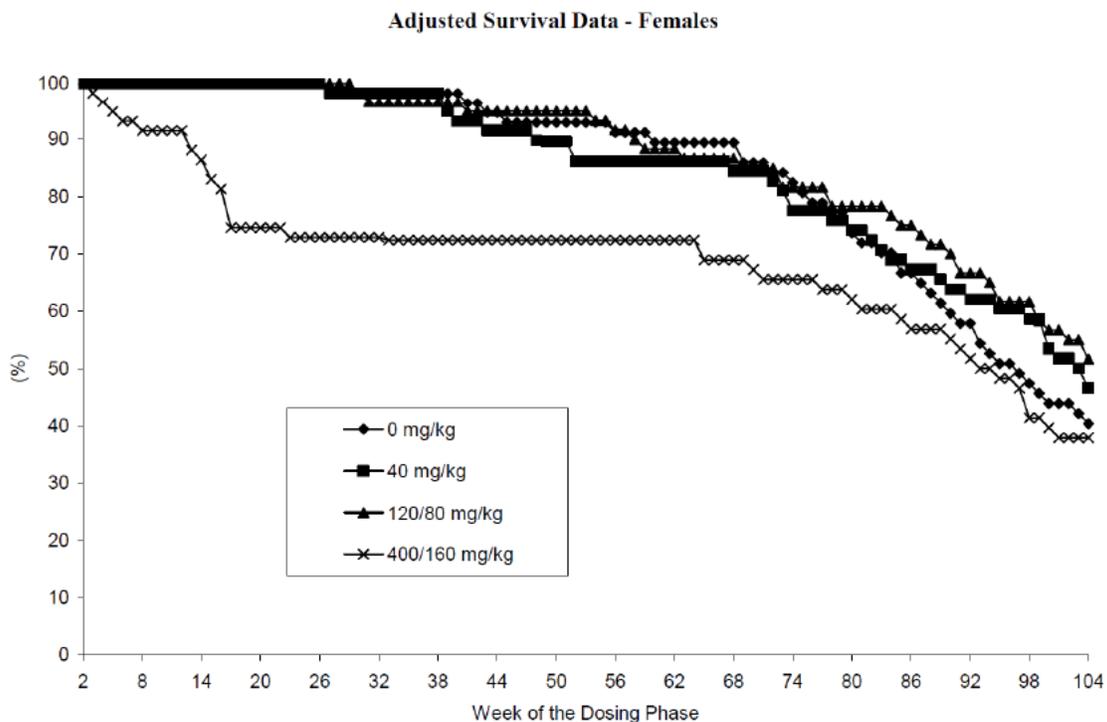
Macroscopically, three males and six females were noted to have gas distention of the gastrointestinal tract. There were no microscopic findings that correlate with the respiratory distress and/or distention of the gastrointestinal tract. The cause of death was not determined for 3 males and 15 females found dead or sacrificed in moribund condition through day 117 of dosing. One female and three males died of gavage-related trauma through day 117 of dosing. The death of one male on day 107 of the dosing phase was determined to be due to renal disease.

The Sponsor reported that after the dose reduction in males and females, survival in the mid- and high-dose groups was comparable with control and low-dose groups. The survival rates are summarized in the table and figures below (taken from the Sponsor's study report).

Adjusted Survival Rates (%) for Toxicity Animals

Dose (mg/kg/day)	Sex	Male				Female			
	0	25	70/50	200/100	0	40	120/80	400/160	
Week 17	98	100	98	93	100	100	100	75	
Week 26	97	98	97	93	100	100	100	73	
Week 52	95	91	86	86	93	86	95	72	
Week 78	78	71	72	79	77	76	78	64	
Week 90	69	60	61	70	60	64	70	55	
Week 104	55	40	40	41	40	47	52	38	





The analysis in the FDA statistical review by Dr. Mohammad Atiar Rahman dated 2/11/2014 showed 32/60 (53%), 23/60 (38%), 23/60 (38%), and 23/60 (38%) survival in male mice, and 23/60 (38%), 27/60 (45%), 31/60 (52%), and 22/60 (37%) survival in female mice in the control, low, middle, and high dose groups, respectively. The analysis showed no statistically significant dose-response relationship in mortality across the treatment groups in either males ($p=0.0109$) or females ($p=0.0998$). Pair-wise comparison also did not show a statistically significant difference in mortality rates among treatment groups in either sex.

Clinical Signs

Animals were observed twice daily for abnormalities, and signs of pain or distress. Detailed observations were performed once during the predose phase, before dosing on day 1, weekly thereafter, and on the day of terminal sacrifice.

The following noteworthy clinical signs were observed.

Clinical Signs in the 2-Year Carcinogenicity Study in Mice

Clinical signs	Sex	Dose (mg/kg/day)			
		0 M 0 F	25 M 40 F	70/50 M 120/80 F	200/100M 400/160 F
Number/sex/group		60	60	60	60
Appearance					
<i>Convulsions (< 1 min)</i>	M	2	1	4	5
<i>Midline ventral abdomen swollen</i>	M	6	8	9	13
<i>Entire abdomen swollen</i>	F	0	0	1	5
Behavior					
<i>Hypoactive</i>	M	5	7	6	10
<i>Sensitive to touch</i>	F	2	3	4	7
Excretion					
<i>Few feces</i>	M	3	6	4	7
Eye					
<i>squinted</i>	M	1	2	3	5
	F	1	2	1	4
Mass					
	M	14	25	17	12
	F	5	3	4	4
Respiration					
<i>Labored</i>	F	7	2	2	12
<i>Irregular</i>	M	1	6	5	4
Skin and Pelage					
<i>Cold to touch</i>	M	0	1	0	4
<i>Yellow haircoat, perineal area</i>	M	7	7	13	8
<i>Pale ears</i>	F	1	3	6	3

Apparent drug-related clinical signs included abdominal swelling, squinted eyes, and labored and/or irregular respiration. These signs occurred before dose reduction in males and females on days 118 and 117, respectively. There were no drug-related clinical signs after dose reduction.

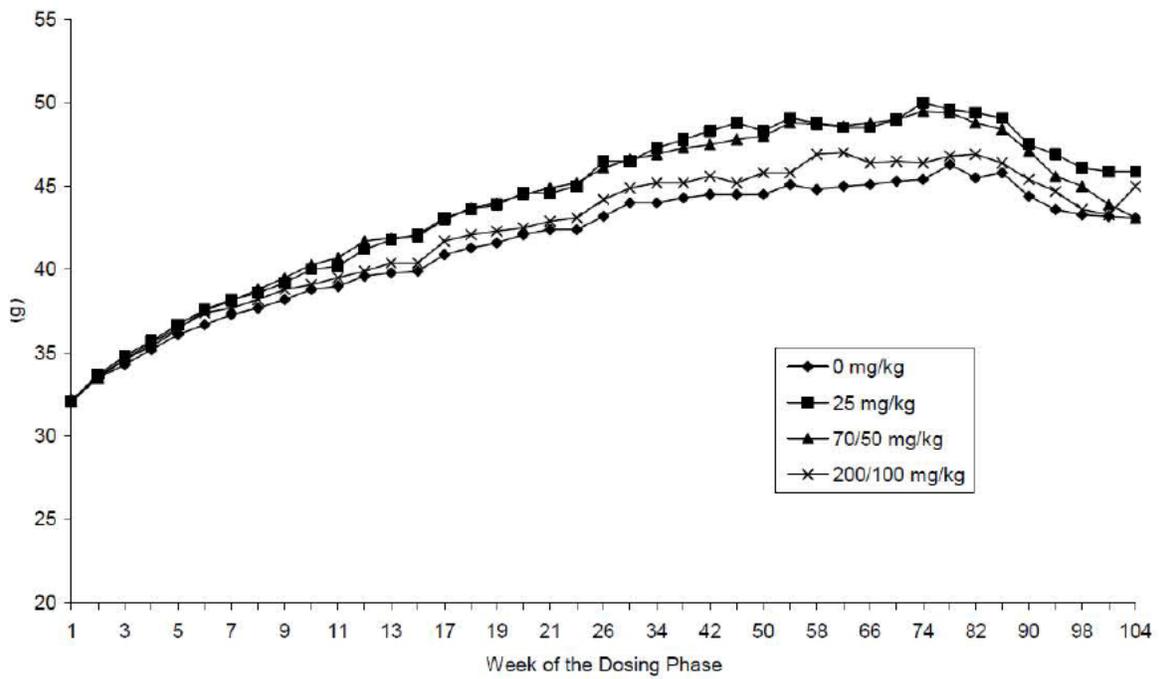
There were no drug-related effects on the incidence of masses.

Body Weights

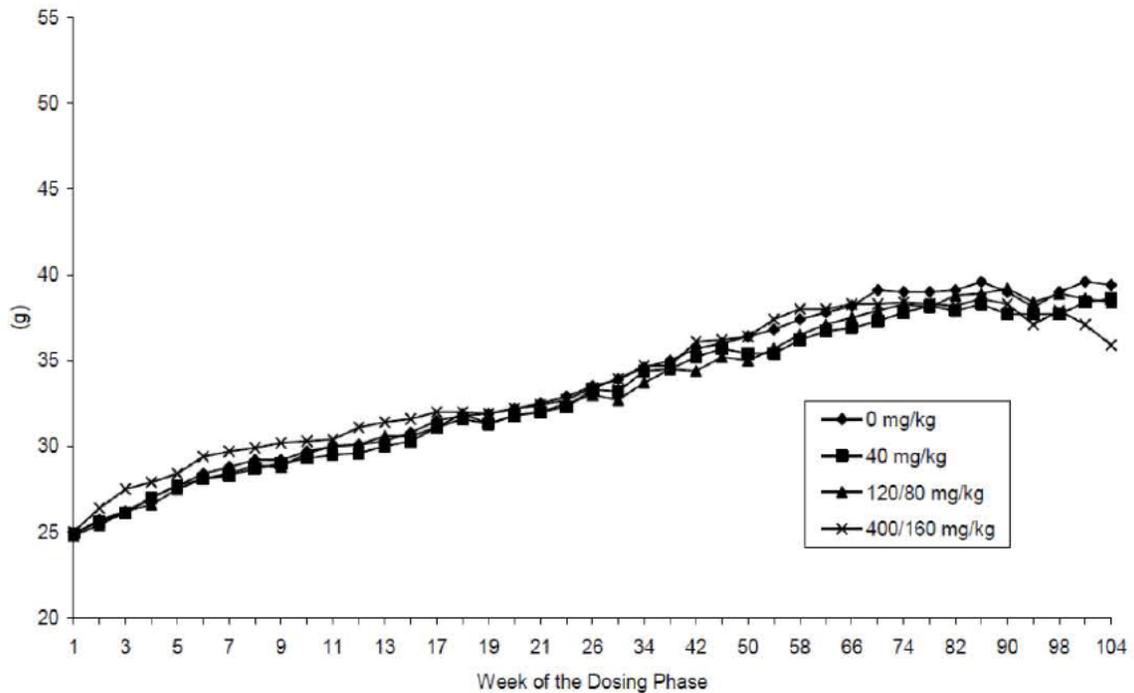
Animals were weighed during the predose phase, prior to dosing on day 1 of the dosing phase, once weekly thereafter on weeks 2 through 14, once at weeks 17, 18, 19, 20, 21, and 22, once every 4 weeks thereafter, and at week 104 of the dosing phase. The changes in body weight parameters are shown below in figures taken from the Sponsor's study report and in the reviewer's table.

Body weight changes in the 2-Year Carcinogenicity Study in Mice

Mean Body Weight Data - Males



Mean Body Weight Data - Females



Changes in Body Weights in the 2-Year Carcinogenicity Study in Mice

MALES	Dose (mg/kg/day)			
	0	25	70/50	200/100
Group	1	2	3	4
Week 1 Weight (g)	32.2	32.1	32.1	32.1
Week 26 Weight (g)	43.2	46.5*	46.1*	44.2
% of Control, Wk 26	100	107.6	106.7	102.3
ΔWk26-Wk1 (g)	11.0	14.4	14.0	12.1
BW Gain, % of Control	100	130.9	127.3	110
Week 54 Weight (g)	45.1	49.1*	48.8*	45.8
% of Control, Wk 54	100	108.9	108.2	101.6
ΔWk54-Wk1 (g)	12.9	17.0	16.7	13.7
BW Gain, % of Control	100	131.8	129.5	106.2
Week 74 Weight (g)	45.4	50.0*	49.5*	46.4
% of Control, Wk 74	100	110.6	109.0	102.2
ΔWk74-Wk1 (g)	13.2	17.9	17.4	14.3
BW Gain, % of Control	100	135.6	131.8	108.3
Week 104 Weight (g)	43.1	45.9	43.1	45.0
% of Control, Wk 104	100	106.5	100	104.4
ΔWk104-Wk1 (g)	10.9	13.8	11.0	12.9
BW Gain, % of Control	100	126.7	100.9	127.6

* Significantly different from controls; p<0.05

FEMALES	Dose (mg/kg/day)			
	0	40	120/80	400/160
Group	1	2	3	4
Week 1 Weight (g)	24.9	24.9	24.8	25.0
Week 26 Weight (g)	33.5	33.3	33.0	33.4
% of Control, Wk 26	100	99.4	98.5	99.7
Δ Wk26-Wk1 (g)	8.6	8.4	8.2	8.4
BW Gain, % of Control	100	97.7	95.3	97.7
Week 54 Weight (g)	36.8	35.4	35.7	37.4
% of Control, Wk 54	100	96.2	97.0	101.6
Δ Wk54-Wk1 (g)	11.9	10.5	10.9	12.4
BW Gain, % of Control	100	88.2	91.6	104.2
Week 74 Weight (g)	39.0	37.8	38.3	38.4
% of Control, Wk 74	100	96.9	98.2	98.5
Δ Wk74-Wk1 (g)	14.1	12.9	13.5	13.4
BW Gain, % of Control	100	91.5	95.7	95.0
Week 104 Weight (g)	39.4	38.6	38.4	35.9
% of Control, Wk 104	100	98.0	97.5	91.1
Δ Wk104-Wk1 (g)	14.5	13.7	13.6	10.9
BW Gain, % of Control	100	94.5	93.8	75.2

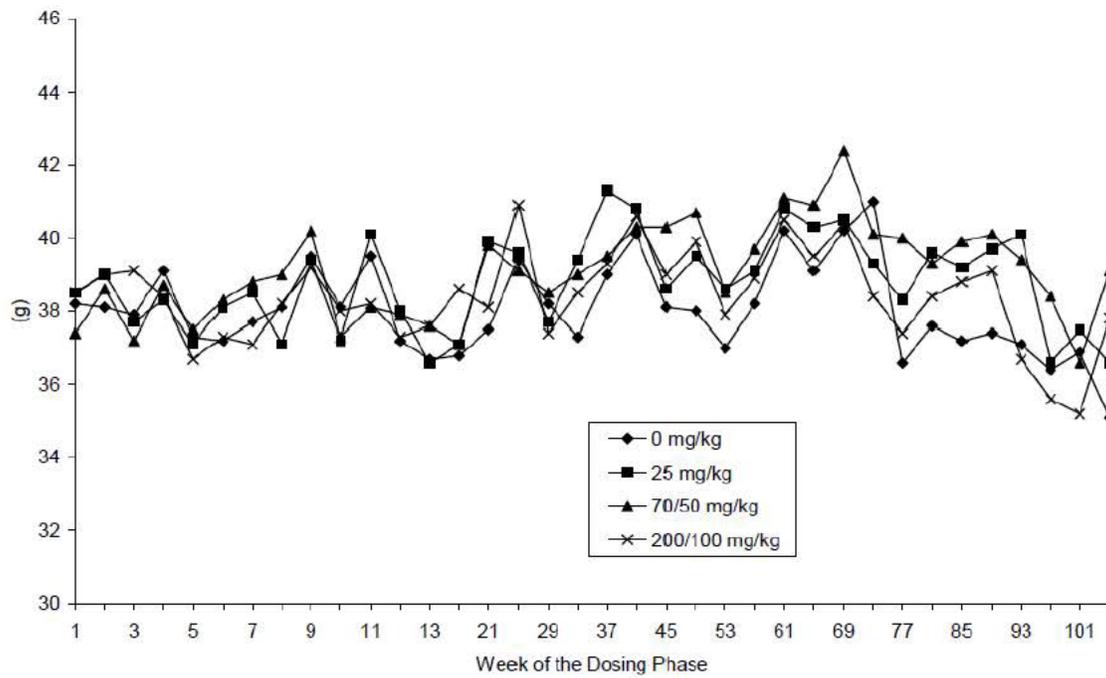
Statistically significant increases in body weight were observed during the treatment period in the 25 and 70/50 mg/kg/day males compared to controls. However, there were no significant changes in body weight in the 400/160 mg/kg/day males compared to controls. At terminal sacrifice, there were no significant changes in the body weight among the treatment groups compared to controls. Therefore, the body weight changes in males are not considered drug-related. In females, there were no statistically significant changes in body weight or bodyweight gains throughout the treatment period.

Feed Consumption

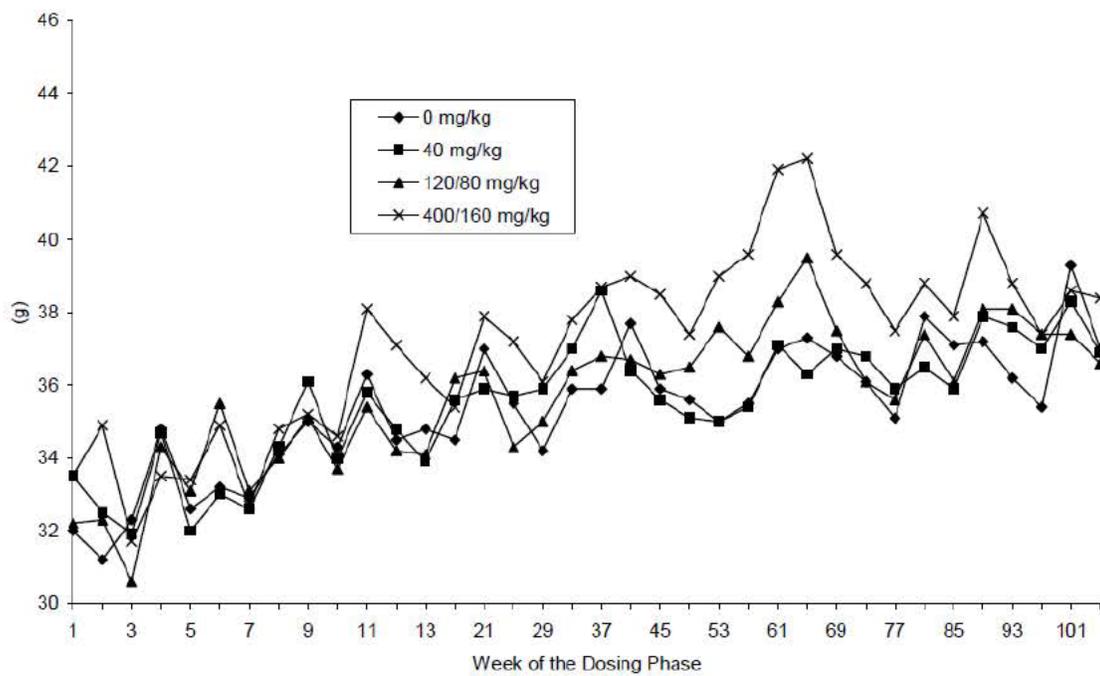
Food consumption was recorded weekly at weeks 1 through 13, every 4 weeks thereafter, and at week 104 during the dosing phase for carcinogenicity animals. As shown in the tables below (taken from the Sponsor's report), there were no meaningful drug-related changes in food consumption, although sporadic, and sometimes statistically significant, changes were noted.

Food Consumption in the 2-Year Carcinogenicity Study in Mice

Mean Food Consumption Data - Males



Mean Food Consumption Data - Females



Gross Pathology

Mortality occurred in males (7 animals) and females (16 animals) treated with 200 or 400 mg/kg/day NKTR-118, respectively, through day 117 of the dosing phase. These animals were found dead or sacrificed in moribund condition with respiratory distress (labored or irregular) and abdominal swelling. Of the 23 animals, 6 females and 3 males were noted to have distention of the gastrointestinal tract with gas. This distention was considered by the Sponsor to be due to swallowed air secondary to the labored breathing noted clinically, and was not considered as drug-related. There were no microscopic findings that correlated with the respiratory distress and/or distention of the gastrointestinal tract.

Histopathology

Preserved tissues from each animal in all 4 main study groups were examined microscopically. The following tissues and organs were collected and preserved (table taken from the study report).

Tissues and Organs Collected

adrenal (2)	optic nerve (2) ^a
aorta	ovary (2)
brain	pancreas
cecum	pituitary gland
cervix	prostate
colon	rectum
duodenum	salivary gland [mandibular (2)]
epididymis (2)	sciatic nerve
esophagus	seminal vesicle
eye (2) ^a	skeletal muscle (thigh)
femur with bone marrow (articular surface of the distal end)	skin/subcutis
gall bladder	spinal cord (cervical, thoracic, and lumbar)
Harderian gland ^a	spleen
heart	sternum with bone marrow
ileum	stomach
jejunum	testis (2) ^a
kidney (2)	thymus
lesions	thyroid (2) with parathyroid
liver	tongue
lung with large bronchi	trachea
lymph node (mandibular)	urinary bladder
lymph node (mesenteric)	uterus
mammary gland	vagina

a Preserved in modified Davidson's fixative.

Peer Review - Yes

A peer review was performed on selected tissues at (b) (4). All testes, all recorded neoplasms, and 10% of animals (all tissues) were examined microscopically.

Neoplastic

In Sponsor's analysis, neoplastic lesions were chosen for statistical analyses if the incidence in at least one of the treated groups was increased or decreased by at least two occurrences compared to the control group. The incidental tumors (i.e., tumors not assigned as the cause of death by the study pathologist) were analyzed by linear logistic regression of tumor prevalence. Fatal and palpable (superficial) tumors were analyzed by the Cox-Tarone binary regression method using the death time or the first palpation time (as applicable) as a surrogate for the tumor onset time. One-sided positive trends in common (background incidence rate > 1%) and rare (background incidence < 1%) tumors (if applicable) defined by the study pathologist were evaluated at the 0.005 and 0.025 significance levels, respectively. High-dose group comparisons in common and rare tumors were evaluated at the 0.01 and 0.05 significance levels, respectively. Other intermediate, pair-wise, and one-sided group comparisons were evaluated at the 5.0% significance level. Benign and malignant neoplastic incidences were evaluated separately and combined, where appropriate.

In the FDA statistical review, the tumor data were analyzed for dose response relationships and pair-wise comparisons of control groups with each of the treated groups; the analyses were performed using the Poly-K method. To adjust for multiple testing, the dose response relationship was tested at levels of $\alpha=0.005$ for common tumors and $\alpha=0.025$ for rare tumors. This method is appropriate for the submission of experiments in two species, in order to keep the false-positive rate at the nominal level of approximately 10%. A rare tumor is defined as one in which the published spontaneous tumor rate is less than 1%. For multiple pair-wise comparisons of treated groups with the control, test levels of $\alpha=0.01$ for common tumors and $\alpha=0.05$ for rare tumors were used, in order to keep the false-positive rate at the nominal level of approximately 10% for the experiments in both species.

Noteworthy neoplastic tumor findings are summarized in the table below.

Neoplastic Tumor Findings in the 2-Year Carcinogenicity Study in Mice

Organ/Tissue	Sex	Doses (mg/kg/day)			
		Control	25 M 40 F	70/50 M 120/80 F	200/100 M 400/160 F
Number / group / sex examined		60	60	60	60
Adrenal Cortex <i>Cortical cell adenoma</i>	M	4	1	1	2
	F	0	0	0	0
Adipose tissue <i>Lipoma</i>	M	0	2	0	0

	F	0	0	0	0
Body (whole/cavity)					
<i>Benign hemangioma</i>	M	0	0	1	0
	F	1	4	4	3
<i>Histiocytic sarcoma</i>	M	0	0	0	1
	F	5	2	0	2
<i>Lymphosarcoma</i>	M	11	10	6	10
	F	23	24	27	10
<i>Hemangiosarcoma</i>	M	2	2	4	0
	F	4	2	3	3
Gland, Harderian					
<i>Adenoma</i>	M	7	6	10	8
	F	5	2	4	4
<i>Carcinoma</i>	M	0	1	1	0
	F	0	2	1	1
Liver					
<i>Hepatocellular adenoma</i>	M	7	8	8	3
	F	2	0	0	1
<i>Hepatocellular carcinoma</i>	M	4	10	8	2
	F	0	0	2	0
Lung					
<i>Bronchiolar-alveolar adenoma</i>	M	15	9	6	9
	F	5	8	10	5
<i>Bronchiolar-alveolar carcinoma</i>	M	5	8	6	7
	F	3	3	2	2
Mammary					
<i>Carcinoma</i>	F	0	0	2	0
Pituitary					
<i>Adenoma</i>	M	2	1	2	0
	F	1	3	2	2
Testis					
<i>Interstitial cell tumor</i>	M	4	1	4	5
Thyroid					
<i>Follicular Cell adenoma</i>	M	2	0	0	0
	F	1	1	1	1
Uterus					
<i>Leiomyoma</i>	F	0	0	2	0
Vagina					
<i>Vaginal polyp</i>	F	1	3	1	1

The Sponsor reported that in males, a borderline significant increase ($p = 0.0497$) in the incidence of liver hepatocellular carcinoma occurred in the 25 mg/kg/day group (10/60, 16.7%) compared to controls (4/60, 6.7%), but without a significant trend ($p=0.3256$). A significant decrease ($p=0.0416$) in the incidence of bronchiolar-alveolar adenoma of the lungs was noted in the 70/50 mg/kg/day males ((6/60, 10%) compared to controls (15/60, 25%), but without a significant trend ($p=0.0932$, negative direction). In females, the whole body/cavity histiocytic sarcoma incidence showed a significant decrease

($p=0.0199$) in the 120/80 mg/kg/day group (0/60) compared to controls (5/60, 8.3%), but without a significant trend ($p=0.0699$, negative direction). The Sponsor reported that there were no other statistically significant changes, in either direction, in any other neoplastic lesion in males or females.

According to the FDA statistical review by Dr. Mohammad Atiar Rahman dated 2/11/2014, none of the tumor incidences were considered to have a statistically significant dose-response relationship in either sex, based on the criteria of adjustment for multiple testing. The pair-wise comparisons also did not show a statistically significant increase in the incidence of the observed tumor types in any treated group compared to their respective controls in either sex. In addition, a request was made by this reviewer to Dr. Rahman for a combined statistical analysis of liver hepatocellular adenoma and carcinoma and lung bronchiolar-alveolar adenoma and carcinoma. No statistically significant changes were found in either of these combinations.

Non Neoplastic

There were no meaningful drug-related non-neoplastic lesions. However, the following microscopic findings were noted.

Non-Neoplastic Findings in the 2-Year Carcinogenicity Study in Mice

Organ/Tissue	Sex	Doses (mg/kg/day)			
		Control	25 M 40 F	70/50 M 120/80 F	200/100 M 400/160 F
Number / group / sex examined		60	60	60	60
Cervix <i>Vessel inflammation</i>	F	0	0	2	2
Epididymis <i>Duct ectasia</i>	M	0	1	1	2
Heart <i>Atrium thrombus</i>	M	1	0	0	4
	F	0	0	0	1
Penis <i>Inflammation</i>	M	0	1	3	1
Tongue <i>Vessel inflammation</i>	M	0	0	1	2
	F	1	4	2	3

The non-neoplastic findings were generally low in frequency and/or lacked dose-dependency. Therefore, these are not considered to be drug-related.

Toxicokinetics

Blood samples (3 animals/sex/time-point) were collected from toxicokinetic animals on day 1 and during weeks 26 and 52 at predose and 0.5, 3, 6, 12, and 24 hr postdose following oral administration. Predose blood samples were not collected from high dose animals during week 52 due to an insufficient number of surviving animals. In the control groups, blood samples were collected at predose, and at approximately 3 hr

post-dose. Plasma samples were analyzed using validated LC-MS/MS methods for quantitation of NKTR-118 and its metabolite, NKTR-118-Glucuronide.

As shown in the table below (taken from the Sponsor's report), plasma NKTR-118 C_{max} and AUC_{0-24hr} increased with dose in a greater than dose proportional manner on day 1 in both males and females. After reducing the middle and high dose at week 17, the increase in plasma C_{max} and AUC values was dose proportional on weeks 26 and 52 in males and females. Females had lower plasma NKTR-118 exposure (approximately 40 to 70%) compared to males, based on a mg/kg/day comparison, on day 1 and on weeks 26 and 52. There was minimal or no accumulation of NKTR-118 in males and females after 52 weeks of NKTR-118 administration. Plasma NKTR-118-Glucuronide exposure (C_{max} and AUC) was generally increased in a greater than dose proportional manner in males, but dose proportionally in females. Females consistently had lower NKTR-118-Glucuronide exposure than males in the middle and high dose groups. There was minimal or no accumulation of NKTR-118-Glucuronide in males and females after 52 weeks of NKTR-118 administration. Exposure of NKTR-118-Glucuronide was consistently greater than that of NKTR-118.

Mean Plasma Toxicokinetic Data Summary

Day/Week	Sex	Dose (mg/kg/day)	T_{max} (hr)	NKTR-118		NKTR-118-Glucuronide	
				C_{max} (ng/mL)	AUC_{0-24hr} (hr•ng/mL)	C_{max} (ng/mL)	AUC_{0-24hr} (hr•ng/mL)
Day 1	Male	25	0.507	1330	2820	1780	3490
		70	0.527	7250	12900	10300	18300
		200	0.530	38500	71000	29400	66200
	Female	40	0.507	1850	3150	3910	6080
		120	0.507	6100	11500	11400	20000
		400	0.500	38100	71500	36800	75100
Week 26	Male	25	0.507	1120	2770	2640	4970
		70/50	0.513	3610	7300	6790	11900
		200/100	0.523	5870	13400	19100	33000
	Female	40	0.500	1450	2560	3640	5800
		120/80	0.500	3120	5900	10100	16800
		400/160	0.500	3610	8110	12900	24000
Week 52	Male	25	0.487	1020	3960	2290	4680
		70/50	0.480	3160	7300	7330	12800
		200/100	0.507	6490	14100	19800	34700
	Female	40	0.493	1720	3000	3840	6140
		120/80	0.500	2790	6010	8410	14000
		400/160	0.507	4430	8860	16300	27900

Dosing Solution Analysis

Stock assay solutions were tested appropriately for stability and drug concentration. Mean concentrations of the 2.5 mg/ml, 4 mg/ml, and 7 mg/ml dosing solutions ranged from 86.0% to 108% of nominal concentrations. Various individual samples were outside the $\pm 10\%$ nominal range but deviations were infrequent and did not affect the

integrity of the study over the 2-year dosing period. Dose formulations are solutions at the concentrations used and, therefore, no homogeneity analysis was performed.

THE FOLLOWING IS THE EXECUTIVE CAC MEETING MINUTES FOR THE SPA:

Executive CAC

Date of Meeting: November 25, 2008

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul C. Brown, Ph.D., OND IO, Member
Barbara A. Hill, Ph.D., DDDP, Alternate Member
David B. Joseph, Ph.D., DGP, Pharmacology Team Leader
Niraj R. Mehta, Ph.D., DGP, Presenting Reviewer

Coordinator: Sam Habet, R.Ph., Ph.D., OND IO, Senior Clinical Pharmacologist/
Science Policy Analyst (Detail)

Author of Draft: Niraj R. Mehta, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions using the eCTD Specifications (June 2008) and the associated Study Data Specifications Document.

IND # 78,781
Date of submission: November 4, 2008
Drug Name: NKTR-118
Sponsor: NEKTAR Therapeutics, Inc.

NKTR-118 (PEG-naloxol) is a pegylated derivative of the opioid antagonist drug naloxone. NKTR-118 binding to peripheral μ -opioid receptors of the GI tract has the potential to reverse opioid-induced effects on bowel function. NKTR-118 is being developed for the treatment of opioid-induced constipation (b)(4)

The anticipated clinical dose will be 100 mg QD. NKTR-118 was positive in a bacterial mutation test, however results of the *in vivo* mouse bone marrow micronucleus assay and the *in vitro* mammalian cell mutation assay were negative.

dose groups.

Mouse and Rat:

- Lastly, the sponsor has proposed to do a histopathologic examination on specific organs/tissues in only the control and high-dose groups, however histopathologic examination of other dose groups will be required under any of the following circumstances:
 - (a) For any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups.
 - (b) For an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group.
 - (c) For an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site, they should look at all relevant tissues for that dose level and the next lower dose level.
 - (d) For an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\

- /Division File/DGP
- /Dr. Joseph/DGP
- /Dr. Mehta/DGP
- /Matthew Scherer/PM/DGP
- /D. Jacobson-Kram, OND IO
- /A. Jacobs, OND IO
- /P. Brown, OND IO
- /SHabet, OND IO

THE FOLLOWING IS THE EXECUTIVE CAC MEETING MINUTES FROM THE FINAL REPORT:

Executive CAC**Date of Meeting:** February 4, 2014**Committee:** David Jacobson-Kram, Ph.D., OND-IO, Chair
Abigail Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Todd Palmby, Ph.D., DHOT, Alternate member
David B. Joseph, Ph.D., DGIEP, Team Leader
Yuk-Chow Ng, Ph.D., DGIEP, Presenting Reviewer**Author of Draft:** Yuk-Chow Ng, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA# 204,760 (IND 78,781)**Drug Name:** NKTR-118 (Naloxegol)**Sponsor:** AstraZeneca**Background:**

Opioid analgesics are the mainstay in the treatment of moderate-to-severe pain; however, their use is frequently associated with opioid-induced constipation. NKTR-118 (naloxegol) is an opioid receptor antagonist that has been developed as an oral treatment for opioid-induced constipation in adult patients with chronic non-cancer pain. NKTR-118 acts as an antagonist at μ - and δ -opioid receptors, and is a weak partial agonist at κ -opioid receptors, with the highest binding affinity at μ -opioid receptors. NKTR-118 (free base) was weakly positive in a bacterial mutation test. However, the oxalate salt of NKTR-118, the API to be used in the commercial formulation, tested negative in the Ames test. The Sponsor proposed that the Ames positive result with the free base was due to a genotoxic degradant. NKTR-118 was negative in the *in vivo* mouse bone marrow micronucleus assay and the *in vitro* L5178Y TK \pm mouse lymphoma mammalian cell gene mutation assay. As part of the nonclinical program, the Sponsor conducted a 2-year oral gavage carcinogenicity study in mice and a 2-year oral gavage carcinogenicity study in rats.

Mouse Carcinogenicity Study:

The doses tested were in accordance with the Executive CAC recommendations (see meeting minutes dated 11/26/2008). For male mice, the Committee recommended doses of 0, 25, 70, and 200 mg/kg/day by oral gavage, based on mortality. For female mice, the Committee recommended doses of 0, 40, 120, and 400 mg/kg/day by oral gavage, based on adverse histopathology observations.

In the 104-week oral carcinogenicity study in CrI:CD1(ICR) mice, males were administered 0 (vehicle), 25, 70/50, or 200/100 mg/kg/day NKTR-118, and females were administered 0 (vehicle), 40, 120/80, or 400/160 mg/kg/day NKTR-118 by oral gavage. The vehicle was water. The dose levels were reduced in males and females on days 118 and 117, respectively, due to increased mortality in the 200 mg/kg/day males and 400 mg/kg/day females. The Executive CAC recommended these reductions in dose levels, in response to an inquiry from the Sponsor (letter to Sponsor dated 6/9/2009). After dose reduction in the mid- and high-dose groups, there were no drug-related effects on clinical signs, body weight, food consumption, or macroscopic and microscopic findings. There was no significant increase in any tumor incidence in either sex.

Rat Carcinogenicity Study:

The doses tested were in accordance with the Executive CAC recommendations (see meeting minutes dated 11/26/2008). The Committee recommended doses of 40, 120, and 400 mg/kg/day by oral gavage, based on the rodent to human plasma AUC ratio exceeding 25-fold.

In the 104-week oral carcinogenicity study in CrI:CD(SD) rats, animals (60/sex/group) were administered 0 (vehicle), 40, 120, or 400 mg/kg/day NKTR-118 by oral gavage. The vehicle was water. The study was terminated on week 93 and 94 for males and females, respectively, due to low survival in the control groups, in accordance with Executive CAC recommendations conveyed on August 2, 2010. According to the FDA statistical review by Dr. Mohammad Atiar Rahman, there was a statistically significant negative dose-response relationship (p=0.0109) in mortality rates in male rats. Pairwise comparisons showed a statistically significant decrease in mortality in the 400 mg/kg/day males and 120 mg/kg/day females, compared to their respective controls. There were no drug-related adverse clinical findings. Decreased bodyweight (87.3% of control value) and bodyweight gain (82.7% of control value) were noted in the 400 mg/kg/day males. According to Dr. Rahman, based on the criteria of adjustment for multiple testing, there was a statistically significant positive trend in the incidence of benign interstitial cell adenoma in testis (0/60, 0/60, 4/60, and 7/60 in the 0, 40, 120, and 400 mg/kg/day males, respectively). Pairwise comparison showed an increase in the 400 mg/kg/day males (p=0.0115), compared to controls, that was not statistically significant based on the common tumor criteria ($\alpha = 0.01$). It is possible that the failure to achieve statistical significance in the pairwise test may be due to the higher mortality rate in the control males, which was factored into the statistical analysis. The following table is taken from Dr. Rahman’s review.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship and/or Pairwise Comparisons of Treated Groups and Control in Rats

Sex	Organ Name	Tumor Name	P_Value				Dose Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
			Control N=60	Low N=60	Med N=60	High N=60				
fff										
Male	Skin/Subcutis	B-Basal Cell Tumor	0	1	0	3	0.0448	0.5119	.	0.1568
	Testis	B-Interstitial Cell Adenoma	0	0	4	7	0.0016*	.	0.0733	0.0115*

In addition, an increase in the incidence of interstitial (Leydig) cell hyperplasia in the males was noted (5%, 8.3%, 20%, and 13.3% in the 0, 40, 120, and 400 mg/kg/day males, respectively). The Sponsor provided evidence that the drug-related incidence of Leydig cell adenoma was mediated by an increase in plasma LH levels.

Executive CAC Recommendations and Conclusions:

Mouse:

1. The Committee concluded that the study was adequate, noting prior Exec CAC review of the protocol.
2. The Committee concluded that there were no treatment-related neoplasms.

Rat:

1. The Committee concluded that the study was adequate, noting prior Exec CAC review of the protocol.
2. The Committee concluded that there was a treatment-related increase in the incidence of benign interstitial cell adenoma in the testis at 400 mg/kg/day.

David Jacobson-Kram, Ph.D.

Chair, Executive CAC

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/NDA 204,760/Division File, DGIEP

/David Joseph/Team leader, DGIEP

/Yuk-Chow Ng/Reviewer, DGIEP

/Maureen Dewey/PM, DGIEP

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9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with NKTR-118 in Rats

Study no.: 7985-106
Study report location: N/A
Conducting laboratory and location: [REDACTED] (b) (4)
Date of study initiation: 11-09-2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: NKTR-118, Lot 149007 (98%), Lot 149008 (98%)

Key Study Findings

- Male rats (22/group) were administered 0 (vehicle), 250, 500, or 1000 mg/kg/day NKTR-118 by oral gavage for at least 28 days prior to mating, during mating, and until necropsy on dosing day 73. Female rats (22/group) were administered 0 (vehicle), 250, 500, or 1000 mg/kg/day NKTR-118 by oral gavage for at least 14 days prior to mating, during mating, and through gestation day 7. The females were necropsied on GD (gestation day) 13.
- In male rats, significant decreases in bodyweight were observed in the 1000 mg/kg/day males during dosing days 35 through 73, with a decrease in bodyweight gain between dosing days 0 and 73, compared to controls. In female rats, there was a transient increase in bodyweight gain in the drug-treated groups between GD 7 and 10, compared to controls, after drug treatment was stopped.
- In male rats, overall food consumptions were comparable between groups during the pre-mating period (28 days); however, transient but significant decreases in food consumption were observed in the 500 and 1000 mg/kg/day males between dosing days 0 and 7. In females, occasional transient changes in food consumption were observed during pre-mating (250, 500, and 1000 mg/kg/day groups) and gestation (500 and 1000 mg/kg/day groups). However, there were no significant changes in overall food consumption between GD 0 to 13.
- In males, there were no drug-related changes in sperm motility or sperm counts. In females, there were no drug-related effects on estrous cycle, percentage pregnancy rate (total pregnant/number mated), the number of corpora lutea, implantation sites, live embryos, dead embryos, resorptions, or pre- and post-implantation losses. There were no abortions or early deliveries; all pregnant dams had viable fetuses.
- The NOAEL for paternal toxicity is considered to be 500 mg/kg/day, based on reduction in bodyweight gain in the 1000 mg/kg/day males. The NOAEL for maternal toxicity is considered to be 1000 mg/kg/day. The NOEL for male fertility is considered to be 1000 mg/kg/day. The NOEL for female fertility and early embryonic development is considered to be 1000 mg/kg/day based on the lack of adverse findings at the high dose.

Methods

Doses:	0 (vehicle), 250, 500, or 1000 mg/kg/day
Frequency of dosing:	once daily
Dose volume:	10 ml/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	solution / in reverse osmosis water
Species/Strain:	Rat/Crl:CD(SD)
Number/Sex/Group:	22
Satellite groups:	None
Study design:	Males were dosed for at least 28 days prior to mating, during mating, and until necropsy (dosing day 73). Females were dosed for at least 14 days prior to mating, during mating, and through gestation day 7 (see Sponsor's table below for design).
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

Study Design of the Fertility and Early Embryonic Development Study

Group ^a Male	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
	Female			
1 (Control)	22	22	0	0
2 (Low)	22	22	250	25
3 (Mid)	22	22	500	50
4 (High)	22	22	1000	100

a Group 1 received control article only.

At the start of treatment, the animals were approximately 9 weeks old, and their bodyweights ranged from 271 to 326 g for males and 178 to 243 g for females.

The Sponsor stated that the high dose selection was based on all available data including those obtained from pharmacology, other toxicology, and kinetics studies, as appropriate. The frequency of administration reflected possible clinical use and the duration of administration was in compliance with the appropriate guideline. It was anticipated that the high dose would show drug-specific effects. Other dose levels were selected at intervals which were expected to reveal any dose related trends. Though priority was given to detecting a dose-related trend, it was expected that the low dose would be a "no observed adverse effect level". The oral gavage route of administration was selected because it is the intended route of administration in humans.

Observations and Results

Mortality

Animals were observed twice daily for mortality. A 1000 mg/kg/day male was found dead on day 30 (day 2 of the mating phase). The animal showed yellow haircoat on a single day (day 28). Macroscopic findings of thickened thymus, pale and tan liver, enlarged spleen, and discolored seminal vesicles were noted. These findings were not observed in other high dose animals, and the death is not considered to be drug-related.

Clinical Signs

Animals were observed twice daily for abnormalities and signs of pain and distress. Cageside observations were performed approximately 1 hour post-dose for the first 2 weeks of dose administration. On non-dosing days, cageside observations were performed in conjunction with the morning mortality check. At each body weight measurement, detailed observations were conducted for each animal.

Yellow haircoat was observed in the 1000 mg/kg/day males and occasional excessive salivation was noted in some of the 500 and 1000 mg/kg/day males. Excessive salivation was observed in the 500 and 1000 mg/kg/day females, and yellow haircoat in the 1000 mg/kg/day females during the pre-mating phase was noted. During gestation, excessive salivation (3/22 500 mg/kg/day females and 1/22 1000 mg/kg/day females) and yellow haircoat (9/22 and 16/22 in the 500 and 1000 mg/kg/day females, respectively) were observed. These occasional clinical observations did not appear to affect the general health of the animals, and are not considered as adverse.

Bodyweight

In male rats, bodyweights were recorded at least once prior to treatment, on the first day of treatment prior to dosing, twice weekly during treatment, and at termination. In female rats, body weights were recorded twice weekly during the pre-mating treatment phase and during mating period. Females that were confirmed to have mated were weighed on GD (gestation day) 0, 3, 7, 10 and 13.

Significant decreases in bodyweight were noted in the 1000 mg/kg/day males on dosing day 24 (-6%) and from days 35 through 73 (-6 to -8%), with a 17% decrease in bodyweight gain between dosing days 0 and 73 (control=243.4 g and 1000 mg/kg/day=202.0 g). In female rats, bodyweight and bodyweight gains were comparable among all groups between dosing days 0 and 14 during the pre-mating phase, as well as during the gestation period. However, bodyweight gains in all drug-treated females were significantly higher between GD 7 and 10 (+30.2% to +55.0%), compared to controls. These changes are likely to be drug-related; however, they are not considered as adverse.

Feed Consumption

Food consumption was measured weekly prior to breeding and for confirmed-mated females on GD 0, 3, 7, 10 and 13. Food consumption was not measured during the

mating period. In males, overall food consumption was comparable between groups during the pre-mating period (28 days); however, transient but significant decreases in food consumption were observed in the 500 mg/kg/day group (-7.3%) and 1000 mg/kg/day group (-14%) between dosing days 0 and 7. In females, food consumption decreased by 8.9%, 6.4%, and 10.2% in the 250, 500, and 1000 mg/kg/day groups, respectively, between dosing days 0 and 7, and by 6.0% both in the 250 and 1000 mg/kg/day groups between dosing days 0 and 14, compared to controls. Significant increases in food consumption were noted during GD 7 to 10 in the 500 mg/kg/day group (+14.4%) and GD 10 to 13 in the 500 and 1000 mg/kg/day group (+15.7% and 12.5%, respectively), compared to controls. There were no significant changes in overall food consumption between GD 0 to 13.

Estrous Cycles

Estrous cycles were determined during the 14-day pre-mating phase. NKTR-118 had no effect on estrous cycles and the majority of the mating confirmation occurred within the first 4 days of mating. Two low-dose females, one mid-dose female, and two high-dose females showed prolonged diestrus (4 or more consecutive days in diestrus) during the pre-mating phase; however all of these females were found to be pregnant at necropsy. The changes in estrous cycles were not considered to be drug-related as three control females also had prolonged diestrus.

Toxicokinetics

Not performed.

Dosing Solution Analysis

The concentration determination of NKTR-118 in the dosing formulation samples was performed in accordance with the validated method. Analyses conducted during the treatment period showed that the concentrations of dose formulations at 25 and 50 mg/mL from Mix 1 and Week 1 (day 7 and backups) were not within $\pm 10\%$ of the nominal concentration (112 to 117% of the mean target). All other dose formulations at 25 and 50 mg/mL were within $\pm 10\%$ of the nominal concentration. Except for Mix 10 (89.1% of the mean target), all dose formulations at 100 mg/mL were within $\pm 10\%$ of the nominal concentration. The Sponsor stated that the variation of dose formulations at 25, 50 and 100 mg/mL did not affect the interpretation of the study results.

Necropsy

Males were terminated on dosing day 73 and females on GD 13, and necropsies were performed. The following organs were weighed: epididymides, prostate, seminal vesicles, and testes. The following organs and tissues were preserved: coagulating gland, epididymis (right and left), lesions, prostate, seminal vesicles, and testes (left and right).

In the 1000 mg/kg/day males, significant increases were noted in right (+11.6%) and left (+11.5%) testis weight/bodyweight ratios, compared to controls. However, there were no changes in absolute testis weight. There were no significant drug-related macroscopic findings. There were no significant necropsy findings in the females. The necropsy findings are summarized in the Sponsor's table below.

Summary of Parental Necropsy Findings

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 500 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
MALES	N	22	22	22	22
THYMUS - THICKENED	N	0	0	0	1
SPLEEN-ENLARGED	N	0	0	0	1
LIVER - PALE AREA(S)	N	0	0	0	1
KIDNEY(S)-DILATED PELVIS(ES)	N	0	0	0	1
SEMINAL VESICLES - DISCOLORED	N	0	0	0	1

N = Number

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 500 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
FEMALES	N	22	22	22	22
KIDNEY(S)-GRANULAR MATERIAL	N	1	0	0	0
URINARY BLADDER-CALCULUS (I)	N	1	0	0	0
URINARY BLADDER-WALLS THICKENED	N	1	0	0	0
PLACENTA(S)-SHARED	N	0	0	0	1
UTERUS - ADDITIONAL IMPLANTATION SITES	N	0	1	0	0

N = Number

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

Animals from respective groups were mated by placing one female in the breeding cage with a male from the same dose group. Once mating occurred, the males and females were separated. The maximum mating period was 3 weeks. During mating, a daily inspection was made for the presence of a retained copulatory plug or obvious copulatory plugs on the tray liner. Females not found with either a retained or dropped copulatory plug were evaluated for vaginal sperm by lavage. The day on which sperm or plug was observed was designated as GD 0. The reproductive indices are summarized in the table below (taken from the Sponsor's study report).

Reproductive Indices

Group	1 0 mg/kg/day	Group 2 250 mg/kg/day	Group 3 500 mg/kg/day	Group 4 1000 mg/kg/day
No. of male/female pairs	22	22	22	22
No. of females confirmed mated	22	22	22	22
Male/Female Copulation Index (%)	100	100	100	100
No. of females pregnant	21	21	20	21
Male/Female Fertility Index (%)	95	95	91	95

The pregnancy rates (total pregnant/number mated) were 95% for the 0, 250 and 1000 mg/kg/day groups, 91% for the 500 mg/kg/day group, and were considered normal. There were no abortions or early deliveries; all pregnant dams had viable fetuses. There were no drug-related effects on the number of corpora lutea, implantation sites, live embryos, dead embryos, resorptions, or pre- and post-implantation losses. The cesarean section data are summarized in the Sponsor's table below. In males, there were no meaningful drug-related changes in sperm motility or sperm counts.

Summary of Cesarean Section Data

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 500 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Females Mated	N	22	22	22	22
Pregnant	N	21	21	20	21
	%	95	95	91	95
Aborted	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Died	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Delivered Early	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Pregnant at C-section	N	21	21	20	21
Dams with Viable Fetuses	N	21	21	20	21
	%	100	100	100	100
Dams with no Viable Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Corpora Lutea	MEAN	17.5	18.2	18.0	17.4
	S.D.	1.9	2.7	3.2	2.6
	N	21	21	20	21
	TOTAL	368	383	359	365
Implantation Sites	MEAN	16.3	17.1	16.8	16.3
	S.D.	1.3	2.3	3.8	2.9
	N	21	21	20	21
	TOTAL	342	359	335	343
Preimplantation Loss	MEAN%	6.6	5.9	8.0	5.9
	S.D.	6.7	6.1	14.3	11.1

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 500 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Pregnant at C-section	N	21	21	20	21
Resorptions: Total	MEAN	0.4	0.5	0.7	0.4
	S.D.	0.7	0.6	0.7	0.6
	N	21	21	20	21
	TOTAL	8	10	13	8
	MEAN%	2.3	2.9	3.9	2.2
	S.D.	4.0	3.8	4.6	3.5
Early	MEAN	0.4	0.4	0.6	0.2
	S.D.	0.7	0.6	0.8	0.4
	N	21	21	20	21
	TOTAL	8	9	11	5
	MEAN%	2.3	2.7	3.4	1.4
	S.D.	4.0	3.8	4.7	2.6
Late	MEAN	0.0	0.0	0.1	0.1
	S.D.	0.0	0.2	0.3	0.5
	N	21	21	20	21
	TOTAL	0	1	2	3
	MEAN%	0.0	0.2	0.5	0.8
	S.D.	0.0	0.9	1.6	2.8
Dead Fetuses	TOTAL	0	0	0	0
Postimplantation Loss	MEAN%	2.3	2.9	3.9	2.2
	S.D.	4.0	3.8	4.6	3.5

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 500 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Pregnant at C-section	N	21	21	20	21
Live Fetuses	MEAN	15.9	16.6	16.1	16.0
	S.D.	1.3	2.4	3.9	2.8
	N	21	21	20	21
	TOTAL	334	349	322	335
	MEAN%	97.7	97.1	96.1	97.8
S.D.	4.0	3.8	4.6	3.5	

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

In summary, the NOAEL for paternal toxicity is considered to be 500 mg/kg/day, based on reduction in bodyweight gain in the 1000 mg/kg/day males. The NOAEL for maternal toxicity is considered to be 1000 mg/kg/day. The NOEL for male fertility, female fertility, and early embryonic development is considered to be 1000 mg/kg/day, based on lack of adverse findings at the high dose.

9.2 Embryonic Fetal Development

Study title: Oral Gavage Study for Effects on Embryo-Fetal Development and Toxicokinetics with NKTR-118 in Rats

Study no.: 7985-108
 Study report location: N/A
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 12-14-2007
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: NKTR-118, lot 149008, 98.3%

Key Study Findings

- Pregnant female rats, 25/group in the main study groups and 6/group in the toxicokinetic groups, were administered 0 (vehicle), 250, 750, or 1000 mg/kg/day NKTR-118 by oral gavage on GD (gestation days) 6-17. C-sections were performed on GD 20.
- Drug-related clinical signs included excessive salivation, urine stain, and brown hair coat. However, they are not considered as adverse since they did not appear to have affected the general health of animals.
- A significant decrease in bodyweight was noted in the 1000 mg/kg/day females during GD 8-18, and significant decreases in bodyweight gain were noted in the 750 and 1000 mg/kg/day females, compared to controls. Significant increases in bodyweight gain were noted in the treatment groups between GD 18 and 20. There were no significant changes in overall bodyweight or bodyweight gain at study termination on GD 20, compared to controls.

- Significant decreases in food consumption was observed between GD 6 and 8 in the 250, 750 and 1000 mg/kg/day groups, between GD 8 and 10 in the 750 and 1000 mg/kg/day groups, and during the entire drug treatment period (GD 6-18) in the 750 and 1000 mg/kg/day groups, compared to controls. The decrease in food consumption during the initial dosing period (GD 6-8) correlated with reduced bodyweight and bodyweight gain.
- No drug-related macroscopic findings were noted when the dams were necropsied. Mean gravid weights, corrected terminal body weights, cesarean section data, mean fetal weights, and number of live fetuses was comparable across groups. There were no drug-related fetal external findings.
- Anorchism, a fetal soft tissue malformation, was noted in a single 1000 mg/kg/day fetus (1/156 or 0.6% compared to 0/150 in controls). This malformation was not seen in historical controls, and the finding is considered as possibly drug-related.
- No fetal skeletal malformations were noted. However, there were significant increases in fetal (5.9%) and litter incidence (28%) of bipartite vertebral centrum in the 1000 mg/kg/day group, compared to the control values (0.6% and 4.0%, respectively). This fetal skeletal variation is considered to be drug-related.
- The no observed adverse effect level (NOAEL) for maternal toxicity is considered to be 750 mg/kg/day, based on the decrease in bodyweight gain (~16%) in the 1000 mg/kg/day group during the treatment period. For embryo-fetal viability and fetal growth, the NOEL is considered to be 1000 mg/kg/day, based on the absence of effects on the c-section parameters at this dose. For embryo-fetal toxicity or teratogenicity, the NOAEL is considered to be 750 mg/kg/day, based on the incidence of anorchism (a soft tissue malformation that was possibly drug-related) in the 1000 mg/kg/day group.

Methods

Doses:	0 (vehicle), 250, 750, or 1000 mg/kg/day
Frequency of dosing:	once daily
Dose volume:	10 ml/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	solution/reverse osmosis water
Species/Strain:	Rat/Crl:CD(SD)
Number/Sex/Group:	25
Satellite groups:	0 (vehicle), 250, 750, or 1000 mg/kg/day for toxicokinetics, 6/group
Study design:	See Sponsor's table below
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

Study Design

Group ^a Females	No. of	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dosing Schedule Days of Gestation
Main-study Animals				
1 (Control)	25	0	0	6-17
2 (Low)	25	250	25	6-17
3 (Mid)	25	750	75	6-17
4 (High)	25	1000	100	6-17
Toxicokinetic Animals				
5 (Control)	6	0	0	6-17
6 (Low)	6	250	25	6-17
7 (Mid)	6	750	75	6-17
8 (High)	6	1000	100	6-17

a Groups 1 and 5 received control article only.

At the start of treatment, the animals were approximately 12 weeks old, and their bodyweights ranged from 220 to 276 g (main-study animals) and from 219 to 262 g (TK animals).

The Sponsor indicated that the high dose selection was based on all available data including those obtained from pharmacology, other toxicology, and kinetics studies, as appropriate. The frequency of administration reflected possible clinical use and the duration of administration was in compliance with the appropriate guideline. It was anticipated that the high dose would show drug-specific effects. Other dose levels were selected at intervals which were expected to reveal any dose related trends. Though priority was given to detecting a dose-related trend, it was expected that the low dose would be a "no observed adverse effect level". The oral gavage route of administration was selected because it is the intended route of administration in humans.

Observations and Results

Mortality

All animals were checked twice daily for mortality. There were no deaths.

Clinical Signs

Animals were checked twice daily for abnormalities, and signs of pain and distress. Cageside observations were performed at approximately 1 hour post-dose (based on the last animal dosed per group) for the main study animals. On non-dosing days, cageside observations were performed for the main study animals in conjunction with the mortality check. At each body weight measurement, detailed observations were

made for each main study animal. The supplier provided documentation as to whether mated females appeared normal on GD 0.

Excessive salivation was noted in the 1000 mg/kg/day group (2/25), urine stain was noted in the 750 and 1000 mg/kg/day groups (5/25 and 8/25, respectively), and brown hair coat was noted in the 1000 mg/kg/day group (2/25). These findings are considered drug-related but not adverse since they did not appear to have affected the general health of animals. There were other clinical findings, including hyperactivity, malocclusion, gasping, and alopecia. However, these signs were either sporadic or were also observed in the control animals, and were not considered as drug-related.

Body Weight

Animals were weighed on days 0, 4, 6, 8, 10, 12, 14, 16, 18, and 20 of gestation. The animal supplier provided the day 0 body weights.

The initial body weight (GD 0) of the control group was 202.8 g and the terminal body weight was 370.8 g (GD 20). Maternal bodyweight decreased significantly in the 750 mg/kg/day group (-4.1%) on GD 8, and in the 1000 mg/kg/day group on GD 8, 10, and 18 (-5.3, -3.8, and -4.8%, respectively), compared to controls. During GD 6 to 8, a significant decrease in bodyweight gain occurred in the 250 mg/kg/day group (-49.1%), and a slight loss of bodyweight was observed in the 750 and 1000 mg/kg/day groups. Bodyweight gain over the entire treatment period (GD 6 to 18) in the 750 and 1000 mg/kg/day groups was reduced by 9.4% and 15.5%, respectively, compared to controls. Significant increases in bodyweight gain were noted in the drug-treated groups (29.2% to 42.5%) between GD 18 and 20, after drug treatment stopped on GD 18, compared to controls. There were no drug-related changes in bodyweight or total bodyweight gain at the end of gestation period.

Maternal Bodyweight During Gestation

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
DAY 0	MEAN	202.8	202.8	203.4	204.3
	S.D.	12.1	10.5	13.2	9.9
	N	25	24	25	25
DAY 4	MEAN	223.4	222.9	224.8	223.2
	S.D.	14.7	12.0	13.2	12.5
	N	25	24	25	25
DAY 6	MEAN	239.4	241.2	242.2	241.8
	S.D.	14.0	10.1	13.5	11.9
	N	25	24	25	25
DAY 8	MEAN	250.8	247.0	240.5*	237.4**
	S.D.	15.3	11.8	12.6	13.0
	N	25	24	25	25
DAY 10	MEAN	261.1	261.8	254.1	251.3*
	S.D.	15.2	12.4	13.0	13.5
	N	25	24	25	25
DAY 12	MEAN	275.6	277.2	272.0	268.5
	S.D.	17.5	14.2	15.9	16.6
	N	25	24	25	25
DAY 14	MEAN	293.5	297.2	290.8	285.8
	S.D.	16.4	16.0	16.9	12.9
	N	25	24	25	25
DAY 16	MEAN	307.8	312.5	308.1	300.4
	S.D.	18.5	16.2	19.6	15.8
	N	25	24	25	25
DAY 18	MEAN	338.4	340.0	331.8	325.4*
	S.D.	20.0	18.5	18.8	17.3
	N	25	24	25	25
DAY 20	MEAN	370.8	382.1	378.1	371.2
	S.D.	23.8	24.0	23.2	22.3
	N	25	24	25	25

ICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

Maternal Bodyweight Gain During Gestation

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
DAYS 0 TO 4	MEAN	20.6	20.1	21.4	18.9
	S.D.	6.1	8.0	6.1	6.2
	N	25	24	25	25
DAYS 4 TO 6	MEAN	16.0	18.2	17.4	18.5
	S.D.	6.1	7.3	5.5	5.2
	N	25	24	25	25
DAYS 6 TO 8	MEAN	11.4	5.8**	-1.8**	-4.4**
	S.D.	4.7	3.9	4.8	5.3
	N	25	24	25	25
DAYS 8 TO 10	MEAN	10.3	14.8**	13.6	14.0*
	S.D.	4.6	4.6	4.7	5.8
	N	25	24	25	25
DAYS 10 TO 12	MEAN	14.6	15.4	17.9	17.2
	S.D.	6.1	6.2	5.2	7.3
	N	25	24	25	25
DAYS 12 TO 14	MEAN	17.8	20.0	18.8	17.3
	S.D.	7.6	8.6	8.5	8.2
	N	25	24	25	25
DAYS 14 TO 16	MEAN	14.3	15.3	17.3	14.6
	S.D.	5.9	4.1	5.2	5.8
	N	25	24	25	25
DAYS 16 TO 18	MEAN	30.6	27.6	23.7**	25.0**
	S.D.	4.7	5.2	9.2	6.4
	N	25	24	25	25
DAYS 18 TO 20	MEAN	32.5	42.0**	46.3**	45.8**
	S.D.	6.8	6.8	8.1	8.3
	N	25	24	25	25

ISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

Feed Consumption

Food consumption was measured at bodyweight measurement intervals for the main study animals beginning on GD 4.

Significant decreases in food consumption were observed between GD 6 and 8 in the 250, 750 and 1000 mg/kg/day groups (-11%, -33%, and -35 %, respectively) and between GD 8 and 10 in the 750 and 1000 mg/kg/day groups (-15% and -18%, respectively), and during the entire treatment period (GD 6-18) in the 750 and 1000 mg/kg/day groups (-8% and -12%, respectively), compared to controls. The decrease in food consumption during GD 6-8 correlated with reduced bodyweight and bodyweight gain.

Toxicokinetics

Blood samples were collected on GD 6 (dosing day 1) and 17 (dosing day 12). The first 3 TK rats per group were bled pre-dose (within 1 hour of dosing), and at 3 and 12 hours post-dose; the second 3 TK rats per group were bled at 0.25, 6, and 24 hours post-dose.

The plasma toxicokinetic parameters of NKTR-118 in female rats during GD 6 and 17 are shown in the Sponsor's table below.

Plasma NKTR-118 Toxicokinetic Parameters During Gestation

Dosing Day	Treatment Group	Dose (mg/kg/day)	C _{max} (µg/mL)	C(24) (µg/mL)	AUC(0-24hr) (hr×µg/mL)	T _{max} (hr)
1	5	0	0.069 [‡]	0.00	0.374	0.250
	6	250	21.2	0.297	180	0.250
	7	750	37.8	10.2	600	3.00
	8	1000	45.8	12.6	728	3.00
12	5	0	0.00	0.00	NR	NR
	6	250	17.6	0.285	206	3.00
	7	750	30.4	1.27	479	6.00
	8	1000	43.3	1.49	599	3.00

[‡] Mean concentrations of plasma NKTR-118, approximately double the lower limit of quantitation (0.30 µg/mL), were found in the control group. NR: not reported with no corresponding expected NKTR-118-Glucuronide concentrations were found (Table 4).

Dosing Day 1=Gestation Day 6, Dosing Day 12=Gestation Day 17. NR: Not reported.

Maximum plasma levels of NKTR-118 were achieved between 0.25 to 6 hours after oral administration on GD 6 and 17 (dosing day 1 and 12, respectively). Plasma exposure (AUC) of NKTR-118 was dose-proportional on GD 6, but less than dose-proportional on GD 17. There was no plasma accumulation of NKTR-118 after repeated dosing. The C_{max} and AUC_{0-24hr} values in the 1000 mg/kg/day animals on GD 17 were 43.3 µg/mL and 599 µg·h/mL, respectively.

The plasma toxicokinetic parameters of NKTR-118-glucuronide, a major metabolite, in female rats during GD 6 and 17 are summarized in the Sponsor's table below.

Plasma NKTR-118-glucuronide Toxicokinetic Parameters during Gestation

Dosing Day	Treatment Group	Dose (mg/kg/day)	C _{max} (µg/mL)	C(24hr) (µg/mL)	AUC(0-24hr) (hr×µg/mL)	T _{max} (hr)
1	6	250	13.8	0.087	64.0	0.250
	7	750	24.0	4.24	234	0.250
	8	1000	25.7	7.04	277	0.250
12	6	250	13.8	0.136	96.2	0.250
	7	750	40.3	0.776	460	3.00
	8	1000	68.5	1.40	770	3.00

Dosing Day1=Gestation Day 6, Dosing Day 12=Gestation Day 17

NKTR-118 was metabolized rapidly to NKTR-118-glucuronide; peak plasma NKTR-118 glucuronide concentrations appeared within 0.25 hr following oral NKTR-118 administration on GD 6. On GD 17, peak plasma NKTR-118-glucuronide concentrations were noted at 3 hr post-dose in the 750 and 1000 mg/kg/day groups. The increase in AUC for NKTR-118-glucuronide was dose-proportional on GD 6 and greater than dose-proportional on GD 17. There was moderate accumulation of NKTR-118-glucuronide (1.5-2.8 fold) in plasma after repeated dosing at all doses. The C_{max} and AUC_{0-24hr} in the 1000 mg/kg/day animals on GD 17 were 68.5 µg/mL and 770 µg·h/mL, respectively.

Dosing Solution Analysis

Stock dosing solutions were tested for stability and drug concentration. The Sponsor reported that mean concentrations of the 25, 75, and 100 mg/ml dosing solutions on GD 6 and 17 were within 10% of the nominal concentrations. Dose formulations are solutions at the concentrations used and, therefore, no homogeneity analysis was performed.

Necropsy

Dams were euthanized on GD 20. The external features of the carcass and the contents of the thoracic, abdominal, and pelvic cavities were examined. Uterus from each gravid animal was excised, and uterine and net body weights were recorded.

There were no drug-related macroscopic findings.

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

Dams were euthanized on GD 20 and a cesarean section was performed. The uterus from each gravid animal was excised, weighed, and examined for the number and placement of live and dead fetuses, the number of early or late resorptions, and any

abnormalities. The right and left ovaries from each gravid female was examined for the number of corpora lutea. The placentas were also examined.

Mean gravid weights, corrected terminal bodyweights, cesarean section data, mean fetal weights and number of live fetuses were comparable across groups. The results are summarized in the Sponsor's tables below. There were no drug-related fetal external findings.

Summary of Uterine and Net Bodyweights (g)

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
GRAVID UTERUS	MEAN	75.67	79.45	79.41	75.40
	S.D.	11.73	8.95	10.62	9.70
	N	25	24	25	25
CORRECTED WEIGHT	MEAN	295.17	302.64	298.71	295.76
	S.D.	19.60	18.43	18.89	18.45
	N	25	24	25	25
NET CHANGE FROM DAY 0	MEAN	92.41	99.84	95.27	91.44
	S.D.	13.17	18.03	13.17	16.02
	N	25	24	25	25

Summary of Cesarean Section Data

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Females Mated	N	25	25	25	25
Pregnant	N	25	24	25	25
	%	100	96	100	100
Aborted	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Died	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Delivered Early	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Pregnant at C-section	N	25	24	25	25
Dams with Viable Fetuses	N	25	24	25	25
	%	100	100	100	100
Dams with no Viable Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Corpora Lutea	MEAN	14.0	14.2	15.0	13.9
	S.D.	3.0	2.0	2.5	2.2
	N	25	24	25	25
	TOTAL	350	341	374	347
Implantation Sites	MEAN	12.4	12.9	13.4	12.5
	S.D.	2.1	1.5	1.8	1.6
	N	25	24	25	25
	TOTAL	309	309	335	313
Preimplantation Loss	MEAN%	10.6	8.8	9.7	8.9
	S.D.	9.7	7.8	8.5	9.6

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01.

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Pregnant at C-section	N	25	24	25	25
Resorptions: Total	MEAN	0.1	0.1	0.4	0.2
	S.D.	0.3	0.4	1.2	0.5
	N	25	24	25	25
	TOTAL	3	3	11	5
	MEAN%	0.9	1.0	3.3	1.5
	S.D.	2.6	3.5	9.4	3.7
Early	MEAN	0.1	0.1	0.4	0.2
	S.D.	0.3	0.3	1.2	0.5
	N	25	24	25	25
	TOTAL	3	2	11	5
	MEAN%	0.9	0.7	3.3	1.5
	S.D.	2.6	2.5	9.4	3.7
Late	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.0	0.2	0.0	0.0
	N	25	24	25	25
	TOTAL	0	1	0	0
	MEAN%	0.0	0.3	0.0	0.0
	S.D.	0.0	1.5	0.0	0.0
Dead Fetuses	TOTAL	0	0	0	0
Postimplantation Loss	MEAN%	0.9	1.0	3.3	1.5
	S.D.	2.6	3.5	9.4	3.7

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Pregnant at C-section	N	25	24	25	25
Live Fetuses	MEAN	12.2	12.8	13.0	12.3
	S.D.	2.1	1.5	2.2	1.6
	N	25	24	25	25
	TOTAL	306	306	324	308
	MEAN%	99.1	99.0	96.7	98.5
	S.D.	2.6	3.5	9.4	3.7
Females	MEAN	6.0	6.3	6.6	6.1
	S.D.	1.8	2.0	2.1	2.3
	N	25	24	25	25
	TOTAL	149	152	164	153
	MEAN%	48.7	49.9	50.4	49.5
	S.D.	12.1	15.5	15.9	17.3
Males	MEAN	6.3	6.4	6.4	6.2
	S.D.	1.9	2.2	2.2	2.1
	N	25	24	25	25
	TOTAL	157	154	160	155
	MEAN%	51.3	50.1	49.6	50.5
	S.D.	12.1	15.5	15.9	17.3
Sex Ratio M:F		51:49	50:50	49:51	50:50

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

Summary of Mean Fetal Weights (g)

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
FETAL WEIGHTS	UNITS: GRAMS				
of all Viable Fetuses	MEAN	3.84	3.84	3.86	3.81
	S.D.	0.23	0.22	0.23	0.25
	N	25	24	25	25
Covariate Adjusted	MEAN	3.83	3.85	3.87	3.80
of Male Fetuses	MEAN	3.92	3.92	3.89	3.92
	S.D.	0.28	0.22	0.28	0.26
	N	25	24	25	25
Covariate Adjusted	MEAN	3.91	3.92	3.90	3.91
of Female Fetuses	MEAN	3.78	3.76	3.80	3.71
	S.D.	0.21	0.25	0.32	0.26
	N	25	24	25	25
Covariate Adjusted	MEAN	3.77	3.76	3.81	3.70

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.

Summary of Fetal External Observations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	25	24	25	25
Fetuses Evaluated	N	306	306	324	308
Live	N	306	306	324	308
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL OBSERVATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
N = Number

Offspring (Malformations, Variations, etc.)

Each fetus (live or dead) was weighed and examined for external abnormalities. Live fetuses were sacrificed and a mid-coronal slice was made in the head of each fetus to evaluate the contents of the cranium. The internal organs of the thoracic and abdominal cavities of all fetuses were examined and the sex of each fetus (live or dead) was determined. Viscera were then removed and carcasses were processed for skeletal examination. Findings were designated as either variations or malformations. Malformations are gross structural changes that are either incompatible with life or may affect the quality of life. Variations are structural deviations which are thought to have no effect on body conformity or the well-being of the animal.

No fetal external findings were noted. Fetal soft tissue variations were the type and frequency commonly seen in this strain of rat, and the incidence of total fetal and litter soft tissue variations were comparable across treatment groups. Anorchism, a fetal soft tissue malformation, was noted in a single 1000 mg/kg/day fetus (1/156, 0.6%), compared to controls (0/150). Because this finding was not observed in the control, low-dose, or mid-dose groups, nor was it observed in the historical controls, the Sponsor concluded that anorchism was possibly drug-related, even though the frequency of occurrence was low.

There were significant increases in skeletal variation parameters. In the 750 and 1000 mg/kg/day groups, there were dose-dependent significant increases in fetal (8.2% and 17%, respectively) and litter (32% and 56%, respectively) incidence of incomplete ossification of skull, compared to controls (fetal 1.9% and litter 8.0%). Other statistically significant changes in variations included: increases in litter incidence of unossified hyoid body in the 1000 mg/kg/day group (68% litter incidence), compared to controls (40%); increases in fetal and litter incidence of bipartite vertebral centrum (5.9% and 28%, respectively) in the 1000 mg/kg/day group, compared to controls (0.6% and 4.0%, respectively); increases in fetal and litter incidence of 5th/6th sternebra incomplete ossification (51% and 96%, respectively) in the 1000 mg/kg/day group, compared to controls (38% and 72%, respectively). However, the Sponsor noted that, except for the increased fetal and litter incidence of bipartite vertebral centrum in the 1000 mg/kg/day group, the incidence of the remaining fetal skeletal variations were within the range of the historical control data. No fetal skeletal malformations were noted. Data for the fetal soft tissue variations and malformations, and fetal skeletal variations and malformations are summarized in the Sponsor's tables below.

Summary of Fetal Soft Tissue Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	25	24	25	25
Fetuses Evaluated	N	150	152	165	156
Live	N	150	152	165	156
Dead	N	0	0	0	0
DILATATION OF LATERAL VENTRICLE(S)					
Fetal Incidence	N	0	1	1	0
	%	0.0	0.7	0.6	0.0
Litter Incidence	N	0	1	1	0
	%	0.0	4.2	4.0	0.0
ABSENT INNOMINATE					
Fetal Incidence -	N	2	0	0	0
	%	1.3	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.0	0.0	0.0	0.0
ADRENAL(S)-DARK					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.7	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	4.2	0.0	0.0
DILATED URETER(S)					
Fetal Incidence -	N	4	1	0	0
	%	2.7	0.7	0.0	0.0
Litter Incidence -	N	2	1	0	0
	%	8.0	4.2	0.0	0.0
TOTAL FETAL SOFT TISSUE VARIATIONS					
Fetal Incidence -	N	4	3	1	0
	%	2.7	2.0	0.6	0.0
Litter Incidence	N	2	3	1	0
	%	8.0	13	4.0	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
- = SIGNIFICANT NEGATIVE TREND.

Summary of Fetal Soft Tissue Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	25	24	25	25
Fetuses Evaluated	N	150	152	165	156
Live	N	150	152	165	156
Dead	N	0	0	0	0
ANORCHISM					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.0
TOTAL FETAL SOFT TISSUE MALFORMATIONS					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.6
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

Summary of Fetal Skeletal Variations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	25	24	25	25
Fetuses Evaluated	N	156	154	159	152
Live	N	156	154	159	152
Dead	N	0	0	0	0
INCOMPLETE OSSIFICATION OF SKULL					
Fetal Incidence +	N	3	9	13	26
	%	1.9	5.8	8.2*	17**
Litter Incidence +	N	2	7	8	14
	%	8.0	29	32*	56**
UNOSSIFIED HYOID BODY					
Fetal Incidence	N	20	25	20	29
	%	13	16	13	19
Litter Incidence +	N	10	11	11	17
	%	40	46	44	68*
SACRAL/CAUDAL VERTEBRAL CENTRUM(A) MINOR FUSION TO ARCH(ES)					
Fetal Incidence -	N	19	22	11	10
	%	12	14	6.9	6.6
Litter Incidence -	N	10	10	7	5
	%	40	42	28	20
INCOMPLETE OSSIFICATION OF VERTEBRAL ARCH(ES)					
Fetal Incidence	N	36	31	37	32
	%	23	20	23	21
Litter Incidence	N	15	13	17	19
	%	60	54	68	76
LESS THAN FOUR CAUDAL VERTEBRAE OSSIFIED					
Fetal Incidence	N	34	22	22*	29
	%	22	14	14	19
Litter Incidence	N	12	11	11	13
	%	48	46	44	52

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.
 N = Number
 - = SIGNIFICANT NEGATIVE TREND
 + = SIGNIFICANT POSITIVE TREND

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	25	24	25	25
Fetuses Evaluated	N	156	154	159	152
Live	N	156	154	159	152
Dead	N	0	0	0	0
BIPARTITE VERTEBRAL CENTRUM(A)					
Fetal Incidence +	N	1	4	3	9
	%	0.6	2.6	1.9	5.9**
Litter Incidence +	N	1	3	3	7
	%	4.0	13	12	28*
25 PRESACRAL VERTEBRAE					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.6	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	4.0	0.0
5TH STERNEBRA UNOSSIFIED					
Fetal Incidence +	N	31	35	43	41
	%	20	23	27	27
Litter Incidence	N	13	19	20	16
	%	52	79*	80*	64
5TH/6TH STERNEBRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence +	N	60	67	65	77
	%	38	44	41	51*
Litter Incidence +	N	18	22	23	24
	%	72	92	92	96*
OTHER STERNEBRA(E) BIPARTITE					
Fetal Incidence	N	1	0	0	0
	%	0.6	0.0	0.0	0.0
Litter Incidence	N	1	0	0	0
	%	4.0	0.0	0.0	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01
 N = Number
 + = SIGNIFICANT POSITIVE TREND

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	25	24	25	25
Fetuses Evaluated	N	156	154	159	152
Live	N	156	154	159	152
Dead	N	0	0	0	0
6TH STERNEBRA UNOSSIFIED					
Fetal Incidence	N %	1 0.6	2 1.3	1 0.6	0 0.0
Litter Incidence	N %	1 4.0	2 8.3	1 4.0	0 0.0
OTHER STERNEBRA (E) INCOMPLETE OSSIFICATION					
Fetal Incidence	N %	2 1.3	2 1.3	4 2.5	1 0.7
Litter Incidence	N %	2 8.0	2 8.3	3 12	1 4.0
OTHER STERNEBRA (E) UNOSSIFIED					
Fetal Incidence	N %	0 0.0	0 0.0	1 0.6	0 0.0
Litter Incidence	N %	0 0.0	0 0.0	1 4.0	0 0.0
5TH/6TH STERNEBRA (E) BIPARTITE					
Fetal Incidence	N %	0 0.0	1 0.6	0 0.0	1 0.7
Litter Incidence	N %	0 0.0	1 4.2	0 0.0	1 4.0
14TH RUDIMENTARY RIB(S)					
Fetal Incidence	N %	5 3.2	3 1.9	6 3.8	3 2.0
Litter Incidence	N %	2 8.0	3 13	4 16	3 12

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	25	24	25	25
Fetuses Evaluated	N	156	154	159	152
Live	N	156	154	159	152
Dead	N	0	0	0	0
WAVY/BENT RIB(S)					
Fetal Incidence	N %	2 1.3	6 3.9	2 1.3	5 3.3
Litter Incidence	N %	2 8.0	5 21	2 8.0	5 20
7TH CERVICAL RIB(S)					
Fetal Incidence	N %	1 0.6	0 0.0	1 0.6	0 0.0
Litter Incidence	N %	1 4.0	0 0.0	1 4.0	0 0.0
13TH RUDIMENTARY RIB(S)					
Fetal Incidence +	N %	0 0.0	0 0.0	1 0.6	2 1.3
Litter Incidence	N %	0 0.0	0 0.0	1 4.0	1 4.0
LESS THAN FOUR METATARSALS OSSIFIED					
Fetal Incidence	N %	1 0.6	0 0.0	0 0.0	0 0.0
Litter Incidence	N %	1 4.0	0 0.0	0 0.0	0 0.0
INCOMPLETE OSSIFICATION OF ISCHIUM(A)					
Fetal Incidence +	N %	0 0.0	2 1.3	4 2.5	3 2.0
Litter Incidence	N %	0 0.0	2 8.3	2 8.0	3 12

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01
 N = Number
 + = SIGNIFICANT POSITIVE TREND

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	25	24	25	25
Fetuses Evaluated	N	156	154	159	152
Live	N	156	154	159	152
Dead	N	0	0	0	0
UNOSSIFIED PUBIS(ES)					
Fetal Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	0.7
Litter Incidence	N	0	0	0	1
	%	0.0	0.0	0.0	4.0
TOTAL FETAL SKELETAL VARIATIONS					
Fetal Incidence	N	113	116	108	114
	%	72	75	68	75
Litter Incidence	N	24	24	23	25
	%	96	100	92	100

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

Summary of Fetal Skeletal Malformations

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 250 MG/KG/DAY	GROUP 3 750 MG/KG/DAY	GROUP 4 1000 MG/KG/DAY
Litters Evaluated	N	25	24	25	25
Fetuses Evaluated	N	156	154	159	152
Live	N	156	154	159	152
Dead	N	0	0	0	0
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

In summary, the NOAEL for maternal toxicity is considered to be 750 mg/kg/day, based on the decrease in bodyweight gain (~16%) in the 1000 mg/kg/day group during the treatment period. For embryo-fetal viability and fetal growth, the NOEL is considered to be 1000 mg/kg/day, based on the absence of effects on the cesarean parameters at this dose. For embryo-fetal toxicity or teratogenicity, the NOAEL is considered to be 750 mg/kg/day, based on the incidence of anorchism (a soft tissue malformation that was possibly drug-related) in the 1000 mg/kg/day group. The dose level of 750 mg/kg/day corresponds to a GD 17 AUC_{0-24hr} of 479 µg•hr/mL and C_{max} of 30.4 µg/mL for the unchanged drug.

Study title: Oral Gavage Study for Effects on Embryo-fetal Development and Toxicokinetics with NKTR-118 in Rabbits

Study no.: 8204391
Study report location: N/A
Conducting laboratory and location: (b) (4)
Date of study initiation: 6-26-2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: NKTR-118, lot 2002279 (99.4%), 200237 (98.1%), 200388 (98.4%)

Key Study Findings

- Pregnant female rabbits were administered 0 (vehicle), 30, 150, or 450 mg/kg/day NKTR-118 by oral gavage during GD (gestation days) 7 to 20 in the main study (20/group) and toxicokinetic groups (3/group).
- There was an abortion in a 450 mg/kg/day dam on GD 28. Food consumption for this dam was low throughout the dosing phase of the study (<100 g/day). This abortion appears to be related to low food consumption rather than a direct effect of NKTR-118 treatment.
- Dose-related fecal changes (few and/or non-formed) were noted in the 150 and 450 mg/kg/day groups, and the findings appear to be related to low food consumption.
- Transient dose-related decreases in bodyweight gain were noted in the 30, 150, and 450 mg/kg/day groups for the GD 7 to 9 interval, compared to controls. The decrease in bodyweight gain correlated with decreases in food consumption in the 150 and 450 mg/kg/day groups for the GD 7 to 9 and GD 9 to 11 intervals. After the drug treatment period, bodyweight gain and food consumption were increased compared to controls.
- There were no drug-related macroscopic findings in the dams and no drug-related effects on cesarean section parameters, including number of corpora lutea, implantation sites, early, middle or late resorptions, sex ratio, fetal weight, or pre- and post- implantation losses.
- There were no-drug related external or visceral variations or malformations in fetuses.
- Fused vertebral arches in 2 fetuses from two 450 mg/kg/day litters were noted. This skeletal malformation had a fetal incidence of 1.6% versus the historical control range of 0-0.7%, and a litter incidence of 12% versus a historical control

range of 0 to 5.9%. However, when the combined incidence of all skeletal malformations was evaluated, there was no dose-dependent incidence of malformations, nor was there a significant increase in malformations in any treatment group. Therefore, the fused vertebral arches in the high-dose group are unlikely to be drug-related.

- The NOAEL for maternal toxicity is considered to be 30 mg/kg/day, based on decreases in bodyweights and food consumption in the 150 and 450 mg/kg/day groups. The NOEL (no observed effect level) for embryo-fetal viability and fetal growth is considered to be 450 mg/kg/day. For developmental toxicity (teratogenicity), the NOEL is considered to be 450 mg/kg/day, based on the lack of dose-dependent findings.

Methods

Doses:	0 (vehicle), 30, 150, 450 mg/kg/day
Frequency of dosing:	once daily
Dose volume:	10 ml/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	solution / reverse osmosis water
Species/Strain:	Rabbit/Hra:(NZW)SPF
Number/Sex/Group:	20
Satellite groups:	Toxicokinetic groups (3/group): 0 (vehicle), 30, 150, 450 mg/kg/day
Study design:	See Sponsor's table below
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

Study Design

Group ^a	No. of Animals	Dose Level		Dose Concentration	Dosing Schedule
	Mated Female	(mg/kg/day)	(μ mol/kg/day)	(mg/mL)	Days of Gestation
Main Study Animals					
1 (Control)	20	0	0	0	7-20
2 (Low)	20	30	46	3	7-20
3 (Mid)	20	150	230	15	7-20
4 (High)	20	450	690	45	7-20
Toxicokinetic Animals					
5 (Control)	3	0	0	0	7-20
6 (Low)	3	30	46	3	7-20
7 (Mid)	3	150	230	15	7-20
8 (High)	3	450	690	45	7-20

a Groups 1 and 5 received vehicle/control article [Reverse-osmosis (RO) Water] only.

At the start of treatment, the animals were 5 months old, and their bodyweights ranged from 2724 to 4438 g.

The Sponsor indicated that the high dose selection was based on all available data including those obtained from pharmacology, other toxicology, and kinetics studies, as appropriate. The frequency of administration reflected possible clinical use and the duration of administration was in compliance with the appropriate guideline. It was anticipated that the high dose would show drug-specific effects. Other dose levels were selected at intervals which were expected to reveal any dose related trends. Though priority was given to detecting a dose-related trend, it was expected that the low dose would be a "no-observed-adverse-effect level". The oral gavage route of administration was selected because it is the intended route of administration in humans.

Observations and Results

Mortality

All animals were checked twice daily for mortality. There were three early terminations or removals from the study. A dam was removed due to mistimed pregnancy, and a 30 mg/kg/day dam was found dead with evidence of gavage trauma based on histopathology evaluation. There was an abortion in a 450 mg/kg/day dam on GD 28. Food consumption for this dam was low throughout the dosing phase of the study (<100 g/day). The Sponsor noted that this abortion appears to be related to low food consumption rather than a direct effect of NKTR-118 treatment.

Clinical Signs

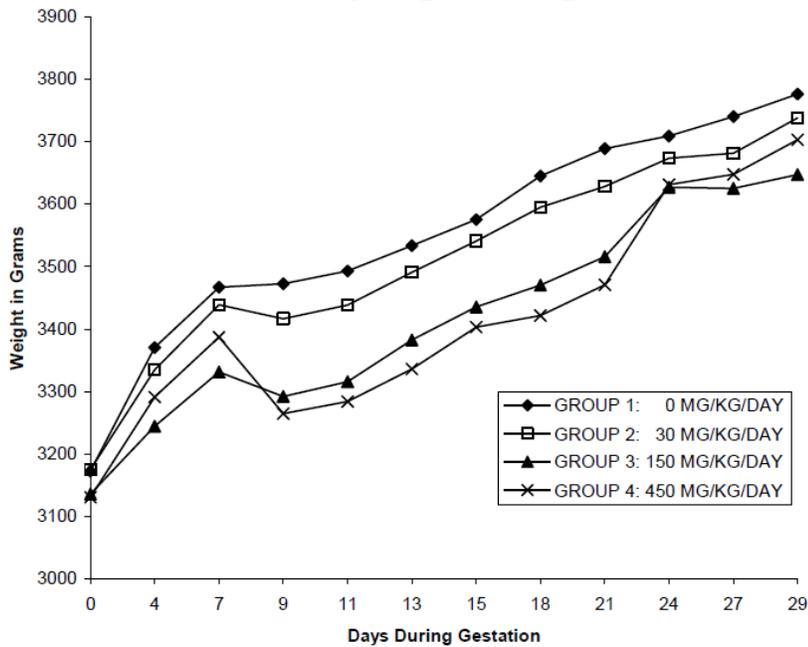
All animals were checked twice daily for abnormalities, and signs of pain and distress. Detailed observations were performed at each body weight measurement interval for main study animals. Daily cageside observations were performed on all main study animals. Post-dose observations were made for each main study animal on GD 7 to 20 at approximately 1 hour post-dose, based on the last animals dosed per group per study room.

Fecal changes (few and/or non-formed) were noted in the 150 and 450 mg/kg/day groups, and the findings appear to be related to low food consumption. The incidence of the fecal findings increased with dose, and is considered to be drug-related.

Bodyweight

Animals were weighed on days 0, 4, 7, 9, 11, 13, 15, 18, and 20 of gestation for main study and toxicokinetic animals, and on days 21, 24, 27, and 29 of gestation for main study animals. The animal supplier provided the GD 0 body weights. The bodyweights and bodyweight gains are shown in the Sponsor's figure and tables below, respectively.

Maternal Bodyweight During Gestation



Maternal Bodyweight Gain During Gestation

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
DAYS 4 TO 7	MEAN	96.5	104.2	87.2	95.9
	S.D.	52.9	39.7	55.1	28.8
	N	19	17	17	18
DAYS 7 TO 9	MEAN	5.6	-22.6	-39.5**	-122.8**
	S.D.	33.0	27.6	29.1	57.8
	N	19	17	17	18
DAYS 9 TO 11	MEAN	20.4	22.2	23.8	19.4
	S.D.	37.7	30.0	36.0	43.9
	N	19	17	17	18
DAYS 11 TO 13	MEAN	40.6	52.3	66.8	51.9
	S.D.	37.0	30.5	34.8	43.3
	N	19	17	17	18
DAYS 13 TO 15	MEAN	42.2	50.3	53.0	67.4*
	S.D.	28.3	29.6	30.6	76.1
	N	19	17	17	18
DAYS 15 TO 18	MEAN	69.6	53.9	35.0	18.5
	S.D.	37.7	41.9	56.4	78.8
	N	19	17	17	18
DAYS 18 TO 21	MEAN	44.1	50.8	45.4	48.9
	S.D.	43.4	36.6	43.8	67.8
	N	19	16	17	18
DAYS 21 TO 24	MEAN	19.9	45.2	111.2**	160.8**
	S.D.	60.6	28.0	37.1	58.6
	N	19	16	17	18

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

Maternal Bodyweight Gain During Gestation (cont.)

DOSE LEVEL		GROUP 1	GROUP 2	GROUP 3	GROUP 4
		0 MG/KG/DAY	30 MG/KG/DAY	150 MG/KG/DAY	450 MG/KG/DAY
DAYS 24 TO 27	MEAN	31.3	8.4	-1.6	16.5
	S.D.	42.9	31.7	90.9	90.4
	N	19	16	17	18
DAYS 27 TO 29	MEAN	35.9	56.3	22.0	25.2
	S.D.	56.7	36.5	39.5	76.0
	N	19	16	17	17
DAYS 7 TO 29	MEAN	309.7	312.6	316.0	317.4
	S.D.	106.9	106.6	106.0	166.8
	N	19	16	17	17
DAYS 4 TO 29	MEAN	406.2	416.7	403.2	412.5
	S.D.	135.6	123.3	131.3	183.2
	N	19	16	17	17
DAYS 7 TO 21	MEAN	222.5	202.7	184.5	83.4
	S.D.	62.2	84.5	96.0	256.6
	N	19	16	17	18
DAYS 21 TO 29	MEAN	87.2	109.9	131.5	205.9**
	S.D.	98.5	80.6	105.9	166.1
	N	19	16	17	17

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

Decreased bodyweight was noted in the 450 mg/kg/day group on GD 21 (-6%, not statistically significant), the end of the treatment period, compared to controls. Dose-related bodyweight loss was noted in the 30, 150, and 450 mg/kg/day groups (-22.6, -39.5, and -122.8 g, respectively) for the GD 7 to 9 interval, whereas the control group showed a slight increase in bodyweight (+5.6 g). A significant increase in bodyweight gain was noted in the 450 mg/kg/day group during GD 13 to 15 (+59.7%). After the drug-treatment period, bodyweight gain increased in the 150 and 450 mg/kg/day groups (+459% and +708%, respectively) for the GD 21 to 24 interval, and in the 450 mg/kg/day group during GD 21 to 29 (+136%), compared to controls. There were no significant drug-related changes in bodyweight at termination (end of the gestation). The changes in bodyweight and bodyweight gain were transient and are not considered as adverse.

Feed Consumption

Food consumption was measured on gestation days 4, 6, 7, 9, 11, 13, 15, 17, 18, 20, 21, 23, 24, 26, 27, and 29 for main study animals.

Significant decreases in food consumption were noted in the 150 and 450 mg/kg/day groups (-24.1% and -41.1%, respectively) for the GD 7 to 9 interval, and in the 450 mg/kg/day group (-19.2%) for the GD 9 to 11 interval, compared to controls. After the drug treatment period, significant increases in food consumption were noted in the 150 and 450 mg/kg/day groups for the GD 21 to 23 interval (+30.9% and +25.6%, respectively), GD 23 to 24 interval (+42.6% and +62.1%, respectively), and in the 450 mg/kg/day group for the GD 24 to 26 interval (+39.6%) and the GD 21 to 29 interval (32.4%). The changes in food consumption are considered to be drug-related, and correlated with the changes in bodyweight gains during gestation.

Toxicokinetics

Blood samples (3/group) were collected from toxicokinetic animals via a medial auricular artery on GD 7 and 20 pre-dose (within 1 hour prior to dosing) and at approximately 0.5, 3, 6, 12, and 24 hours post-dose.

Mean values of plasma toxicokinetic parameters of NKTR-118 in pregnant rabbits on GD 7 and 20 are shown in the Sponsor's table below.

Toxicokinetics of NKTR-118 in Pregnant Rabbits

Day	Dose (mg/kg/day)	TK Parameter Value (Mean \pm SD) [‡]			
		AUC(0-24hr) hr• μ g/mL	C24hr μ g/mL	Cmax μ g/mL	Tmax hr
GD 7	30	2.88 \pm 0.912	0.0187 \pm 0.00643	1.29 \pm 0.517	0.570
	150	25.5 \pm 4.94	0.0707 \pm 0.0304	13.1 \pm 3.86	0.550
	450	110 \pm 35.1	0.316 \pm 0.0661	49.8 \pm 18.6	0.550
GD 20	30	3.53 \pm 1.44	0.0287 \pm 0.022	1.58 \pm 0.568	0.500
	150	26.2 \pm 13.2	0.115 \pm 0.0429	12.0 \pm 9.13	0.500
	450	135 \pm 24.7	0.773 \pm 0.225	52.4 \pm 18.5	0.500

[‡] Tmax value is reported as median.

Maximum plasma levels of NKTR-118 were achieved around 0.5 hour after oral administration on GD 7 and GD 20. Plasma exposure to NKTR-118 (AUC) was slightly greater than dose-proportional on GD 7 and GD 20. There was no accumulation of NKTR-118 in plasma after repeated dosing. The C_{max} and AUC_{0-24hr} values in the 450 mg/kg/day group on GD 20 were 52.4 μ g/mL and 135 μ g•h/mL, respectively.

Mean values of plasma toxicokinetic parameters of NKTR-118-glucuronide, a major metabolite, are shown in the Sponsor's table below.

Toxicokinetics of NKTR-118-glucuronide in Pregnant Rabbits

Day	Dose (mg/kg/day)	TK Parameter Value (Mean ± SD) [‡]			
		AUC(0-24hr) hr•µg/mL	C _{24hr} µg/mL	C _{max} µg/mL	T _{max} hr
GD 7	30	27.2 ± 4.66	0.261 ± 0.0917	10.4 ± 1.29	0.570
	150	144 ± 31.4	1.31 ± 0.544	50.1 ± 18.4	0.550
	450	771 ± 283	5.81 ± 3.40	220 ± 149	0.550
GD 20	30	37.6 ± 7.60	0.542 ± 0.291	13.6 ± 1.64	0.500
	150	206 ± 19.5	1.89 ± 1.05	68.9 ± 15.5	0.500
	450	1270 ± 272	13.2 ± 5.91	318 ± 164	0.500

[‡] T_{max} value is reported as median.

NKTR-118 was metabolized rapidly to NKTR-118-glucuronide; peak plasma NKTR-118-glucuronide concentrations on GD 7 and 20 appeared within 0.5 hr following oral NKTR-118 administration at all doses. Plasma exposure to NKTR-118 glucuronide was greater than dose-proportional. There was a small accumulation of NKTR-118-glucuronide in plasma (1.4- to 1.6-fold) after repeated dosing at all doses. The C_{max} and AUC_{0-24hr} in the 450 mg/kg/day group on GD 20 were 318 µg/mL and 1270 µg·h/mL, respectively.

Dosing Solution Analysis

Stock assay solutions were tested appropriately for stability and drug concentration. Mean concentrations of the 3, 15, and 45 mg/ml dosing solutions on GD 7 and 20 ranged from 101% to 106% of nominal concentrations. Dose formulations are solutions at the concentrations used and, therefore, no homogeneity analysis was performed.

Necropsy

All females which died or were sacrificed prior to the scheduled cesarean section were examined macroscopically for abnormalities of the thoracic, abdominal, and cervical viscera. The uterus and ovaries were examined for implantations and corpora lutea, respectively. The placenta or amniotic sac was examined for any abnormalities. An evaluation of the uterus was made to identify early- or late-resorbing fetuses, dead fetuses, or normally developed fetuses. In addition, a histologic evaluation was performed on the lungs of the rabbit that died on GD 18. For dams sacrificed at scheduled c-section, the uterus from each gravid animal was excised, and uterine and net body weights were recorded.

There were no drug-related macroscopic findings in the dams. In the 450 mg/kg/day dam that aborted, liver with a prominent reticular pattern was noted. This finding

correlated with the low food consumption noted in this animal. Decreases in gravid uterine weight were noted in the 150 and 450 mg/kg/day groups (6.3 and 7.0%, respectively). However, the decrease was not statistically significant, and there were no correlated effects on resorptions, post-implantation loss, or on the mean number of live fetuses. In addition, the mean gravid uterine weights (471.0 to 467.4 g) were within the historical control range (420.8 to 547.3 g). There were no drug-related effects on either corrected mean body weights (terminal body weight minus gravid uterine weight) or net weight changes (corrected weight minus day 4 body weight). Uterine and net body weight data are summarized in the Sponsor's table below.

Summary of Uterine and Net Body Weights

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
GRAVID UTERUS	MEAN	502.81	502.75	471.02	467.39
	S.D.	63.50	111.34	88.76	123.87
	N	19	16	17	17
CORRECTED WEIGHT	MEAN	3273.93	3235.81	3176.51	3236.08
	S.D.	297.12	358.54	255.77	297.30
	N	19	16	17	17
NET CHANGE FROM DAY 4	MEAN	-96.60	-86.06	-67.85	-54.92
	S.D.	147.56	99.55	142.49	138.60
	N	19	16	17	17

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

CORRECTED WEIGHT = TERMINAL BODY WEIGHT MINUS GRAVID UTERINE WEIGHT
NET WEIGHT CHANGE FROM DAY 4 = CORRECTED WEIGHT MINUS DAY 4 BODY WEIGHT

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

Dams were euthanized on GD 29. The external features of the carcass and the contents of the thoracic, abdominal, and pelvic cavities were examined. A cesarean section was performed, and the uterus from each gravid animal was excised, weighed, and examined for the number and placement of live and dead fetuses, the number of early or late resorptions, and any abnormalities. The right and left ovaries from each gravid female was examined for the number of corpora lutea. The placentas were also examined.

Most parameters, including corpora lutea, implantation sites, resorptions (early or late), sex ratio, fetal weight, and the pre- and post-implantation losses in each treatment group, were comparable to that of the control group. However, there was a slight decrease in the number of live fetuses per litter in the 150 and 450 mg/kg/day dams (7.7 and 7.5, respectively), compared to controls (8.7). However, both means are within the historical control range (6.6 to 9.3). The data are summarized in the Sponsor's table below.

Summary of Cesarean Section Data in the Embryo-fetal Developmental Study in Rabbits

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Females Mated	N	20	20	20	20
Pregnant	N	19	18	17	18
	%	95	90	85	90
Aborted	N	0	0	0	1
	%	0.0	0.0	0.0	5.0
Died	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Delivered Early	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Pregnant at C-section	N	19	16	17	17
Dams with Viable Fetuses	N	19	16	17	17
	%	100	100	100	100
Dams with no Viable Fetuses	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Corpora Lutea	MEAN	9.5	9.4	9.2	8.8
	S.D.	1.4	1.7	1.5	1.5
	N	19	16	17	17
	TOTAL	181	151	157	149
Implantation Sites	MEAN	8.8	8.9	8.0	7.9
	S.D.	1.4	1.7	1.7	1.7
	N	19	16	17	17
	TOTAL	168	142	136	134
Preimplantation Loss	MEAN%	6.8	5.8	12.7	10.0
	S.D.	9.3	7.5	18.8	14.5

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Pregnant at C-section	N	19	16	17	17
Resorptions: Total	MEAN	0.2	0.4	0.3	0.4
	S.D.	0.4	0.9	1.0	0.7
	N	19	16	17	17
	TOTAL	3	7	5	7
	MEAN%	1.6	6.1	3.3	7.4
	S.D.	3.9	15.3	10.9	16.7
Early	MEAN	0.1	0.4	0.1	0.3
	S.D.	0.3	0.9	0.3	0.6
	N	19	16	17	17
	TOTAL	2	6	2	5
	MEAN%	1.1	5.6	1.3	6.1
	S.D.	3.3	15.4	3.7	16.4
Late	MEAN	0.1	0.1	0.2	0.1
	S.D.	0.2	0.2	0.7	0.3
	N	19	16	17	17
	TOTAL	1	1	3	2
	MEAN%	0.5	0.6	2.0	1.2
	S.D.	2.3	2.3	8.1	3.5
Dead Fetuses	TOTAL	0	0	0	0
Postimplantation Loss	MEAN%	1.6	6.1	3.3	7.4
	S.D.	3.9	15.3	10.9	16.7

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Pregnant at C-section	N	19	16	17	17
Live Fetuses	MEAN	8.7	8.4	7.7	7.5
	S.D.	1.3	2.1	1.8	2.0
	N	19	16	17	17
	TOTAL	165	135	131	127
	MEAN%	98.4	93.9	96.7	92.6
	S.D.	3.9	15.3	10.9	16.7
Females	MEAN	4.7	3.4	3.2	3.2
	S.D.	2.0	1.5	1.5	1.4
	N	19	16	17	17
	TOTAL	89	55	54	55
	MEAN%	52.6	40.7	40.6	46.8
	S.D.	19.0	11.8	16.2	22.0
Males	MEAN	4.0	5.0	4.5	4.2
	S.D.	1.4	1.5	1.4	2.0
	N	19	16	17	17
	TOTAL	76	80	77	72
	MEAN%	47.4	59.3	59.4	53.2
	S.D.	19.0	11.8	16.2	22.0
Sex Ratio	M:F	46:54	59:41	59:41	57:43

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

Offspring (Malformations, Variations, etc.)

Each fetus (live or dead) was weighed and examined for external abnormalities. Live fetuses were sacrificed, and a mid-coronal slice was made in the head of each fetus to evaluate the contents of the cranium. The internal organs of the thoracic and abdominal cavities of all fetuses were examined, and the sex of each fetus (live or dead) was determined. Viscera were removed and carcasses were processed for skeletal examination. Findings were designated as either variations or malformations. Malformations are gross structural changes that are either incompatible with life or may affect the quality of life. Variations are structural deviations which are thought to have no effect on body conformity or the well-being of the animal.

There were no drug-related external variations or malformations.

Fetal visceral variations and malformations: Sporadic variations included left carotid arising from the innominate artery, accessory subclavian, small or missing intermediate lobe of the lung, small gallbladder, and distended stomach. These findings were either not dose-related, observed in both treatment and control groups, and/or were within historical ranges, and therefore are not considered to be drug-related. Fetal visceral malformations included hydrocephaly in a 30 mg/kg/day fetus and gallbladder agenesis in a 150 mg/kg/day fetus. These malformations are not considered drug-related because they were not dose-dependent and/or were within historical control ranges. Therefore, there were no drug-related fetal visceral variations or malformations.

Fetal skeletal variations: There were dose-related increases in percent fetal and litter incidence for the following variations: 26 presacral vertebrae, 6th sternbrae unossified, and 13th full rib (fetal incidence only). The incidence of 26 presacral vertebrae in fetuses and litters at 450 mg/kg/day were 22 and 59%, respectively, and were within historical ranges of 1.4 to 31% for fetuses and 13 to 84% for litters. For 6th sternbrae unossified and 13th full rib, fetal percent incidences were 20% and 62%, respectively, versus

historical ranges of 0.7 to 19% and 19 to 56%, respectively. Litter incidences, however, were within historical ranges for both parameters. The Sponsor noted that since the litter incidences were within the historical control range and the number of pregnant does was decreased in both of these treatment groups due to a high percentage of non-pregnant does, these findings were not considered drug-related. Other fetal variations were considered incidental because they were only observed in one fetus, the concurrent control incidence was greater than that observed in treated groups, and/or there was no evidence of a dose response.

Fetal skeletal malformations: The following malformations were noted: a fused skull bone in one 30 mg/kg/day fetus; fused vertebral arches in two fetuses from two 450 mg/kg/day litters; vertebral anomaly with/without associated rib anomaly in two fetuses from two 30 mg/kg/day litters and in one 450 mg/kg/day fetus; major fusion of the sternbrae in one control fetus and two fetuses in two 150 mg/kg/day litters; and one 30 mg/kg/day fetus with forked/fused ribs. With the exception of the fused vertebral arches, the Sponsor did not consider the malformations to be drug-related because they were only observed in a single fetus, there was no dose-dependency, and/or the incidences observed were within historical control ranges. Fused vertebral arches had a fetal incidence of 1.6% in the 450 mg/kg/day group, as compared to the historical control range of 0-0.7%, and a litter incidence of 12%, as compared to a historical control range of 0 to 5.9%. However, when the combined incidence of all skeletal malformations are considered, the malformations do not appear to be dose-dependent, nor is there a significant increase in any treatment group. Some of the malformations occurred in the control, low-dose, and middle-dose groups, but not in the high dose group. Therefore, the fused vertebral arches are not considered to be drug-related.

The fetal weight, variations, and malformations data are summarized in the Sponsor's tables below.

Summary of Fetal Rabbit Weights

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
FETAL WEIGHTS UNITS: GRAMS					
of all Viable Fetuses	MEAN	39.83	40.21	41.60	42.52
	S.D.	4.16	3.41	5.12	5.27
	N	19	16	17	17
	Covariate Adjusted MEAN	40.71	40.72	41.04	41.62
of Male Fetuses	MEAN	39.97	40.21	41.73	42.42
	S.D.	4.99	3.58	6.19	5.23
	N	19	16	17	16
	Covariate Adjusted MEAN	40.79	40.63	40.93	41.90
of Female Fetuses	MEAN	39.46	40.22	40.38	41.40
	S.D.	3.95	3.96	4.07	6.11
	N	18	16	16	17
	Covariate Adjusted MEAN	40.31	40.61	40.01	40.49
STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01.					

Summary of External Variations in Fetal Rabbits

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Litters Evaluated	N	19	16	17	17
Fetuses Evaluated	N	165	135	131	127
Live	N	165	135	131	127
Dead	N	0	0	0	0
TOTAL FETAL EXTERNAL VARIATIONS					
Fetal Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
Litter Incidence	N	0	0	0	0
	%	0.0	0.0	0.0	0.0

Summary of External Malformations in Fetal Rabbits

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Litters Evaluated	N	19	16	17	17
Fetuses Evaluated	N	165	135	131	127
Live	N	165	135	131	127
Dead	N	0	0	0	0
UMBILICAL HERNIA					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.7	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	6.2	0.0	0.0
TOTAL FETAL EXTERNAL MALFORMATIONS					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.7	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	6.2	0.0	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

Summary of Soft Tissue Variations in Fetal Rabbits

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Litters Evaluated	N	19	16	17	17
Fetuses Evaluated	N	165	135	131	127
Live	N	165	135	131	127
Dead	N	0	0	0	0
LEFT CAROTID ARISES FROM THE INNOMINATE ARTERY					
Fetal Incidence +	N	6	1	3	11
	%	3.6	0.7	2.3	8.7
Litter Incidence +	N	4	1	3	6
	%	21	6.2	18	35
ACCESSORY SUBCLAVIAN					
Fetal Incidence	N	2	3	2	1
	%	1.2	2.2	1.5	0.8
Litter Incidence	N	1	3	2	1
	%	5.3	19	12	5.9
INTERMEDIATE LOBE OF LUNG SMALL/MISSING					
Fetal Incidence +	N	7	6	4	11
	%	4.2	4.4	3.1	8.7
Litter Incidence	N	3	4	1	5
	%	16	25	5.9	29
GALL BLADDER SMALL					
Fetal Incidence +	N	0	0	0	1
	%	0.0	0.0	0.0	0.8
Litter Incidence +	N	0	0	0	1
	%	0.0	0.0	0.0	5.9
STOMACH DISTENDED					
Fetal Incidence	N	1	0	0	1
	%	0.6	0.0	0.0	0.8
Litter Incidence	N	1	0	0	1
	%	5.3	0.0	0.0	5.9

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

Summary of Soft Tissue Variations in Fetal Rabbits (cont.)

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Litters Evaluated	N	19	16	17	17
Fetuses Evaluated	N	165	135	131	127
Live	N	165	135	131	127
Dead	N	0	0	0	0
TOTAL FETAL SOFT TISSUE VARIATIONS					
Fetal Incidence +	N	15	10	9	24
	%	9.1	7.4	6.9	19*
Litter Incidence	N	8	7	5	11
	%	42	44	29	65

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01
 N = Number
 + = SIGNIFICANT POSITIVE TREND

Summary of Soft Tissue Malformations in Fetal Rabbits

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Litters Evaluated	N	19	16	17	17
Fetuses Evaluated	N	165	135	131	127
Live	N	165	135	131	127
Dead	N	0	0	0	0
INTERNAL HYDROCEPHALY					
Fetal Incidence	N	0	1	0	0
	%	0.0	0.7	0.0	0.0
Litter Incidence	N	0	1	0	0
	%	0.0	6.2	0.0	0.0
GALL BLADDER AGENESIS					
Fetal Incidence	N	0	0	1	0
	%	0.0	0.0	0.8	0.0
Litter Incidence	N	0	0	1	0
	%	0.0	0.0	5.9	0.0
TOTAL FETAL SOFT TISSUE MALFORMATIONS					
Fetal Incidence	N	0	1	1	0
	%	0.0	0.7	0.8	0.0
Litter Incidence	N	0	1	1	0
	%	0.0	6.2	5.9	0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P<0.05 ** = P<0.01

Summary of Skeletal Variations in Fetal Rabbits

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Litters Evaluated	N	19	16	17	17
Fetuses Evaluated	N	165	135	131	127
Live	N	165	135	131	127
Dead	N	0	0	0	0
ANGULATED HYOID WING(S)					
Fetal Incidence	N %	6 3.6	4 3.0	4 3.1	3 2.4
Litter Incidence	N %	5 26	3 19	4 24	2 12
ACCESSORY BONE(S) IN SKULL					
Fetal Incidence	N %	1 0.6	0 0.0	1 0.8	0 0.0
Litter Incidence	N %	1 5.3	0 0.0	1 5.9	0 0.0
UNOSSIFIED HYOID BODY					
Fetal Incidence	N %	0 0.0	2 1.5	0 0.0	0 0.0
Litter Incidence	N %	0 0.0	2 13	0 0.0	0 0.0
26 PRESACRAL VERTEBRAE					
Fetal Incidence +	N %	4 2.4	4 3.0	21 16**	28 22**
Litter Incidence +	N %	4 21	3 19	8 47	10 59*
LESS THAN SIXTEEN CAUDAL VERTEBRAE OSSIFIED					
Fetal Incidence	N %	0 0.0	2 1.5	2 1.5	1 0.8
Litter Incidence	N %	0 0.0	1 6.2	2 12	1 5.9

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Litters Evaluated	N	19	16	17	17
Fetuses Evaluated	N	165	135	131	127
Live	N	165	135	131	127
Dead	N	0	0	0	0
24 PRESACRAL VERTEBRAE					
Fetal Incidence	N %	0 0.0	1 0.7	0 0.0	2 1.6
Litter Incidence	N %	0 0.0	1 6.2	0 0.0	2 12
MISALIGNED OR BIPARTITE DISTAL CAUDAL VERTEBRA(E)					
Fetal Incidence	N %	0 0.0	1 0.7	0 0.0	0 0.0
Litter Incidence	N %	0 0.0	1 6.2	0 0.0	0 0.0
5TH/6TH STERNEBRA(E) INCOMPLETE OSSIFICATION					
Fetal Incidence	N %	37 22	15 11**	12 9.2**	16 13*
Litter Incidence	N %	15 79	11 69	7 41*	10 59
6TH STERNEBRA UNOSSIFIED					
Fetal Incidence +	N %	6 3.6	3 2.2	11 8.4	26 20**
Litter Incidence +	N %	5 26	3 19	5 29	9 53
5TH STERNEBRA UNOSSIFIED					
Fetal Incidence	N %	18 11	9 6.7	5 3.8*	7 5.5
Litter Incidence	N %	8 42	5 31	4 24	6 35

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Litters Evaluated	N	19	16	17	17
Fetuses Evaluated	N	165	135	131	127
Live	N	165	135	131	127
Dead	N	0	0	0	0
MINOR FUSION OF STERNEBRAE					
Fetal Incidence	N %	0 0.0	2 1.5	0 0.0	0 0.0
Litter Incidence	N %	0 0.0	2 13	0 0.0	0 0.0
STERNEBRA (E) ASYMMETRICALLY OSSIFIED					
Fetal Incidence	N %	1 0.6	1 0.7	0 0.0	0 0.0
Litter Incidence	N %	1 5.3	1 6.2	0 0.0	0 0.0
5TH/6TH STERNEBRA (E) BIPARTITE					
Fetal Incidence	N %	2 1.2	1 0.7	3 2.3	1 0.8
Litter Incidence	N %	2 11	1 6.2	3 18	1 5.9
13TH RUDIMENTARY RIB(S)					
Fetal Incidence	N %	29 18	21 16	28 21	20 16
Litter Incidence	N %	14 74	11 69	12 71	13 76
13TH FULL RIB(S)					
Fetal Incidence +	N %	39 24	32 24	56 43**	79 62**
Litter Incidence	N %	14 74	13 81	16 94	16 94

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Litters Evaluated	N	19	16	17	17
Fetuses Evaluated	N	165	135	131	127
Live	N	165	135	131	127
Dead	N	0	0	0	0
13TH UNILATERAL FULL RIB					
Fetal Incidence	N %	5 3.0	2 1.5	3 2.3	5 3.9
Litter Incidence	N %	5 26	2 13	2 12	3 18
TOTAL FETAL SKELETAL VARIATIONS					
Fetal Incidence +	N %	111 67	79 59	101 77*	114 90**
Litter Incidence -	N %	19 100	16 100	17 100	16 94

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

Summary of Skeletal Malformations in Fetal Rabbits

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Litters Evaluated	N	19	16	17	17
Fetuses Evaluated	N	165	135	131	127
Live	N	165	135	131	127
Dead	N	0	0	0	0
SKULL BONE(S) FUSED					
Fetal Incidence	N %	0 0.0	1 0.7	0 0.0	0 0.0
Litter Incidence	N %	0 0.0	1 6.2	0 0.0	0 0.0
FUSED ARCHES					
Fetal Incidence +	N %	0 0.0	0 0.0	0 0.0	2 1.6
Litter Incidence +	N %	0 0.0	0 0.0	0 0.0	2 12
VERTEBRAL ANOMALY WITH/WITHOUT ASSOCIATED RIB ANOMALY					
Fetal Incidence	N %	0 0.0	2 1.5	0 0.0	1 0.8
Litter Incidence	N %	0 0.0	2 13	0 0.0	1 5.9
MAJOR FUSION OF STERNEBRAE					
Fetal Incidence	N %	1 0.6	0 0.0	2 1.5	0 0.0
Litter Incidence	N %	1 5.3	0 0.0	2 12	0 0.0
FORKED/FUSED RIB(S)					
Fetal Incidence	N %	0 0.0	1 0.7	0 0.0	0 0.0
Litter Incidence	N %	0 0.0	1 6.2	0 0.0	0 0.0

STATISTICAL ANALYSES WERE CONDUCTED. IF SIGNIFICANT DIFFERENCES OCCUR, THEY ARE DENOTED AS FOLLOWS: * = P≤0.05 ** = P≤0.01

DOSE LEVEL		GROUP 1 0 MG/KG/DAY	GROUP 2 30 MG/KG/DAY	GROUP 3 150 MG/KG/DAY	GROUP 4 450 MG/KG/DAY
Litters Evaluated	N	19	16	17	17
Fetuses Evaluated	N	165	135	131	127
Live	N	165	135	131	127
Dead	N	0	0	0	0
TOTAL FETAL SKELETAL MALFORMATIONS					
Fetal Incidence	N %	1 0.6	3 2.2	2 1.5	3 2.4
Litter Incidence	N %	1 5.3	3 19	2 12	3 18

In summary, the NOAEL for maternal toxicity in rabbits is considered to be 30 mg/kg/day, based on decreases in bodyweights and food consumption in the 150 and 450 mg/kg/day groups. The NOEL for embryo-fetal viability and fetal growth is considered to be 450 mg/kg/day. For developmental toxicity (teratogenicity), the NOEL is considered to be 450 mg/kg/day, based on the lack of dose-dependent findings. Mean values of toxicokinetic parameters that correspond to the NOEL and NOAEL on GD 20 are summarized in the Sponsor's table below.

Toxicokinetic Parameters Corresponding to Reproductive Parameters in Rabbits

	Reproductive parameter NOEL/NOAEL	Dose Level (mg/kg/day)	TK Parameter Value (Mean ± SD) GD 20	
			C _{max} μg/mL	AUC(0-24hr) hr•μg/mL
NKTR-118	Maternal Toxicity (NOEL)	30	1.58 ± 0.568	3.53 ± 1.44
	Maternal Toxicity (NOAEL)	450	52.4 ± 18.5	135 ± 24.7
	Embryo/Fetal Viability (NOEL)	450	52.4 ± 18.5	135 ± 24.7
	Fetal Growth (NOEL)	450	52.4 ± 18.5	135 ± 24.7
	Developmental Toxicity/Teratology (NOEL)	150	12.0 ± 9.13	26.2 ± 13.2

9.3 Prenatal and Postnatal Development**Study title: NKTR-118: Oral (gavage) Pre- and Post-Natal Development Study in the Rat**

Study no.: ASU0140
 Study report location: N/A
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 02-01-2012
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: NKTR-118(free base), lot 1007, 97.8%

Key Study Findings

- Mated female rats (25/group) were administered 0 (vehicle), 50, 250, or 500 mg/kg/day NKTR-118 by oral gavage from day 6 of gestation to day 20 of lactation, inclusive. F1 generation litters were randomly culled to 8 pups (4/sex) on day 4 of lactation. After weaning, selected F1 generation animals were allowed to mature untreated and the effects of NKTR-118 on growth, development, behavior, and reproductive performance were evaluated. The selected F1 males and females from each dose group were mated on week 7 of the F1 generation (after weaning, male and female pups in each group were selected for detailed examinations and rearing to sexual maturity; the week of selection was designated as week 0 of the F1 generation by the Sponsor). The F0 females were sacrificed on day 21 of lactation (weaning of litters). A necropsy was conducted on all F1 generations pups culled, prematurely killed, found dead during lactation, and unselected pups after weaning. Mated F1 generation adult

males were necropsied approximately 2 weeks after completion of the mating period. Mated F1 generation females were sacrificed on GD 13.

- There were no drug-related effects on overall bodyweight or bodyweight gain in F0 females during gestation or lactation. The transient decreases in bodyweight gain during gestation were not considered adverse. Decreases in food consumption were noted in all drug-treated groups on GD 6 to 9, on GD 12 in the 500 mg/kg/day group, and on lactation days 4 through 14 in the 250 and 500 mg/kg/day groups.
- There were no drug-related maternal necropsy findings in the F0 females, and there were no drug-related effects on pregnancy or litter parameters.
- In F1 generation pups, significant increases in organ weight/bodyweight ratios in brain, liver, and testes were noted. However, there were no drug-related macroscopic or microscopic findings.
- In F1 generation animals, one 500 mg/kg/day male was found dead on day 8 of the F1 generation. The death was not considered drug-related due to the absence of clinical signs in life or macroscopic abnormalities at necropsy. One 500 mg/kg/day male was euthanized on day 66 of the F1 generation due to swollen hind limb, which was considered to be the result of an injury.
- There were no drug-related clinical signs in the F1 generation animals either during lactation or post-weaning.
- In the F1 generation animals, bodyweight and bodyweight gains were lower throughout lactation in the 250 and 500 mg/kg/day males and females, compared to controls. However, the Sponsor noted that the bodyweight gains were within the Sponsor's historical background ranges for this strain.
- There were no drug-related effects on physical development of the pups during lactation.
- In post-weaning F1 generation males, bodyweight and bodyweight gains in all drug-treated groups decreased significantly compared to controls; the decrease in bodyweight gain in the 500 mg/kg/day males was significant during the entire post-weaning period. There were no drug-related effects on bodyweight or bodyweight gain in the F1 females. Although administration of 50 to 500 mg/kg/day NKTR-118 to F0 females produced significant decreases in bodyweight and bodyweight gains in F1 generation males during the post-weaning period, these effects were small and are not considered to be adverse.
- There were no drug-related adverse effects on learning and memory, auditory startle reflex, or locomotor activity in F1 generation post-weaning animals.

- Significant increases in organ/bodyweight ratios in brain, testes, and epididymides were noted in the F1 generation adult males. The increases can be attributed to lower terminal weight observed in the treatment groups. There were no drug-related macroscopic or microscopic findings in the F1 generation adult males.
- There were no drug-related effects on mating performance or fertility in the maternal NKTR-118-treated F1 generation animals.
- There were no drug-related effects on any of the pregnancy parameters, except for an increase in the mean number of corpora lutea per female in the 500 mg/kg/day group. However, the magnitude of the increase was small, and it was not considered as adverse.
- Plasma NKTR-118 levels were roughly dose-proportional in the dams and pups at 3 hours after dosing on day 20 of lactation. The maternal/pup plasma NKTR-118 ratio ranged from 180 to 311 fold.
- The NOAEL for maternal effects and F1 generation offspring was considered to be 500 mg/kg/day.

Methods

Doses:	0 (vehicle), 50, 250, or 500 mg/kg/day
Frequency of dosing:	once daily
Dose volume:	5 ml/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	solution/deionized purified water
Species/Strain:	Rat/Crl:CD(SD)
Number/Sex/Group:	25
Satellite groups:	None
Study design:	Mated females received NKTR-118 from day 6 of gestation to day 20 of lactation, inclusive. Selected F1 generation animals were then allowed to mature untreated, and the effects on growth, development, behavior, and reproductive performance were evaluated. The design of the study is summarized in the Sponsor's table below.
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

Study Design

Group	Animals	Animal reference numbers	Test item	Daily dose levels	
				(mg/kg)	(μ mol/kg)
1	25	1 - 25	Vehicle	0	0
2	25	26 - 50	NKTR-118	50	80
3	25	51 - 75	NKTR-118	250	380
4	25	76 - 100	NKTR-118	500	770

All dose levels are expressed in terms of the parent form of NKTR-118. The dose levels in μ mol/kg are approximate.

In a dose range-finding pre- and post-natal study in rats (Study No. 3270WR), animals (6/group) were administered 0 (vehicle), 50, 250 or 750 mg/kg/day NKTR-118 (oxalate salt) from day 6 of gestation to day 7 of lactation, inclusive. At the start of treatment, animals were 11 to 13 weeks old, and their bodyweights ranged from 249 to 257 g. There were no deaths. At dose levels of 750 mg/kg/day and below, NKTR-118 was generally well tolerated. Initial bodyweight loss and reduced food consumption was noted at 250 and 750 mg/kg/day; however, there were no apparent effects on parturition, pup survival, or pup development. Therefore, the Sponsor indicated that for the current study, the selected high dose (500 mg/kg/day) was expected to cause minimal reductions in maternal bodyweight, bodyweight gain, and food consumption, and was estimated to produce maternal plasma exposure (AUC) approximately 1100 times the maximum human therapeutic exposure (based on data from non-pregnant female rats given NKTR-118 free base). The low dose (50 mg/kg/day) was expected to be a NOEL, and was estimated to provide a maternal plasma AUC approximately 70 times the predicted human AUC. The intermediate dose (250 mg/kg/day) was selected to investigate the dose response. This dose was expected to have potential to cause slight changes in maternal bodyweight gain during early gestation, and was estimated to provide plasma exposure approximately 550 times the predicted human therapeutic exposure. At the start of treatment, animals were 10 to 12 weeks old, and their bodyweights were approximately 270 g.

Observations and Results

F₀ Dams

- Survival:** All animals were observed twice daily for mortality. A 500 mg/kg/day female was found dead on GD 12 after dosing. Necropsy showed that the death was most likely due to dosing trauma. A control group female and a 250 mg/kg/day female were sacrificed on day 1 of lactation after the death of their litters.
- Clinical signs:** All F₀ animals were observed daily after the initiation of dosing for signs of toxicity or changes in behavior and appearance. Occasional incidence of excessive salivation was noted, and they are not considered as adverse.
- Body weight:** Body weights were recorded on GD 0 and daily from GD 6, throughout gestation, and on days 0, 1, 4, 10, 14, 17, and 21 of lactation. Bodyweights in all groups were comparable throughout the gestation and lactation periods. However, significant decreases in bodyweight gain, compared to controls, were observed on GD 6 to 7 in all drug-treated groups (+2.8g, -0.2g, -3.2g, -6.4g in the 0, 50, 250, and 500 mg/kg/day groups, respectively). Significant increases in maternal bodyweight gain were noted on GD 9 to 12 in the 250 and 500 mg/kg/day groups (+17.5g, +22.7g, +22.2g in the 0, 250, and 500 mg/kg/day groups, respectively). Maternal bodyweight gains in the 500 mg/kg/day group during the entire gestation period were slightly, but not significantly, lower than the controls. There were no drug-related effects in bodyweight gain in the 250 and 500 mg/kg/day F₀ females during the lactation period. Therefore, there were no drug-related effects on overall bodyweight or bodyweight gain in F₀ females during the gestation or lactation period. The transient decrease in bodyweight gain during gestation is not considered as adverse.
- Feed consumption:** Food consumption was measured on GD 6 to 9, 9 to 12, 12 to 15, and 15 to 20, and on days 1 to 4, 4 to 7, 7 to 10, and 10 to 14 of lactation. Decreases in food consumption were noted in all drug-treated groups on GD 6 to 9 (-9.3%, -14.7%, and -26.9% in the 50, 250, and 500 mg/kg/day groups, respectively), on GD 12 in the 500 mg/kg/day group (-8.7%), and on lactation days 4 through 14 in the 250 and 500 mg/kg/day groups (-6.5% to -12.8%, respectively), compared to controls. The decrease

- in food consumption is considered drug-related.
- Uterine content:** There were no drug-related effects on pregnancy or litter parameters, including during of gestation, number of pups born, live birth index, viability index, lactation index, and sex ratio at birth. There was a very small but statistically significant decrease in cumulative survival index in the 500 mg/kg/day group (92.2%), compared to controls (95.1%). The results are summarized in the Sponsor's table below.
- Necropsy observation:** The F0 females were sacrificed on day 21 of lactation. Females that were not pregnant were sacrificed on day 26 post-mating. Females were also sacrificed if all pups in their litter died prior to day 21 of lactation. There were no drug-related maternal necropsy findings.
- Toxicokinetics:** Blood samples were collected from dams (3/group) and pups prior to dosing, and at 3 and 6 hours after dosing on days 4, 14, and 20 of lactation. For the pups, blood samples were obtained from 3 litters per group per time point, where possible. On days 4 and 14 of lactation, blood samples were pooled from preferably 1 male and 1 female pup. On day 20 of lactation, 3 animals per group from different litters were sampled to include preferably at least 1 male and 1 female blood sample, where possible. Systemic exposure of NKTR-118 was demonstrated in the dams and pups. Plasma NKTR-118 levels were roughly dose-proportional in the dams and pups at 3 hours after dosing on day 20 of lactation. The maternal/pup plasma NKTR-118 ratio ranges from 180 to 311-fold. The results are summarized in the Sponsor's table below.
- Dosing Solution Analysis:** The analysis confirmed the NKTR-118 concentrations of the formulations were prepared within the range of 101% to 103% of the nominal concentration.

Pregnancy and Litter Data in F0 Generation Females and F1 Generation Litters

Group	:	1	2	3	4
Test item	:	Control		NKTR-118	
Dose (mg/kg/day)	:	0	50	250	500

Group	Number littered (N)	Gestation# index %	Mean duration# of gestation (days) ± S.D.	Mean number# of pups born ± S.D.	Mean# live birth index %	Mean# viability index %	Mean# lactation index %	Mean# cumulative survival index %	Sex ratio# at birth M : F
1	24	95.8	21.9 ± 0.5	13.2 ± 2.6(23)	97.6(23)	97.1(23)	98.8(20)	95.1(20)	52.8 : 47.2
2	24	100.0	22.2 ± 0.5	13.1 ± 1.8	99.1	99.7	99.4(21)	98.7(21)	58.5 : 41.5
3	24	100.0	22.0 ± 0.4	13.5 ± 2.5	89.9	98.8(23)	94.6(19)	82.6(20)	54.1 : 45.9
4	24	100.0	22.0 ± 0.6	13.0 ± 2.6	98.5	97.9	94.6(21)	92.2(21)*	52.4 : 47.6

Gestation index (%) = $\frac{\text{number of pregnant females with live pups born}}{\text{number of pregnant females}} \times 100$

Live birth index (%) = $\frac{\text{number of pups alive on Day 1 of lactation}}{\text{total number of pups born}} \times 100$

Viability index (%) = $\frac{\text{number of pups alive on Day 4 of lactation}}{\text{number of pups born alive}} \times 100$

Lactation index (%) = $\frac{\text{number of pups alive on Day 21 of lactation}}{\text{number of pups present after culling}} \times 100$

Cumulative survival index = $\frac{\text{no. of pups alive Day 21 of L}}{\text{no. pups present after culling}} \times \frac{\text{no. of pups alive Day 4 of L}}{\text{no. pups born}} \times 100$

Toxicokinetics Parameters in F0 Generation Females and F1 Generation Litters

Group	Dose (mg/kg/day)	Day of study	No of animals ^a	Timepoint (hours post-dose)	Plasma concentration (µmol/L)				Maternal/pup concentration ratio
					Maternal		Pups		
					Mean	SD	Mean	SD	
2	50	Day 4 of Lactation	3, 3	Pre-dose	0.0669	0.0409	0.0812	0.0842	0.824
			3, 3	3	2.76	0.795	0.0836	0.0199	33.1
			3, 3	6	2.39	0.977	0.100	0.0757	23.9
3	250	Day 4 of Lactation	3, 3	Pre-dose	0.346	0.197	0.618	0.292	0.560
			3, 3	3	17.9	2.75	0.594	0.224	30.2
			3, 3	6	14.4	4.81	0.852	0.555	16.9
4	500	Day 4 of Lactation	3, 3	Pre-dose	1.10	0.465	0.778	0.264	1.41
			3, 3	3	21.6	7.17	1.16	1.24	18.6
			3, 3	6	31.2	5.11	1.30	0.283	23.9
2	50	Day 14 of Lactation	3, 3	Pre-dose	0.0169	0.0227	0.00184	0.00165	9.18
			3, 3	3	3.77	2.64	0.0143	0.00674	263
			3, 3	6	1.04	0.367	0.0150	0.00537	69.2
3	250	Day 14 of Lactation	3, 3	Pre-dose	0.143	0.0969	0.0303	0.0210	4.73
			3, 3	3	11.6	3.21	0.519	0.729	22.4
			3, 3	6	17.2	3.06	0.108	0.0432	159
4	500	Day 14 of Lactation	3, 3	Pre-dose	0.498	0.294	0.235	0.167	2.12
			3, 3	3	23.6	5.78	0.154	0.166	153
			3, 3	6	23.5	3.21	0.278	0.0785	84.6
Group	Dose (mg/kg/day)	Day of study	No of animals ^a	Timepoint (hours post-dose)	Plasma concentration (µmol/L)				Maternal/pup concentration ratio
					Maternal		Pups		
					Mean	SD	Mean	SD	
2	50	Day 20 of Lactation	3, 3	Pre-dose	0.0106	0.00518	NC	NC	NC
			3, 3	3	2.60	0.673	0.00889	0.00989	293
			3, 3	6	1.17	0.405	0.0258	0.0293	45.4
3	250	Day 20 of Lactation	3, 3	Pre-dose	0.151	0.0273	0.0104	0.00682	14.5
			3, 3	3	14.9	3.21	0.0477	0.0219	311
			3, 3	6	12.9	3.42	0.0257	0.0183	500
4	500	Day 20 of Lactation	3, 3	Pre-dose	0.594	0.148	0.0214	0.0140	27.8
			3, 3	3	27.6	5.68	0.154	0.0318	180
			3, 3	6	25.8	4.44	0.299	0.375	86.3

F₁ Generation (After weaning, 20 male and 20 female pups in each group were selected for detailed post-weaning examinations and rearing to sexual maturity. The week of selection was designated week 0 of the F₁ generation by the Sponsor.)

Survival: Litter size was recorded after the completion of parturition and daily thereafter. All pups were examined on day 1 of lactation for malformations. Any pups observed with debilitating malformations were killed. A necropsy was performed, where possible, on any pups found dead, dying or killed prematurely during lactation, and on unselected pups after weaning. On day 4 of lactation, each litter was randomly culled to 8 pups (4 animals/sex). One litter from the control and 250 mg/kg/day groups died (i.e. 100% mortality) during early lactation, but these deaths were not considered to be drug-related. There were no drug-related effects on pregnancy or litter parameters, including duration of gestation, number of pups born, live birth index, viability index, lactation index, and sex ratio at birth. There was a small, but statistically significant, decrease in cumulative survival index in the 500 mg/kg/day group (92.2%), compared to controls (95.1%). There were no significant drug-related effects on survival during lactation.

Post-weaning F1 generation animals were examined twice daily for morbidity and mortality. One 500 mg/kg/day male was found dead on day 8 of the F1 generation. The death was not considered drug-related because of the absence of clinical signs in life or macroscopic abnormalities at necropsy. One 500 mg/kg/day male was euthanized on day 66 of the F1 generation due to swollen hind limb, which was considered to be due to an injury. The morbidity and mortality were not considered to be drug-related.

Clinical signs: The pups were examined daily during lactation for clinical signs. There were no drug-related clinical signs during lactation.

Post-weaning F1 generation animals were examined twice daily for clinical signs. There were no drug-related clinical signs post-weaning.

Bodyweight: Pups were weighed on days 1, 4, 7, 14 and 21 of lactation. Bodyweights were reduced in the 250 and 500 mg/kg/day groups by up to 13.3% and 12.2%, respectively, throughout lactation, compared to controls. Bodyweight gains during the lactation

period were also significantly lower in the 250 and 500 mg/kg/day groups (-11.0% and -12.2%, respectively). However, the Sponsor noted that the gains in males and females were within the Sponsor's historical background ranges for this strain (male pups = 42.6 to 48.4 g; female pups=41.0 to 46.9 g). Therefore, the reductions in bodyweight and bodyweight gain during lactation are not considered to be adverse. The bodyweight and bodyweight gain data are summarized in the Sponsor's tables below.

Bodyweight of the post-weaning F1 generation males was recorded once weekly through necropsy. Female bodyweights were recorded once weekly until paring with males, and then on days 0, 7, and 13 of gestation. Bodyweight in all male drug-treated groups decreased significantly at necropsy, up to a 12.3% decrease in the 500 mg/kg/day males, compared to controls. Post-weaning bodyweight gains in all male drug-treated groups also decreased significantly (e.g. 12.2% overall decrease at necropsy in the 500 mg/kg/day males). There were no drug-related effects on bodyweight or bodyweight gain in the females. The decreased bodyweight and bodyweight gains in F1 generation males during the post-weaning period were small effects, and are not considered to be adverse. The results are summarized in the Sponsor's table below.

- Feed consumption: Not measured.
- Physical development: The following parameters were evaluated: pinna detachment, eye lid separation, static righting reflex, startle response, pupillary light reflex, and incisor eruption. There were no drug-related effects on physical development of the pups during lactation. Balanopreputial separation and vaginal opening in pre-weaning were similar between all groups.
- Neurological assessment: Learning test: During weeks 1 and 2 of the F1 generation, selected pups were tested for learning potential and memory retention using a water-filled E-shape maze system. Percent correct turn and exit time were recorded. There were no drug-related effects on E-Maze learning test in the post-weaning animals.
- Auditory startle reflex: During week 0 of the F1 generation, the auditory startle reflex of the selected pups was assessed. Positive and negative responses were recorded. There were no drug-related effects on auditory startle reflex in the post-weaning animals.
- Locomotor activity: During week 0 of the F1 generation, motor activity of the selected pups was assessed using an automated infra-red beam activity monitoring system. Activity counts over 10 minute periods were recorded. Increased activity during the initial 10 minutes of the assessment was noted in post-weaning animals from drug-treated F0 females, compared to controls. However, the increased activity was transient, and is not considered as adverse.
- There were no drug-related adverse effects on learning and memory, auditory startle reflex, or locomotor activity during lactation.
- Reproduction: The selected F1 generation males and females from each dose group were mated on week 7 of the F1 generation (approximately 11 weeks of age) for up to 10 days. Vaginal smears were taken daily, and the stage of the estrous cycle and presence of sperm were recorded. The time course of mating, fertility, and mating performance, including copulation and

fertility indices were assessed. There were no drug-related effects on mating performance or fertility in the maternal drug-treated F1 generation animals. The results are summarized in the Sponsor's table below.

Mated females were sacrificed on GD 13. There were 19, 20, 20 and 19 pregnant females at scheduled necropsy in the control, 50, 250, and 500 mg/kg/day groups, respectively. There were no drug-related effects on fertility or mating parameters. The data are summarized in the Sponsor's table below.

Pregnancy parameters, including number of corpora lutea, implantations and live embryos, and the incidence of pre- and post-implantation losses were assessed. The only drug-related effect was an increase in the mean number of corpora lutea per female at 500 mg/kg/day. The magnitude of the increase was small, and the Sponsor considered the increase to be spontaneous. The data are summarized in the Sponsor's table below.

Other: In F1 generation pups, a necropsy was conducted on all pups culled, prematurely killed, found dead during lactation, and unselected pups after weaning. For all unselected pups (male and female) necropsied after weaning, body weight, brain weight, and liver weight were recorded. In addition, for all unselected male pups necropsied after weaning, the testes, epididymides and prostate weights were recorded. These tissues were retained for histopathology examination. Statistically significant increases in organ weight/bodyweight ratios for brain and liver in both sexes, and for testes were noted. However, there were no drug-related macroscopic or microscopic findings in these animals.

F1 generation adult males were sacrificed approximately 2 weeks after completion of the mating period, and a necropsy was performed. Bodyweight and brain, liver, testes, epididymides and prostate weights were recorded and the tissues were examined microscopically. Statistically significant increases in organ/bodyweight ratios for brain, testes, and epididymides were noted in the F1 generation adult males. The Sponsor attributed this increase to lower terminal weight observed in the treatment groups. There were no drug-related macroscopic or microscopic findings in the F1 generation adult males.

Therefore, there were no macroscopic or microscopic findings in the F1 generation pups or adults that are related to maternal administration of NKTR-118.

Bodyweight and Bodyweight Gain in F1 Generation Rats During Lactation

Males

Body weight# (Day of age)	Group			
	1	2	3	4
N	23	24	23	24
1	7.4 ± 0.8	7.3 ± 0.5	6.8 ± 0.6**	6.8 ± 0.6**
4	10.8 ± 1.7	10.6 ± 0.8	9.6 ± 1.3**	9.8 ± 1.4**
7	18.1 ± 2.7	17.9 ± 1.5	15.7 ± 1.9***	15.9 ± 2.0***
14	36.7 ± 4.0	35.4 ± 3.3	33.0 ± 3.5***	32.3 ± 3.5***
21	59.1 ± 6.2	57.6 ± 6.1	52.9 ± 5.3***	52.3 ± 6.2***

Body weight gain (g)# (Day 1 to 21)	51.8 ± 5.8	50.3 ± 5.8	46.1 ± 5.2***	45.5 ± 5.9***
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N = number of litters in mean

= statistically analysed * = p < 0.05 ** = p < 0.01 *** = p < 0.001

Females

Body weight# (Day of age)	Group			
	1	2	3	4
N	23	24	23	24
1	6.9 ± 0.7	7.0 ± 0.6	6.5 ± 0.8*	6.5 ± 0.7*
4	10.2 ± 1.5	10.1 ± 0.9	9.2 ± 1.6*	9.3 ± 1.4*
7	17.2 ± 2.6	17.0 ± 1.5	15.2 ± 2.0**	15.3 ± 2.0**
14	35.3 ± 4.1	34.5 ± 3.6	32.0 ± 3.0**	31.5 ± 3.9***
21	56.3 ± 6.2	55.6 ± 5.2	51.7 ± 5.3**	50.6 ± 6.3**

Body weight gain (g)# (Day 1 to 21)	49.5 ± 5.8	48.6 ± 4.9	45.2 ± 4.9**	44.1 ± 6.0**
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N = number of litters in mean

= statistically analysed * = p < 0.05 ** = p < 0.01 *** = p < 0.001

Bodyweight in F1 Generation Rats Post-Weaning

Group : 1 2 3 4
 Test item : Control NKTR-118
 Dose (mg/kg/day) : 0 50 250 500

Group sex		Day number							
		0#	7	14	21	28	35	42	49
1M	Mean	78.0	132.4	201.5	269.6	338.3	392.5	436.8	477.1
	S.D.	9.5	16.2	21.5	24.1	30.8	35.4	44.7	47.2
	N	20	20	20	20	20	20	20	20
2M	Mean	76.7	129.4	196.1	262.7	325.4	377.0	419.8	451.6
	S.D.	8.4	10.9	12.5	14.1	18.4	22.8	26.7	31.4
	N	20	20	20	20	20	20	20	20
3M	Mean	71.5*	120.9	186.5	252.5	318.8	371.7	412.6	450.8
	S.D.	7.5	10.1	14.5	16.4	20.0	22.6	24.9	27.7
	N	20	20	20	20	20	20	20	20
4M	Mean	69.5**	118.7	181.3	247.6	308.8	358.8	396.9	432.0
	S.D.	9.1	12.6	18.0	22.7	28.9	33.6	39.2	41.5
	N	20	20	19	19	19	19	19	19

- statistically analysed * = p < 0.05 ** = p < 0.01 *** = p < 0.001

Group : 1 2 3 4
 Test item : Control NKTR-118
 Dose (mg/kg/day) : 0 50 250 500

Group sex		Day number				
		56	63	70	77	84#
1M	Mean	500.3	529.6	557.3	577.9	595.3
	S.D.	49.3	53.6	56.2	57.2	62.3
	N	20	20	20	20	20
2M	Mean	473.4	501.0	522.0	536.1	550.3**
	S.D.	33.1	32.8	37.9	40.7	44.8
	N	20	20	20	20	20
3M	Mean	470.9	499.5	517.6	533.8	546.3**
	S.D.	29.1	31.7	32.5	35.0	37.7
	N	20	20	20	20	20
4M	Mean	454.9	479.8	496.3	511.3	522.2***
	S.D.	44.1	42.2	42.0	42.5	45.1
	N	19	19	18	18	18

- statistically analysed * = p < 0.05 ** = p < 0.01 *** = p < 0.001

Group : 1 2 3 4
 Test item : Control NKTR-118
 Dose (mg/kg/day) : 0 50 250 500

Group sex		Day pre-pairing							
		0#	7#	14#	21#	28#	35#	42#	49#
1F	Mean	74.4	116.1	157.4	187.7	215.2	236.0	251.0	266.1
	S.D.	7.9	11.7	11.6	12.6	14.2	17.0	21.7	24.1
	N	20	20	20	20	20	20	20	20
2F	Mean	71.9	113.3	154.0	185.7	211.3	232.3	248.0	262.4
	S.D.	8.8	9.2	9.2	10.0	13.3	15.1	16.0	17.6
	N	20	20	20	20	20	20	20	20
3F	Mean	69.7*	111.0	154.8	187.6	213.8	234.5	250.2	264.1
	S.D.	7.1	11.1	14.0	16.9	19.9	19.0	21.5	23.7
	N	20	20	20	20	20	20	20	20
4F	Mean	66.3**	107.3*	149.1*	182.2	208.6	227.4	243.8	258.4
	S.D.	7.9	9.9	13.0	15.4	19.2	19.9	19.9	21.1
	N	20	20	20	20	20	20	20	20

- statistically analysed * = p < 0.05 ** = p < 0.01 *** = p < 0.001

Bodyweight Gain in F1 Generation Rats Post-Weaning

Group : 1 2 3 4
 Test item : Control NKTR-118
 Dose (mg/kg/day) : 0 50 250 500

Group sex		Day number							
		Gain# 0-7	Gain# 7-14	Gain# 14-21	Gain# 21-28	Gain# 28-35	Gain# 35-42	Gain# 42-49	Gain# 49-56
1M	Mean	54.4	69.2	68.1	68.7	54.3	44.3	40.3	23.2
	S.D.	8.2	7.5	6.7	10.9	8.0	11.3	5.7	9.2
	N	20	20	20	20	20	20	20	20
2M	Mean	52.7	66.8	66.6	62.7	51.6	42.9	31.8*	21.8
	S.D.	4.3	4.3	6.3	6.4	6.5	7.2	7.8	6.2
	N	20	20	20	20	20	20	20	20
3M	Mean	49.5*	65.6*	66.0	66.4	52.9	40.9	38.2*	20.2
	S.D.	4.2	5.3	4.9	7.5	6.6	7.1	5.4	6.4
	N	20	20	20	20	20	20	20	20
4M	Mean	49.2*	63.1**	66.4	61.2**	50.1	38.1*	35.1*	22.9
	S.D.	4.8	6.1	6.6	7.8	7.3	7.3	5.7	6.2
	N	20	19	19	19	19	19	19	19

- statistically analysed * = p < 0.05 ** = p < 0.01 *** = p < 0.001

Group : 1 2 3 4
 Test item : Control NKTR-118
 Dose (mg/kg/day) : 0 50 250 500

Group sex		Day number				
		Gain# 56-63	Gain# 63-70	Gain# 70-77	Gain# 77-84	Gain# 0-84
1M	Mean	29.3	27.7	20.6	17.5	517.4
	S.D.	6.3	5.4	9.2	8.9	60.4
	N	20	20	20	20	20
2M	Mean	27.7	21.0**	14.1*	14.2	473.6**
	S.D.	8.1	8.4	6.9	7.3	43.8
	N	20	20	20	20	20
3M	Mean	28.6	18.2***	16.2*	12.5*	474.8**
	S.D.	7.3	7.4	6.3	8.4	38.8
	N	20	20	20	20	20
4M	Mean	24.8	20.3***	14.9*	10.9*	454.1***
	S.D.	6.5	5.3	5.7	5.4	41.3
	N	19	18	18	18	18

- statistically analysed *= $p < 0.05$ **= $p < 0.01$ ***= $p < 0.001$

Group : 1 2 3 4
 Test item : Control NKTR-118
 Dose (mg/kg/day) : 0 50 250 500

Group sex		Day pre-pairing							
		Gain# 0-7	Gain# 7-14	Gain# 14-21	Gain# 21-28	Gain# 28-35	Gain# 35-42	Gain# 42-49	Gain# 0-49
1F	Mean	41.7	41.4	30.3	27.5	20.9	15.0	15.1	191.7
	S.D.	6.1	6.0	6.3	4.9	7.7	6.8	6.1	25.5
	N	20	20	20	20	20	20	20	20
2F	Mean	41.4	40.7	31.7	25.6	21.0	15.8	14.4	190.5
	S.D.	4.7	4.2	5.1	8.0	6.1	5.6	5.8	15.4
	N	20	20	20	20	20	20	20	20
3F	Mean	41.3	43.8	32.8	26.2	20.7	15.7	13.9	194.4
	S.D.	5.1	4.9	4.4	6.3	7.0	4.9	5.2	20.6
	N	20	20	20	20	20	20	20	20
4F	Mean	41.0	41.8	33.1	26.5	18.8	16.4	14.7	192.2
	S.D.	3.7	4.7	6.3	6.3	4.9	7.0	4.2	15.7
	N	20	20	20	20	20	20	20	20

- statistically analysed

Time Course of Mating in F1 Generation Females

Group : 1 2 3 4
 Test item : Control NKTR-118
 Dose (mg/kg/day) : 0 50 250 500

Group	Number paired	N	Number of females mating on day after pairing																	Mean number# of days taken to mate ± S.D.				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		18	19	20	
1	20	20	1	5	7	4	1							1			1							4.0 ± 3.3
2	20	20	6	6	3	3	2																	2.5 ± 1.4
3	20	20	8	4	6	2																		2.1 ± 1.1
4	20	20	5	2	3	6	2								1						1			4.1 ± 4.0

N = number of animals in mean
 # = statistically analysed

Fertility and Mating Data in F1 Generation Adults

Group : 1 2 3 4
 Test item : Control NKTR-118
 Dose (mg/kg/day) : 0 50 250 500

Group	Sex	Number paired	Number mated	Number fertile	Copulation# index	Fertility# index
1	M	20	18	17	90.0	94.4
2	M	20	20	20	100.0	100.0
3	M	20	20	20	100.0	100.0
4	M	19	17	16	89.5	94.1

Group	Sex	Number paired	Number mated	Number fertile	Copulation# index	Fertility# index
1	F	20	20	19	100.0	95.0
2	F	20	20	20	100.0	100.0
3	F	20	20	20	100.0	100.0
4	F	20	20	19	100.0	95.0

= statistically analysed

Pregnancy Data in F1 Generation Females

Group : 1 2 3 4
 Test item : Control NKTR-118
 Dose (mg/kg/day) : 0 50 250 500

Number of females:	Group 1	Group 2	Group 3	Group 4
In group	20	20	20	20
Not pregnant (%)	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)
Died/killed	0	0	0	0
Survived to scheduled kill	1	0	0	1
Pregnant (%)	19 (95.0)	20 (100.0)	20 (100.0)	19 (95.0)
Died/killed/aborted	0	0	0	0
with total resorptions	2	0	0	0
with live foetuses at scheduled kill	17	20	20	19

Uterine and Implantation Data in F1 Generation Females

Group	:	1	2	3	4
Test item	:	Control		NKTR-118	
Dose (mg/kg/day)	:	0	50	250	500

	Group 1	Group 2	Group 3	Group 4
Number of females with implantations at scheduled kill	19	20	20	19
Number of corpora lutea	307	335	335	351
Mean number per female#	16.2	16.8	16.8	18.5*
Standard deviation	2.8	1.5	2.0	4.7
Number of implantations	267	322	326	312
Mean number per female#	14.1	16.1	16.3	16.4
Standard deviation	5.4	1.3	2.4	2.6
Mean % pre-implantation loss#	14.9	3.7	3.0	8.1
Number of early embryo/foetal deaths	15	19	26	22
Number of dead embryos	0	0	0	0
Mean % post-implantation loss#	14.8	6.0	10.0	6.8
Number of live embryos	252	303	300	290
Mean number per female#	13.3	15.2	15.0	15.3
Standard deviation	5.5	1.8	3.9	2.5
Mean % of implantations	85.2	94.0	90.0	93.2

= statistically analysed

* = p < 0.05

** = p < 0.01

*** = p < 0.001

F₂ Generation (No F₂ generation animals were evaluated)

Survival: N/A

Body weight: N/A

External evaluation: N/A

Male/Female ratio: N/A

Other: N/A

10 Special Toxicology Studies**NKTR-118: 14-day Oral (gavage) Study in Male Sprague-Dawley Rats to Investigate Cytochrome P450 Enzyme Induction (Study No.: 3368DR)****Objective:** The objective of this study was to investigate whether cytochrome P450 induction is a potential mechanism contributing to increased incidence of Leydig cell

tumors in the 2-year oral carcinogenicity study in rats. The Sponsor suggested that CYP induction may lead to increased testosterone clearance and subsequent increase in the incidence of Leydig cell tumors.

Methods: In the main study, male Sprague Dawley rats (CrI:CD(SD)) (10/group), 6-7 weeks of age, were treated with 0 (vehicle), 400, or 800 mg/kg/day NKTR-118 by oral gavage for 14 days. The 400 mg/kg/day and 800 mg/kg/day doses represent the highest dose used in the 2-year oral rat carcinogenicity study and the 26-week oral rat chronic toxicity study, respectively. In the toxicokinetic groups, rats were treated with 0 (3/group), 400 (5/group), or 800 mg/kg/day (5/group) NKTR-118 by oral gavage for 14 days. Animals were observed daily and physical examination was conducted at least once weekly. Body weights were recorded at randomization and daily from week -1 to day 15. Food consumption in the main study animals was recorded daily for each cage of animals. Main study animals were sacrificed for scheduled necropsy one day after the final dose was given. The body weights and macroscopic abnormalities were recorded. The liver was weighed intact. Microsomes were prepared from the liver samples, and levels of CYP1A, CYP2B, CYP3A and CYP4A were determined by an enzyme-linked immunoassay (ELISA).

Blood samples in the TK group were collected from the tail vein from animals (3/dose/time-point) at 0.25, 3, 6, 12, and 24 hours post-dose on days 1 and 14. Blood samples from control animals were collected at 0.25 and 24 hours post-dose.

Results: There were no deaths. Clinical signs were limited to increased incidence and frequency of salivation in the 400 and 800 mg/kg/day animals starting from day 5. There were no meaningful drug-related effects on body weight or bodyweight gain.

Food consumption was decreased in the 800 mg/kg/day group on day 1 (-13%) and day 2 (-21%) and on occasions during the remainder of the dosing period, compared to pre-dose values. However, at sacrifice there was only an 8% decrease in food consumption in the 800 mg/kg/day group compared to controls. There were no drug-related changes in organ weights or macroscopic and microscopic findings.

There was no induction of hepatic microsomal CYP1A, CYP2B, CYP3A, or CYP4A proteins in either treatment group, compared to the control group. The results are summarized in the following table taken from the Sponsor's study report.

Table 2: Group mean concentrations of CYP protein in liver microsomes

Group	CYP1A		CYP2B		CYP3A		CYP4A	
	Conc. pmol/ mg	Fold Change	Conc. pmol/ mg	Fold Change	Conc. pmol/ mg	Fold Change	Conc. pmol/ mg	Fold Change
Male Animals								
1	20.00	1.00	152.19	1.00	78.56	1.00	23.36	1.00
2	11.92	0.60	64.57	0.42	53.68	0.68	25.11	1.08
3	11.15	0.56	73.23	0.48	65.57	0.83	28.49	1.22

Fold change compared to the concentration seen in the relevant controls (no change = 1.00).

Systemic NKTR-118 exposure was demonstrated in all rats receiving NKTR-118. The exposure of NKTR-118, in terms of C_{max} and AUC_{0-24} , generally increased with dose in a greater than dose proportional manner. Potential accumulation of NKTR-118 was observed after multiple dosing. The TK data are summarized in the table below (taken from the Sponsor's study report).

Table 1 Summary of toxicokinetics

	NKTR-118			
	400 mg/kg/day		800 mg/kg/day	
	Day 1	Day 14	Day 1	Day 14
T_{max} (h)	0.25	6.0	0.25	0.25
Mean C_{max} $\mu\text{mol/L}$	15.0	20.9	44.9	60.0
Mean $AUC(0-24h)$ $\mu\text{mol}\cdot\text{hr/L}$	112	224	371	611

Conclusions: There was no induction of hepatic microsomal CYP1A, CYP2B, CYP3A, or CYP4A in male Sprague-Dawley rats treated with 400 or 800 mg/kg/day NKTR-118 for 14 days. The maximum levels of exposure to NKTR-118 in these animals (C_{max} = 60.0 $\mu\text{mol/L}$, AUC_{0-24} = 611 $\mu\text{mol}\cdot\text{h/L}$) on day 14 are comparable to those achieved in the 2-year oral carcinogenicity study in rats at the highest dose level in males (C_{max} = 48.8 $\mu\text{mol/L}$, AUC_{0-24} = 364 $\mu\text{mol}\cdot\text{h/L}$).

Analysis of Luteinizing Hormone (LH) and Testosterone After a Single Intravenous Bolus Dose of Naloxone in Male Han Wistar and Sprague Dawley Rats (Study No.: 3311KR)

Objectives: The purpose of this study was to identify a rat model suitable for investigating opioid-mediated changes in systemic luteinizing hormone (LH) and

testosterone levels after a single bolus injection of naloxone, an opioid receptor antagonist.

Methods: Male Han Wistar and Sprague Dawley rats were surgically cannulated in the femoral vein (for sample collection) and the jugular vein (for dosing) and allowed at least 7 days of recovery prior to dosing on day 1. The surgeries were performed at the vendor's facility (b) (4). On day -1 (one day prior to dosing), the rats were checked for general health, body weight and catheter patency. The rats were anesthetized with isoflurane on day -1, placed in jackets, and attached to a tether/swivel system. The rats were housed in their home cage during the dosing period. The study design is summarized in the table below (taken from the Sponsor's study report).

Study Design

Table 1 Dose groups and animals

Group	Compound/ Vehicle	Animal reference numbers	Route	Dose levels (mg/kg)	Dose volume (mL/kg)	Concentration of dosing formulation (mg/mL)
1	Vehicle (HW)	1-5M	IV	0	2	0
2	Vehicle (SD)	6-10M	IV	0	2	0
3	Naloxone (HW)	11-15M	IV	2	2	1
4	Naloxone (SD)	16-20M	IV	2	2	1

HW: Han Wistar rats

SD: Sprague Dawley

On day 1 (the day of dosing), animals in Groups 1 and 2 were administered an IV bolus of vehicle solution at 2 mL/kg. Animals in Groups 3 and 4 were administered an IV bolus dose of 2 mg/kg naloxone. Blood samples were collected from the femoral vein for LH and testosterone analysis.

Results and Conclusions: Intravenous administration of naloxone (2 mg/kg) in SD rats resulted in a statistically significant increase in LH, with peak plasma concentrations at approximately 60 minutes post-dose and a subsequent statistically significant increase in peak testosterone concentration at approximately 90 minutes post-dose.

There was an increase in systemic LH in HW rats following IV administration of naloxone. However, the increase was not statistically significant and was not as robust as that in the SD strain. The Sponsor suggested that the absence of a statistically significant LH response in the HW rats may be due to high background levels of LH in plasma, which in combination with the blood sampling regimen used, may have prevented the detection of a transient increase in LH. The Sponsor indicated that despite the lack of a significant LH response, a testosterone response was detected.

The dual cannulated Sprague Dawley rat model was judged by the Sponsor to be suitable to investigate opioid-induced changes in plasma LH and testosterone concentrations up to 3-5 times the baseline levels.

Investigative Maximum Tolerated Dose of Methylnaltrexone and Analysis of Luteinizing Hormone (LH) and Testosterone by a Single Intravenous Bolus Injection in Sprague Dawley and Han Wistar Rats (Study No.: 3381KR)

In a study that was almost identical to the study described above (Study No.: 3311KR), the dual cannulated Sprague Dawley and Han Wistar rat models were tested using methylnaltrexone, another peripheral opioid antagonist. Intravenous administration of 3 mg/kg methylnaltrexone in SD and HW rats resulted in statistically significant increases in LH (peak plasma concentrations at ~10 minutes post-dose), with no significant changes in testosterone plasma levels in either strain. Strain comparisons also did not yield any significant differences in the magnitude of changes in LH and testosterone levels in animals treated with methylnaltrexone. The Sponsor concluded that the dual cannulated SD rat model is more sensitive in detecting changes in systemic LH than testosterone. Based on the results of this study and data obtained from the previous study (No. 3311KR), the Sponsor concluded that the dual cannulated SD rat model is suitable for investigating opioid-mediated changes in systemic LH.

Analysis of Luteinizing Hormone (LH) and Testosterone After Intravenous (IV) Infusion of Naloxegol in Male Sprague Dawley Rats (Study No.: 3533KR)

Objectives: In a 2-year oral NKTR-118 carcinogenicity study in rats, it was demonstrated that long term exposure of NKTR-118 was associated with a dose-dependent increase in the incidence of Leydig cell adenoma and hyperplasia. The purpose of this study was to investigate, in a dual cannulated male Sprague-Dawley rat model, whether an acute high dose naloxegol, at an exposure similar to that in the 2-year carcinogenicity study, affects LH and testosterone secretion. The Sponsor's hypothesis is that a prolonged increase in LH leads to Leydig cell tumor formation in male rats.

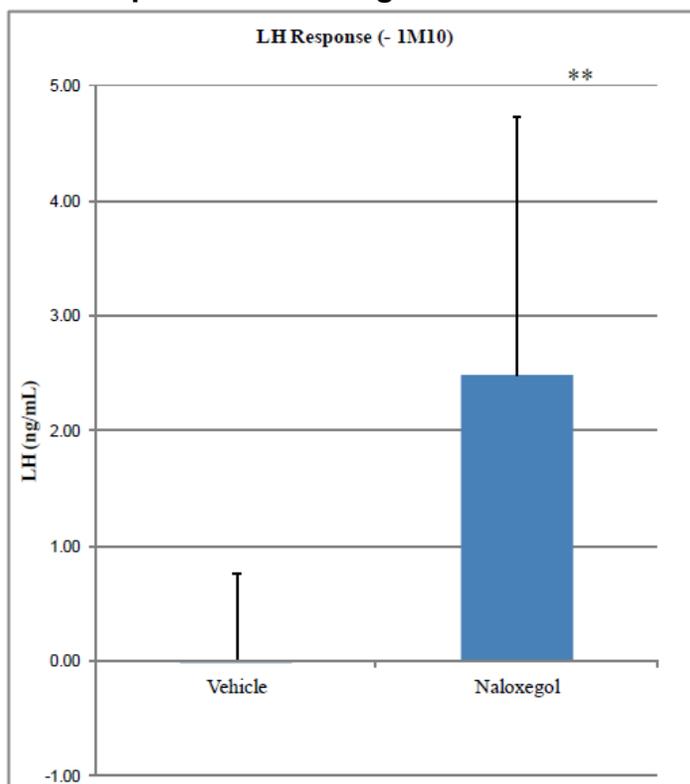
Methods: Male Sprague Dawley rats were surgically cannulated in the femoral vein (for sample collection) and the jugular vein (for dosing) and allowed at least 7 days of recovery prior to dose day 1. On day -1, the animals were placed in jackets and attached to a tether/swivel system in individual cages. On day 1, animals (10/group) were administered 0 (vehicle) or 80 mg/kg NKTR-118 by IV infusion via the jugular vein cannula over a period of 10 minutes. Blood samples were collected at 30 and 5 minutes pre-dose and at 5, 10, 15, 30, 45, 60, and 180 minutes post IV dosing for LH and testosterone analysis. In the toxicokinetic groups, blood samples were collected from the control animals (10/group) at the 60-minute time point, and from the NKTR-118 treated animals (10/group) at the 10, 60, and 120 post-dose. Plasma concentrations of NKTR-118 were measured by solid phase extractions and LCMS/MS using a validated assay.

Results: There were no deaths during the study. A 10-minute infusion of naloxegol at 80 mg/kg was well tolerated and the animals did not display any adverse effects. The LH response was determined by normalizing to the 5-minute pre-dose time point (post-dose concentration minus the 5 minute pre-dose concentration). LH levels were statistically higher compared to controls at the 15 minute (3.37 ng/ml, $p=0.034$) and 45 minute (2.2 ng/ml, $p=0.041$) time points. The results are summarized in the table below (taken from the Sponsor's report).

Table 3 Statistical data of LH response (post-dose minus 5-minute pre-dose)

Time (Minutes)	t Value	Pr > t	Significance
5	0.69	0.5018	
10	0.14	0.8911	
15	3.37	0.0034	**
30	-0.16	0.8727	
45	2.2	0.041	*
60	1.91	0.0721	
180	-0.22	0.8265	

Summation of the levels at all the individual time points was used to determine LH response. All animals were included in the analysis with the exception of one animal due to a missing data point. As shown in the table below (taken from the Sponsor's report), the naloxegol-treated group showed significantly higher plasma concentrations of LH ($p=0.0015$), compared to controls.

LH response in Naloxegol-Treated SD Rats

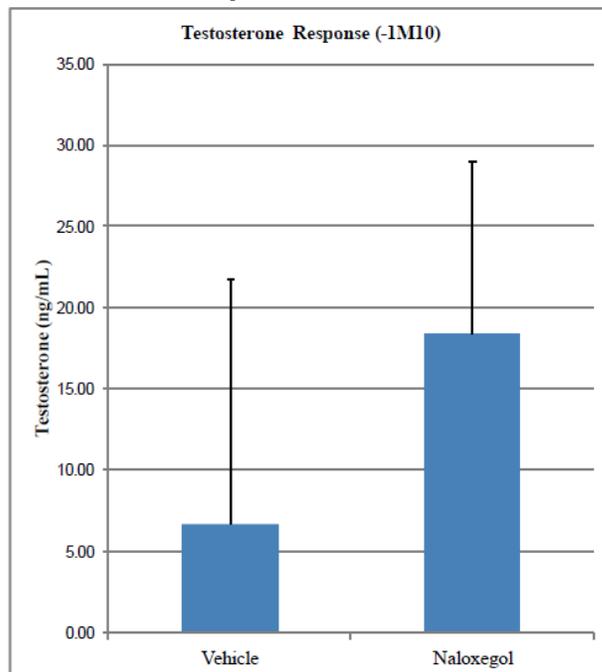
The testosterone response also was determined by subtracting the 5-minute pre-dose concentration from the 5, 10, 15, 30, 45, 60, and 180-minute post-dose values, followed by summation of all the individual time points. All animals were included in the analysis with the exception of one animal, in order to be in line with that of the LH analysis. Statistically significant increases in testosterone levels, compared to controls, were noted at the 45 minute (2.18 ng/ml, $p=0.0442$) and 180 minute (2.15 ng/ml, $p=0.0472$) time points, as shown in the Sponsor's table below.

Table 4 Statistical data of testosterone response (post-dose minus 5-minute pre-dose)

Time (Minutes)	t Value	Pr > t	Significance
5	-1.28	0.22	
10	-0.6	0.5546	
15	0.28	0.7806	
30	0.74	0.4726	
45	2.18	0.0442	*
60	2.01	0.0618	
180	2.15	0.0472	*

However, summation of the testosterone levels from all the individual time points showed an increase that was not statistically significant.

Testosterone Response in NKTR-118-Treated Rats



Mean plasma NKTR-118 concentrations in the control and NKTR-118-treated rats are summarized in the Sponsor’s table below.

Table 5 Summary of mean plasma concentrations (±SD) of naloxegol in SD rats after 10-minute IV infusion

Group	Dose level (mg/kg)	Sample time point (Minutes)	Plasma concentration (µmol/L)
1	0	60	NQ
2	80	10	55.6 ± 8.03
		60	5.83 ± 1.28
		120	2.05 ± 0.57

NQ Not Quantifiable

As shown in the Sponsor’s table below, the intravenous dosing reproduced the maximum plasma NKTR-118 concentrations observed at week 52 in the 400 mg/kg/day males in the 2-year rat carcinogenicity study. This group showed an increased incidence of benign interstitial cell adenoma of the testis (b) (4) Study No. 7985-111). However, in the present study, plasma NKTR-118 following infusion declined rapidly and did not reproduce the total exposure observed in the carcinogenicity study.

Table 3 Comparison of C_{max} values (mean ± SD; n) between 3533KR and LS-2008-007 in male rats

	3533KR IV infusion 80 mg/kg	LS-2008-007 oral chronic dosing at 400 mg/kg*
C _{max} (μmol/L)	55.6 ± 8.03; 10	55.5 ± 9.48; 3
AUC _∞ (μmol.h/L)	35.3 ± 5.58; 10	414 [§]

* Data converted using 652 ng/mL is equivalent to 1 μmol/L
[§] Composite AUC value

Conclusions: The opioid system exerts a tonic inhibition on the gonadotropin-releasing hormone (GnRH) neurons. A release of this inhibition, for example by opioid receptor antagonists such as naloxone, causes a change in GnRH release and, consequently, pituitary LH secretion. Male rats are known to be sensitive to changes in LH. The Sponsor hypothesized that, as a result of chronic exposure to NKTR-118, rats develop Leydig cell tumors in response to prolonged increase in LH. The results from this study demonstrate that plasma LH increased significantly after a high dose (80 mg/kg) infusion of NKTR-118. Plasma levels of testosterone also increased, but to a lesser extent. Taken together, the results are consistent with the hypothesis that the drug-related increase in the incidence of benign Leydig cell adenoma and hyperplasia observed in the carcinogenicity study in rats may be due to NKTR-118-induced hormonal changes.

11 Integrated Summary and Safety Evaluation

Opioid analgesics are the mainstay in the treatment of moderate-to-severe pain; however, their use is frequently associated with opioid-induced constipation (OIC). Naloxegol (NKTR-118) is an opioid receptor antagonist that has been developed as an oral treatment for opioid-induced constipation in adult patients with chronic non-cancer pain. Naloxegol is a PEGylated derivative of naloxone. The proposed dose regimen is 25 mg orally once daily, or 12.5 mg once daily for patients who are taking moderate CYP3A4 inhibitors.

Primary pharmacological action of naloxegol was assessed in a number of *in vitro* and *in vivo* assays. Competitive receptor-ligand binding studies were performed in CHO cells expressing cloned human opioid receptors. Naloxegol binds to μ-, δ- and κ-opioid receptors, with highest affinity at the human μ-opioid receptor (K_i = 33.8 nM); the naloxegol affinity for μ-opioid receptors was 20-fold lower than that of naloxone. Reduced binding affinity for naloxegol, compared to naloxone, was also observed at the human κ- and δ-opioid receptors (47-fold and 5-fold lower, respectively). For naloxegol, the rank order of affinity for opioid receptors was μ>δ>κ, whereas the rank order for naloxone was μ>κ>δ. It is noted that the naloxegol binding affinity is not markedly different among the cloned human opioid receptors, with a μ receptor affinity that exceeds the δ and κ receptor affinity by only 1.6- and 5.5-fold, respectively. Opioid receptor affinities for naloxegol and methylnaltrexone, a marketed peripherally-acting μ-opioid receptor antagonist, were compared in membrane preparations of cells

expressing cloned human μ - and δ -opioid receptors and cloned rat κ -opioid receptors. Naloxegol was 3- and 9.4-fold more potent than methylnaltrexone at human μ - and δ -opioid receptors, respectively, and naloxegol was equipotent with methylnaltrexone at rat κ -opioid receptors. The rank order of affinity for opioid receptors was $\mu=\kappa>\delta$ for naloxegol, whereas the rank order for methylnaltrexone was $\kappa>\mu>\delta$. In opioid receptor functional assays that measured [35 S]GTP γ S binding to receptor-associated G-proteins, naloxegol alone had no effect on the binding of [35 S]GTP γ S from interactions with the δ -opioid receptor, while it decreased the response to a reference δ -opioid receptor agonist. This indicates that naloxegol is a neutral or pure antagonist, and has no effect on the constitutive activity of the δ -opioid receptor. By contrast, naloxegol alone induced a concentration-dependent increase in κ -opioid receptor activity in the [35 S]GTP γ S assay; however, the maximum response induced was only 39% of the reference agonist. Therefore, naloxegol is an antagonist of the δ -opioid receptor, but exhibits partial agonist activity at the κ -opioid receptor in this receptor function assay. In the field-stimulated rabbit vas deferens, the reference κ -opioid receptor agonist produced a decrease in the twitch contraction amplitude, whereas naloxegol at concentrations up to 10 μ M did not. Thus, naloxegol exhibited no apparent functional agonist activity at κ -opioid receptors in this *ex vivo* assay. Naloxegol was further characterized for its competitive antagonism at human μ -opioid receptors in Schild-type experiments to evaluate the ability to elicit rightward-shifts in the concentration-response curve of morphine in [35 S]GTP γ S binding assays. Both naloxegol and methylnaltrexone elicited parallel rightward-shifts in the morphine dose-response curve with no reduction in E_{max} . Naloxegol was 3.4-fold more potent than methylnaltrexone as an antagonist at human μ -opioid receptors. The difference in functional μ -opioid receptor antagonist potency was comparable to that seen in the μ -opioid receptor binding assay.

Naloxegol at 90 mg/kg po produced complete inhibition of morphine-induced impairment of GI transit in rats, whereas naloxone as low as 10 mg/kg po produced a similar effect. ED_{50} values derived from the dose-response modeling of the antagonist effect against morphine in the GI tract were 23.1 and 0.69 mg/kg for NKTR-118 and naloxone, respectively. Naloxegol at doses of 10 and 30 mg/kg produced no significant reversal of analgesia. However, partial reversal of analgesia was observed at 90 mg/kg. By contrast, naloxone produced a complete reversal of analgesia at 10 mg/kg, and partial reversal of analgesia was noted at doses starting at 1 mg/kg. A comparison of the effects on GI transit and analgesia shows that the dose-response curves for naloxone overlap, indicating that there is little or no separation between the central and peripheral antagonist effects of naloxone. By contrast, there was a small separation between the dose curves for central and peripheral antagonist effects of naloxegol.

The secondary pharmacology profile of naloxegol was assessed in a panel of 327 *in vitro* radioligand binding and enzyme assays. Naloxegol at 10 μ M inhibited 98%, 97%, and 90% of the radioligand binding at μ , κ , and δ opioid receptors, respectively. Naloxegol produced no significant activity in the rest of the 324 radioligand binding and enzyme assays at a concentration of 10 μ M. Naloxegol also exhibited insignificant

effects on seven cardiac ion channels at concentrations up to 100 μM . Therefore, naloxegol appears to be relatively specific for opioid receptors. Potential effects of naloxegol on the central nervous system (CNS), including effects on abuse liability and drug dependence, were evaluated in a battery of *in vivo* safety pharmacology and nonclinical toxicology studies. The effects of naloxegol on gross behavioral and physiological parameters were assessed using a modified Irwin test in conscious rats. No changes in behavioral scoring indicative of CNS adverse effects were observed at doses up to 1000 mg/kg (347 times the human C_{max} at the MRHD). The behavioral effects of naloxegol were also assessed during a 28-day oral toxicity study in rats. No statistically significant effects were seen at doses up to 500 mg/kg (up to 796 times the human AUC at the MRHD (maximum recommended human dose)).

Potential proconvulsant and analgesic effects of naloxegol were assessed using a rat PTZ (pentylenetetrazole) model and a mouse grid stimulation analgesia model, respectively. Naloxegol produced no proconvulsant or analgesic effects at doses up to 1000 mg/kg. Based on similar studies using the same dose and species, 1000 mg/kg naloxegol is estimated to give an exposure multiple of 347- and 289-times the human C_{max} at the MRHD for the rat PTZ proconvulsant and mouse analgesia models, respectively. The abuse potential of naloxegol was assessed using a battery of animal models consisting of drug discrimination, self-administration, and drug dependence/withdrawal studies (these studies were reviewed by Katherine Bonson of the Controlled Substance Staff). The potential of naloxegol to produce psychoactive effects of the type mediated by centrally acting μ -opioid receptor agonists was assessed using a rat morphine drug discrimination assay. Naloxegol produced no morphine-like discriminative effects up to 1000 mg/kg; this dose is estimated to give an exposure multiple of 347 times the human C_{max} at the MRHD. Naloxegol produced a dose-dependent reduction in the psychoactive effects of morphine at 30 and 300 mg/kg, showing that it acts as a μ -opioid antagonist with considerable CNS exposure of naloxegol in rats at these doses. Based on similar studies using the same dose and species, 30 and 300 mg/kg are estimated to give exposure multiples of 15- and 112-times the human C_{max} at the MRHD. The physical dependence liability of naloxegol was investigated in rats by assessing withdrawal symptoms during and following a 15-day dosing period. Naloxegol produced no withdrawal signs suggestive of physical dependence at doses up to 500 mg/kg, with an exposure multiple 266 times the human C_{max} at the MRHD. The abuse potential of naloxegol was also tested in a rat intravenous self-administration assay. The amount of naloxegol self-administered was not different from vehicle at doses up to 30 mg/kg. This is estimated to give an exposure multiple 146 times the human C_{max} at the MRHD.

The effects of naloxegol on the cardiovascular system were assessed using a variety of *in vitro*, *ex vivo*, and *in vivo* assays measuring a number of cardiovascular parameters. The IC_{50} for the inhibition of the hERG potassium current was $>300 \mu\text{M}$. Naloxegol was inactive at 7 cardiac ion channels, and had no effect on contractility parameters in canine ventricular myocytes at up to 100 μM . In addition, naloxegol at up to 10 μM produced no significant effects on cardiac contractile function in a rat isolated heart model. Taken together, these *in vitro* and *ex vivo* data suggest that naloxegol produces

insignificant direct effects on cardiac ion channels or contractile function of the cardiac cells or isolated hearts. In the conscious dog telemetry model, oral doses of naloxegol >5 mg/kg were associated with moderate decreases in arterial blood pressure, left ventricular systolic pressure, and indices of cardiac contractility and relaxation, compared to controls. The NOEL for cardiovascular effects was considered to be 5 mg/kg. This dose corresponds to a measured C_{max} of 0.152 $\mu\text{mol/L}$ or 99 ng/ml, which is close to the measured human C_{max} of 80 ng/ml at the MRHD. No cardiovascular effects, including blood pressure and ECG parameters, were noted at doses up to 500 mg/kg in the 14-day, 1-month, and 9-month repeated-dose toxicology studies in dogs.

The effect of naloxegol on respiratory parameters was assessed in conscious rats using whole body plethysmography. Naloxegol produced no effects on any respiratory parameters at doses up to 1000 mg/kg (347 times the human C_{max} at the MRHD). In addition, naloxegol had no effects on respiratory parameters measured in a 1-month repeated dose toxicology study in dogs at doses up to 500 mg/kg/day (193 times the human AUC at the MRHD).

The effects of naloxegol on the gastrointestinal system were evaluated by measuring effects on gastric emptying and small intestinal transport in conscious rats. Naloxegol at ≥ 100 mg/kg produced a significant increase in stomach weight, and at 1000 mg/kg/day completely inhibited intestinal transport. The NOEL for decreased gastric emptying and decreased intestinal transport was 30 mg/kg and 300 mg/kg, respectively. These doses correspond to estimated C_{max} values of 1220 (15 times the human C_{max} at the MRHD) and 9000 ng/mL (113 times the human C_{max} at the MRHD), respectively.

Potential effects of naloxegol on the renal system were assessed in rats. Dose-related changes in clinical chemistry parameters were seen in urine at doses above 100 mg/kg. However, the effects at doses up to 300 mg/kg were small, and not considered to be adverse. Therefore, the NOAEL is considered to be 300 mg/kg. The doses correspond to estimated C_{max} values of 9000 ng/mL (113 times the human C_{max} at the MRHD).

The pharmacokinetics of naloxegol was assessed in a battery of *in vitro* and *in vivo* studies. Comparative studies in Caco2 cells showed that naloxegol, a PEGylated derivative of naloxone, had reduced permeability compared to naloxone, and that naloxegol was a Pgp efflux substrate, unlike naloxone. An *in situ* permeability study in rat jejunal sections suggested that naloxegol exhibited moderate intestinal permeability. Single dose pharmacokinetic studies showed a high volume of distribution and clearance leading to half-lives of approximately 3, 6 and 5 hours in rat, dog and monkey respectively. The bioavailability ranged from 21% in dogs to 2% in monkeys. In repeated-dose safety studies, high exposures to naloxegol were achieved in all species. Exposure was higher in male mice and female rats, but there was no sex-related difference in dogs. Plasma exposure in all species was dose proportional or sometimes more than dose proportional at high doses. There was only small accumulation, generally less than 2-fold, on repeated dosing.

Plasma protein binding of naloxegol was low in all species examined, ranging from 4.2% in humans to 14.1% in mice, 20.8% in rats, and 9.7% in monkeys; it was concentration dependent only in dogs. Because the fraction bound to plasma protein was less than 50% in all cases, the Sponsor calculated exposure margins using total plasma concentrations. There was little association of naloxegol-related radioactivity with cellular components of the blood in rats and dogs. The distribution of naloxegol-related radioactivity in rats was studied by quantitative whole body autoradiography after oral administration of [¹⁴C]NKTR-118. There was wide distribution of total radioactivity in all tissues; the highest levels were in the liver, kidney, glandular tissues, and pigmented tissues. Radioactivity was retained in eyes and skin, suggesting that naloxegol binds to melanin. It is noted that naloxegol does not absorb light within the range of 290-700 nm, and therefore, even though naloxegol is distributed in eyes and skin in rats, there is no concern about the potential for phototoxicity. Distribution of radioactivity into the brain and spinal cord was low, suggesting relatively low CNS penetration of naloxegol in rats. *In vivo* single-pass brain penetration study in rats showed that the penetration of naloxegol was approximately 15 times slower than that of naloxone. The data in pregnant female rats showed distribution of radioactivity in the placenta and fetal tissues, suggesting trans-placental transfer of naloxegol and/or its metabolites. Radioactivity in fetal liver and fetal uvea was lower than that in the mother, and radioactivity in the fetal brain was lower than that in the liver or uveal tract. Measurements using a preliminary assay for naloxegol in rat milk showed high concentrations of naloxegol in milk (NKTR-118: Verification of a Method for the Determination of NKTR-118 in Rat Milk by HPLC with MS/MS Detection and Exploratory Analysis of NKTR-118 in Rat Milk Samples from Sequani Limited Study Number ASU0139), suggesting efficient transfer of naloxegol into milk.

Metabolism studies using cultured hepatocytes and *in vivo* cross-species comparison of metabolic profiles of naloxegol in humans, dogs, rats, and mice showed that all metabolites detected in humans were also present in the animal species tested. In animal studies, naloxegol was metabolized mainly through O-glucuronidation, N-dealkylation, and shortening of the PEG-chain. In humans, naloxegol was metabolized mainly through shortening of the PEG-chain, N-dealkylation, and oxidation. In plasma and urine, seventeen and sixteen metabolites were identified, respectively. The most abundant component in both plasma and urine was naloxegol, at approximately 80% of the mass chromatogram, with no other component representing >10% of the mass chromatogram. The Sponsor noted that the metabolism of the PEG chain produces structures with terminal hydroxyl groups or terminal carboxylic acids with chain lengths of 1 to 7 ethylene glycol units, but not complete removal of the chain. Repeated-dose studies showed that the four most abundant circulating metabolites in humans were M1, M7, M10, and M13. AUC multiples of 6- to 205-fold of these metabolites were demonstrated at the NOAELs of the repeated-dose animals studies. Naloxegol glucuronide, which is a major metabolite in animals, was not present at significant levels in humans. *In vitro* studies with expressed enzymes showed that the 7 primary metabolites observed were mainly formed by CYP3A4/5.

After oral administration of naloxegol to rats and dogs, there was high total recovery of radioactivity (>93%) in excreta. The elimination was mostly fecal (63-80% dose), with urinary elimination accounting for 25% of the dose in dogs and 19% and 30% in male and female rats, respectively. The recovery was largely complete by 24 hours in rat and approximately 70% was recovered by 48 hours in dog. There was high biliary elimination in cannulated male rats (66% dose), which together with a 13% urinary excretion indicated that about 80% of the dose was absorbed. It was noted that in humans, the mean total recovery was about 84%, with 68% recovered in feces and 16% in urine.

No lethality was observed in acute toxicity or single-dose PK studies using doses up to 2000 mg/kg po in mice and rats, 100 mg/kg IV in rats, 20 mg/kg po in dogs, or 0.4 mg/kg IV in dogs. It is noted that the drug substance in the to-be-marketed drug product (Movantik) will be naloxegol oxalate, whereas naloxegol free base was used in almost all of the toxicology studies. The Sponsor chose the oxalate salt for marketing due to its improved stability relative to the free base. In an oral tolerability study in Sprague Dawley rats, animals (3/sex/group) were administered 0 (vehicle), 500, 1000, 1500, or 2000 mg/kg NKTR-118 free base, or 500 or 1000 mg/kg NKTR-118 oxalate by oral gavage. The dose levels for the oxalate salt groups are expressed as free base equivalent. Notable clinical signs included decreased motor activity, half-shut eyes, pilo-erection, and respiration changes in the 2000 mg/kg NKTR-118 free base group, and half-shut eyes and pilo-erection in male rats given 1000 mg/kg NKTR-118 oxalate. Exposure to NKTR-118 was generally similar after dosing with 500 or 1000 mg/kg of NKTR-118 free base or NKTR-118 oxalate. Thus, NKTR-118 free base and NKTR-118 oxalate showed similar effects in rats under the study conditions. Oxalate, or oxalic acid, is a substance of endogenous origin which is present in all mammalian species and plants. It is estimated that the average daily dietary intake in an individual is about 150 mg/day. Daily oxalate intake from a tablet containing 28.5 mg naloxegol oxalate is 3.5 mg, or less than 3% of the daily average individual dietary intake. Therefore, there is no safety concern regarding the use of the oxalate salt of naloxegol in the commercial formulation.

Subchronic and chronic toxicity studies were conducted in rats, mice, and dogs. In the 7-day oral toxicity study in Sprague-Dawley rats, animals (5/sex/group) were administered 0 (vehicle), 100, 500, or 1000 mg/kg/day NKTR-118 by oral gavage. Animals in the toxicokinetic group (5/sex/group) were administered 100 or 1000 mg/kg/day NKTR-118. Increased adrenal weights in the 1000 mg/kg/day group correlated with histopathological findings of vacuolation of the adrenal cortical cells (also observed in the 500 mg/kg/day males). Increased liver weights in the 500 and 1000 mg/kg/day group (both sexes) were associated with diffuse hepatocellular hypertrophy (females only). The NOEL was considered to be 100 mg/kg/day, which corresponds to the AUC_{0-24hr} of 13,100 and 53,400 ng·hr/mL, and C_{max} of 2,370 and 5,080 ng/ml in males and females, respectively.

In the 1-month oral toxicity study in Sprague-Dawley rats, main study animals (10/sex/group) were administered 0 (vehicle), 50, 150, or 500 mg/kg/day NKTR-118 by

oral gavage for 28 days. Apparent drug-related increases in cholesterol levels were noted in the 150 mg/kg/day males and 500 mg/kg/day males and females, and higher triglyceride levels were noted in all drug-treated groups. The changes were reversible at the end of the 14-day recovery period. The NOAEL was considered to be 500 mg/kg/day NKTR-118, which corresponds to the AUC_{0-24hr} of 262,660 and 372,020 ng·hr/mL, and C_{max} of 23,444 and 36,341 ng/ml NKTR-118 in males and females, respectively, on day 28.

In order to support dose selection in a carcinogenicity study in rats, a 3-month oral toxicity study in rats was conducted to establish a maximum tolerated dose. Animals (10/sex/group) were treated with 0 (vehicle), 50, 400, 600, or 800 mg/kg/day NKTR-118. There were five deaths: one 50 mg/kg/day male, one 800 mg/kg/day male, one 400 mg/kg/day male and female, and one 600 mg/kg/day female. The deaths were not considered as drug-related due to a clear lack of dose-dependence. Clinical signs included clear oral discharge, yellow staining of the perineal area, and rough haircoat. Two females given 400 and 800 mg/kg/day were observed with palpable masses starting on days 92 and 77, respectively, of the dosing phase. These masses were diagnosed as neoplasms based on microscopic examination, and classified as a mammary carcinoma in the 400 mg/kg/day female and a myxoma of the skin in the 800 mg/kg/day female. Decreases in bodyweight gain in the 400, 600, and 800 mg/kg/day males were not associated with significant changes in food consumption. In addition, clinical chemistry results showed significant increases in cholesterol levels in males and females dosed with ≥ 400 mg/kg/day of NKTR-118. There were no macroscopic or microscopic findings. Based on the decrease in bodyweight gain (12%) in the 800 mg/kg/day males, the NOAEL and MTD in males was determined to be 600 mg/kg/day. The NOAEL for females was 800 mg/kg/day based on a lack of drug-related adverse findings, and a MTD could not be defined in females.

In the 26-week oral toxicity study in rats, animals (15/sex/group) were treated orally with 0 (vehicle), 50, 200, or 800 mg/kg/day NKTR-118 for 26 weeks. Recovery animals (10/sex/group) were treated orally with 0 or 800 mg/kg/day NKTR-118 for 26 weeks, followed by a 4-week recovery period. There were 8 unscheduled deaths; however, none of the deaths appear to be drug-related. Drug-related clinical signs included colored and rough hair coats, minor skin lesions, and hunched appearance in the 800 mg/kg/day group, excessive salivation in the 200 and 800 mg/kg/day groups, and abnormal respiration in the 200 and 800 mg/kg/day males; these clinical signs were reversible after the recovery period. Bodyweight and bodyweight gains at the end of the dosing phase were reduced by 9.4% and 14.5%, respectively, in the 800 mg/kg/day males. The bodyweight changes were not correlated with any decrease in food consumption. Increases in cholesterol levels, up to 75% compared to controls, were noted in the 200 and 800 mg/kg/day groups. Increases in liver weight in the 200 and 800 mg/kg/day females correlated with dose-dependent hepatocellular hypertrophy. The increase in liver weight in the females was partially reversible after the recovery period. Other microscopic findings included pancreatic atrophy in the 800 mg/kg/day group, and dilatation of the uterus in the 800 mg/kg/day females; the changes were partially reversed after the recovery period. Carcinoma of the mammary gland was

noted in one female in each of the 200 and 800 mg/kg/day group. A metastatic carcinoma of unknown origin was noted in the mandibular lymph node of one main study 800 mg/kg/day female, and a tumor (astrocytoma) was noted in the brain of one main study 800 mg/kg/day female. Pituitary adenoma of the pars distalis was noted in the 800 mg/kg/day group (1 male and 1 female). The NOAEL is considered to be 50 mg/kg/day based on observed changes in clinical signs, cholesterol levels, and pancreatic atrophy in males and females, and microscopic liver findings in females at doses \geq 200 mg/kg/day.

In order to support dose selection in a carcinogenicity study, a 3-month oral toxicity study in mice was conducted to establish a maximum tolerated dose. Animals (10/sex/group) were treated with 0 (vehicle), 50, 400, 600, or 800 mg/kg/day NKTR-118. A total of 9 main study animals were found dead or sacrificed prematurely (two each of the 800 mg/kg/day males and females and four males and one female given 600 mg/kg/day). In the TK groups, 28 males and females were found dead or sacrificed prematurely (1/57 females at 50 mg/kg/day, 8/114 mice at 400 mg/kg/day, 5/114 mice at 600 mg/kg/day, and 13/114 mice at 800 mg/kg/day). Other predominant clinical signs including few feces and rough haircoat occurred in two 400 mg/kg/day males, one 600 mg/kg/day male, and eight 800 mg/kg/day males. A decrease in body weight gain was observed in males dosed at \geq 400 mg/kg/day. Food consumption in the 800 mg/kg/day males and females decreased by 34% and 16% respectively, during the last week of the dosing phase. Significant histopathology findings included mild to marked, myeloid hyperplasia of bone marrow (femur) in the 400, 600, and 800 mg/kg/day males, and 600 and 800 mg/kg/day females. In addition, mild to marked myeloid hyperplasia of bone marrow (sternum) was observed in the 400, 600, and 800 mg/kg/day males and females. Other microscopic findings included minimal to slight necrosis of the liver in the 800 mg/kg/day males and 400 mg/kg/day females, and necrosis of the spleen was noted in the 800 mg/kg/day males. Thymic necrosis was noted in the 800 mg/kg/day males and females. Based on the toxicity, mortality, and bodyweight gain decreases (\geq 10%), the NOAEL and MTD in males was determined to be greater than 50, but less than 400 mg/kg/day. Based on toxicity and mortality, the NOAEL and MTD in females was <600 mg/kg/day.

In the 14-day oral toxicity study in Beagle dogs, animals (3/sex/group) were administered 0 (vehicle), 25, 75, or 200 mg/kg/day NKTR-118 by oral gavage. Drug-related clinical signs were limited to soft stool and diarrhea in all drug-treated groups. There were no drug-related changes in bodyweight, food consumption, ECG, blood pressure, clinical pathology parameters, or organ weights, and there were no drug-related macroscopic or microscopic findings. The NOAEL was considered to be >200 mg/kg/day, corresponding to an AUC_{0-24hr} of 24,421 and 19,297 ng·hr/mL and C_{max} of 20,144 and 16,600 ng/mL, in male and female dogs, respectively.

In another 14-day oral toxicity study in Beagle dogs, animals (3/sex/group) were administered 0 (vehicle), 200, or 500 mg/kg/day NKTR-118 by oral gavage. Drug-related clinical signs were limited to abnormal (loose) feces in the 200 and 500 mg/kg/day groups and excess salivation in the 500 mg/kg/day group. The NOAEL was

considered to be 500 mg/kg/day, the highest dose tested. This dose corresponds to an AUC_{0-24hr} of 90,743 and 65,696 ng·hr/mL and C_{max} of 103,949 and 55,797 ng/mL in male and female dogs, respectively.

In the 1-month oral toxicity study in Beagle dogs, animals (4/sex/group) were administered 0 (vehicle), 50, 150, or 500 mg/kg/day NKTR-118 by oral gavage. Reversibility of the treatment effects was evaluated over a 14-day recovery period (2/sex for the control and high-dose groups). There were no changes in any of the clinical pathology, ophthalmology, respiratory, or ECG parameters, and there were no macroscopic findings. The NOAEL was considered to be 150 mg/kg/day, based on excessive salivation, emesis, and decreased bodyweight gain at 500 mg/kg/day. The NOAEL corresponds to an AUC_{0-24hr} of 14,657 and 11,548 ng·hr/mL and C_{max} of 12,492 and 14,061 ng/mL in male and female dogs, respectively.

In the 39-week oral toxicity study in beagle dogs, animals (6/sex/group) were treated orally with 0 (vehicle), 20, 200, or 500 mg/kg/day NKTR-118 for 39 weeks. Recovery animals (4/sex/group) were treated orally with 0 or 500 mg/kg/day NKTR-118 for 39 weeks, followed by a 4-week recovery period. NKTR-118 was generally well tolerated. There were no deaths. Drug-related clinical signs included tremors, ataxia, hypoactive behavior, and skin lesions in the 500 mg/kg/day group, retching and increased incidence of emesis in the 200 and 500 mg/kg/day groups, and dose-dependent excessive salivation. These clinical signs were reversible after the recovery period. Sinus tachycardia was observed in the control, 200, and 500 mg/kg/day groups, and was not considered as drug-related. Increases in cholesterol levels (up to 51%), were observed in the 200 mg/kg/day males and females and the 500 mg/kg/day males. The NOAEL is considered to be 200 mg/kg/day, based on tremors, ataxia, and hypoactive behavior in the 500 mg/kg/day animals.

Naloxegol free base was negative in the *in vitro* L5178Y TK^{+/-} mouse lymphoma mammalian cell gene mutation assay and *in vivo* mouse micronucleus assay, but was positive in the Ames test. However, naloxegol oxalate, which is protected from oxidative degradation, was negative in the Ames test. (b) (4), a degradation product in various batches of naloxegol free base, showed strong mutagenic activity. Therefore, it was concluded that naloxegol itself was not mutagenic, and that the mutagenic activity observed with naloxegol free base can be attributed to (b) (4) a degradation product of naloxegol.

In the oral carcinogenicity study in Crl:CD(SD) rats, animals (60/sex/group) were administered 0, 40, 120, or 400 mg/kg/day NKTR-118 by oral gavage. The study was terminated on week 93 and 94 for males and females, respectively, due to low survival in the control groups. A statistically significant increase in the survival rate of the 400 mg/kg/day males and 120 mg/kg/day females was noted, when compared to controls. There were no drug-related adverse clinical findings. Decreased body weight (87.3% of control value) and bodyweight gain (82.7% of control value) were noted in the 400 mg/kg/day males. There was a dose-dependent statistically significant increase in the incidence of benign interstitial (Leydig) cell adenoma of the testis (0/60, 0/60, 4/60, and

7/60 in the 0, 40, 120, and 400 mg/kg/day males, respectively; $p=0.0016$ for trend test, significant based on either the rare or common tumor criteria). A pair-wise comparison showed a statistically significant increase in the incidence in the 400 mg/kg/day males, compared to controls ($p=0.0115$, significant based on the rare tumor criteria). In addition, the Sponsor reported a significant increase in the incidence of Leydig cell hyperplasia in the 120 mg/kg/day males, significant decreases in the incidence of pituitary tumors in the 120 mg/kg/day females and 400 mg/kg/day males and females, and significant decreases in the incidence of mammary tumors in the 120 and 400 mg/kg/day females. Therefore, the neoplastic no observed effect level (NOEL) for NKTR-118 in rats is considered to be 120 mg/kg/day in males and 400 mg/kg/day in females, based on increased incidence of interstitial cell adenoma of the testis in the 400 mg/kg/day males. The Sponsor considered the NOEL in males to be 40 mg/kg/day due to increased incidence of Leydig cell hyperplasia in the 120 mg/kg/day males.

The Sponsor provided a study which showed that infusion of naloxegol in male rats produced a transient elevation of plasma LH concentration, which suggests that the incidence of Leydig cell adenomas was hormonally mediated. However, the potential risk of Leydig cell tumor development in humans due to chronic elevation of LH levels is not understood. Nevertheless, it is reasonable to conclude that the Leydig cell neoplasms in rats are unlikely to be relevant to tumor risk in humans based on the extremely high rat to human AUC multiple (818) which produced the statistically significant increase in this tumor.

In the 104-week oral carcinogenicity study in Crl:CD1(ICR) mice, males were administered 0, 25, 70/50, or 200/100 mg/kg/day NKTR-118, and females were administered 0, 40, 120/80, or 400/160 mg/kg/day NKTR-118 by oral gavage. The dose levels were reduced in males and females on days 118 and 117, respectively, due to increased mortality in the 200 mg/kg/day males and 400 mg/kg/day females. After dose reduction in the mid- and high-dose groups, there were no drug-related effects on clinical signs, body weight, food consumption, or macroscopic and microscopic findings. There was no significant increase in any tumor incidence in either sex. Therefore, the neoplastic no observed effect level (NOEL) for NKTR-118 in mice is considered to be 100 mg/kg/day for males and 160 mg/kg/day for females.

The Sponsor examined the possibility that the NKTR-118-related increase in the incidence of benign Leydig cell tumors in rats was due to chronic exposure to elevated levels of plasma luteinizing hormone. Using a dual cannulated Sprague Dawley rat model, the Sponsor demonstrated that plasma LH increased significantly after a 10-minute infusion of NKTR-118 at 80 mg/kg. This intravenous dose produced a maximum plasma NKTR-118 concentration that was comparable to that observed in the 400 mg/kg/day group at week 52 in the rat carcinogenicity study. Plasma levels of testosterone also increased, but to a lesser extent. Taken together, the results support the contention that the observed drug-related increase in the incidence of benign Leydig cell adenoma and hyperplasia in the 2-year oral carcinogenicity study in rats may have been due to drug-induced hormonal changes (i.e. elevated LH levels).

In reproductive toxicity studies, NKTR-118 was evaluated in a segment 1 fertility and early embryonic developmental study in rats. Male rats were administered 0 (vehicle), 250, 500, or 1000 mg/kg/day NKTR-118 by oral gavage for at least 28 days prior to mating, during mating, and until necropsy on dosing day 73. Female rats were administered 0 (vehicle), 250, 500, or 1000 mg/kg/day NKTR-118 by oral gavage for at least 14 days prior to mating, during mating, and through gestation day 7. The females were necropsied on GD (gestation day) 13. In male rats, significant decreases in bodyweight were observed at 1000 mg/kg/day during dosing days 35 through 73, with a decrease in bodyweight gain between dosing days 0 and 73, compared to controls. In female rats, there was a transient increase in bodyweight gain in the treatment groups between GD 7 and 10, compared to controls. Transient changes in food consumption in males and females were observed. There were no drug-related effects on estrous cycles, percentage pregnancy rates (total pregnant/number mated), the number of corpora lutea, implantation sites, live embryos, dead embryos, resorptions, or pre- and post-implantation losses. There were no abortions or early deliveries; all pregnant dams had viable fetuses. The NOAEL for paternal toxicity is considered to be 500 mg/kg/day, based on reduction in bodyweight gain in the 1000 mg/kg/day males. The NOAEL for maternal toxicity was considered to be 1000 mg/kg/day. The NOEL for male fertility and for female fertility and early embryonic development was considered to be 1000 mg/kg/day, based on the lack of adverse findings at this high dose.

Developmental toxicity of NKTR-118 was studied in rats and rabbits. In the segment 2 embryo-fetal developmental study in rats, pregnant rats were administered 0 (vehicle), 250, 750, or 1000 mg/kg/day NKTR-118 by oral gavage on GD 6-17. C-sections were performed on GD 20. A drug-related decrease in maternal bodyweight was noted in the 1000 mg/kg/day females during GD 8-18, and significant decreases in bodyweight gain were noted in the 750 and 1000 mg/kg/day females, compared to controls. A significant increase in bodyweight gain was noted in the treatment groups after drug treatment had stopped on GD 18. There were no drug-related significant changes in overall bodyweight or bodyweight gain at study termination on GD 20. A drug-related decrease in food consumption was observed between GD 6 and 8 in all drug-treated groups, between GD 8 and 10 in the 750 and 1000 mg/kg/day groups, and during the entire treatment period (GD 6-18) in the 750 and 1000 mg/kg/day groups, compared to controls. The decrease in food consumption during the initial dosing period (GD 6-8) correlated with reduced bodyweight and bodyweight gain. Maternal plasma exposure to NKTR-118 was dose-proportional on GD 6 and 17. There was no plasma accumulation of NKTR-118 after repeated dosing. NKTR-118 was metabolized rapidly to NKTR-118-glucuronide. There was moderate accumulation of NKTR-118-glucuronide (1.5-2.8 fold) in plasma after repeated dosing at all doses. Mean gravid weights, corrected terminal bodyweights, cesarean section data, mean fetal weights, and number of live fetuses were comparable across the treatment groups. There were no drug-related effects on fetal weight or fetal external findings. Anorchism, a soft tissue malformation, was noted in a single 1000 mg/kg/day fetus (1/156 or 0.6% compared to 0/150 in controls). Because this malformation was not seen in historical controls, it is considered as possibly drug-related. There were significant increases in fetal and litter incidence of bipartite vertebral centrum (variation) in the 1000 mg/kg/day group. No fetal skeletal

malformations were noted. The NOAEL for maternal toxicity is considered to be 750 mg/kg/day, based on a decrease in bodyweight gain (~16%) during the treatment period. For embryo-fetal viability and fetal growth, the NOEL is considered to be 1000 mg/kg/day, based on the absence of effects on the cesarean section parameters at this dose. For embryo-fetal toxicity or teratogenicity, the NOAEL is considered to be 750 mg/kg/day, based on the incidence of anorchism in the 1000 mg/kg/day group.

In the segment 2 embryo-fetal developmental study in rabbits, pregnant female rabbits were administered 0 (vehicle), 30, 150, or 450 mg/kg/day NKTR-118 by oral gavage on GD 7 to 20. Dams were sacrificed on GD 29. There was an abortion in a 450 mg/kg/day dam on GD 28. Food consumption for this dam was low throughout the dosing phase of the study (<100 g/day). This abortion appears to be related to low food consumption rather than a direct effect of NKTR-118 treatment. Transient dose-related decreases in bodyweight gain were noted in the 30, 150, and 450 mg/kg/day groups for the GD 7 to 9 interval, compared to controls. The decrease in bodyweight gain correlated with decreases in food consumption in the 150 and 450 mg/kg/day groups for the GD 7 to 9 and GD 9 to 11 intervals. After the drug treatment period, bodyweight gain and food consumption increased compared to controls. There were no drug-related macroscopic findings in the dams or effects on cesarean section parameters. There were no drug-related external or visceral variations or malformations in fetuses. Fused vertebral arches were noted in 2 fetuses from two 450 mg/kg/day litters. The fetal and litter incidence of this malformation was higher than historical controls. However, when the combined incidence of all skeletal malformations was evaluated, there was no dose-dependent incidence of malformations, nor was there a significant increase in malformations in any treatment group. Therefore, the fused vertebral arches are not considered to be drug-related. The NOAEL for maternal toxicity is considered to be 30 mg/kg/day, based on decreases in bodyweights and food consumption in the 150 and 450 mg/kg/day groups. The NOEL for embryo-fetal viability and fetal growth was considered to be 450 mg/kg/day, based on the absence of effects on the cesarean section parameters. For developmental toxicity (teratogenicity), the NOEL was 450 mg/kg/day, based on the lack of dose-dependent finding.

In the segment 3 pre- and postnatal developmental study in rats, mated female rats were administered 0 (vehicle), 50, 250, or 500 mg/kg/day NKTR-118 by oral gavage from day 6 of gestation to day 20 of lactation, inclusive. There were no drug-related effects on overall bodyweight or bodyweight gain in the F0 females during gestation or lactation. Decreases in food consumption, compared to controls, were noted in all drug-treated groups on GD 6 to 9, in the 500 mg/kg/day group on GD 12, and in the 250 and 500 mg/kg/day groups on lactation days 4 through 14. There were no drug-related maternal necropsy findings in the F0 females or effects on pregnancy or litter parameters. There were no drug-related effects on physical development of the F1 generation pups during lactation. In post-weaning F1 generation males, bodyweight and bodyweight gains in all drug-treated groups were decreased compared to controls. The decrease in bodyweight gain in the 500 mg/kg/day males was significant during the entire post-weaning period. However, these effects were relatively small and were not considered to be adverse. There were no drug-related effects on bodyweight or

bodyweight gain in the F1 females. There were no drug-related adverse effects on learning and memory, auditory startle reflex, or locomotor activity in F1 generation post-weaning animals. There were no drug-related effects on mating performance, fertility, or pregnancy parameters in the maternal NKTR-118 treated F1 generation animals. Plasma NKTR-118 levels were roughly dose-proportional in the dams and pups at 3 hours after dosing on day 20 of lactation. The maternal/pup plasma NKTR-118 ratios ranged from 180- to 311-fold. The NOAEL for maternal effects and the F1 generation offspring was considered to be 500 mg/kg/day.

In summary, naloxegol, a PEGylated derivative of naloxone, acts as an antagonist at μ - and δ -opioid receptors, and is a weak partial agonist at κ -opioid receptors, with the highest binding affinity at μ -opioid receptors. It is a substrate of the P-glycoprotein (P-gp) transporter, which reduces its ability to cross the blood-brain barrier. Naloxegol functions as a μ -opioid receptor antagonist in the gastrointestinal tract with reduced CNS effects. All human metabolites of naloxegol were also present in the nonclinical studies. Toxicology studies in rats, mice, and dogs show that naloxegol is well tolerated in all three species at exposure levels (AUC) significantly higher (50 to 1000-fold) than the exposure at the MRHD. In the rat carcinogenicity study, naloxegol produced an increased incidence of interstitial cell adenoma in testes. However, this was likely due to an indirect hormonal mechanism (i.e. elevated plasma LH), and is unlikely relevant to tumor risk in humans due to the extremely high rat to human AUC multiple (818) that produced the statistically significant increase in this tumor. Naloxegol had no effects on mating or fertility in rats. No developmental toxicity was observed in rats or rabbits in either the segment 2 or segment 3 studies.

Toxicity Study	Species	NOAEL (mg/kg/day)	Exposure (AUC) at NOAEL (ng·hr/mL) ^a	Multiples of MRHD ^b
13-week oral	Mouse	M: <400 F: <600	M: <16,300 F: <44,600	M: <49 F: <135
13-week oral	Rat	M: 600 F: 800	M: 337,000 F: 580,000	M: 1021 F: 1758
26-week oral	Rat	M & F: 50	47,300	143
39-week oral	Dog	M & F: 200	55,900	169
Segment 1 Reproductive	Rat	M & F: 1000	N/A	>1000 ^c
Segment 2 Reproductive	Rat	F: 750	479,000	1452
	Rabbit	F: 450	135,000	409

a: Average of male and female AUC was used when NOAEL for males and females was the same

b: Human exposure at MRHD (25 mg/day): AUC = 330 ng·h/mL; C_{max} = 80 ng/mL

c: Based on AUC from a 3-month toxicity study in rats

Recommendations:

From a nonclinical standpoint, this application should be approved.

Suggested labeling:

The labeling should be changed as described in the "EXECUTIVE SUMMARY" section of this review.

REVIEWER SIGNATURE _____

YUK-CHOW NG, PH.D.
PHARMACOLOGIST
Division of Gastroenterology and Inborn Errors Products

SUPERVISOR SIGNATURE _____

DAVID B. JOSEPH, PH.D.
LEAD PHARMACOLOGIST
DIVISION OF GASTROENTEROLOGY AND INBORN ERRORS PRODUCTS

cc:
ORIG NDA 204,760
DGIEP
DGIEP/PM
DGIEP/DR. JOSEPH
DGIEP/DR. NG
DGIEP/DR. PETERSON
DGIEP/DR. RAJPAL
OND IO/DR. JACOBS

R/D INIT.: D. JOSEPH 5/6/14

12 Appendix/Attachments

None

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/s/

YUK-CHOW NG

05/15/2014

From a nonclinical standpoint, this application should be approved.

DAVID B JOSEPH

05/15/2014

I concur.

Comments on NDA 204760 Movantil (naloxegol oxalate) tablets

Date: May 13, 2014

From: Abigail Jacobs, AD

1. I concur that there are no pharm-tox approval issues.
2. I concur that there was no nonclinical drug-related teratogenicity.
3. I have discussed some issues with the pharm-tox team leader and they were addressed appropriately.

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/s/

ABIGAIL C JACOBS
05/13/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 204,760

Applicant: AstraZeneca

Stamp Date: 9/16/13

Drug Name: Movantik
(naloxegol)

NDA Type: 505(b)(1)

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		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement
010908

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?			N/A (see review from the Controlled Substance Staff)
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

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

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

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

Yuk-Chow Ng, Ph.D.	10-24-2013
_____ Reviewing Pharmacologist	_____ Date
David B. Joseph, Ph.D.	10-27-2013
_____ Team Leader/Supervisor	_____ Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

YUK-CHOW NG
10/29/2013
The application is fileable.

DAVID B JOSEPH
10/30/2013

**PHARMACOLOGIST'S REVIEW OF IND 78,781
(Supporting Document # 021 Dated November 4, 2008)**

Reviewer: Niraj R. Mehta, Ph.D.
Pharmacologist, DGP

Sponsor and Address: NEKTAR Therapeutics, Inc.
San Carlos, CA

Drug: PEG-Naloxol

Other Names: NKTR-118

Drug Class: selective peripheral opioid antagonist

Date of Submission: November 4, 2008

Date of CDER CDR Receipt: November 4, 2008

Date of Review: May 19, 2010

Submission Contents: This amendment contains a protocol for a carcinogenicity study in rats (submitted for special protocol assessment) and a 3-month oral toxicity study in rats.

Introduction: The carcinogenicity study protocol and the 3-month oral toxicity study in rats are reviewed below.

**Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Proposals**

Application (IND/NDA) number: IND 78,781

Submission date and number: November 4, 2008, Supporting Document #21

Division: Gastroenterology Products

Project manager: Matthew Scherer

CAS#: none

Drug name: PEG-Naloxol

Pharmacological Classification: Selective peripheral opioid antagonist

Sponsor/Applicant: NEKTAR Therapeutics, Inc.
San Carlos, CA

Sponsor/Applicant contact name: Ylan Tran, M.Sc.

Sponsor/Applicant telephone and fax number: 650-631-5065 (Phone)
650-620-5425 (Fax)

Date submitted (stamp date): November 4, 2008

45-day date (from submission stamp date): December 19, 2008

P/T Reviewer(s): Niraj R. Mehta, Ph.D.

Date Review Completed: May 19, 2010

Date of Executive CAC review: November 25, 2008

Executive CAC members: David Jacobson-Kram (Chair), Abby Jacobs, Paul Brown, and Barbara Hill.

Summary of Proposal for Review:

Species/strain: rat/Crl:CD®(SD)

Number/sex/dose: 60/sex/group

Route: oral

Table 3: Cross-Species Comparison of Identified Metabolites in Human, Rat, and Mouse Plasma (% of parent NKTR-118 in the sample)

#	Metabolite	MW (Da)	Human (%)	Rat (%)	Mouse (%)
1	HO-PEG4-naloxol	505.27	6.7 ± 0.9	0.9	ND
2	HO-PEG5-naloxol	549.26	3.5 ± 2.1	1.3	1.1
3	Carboxymethyl-PEG4-naloxol	563.3	3.3 ± 1.2	0.3	2
4	N-Dealkylated mPEG7-naloxol	611.3	3.1 ± 0.6	15.2	1.1
5	Carboxymethyl-PEG3-naloxol	519.23	2.9 ± 1.0	0.6	0.7
6	HO-PEG3-naloxol	461.23	2.7 ± 0.7	0.8	5.3
7	2-Hydroxyl mPEG7-naloxol	667.27	2.5 ± 0.7	1.6	0.4
8	Carboxymethyl-PEG2-naloxol	475.16	1.8 ± 0.7	1.2	0.6
9	HO-PEG6-naloxol	593.33	1.7 ± 0.3	1.9	1.6
10	HO-PEG2-naloxol	417.2	1.4 ± 0.6	1.1	ND
11	Carboxymethyl-PEG5-naloxol	607.27	1.0 ± 0.3	0.5	1.6
12	Carboxymethyl-PEG6-naloxol	651.26	0.8 ± 0.3	0.5	1.3
13	PEG7-naloxol-cysteine	770.23	0.5 ± 0.4	0.4	ND
14	Demethylated PEG7-naloxol	637.29	0.3 ± 0.3	0.6	3.6
15	PEG7-naloxol-glucuronide	827.21	ND	21.3	28.9
16	N-Dealkylated 2-hydroxyl mPEG7-naloxol	627.23	ND	1.6	ND
17	PEG7-naloxol-glutathione	957	ND	0.2	ND

Note: All metabolite identification work was coupled with a semi-quantitative approach that assumed ionization efficiencies among the NKTR-118 related analytes studied were similar. Values (% of parent NKTR-118 in the sample) sorted by relative abundance in human plasma. Mean±SD (n=6) for human values, single values for rat, and mean (n=2) for mouse values. Samples used for analysis were 0.5, 0.5, and 0.25 hr post dose for human (250 mg oral dose), rat (100 mg/kg oral dose), and mouse (5 mg/kg dose), respectively. ND=not detected. MW (Da) is analyte molecular weight in Daltons.

Upon the Agency’s request, the sponsor submitted absolute plasma concentrations of the metabolites shown in the table above. The rat and human plasma concentrations were taken 0.5 hr after a single dose of NKTR-118. The 0.5 hr time-point corresponds to the T_{max} of the NKTR-118. However, this may or may not represent the time-point at which the peak concentration of individual metabolites is observed. Although some of the metabolites are present in higher concentrations in humans compared to rats, the 250 mg dose is 2.5-fold greater than the maximum proposed dose in humans, 100 mg QD. In addition, the rat plasma concentrations in the table below represent levels after a 100 mg/kg dose, which is a significantly lower dose than the highest doses tested in previous oral toxicity studies in rats (800 mg/kg/day), and the highest proposed dose for the 2-year carcinogenicity in rats, 400 mg/kg/day. Therefore, even though the table below shows that certain metabolites of NKTR-118 are present in higher concentrations in human plasma compared to rat plasma, metabolite concentrations in rat plasma might be similar to or even higher than concentrations measured in human plasma if the doses administered in humans and rats were more appropriate. Concentrations of selected metabolites of NKTR-118 in rat and human plasma are summarized in the table below.

Rat (100 mg/kg NKTR-118) & Human (250 mg NKTR-118) Plasma metabolite concentrations after 1st dose			
#	Metabolite (Same number as noted in table above)	Rat Plasma Concentration @ 0.5 hr (T _{max} of NKTR-118)	Human Plasma Concentration @ 0.5 hr (T _{max} of NKTR-118)
1	HO-PEG4-naloxol	37.9 ng/mL	66.4 ng/mL
2	HO-PEG5-naloxol	54.8 ng/mL	34.7 ng/mL
3	Carboxymethyl-PEG4-naloxol	12.6 ng/mL	32.7 ng/mL

5	Carboxymethyl-PEG3-naloxol	25.3 ng/mL	28.73 ng/mL
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The doses tested in the 13-week dose range finding study were 50, 200, 400, 600 and 800 mg/kg/day. Based on the body weight gain decreases $\geq 10\%$ in the 13-week oral toxicity study in rats, the NOAEL (no observed adverse effect level) and the MTD (maximum tolerated dose) in males was 600 mg/kg/day, which corresponded to mean $AUC_{(0-24hr)}$ and C_{max} values of 337 hr· μ g/mL and 36.2 μ g/mL, respectively, measured at day 89 of the dosing phase. Since no test article-related adverse effects were observed in females, the NOAEL for females in the 13-week oral toxicity study was 800 mg/kg/day, which corresponded to mean $AUC_{(0-24hr)}$ and C_{max} values of 580 hr· μ g/mL and 56.6 μ g/mL, respectively, measured at day 89 of the dosing phase. A MTD could not be defined in females, since there was no observed test article-related toxicity in female rats. For the proposed carcinogenicity study, the recommended high dose is 400 mg/kg/day, based on the rodent to human AUC ratio. The high dose is 3.3-fold greater than the recommended intermediate dose level (120 mg/kg/day). The intermediate dose is 3-fold greater than the recommended low dose (40 mg/kg/day).

CAC Concurrence (y/n): No

CAC Recommendations: The Committee recommended doses of 40, 120, and 400 mg/kg/day by oral gavage, based on the rodent to human plasma AUC ratio of NKTR-118 exceeding 25-fold. Furthermore, the sponsor should use one (constant) dose volume for all dose groups

Comments: The sponsor has proposed to do a histopathologic examination on specific organs/tissues in only the control and high-dose groups, however histopathologic examination of other dose groups will be required under any of the following circumstances:

- ◆ For any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups.
- ◆ For an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group.
- ◆ For an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site, they should look at all relevant tissues for that dose level and the next lower dose level.
- ◆ For an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

Protocol Entitled “2-year Oral Gavage Carcinogenicity and Toxicokinetic Study with NKTR-118 in Rats”

Study # (b) (4) **7985-111 (LS-2008-007)**

Methods: Crl:CD®(SD) rats will be treated orally with 0 (vehicle control), 40, (b) (4) or 400 mg/kg/day NKTR-118 for 104 weeks (60 rats/sex/group). The drug will be administered as a solution of NKTR-118 in reverse osmosis water. Animals in Groups 1 and 5 will be dosed at a volume of 4 mL/kg and animals in Groups 2 through 4 and 6 through 8 will be dosed at the volume of 10 mL/kg (see table below). Food and water will be available to the study animals *ad libitum*. The following parameters will be recorded: mortality, clinical signs, bodyweight, food consumption, clinical pathology (blood films), gross pathology, bone marrow smears, and histopathology. In the toxicokinetic groups, blood samples will be collected from three animals/sex/cohort on day 1 and during Weeks 26 and 52 of the dosing phase. Group 5 (vehicle control) will have blood collected approximately 3 hours postdose, and Groups 6 through 8 (test article-treated) will have blood collected approximately 0.5, 3, 6, 12, and 24 hours postdose (see table below). Histopathologic examination will be performed on the following organs/tissues in the control and high-dose groups, and main study animals that are found dead or sacrificed prematurely: adrenal, aorta, brain, cecum, cervix, colon, duodenum, epididymis, esophagus, eye, femur with bone marrow, Harderian gland, heart, ileum, jejunum, kidney, lesions, liver, lung, lymph nodes (mandibular, mesenteric), mammary gland (females only), optic nerve, ovary, pancreas, pituitary gland, prostate, rectum, salivary gland (mandibular), sciatic nerve, seminal vesicle, skeletal muscle (thigh), skin/subcutis, spinal cord (cervical, thoracic, lumbar), spleen, stomach (glandular and non-glandular), testis, thymus, thyroid (with parathyroid), tongue, trachea, urinary bladder, uterus, and vagina. The protocol states that hematoxylin and eosin-stained tissue sections will be prepared from all of the above organs and tissues.

Group ^a	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
	Male	Female		
Carcinogenicity Animals				
1 (Control)	60	60	0	0
2 (Low)	60	60	40	10
3 (Mid)	60	60	150	15
4 (High)	60	60	400	40
Toxicokinetic Animals^b				
5 (Control)	7	7	0	0
6 (Low)	13	13	40	10
7 (Mid)	13	13	150	15
8 (High)	13	13	400	40

a Groups 1 and 5 will receive control article only.

b Toxicokinetic animals included solely for the purpose of blood sample collections. Cohorts of 3/sex/group each to be bled twice at each sample-collection interval (for test article groups, 9/sex/group designated for sample collections). The Control group will consist of 3 animals/sex bled at two intervals. An additional 4 animals/sex/group will be included in the study design to be used as replacements to compensate for possible mortality.

Dose Selection: High dose selection was based on toxicity and toxicokinetic results from the 3-month toxicity study, as well as pharmacokinetic considerations relative to the maximum human

clinical dose. The sponsor stated that the high dose, 400 mg/kg/day, would provide a 236-fold safety factor based on plasma NKTR-118 AUC in humans at the maximum proposed clinical dose of 100 mg QD. The Sponsor cited the ICH Guidance S1C(R2), “Dose Selection for Carcinogenicity Studies of Pharmaceuticals & Limit Dose” to support the high dose selection. ICH Guidance S1C(R2) recommends a high dose in rodents that produces a rodent to human plasma AUC ratio of 25 for the parent compound and/or metabolites.

The low dose of 40 mg/kg/day is lower than the low dose of 50 mg/kg/day tested in the 3-month dose ranging study, therefore the low dose would not be expected to produce adverse effects. The sponsor stated that a mid-dose of ^{(b) (4)} mg/kg/dose was selected ^{(b) (4)}

In the 13-week oral toxicity study in rats, there were a total of five toxicity (main phase) and toxicokinetic animals that were found dead or sacrificed prematurely; one 50 mg/kg/day male, one 800 mg/kg/day male, one toxicokinetic male and female given 400 mg/kg/day, and one toxicokinetic female given 600 mg/kg/day of test article. The summary of animals that died prior to scheduled sacrifice or were sacrificed prematurely is provided in the table below.

Dose in mg/kg/day (Main phase/TK)	Sex	Day of Sacrifice	Clinical Symptoms	Gross Pathology (GP:)/ Histopathology (HP:)
50 (main phase)	M	Moribund Day 78	Red discharge in pan, & limited use of hind limb (swollen)	<u>GP:</u> Bilaterally large kidneys with dark red fluid in renal pelvis, & distended urinary bladder. <u>HP:</u> Multiple renal cysts/distended renal pelvis, & min. lymphocytic infiltrate of epididymis
400 (TK)	M	Moribund Day 19	Hunched posture, red haircoat and nose, & red discharge in pan	No changes
400 (TK)	F	Moribund Day 78	Head tilt & hyperactivity	<u>GP:</u> Small optic nerves
600 (TK)	F	Moribund Day 61	thin appearance, hunched posture, rough/yellow haircoat, hypoactivity, & sensitivity to touch	<u>GP:</u> Distended urinary bladder
800 (main phase)	M	Moribund Day 60	General debilitation, head tilt & ataxic, hunched, & sensitive to touch	<u>GP:</u> None <u>HP:</u> Inflammation in meninges surrounding brain and spinal cord, macrophage infiltrate in lungs, thymus atrophy, kidney inflammation/ cell infiltration, & prostate inflammation

In the 3-month study, the most common test article-related clinical signs were clear oral discharge and yellow staining of the perineal area. Rough haircoat was observed in 5/10 males and 2/10 females in the 800 mg/kg/day dose group. Two females given 400 and 800 mg/kg/day were observed with palpable masses starting on days 92 and 77 of the dosing phase. These masses were diagnosed as neoplasms, based on microscopic examination, and classified as a mammary carcinoma in the 400 mg/kg/day female, and a myxoma of the skin in the 800 mg/kg/day female.

There was a dose-dependent reduction in body weight gain in males (-8%, -9%, and -12% decrease relative to controls at 400, 600, and 800 mg/kg/day, respectively) without a significant change in food consumption. Females gained weight relative to controls with minor increases in food consumption on specific weeks throughout the dosing phase. In addition, clinical chemistry results showed significant increases in cholesterol levels in males and females dosed with ≥ 400 mg/kg/day of NKTR-118. There were no changes in gross pathology. Significant, dose-dependent increases in liver weights were observed in both males and females, however no concurrent histopathological changes were observed. Toxicokinetic analyses revealed that the C_{max} increased dose-proportionally in males and less than dose proportionally in females. Females had greater exposures than males at all doses. Accumulation of NKTR-118 in male rats was observed. The AUC on day 89 in males was increased by 2.1- to 4.7-fold compared to day 1. Female rats had minimal accumulation from days 1 to 89.

3-Month Oral Gavage Toxicity and Toxicokinetic Study with NKTR-118 in Rats

Key Study Findings: Based on the body weight decreases $\geq 10\%$ male rats, the MTD (maximum tolerated dose) in males was 600 mg/kg/day. A MTD could not be defined in females, since there was no observed test article-related toxicity in female rats.

Study #: (b) (4) No. 7985-101 (LS-2007-028)

EDR 4.2.3.2 pgs. 1-1122 (Serial No. 0022)

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: August 14, 2007 (report dated September 4, 2008)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: Lot# 149006; 98% pure

Methods: The test and control/vehicle articles were administered once daily by oral gavage for at least 94 days as described in the sponsor's table below. The animals were euthanized upon completion of the treatment periods.

10 Page(s) of duplicate copy of e-p 77-88 above has been
Withheld in Full immediately following this page

palpable masses starting on days 92 and 77 of the dosing phase. These masses were diagnosed as neoplasms based on microscopic examination and classified as a mammary carcinoma in the 400 mg/kg/day female and a myxoma of the skin in the 800 mg/kg/day female.

There was a dose-dependent reduction in body weight gain in males (-8%, -9%, and -12% decrease relative to controls at 400, 600, and 800 mg/kg/day, respectively) without a significant change in food consumption. Females gained weight relative to controls with minor increases in food consumption on certain weeks throughout the dosing phase. In addition, clinical chemistry results showed significant increases in cholesterol levels in males and females dosed with ≥ 400 mg/kg/day of NKTR-118. There were no changes in gross pathology. Significant, dose-dependent increases in liver weights were observed in both males and females, however there were no concurrent histopathological changes. Toxicokinetic analyses revealed that the C_{\max} increased dose-proportionally in males and less than dose proportionally in females. Females had greater exposures than males at all doses. Male rats had a 2.1 to 4.7-fold higher AUC on day 89 compared to day 1, whereas female rats had minimal drug accumulation.

Based on the decrease in bodyweight gain (12%) in the 800 mg/kg/day males, the NOAEL (no observed adverse effect level) and the MTD (maximum tolerated dose) in males was 600 mg/kg/day, which corresponded to $AUC_{(0-24hr)}$ and C_{\max} values of 337 hr $\cdot\mu\text{g/mL}$ and 36.2 $\mu\text{g/mL}$, respectively, measured at day 89. Since no test article-related adverse effects were observed in females, the NOAEL for females in the 13-week oral toxicity study was 800 mg/kg/day, which corresponded to $AUC_{(0-24hr)}$ and C_{\max} values of 580 hr $\cdot\mu\text{g/mL}$ and 56.6 $\mu\text{g/mL}$, respectively, measured at day 89. A MTD could not be defined in females, since there was no observed test article-related toxicity in female rats.

SUMMARY AND EVALUATION:

NKTR-118 is a PEGylated derivative of naloxone that is under development for the treatment of opioid-induced constipation (OIC) (b) (4)

NKTR-118 has been evaluated in three Phase 1 studies: a single-dose PK study, and single-dose and multiple-dose safety and tolerability studies. In addition, NKTR-118 has been evaluated in a Phase 2 study. The Sponsor submitted a protocol for a 2-year oral carcinogenicity study in rats. NKTR-118 tested positive in the bacterial reverse mutation assay (Ames test) and negative in the *in vivo* mouse micronucleus test and the L5178Y TK +/- mouse lymphoma assay.

The Sponsor proposed a 2-year carcinogenicity study in rats using oral dose levels of 0 (vehicle), 40, (b) (4) and 400 mg/kg/day NKTR-118. The Sponsor's dose selection was based on the expected rat to human AUC ratio at the anticipated maximum clinical dose (100 mg QD).

The Executive CAC did not concur with the Sponsor's proposed intermediate dose. The Committee recommended doses of 40, 120, and 400 mg/kg/day for both males and females, based on the rodent to human plasma AUC ratio of NKTR-118 exceeding 25-fold. Furthermore, the Committee recommended the use of one (constant) dose volume for all dose groups.

RECOMMENDATIONS:

Executive CAC Recommendations and Conclusions:

1. The Committee did not concur with the Sponsor's proposed intermediate dose. The Committee recommended doses of 40, 120, and 400 mg/kg/day for both males and females, based on the rodent to human plasma AUC ratio exceeding 25-fold.
2. The sponsor has proposed to do a histopathologic examination on specific organs/tissues in only the control and high-dose groups, however histopathologic examination of other dose groups will be required under any of the following circumstances:
 - a. For any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups.
 - b. For an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group.
 - c. For an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site, they should look at all relevant tissues for that dose level and the next lower dose level.
 - d. For an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-78781	ORIG-1	NEKTAR THERAPEUTICS	NKTR 118

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/s/

NIRAJ R MEHTA
05/19/2010

DAVID B JOSEPH
05/19/2010
I concur.

**Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Proposals**

Application (IND/NDA) number: IND 78,781

Submission date and number: November 4, 2008, Supporting Doc # 021

Division: Gastroenterology Products

Project manager: Matthew Scherer

CAS#: none

Drug name: PEG-Naloxol

Pharmacological Classification: Selective peripheral, opioid antagonist

Sponsor/Applicant: NEKTAR Therapeutics, Inc.
San Carlos, CA

Sponsor/Applicant contact name: Ylan Tran, M.Sc.

Sponsor/Applicant telephone and fax number: 650-631-5065 (Phone)
650-620-5425 (Fax)

Date submitted (stamp date): November 4, 2008

45-day date (from submission stamp date): December 19, 2008

P/T Reviewer(s): Niraj R. Mehta, Ph.D.

Date Review Completed: October 22, 2009

Date of Executive CAC review: November 25, 2008

Executive CAC members: David Jacobson-Kram (Chair), Abby Jacobs, Paul Brown, and Barbara Hill.

Summary of Proposal for Review:

Species/strain: mouse/Crl:CD1(ICR)

Number/sex/dose: 60/sex/group

Route: oral

high dose is 2.9-fold greater than the recommended intermediate dose level (70 mg/kg/day). The intermediate dose is 2.8-fold greater than the recommended low dose (25 mg/kg/day).

CAC Concurrence (y/n): n

CAC Recommendations: For male mice, the Committee recommended doses of 0, 25, 70, and 200 mg/kg/day by oral gavage, based on mortality. For female mice, the Committee recommended doses of 0, 40, 120, and 400 mg/kg/day by oral gavage, based on adverse histopathology observations. Furthermore, the sponsor should use one (constant) dose volume for all dose groups.

Comments: The sponsor has proposed to do a histopathologic examination on specific organs/tissues in only the control and high-dose groups, however histopathologic examination of other dose groups will be required under any of the following circumstances:

- ◆ For any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups.
- ◆ For an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group.
- ◆ For an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site, they should look at all relevant tissues for that dose level and the next lower dose level.
- ◆ For an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

Protocol Entitled “2-year Oral Gavage Carcinogenicity and Toxicokinetic Study with NKTR-118 in Mice”

Study # (b) (4) 7985-111 (LS-2008-007)

Methods: Crl:CD1(ICR) mice will be treated orally (b) (4). The drug will be administered as a solution of NKTR-118 in reverse osmosis water. Animals in Groups 1 and 5 will be dosed at a volume (b) (4) and animals in Groups 2 through 4 and 6 through 8 will be dosed at the volume (b) (4) (see table below). Food and water will be available to the study animals *ad libitum*. The following parameters will be recorded: mortality, clinical signs, bodyweight, food consumption, clinical pathology (blood films), gross pathology, bone marrow smears, and histopathology. In the toxicokinetic groups, blood samples will be collected from three animals/sex/cohort on day 1 and during Weeks 26 and 52 of the dosing phase. Group 5 (vehicle control) will have blood collected approximately 3 hours postdose, and Groups 6 through 8 (test article-treated) will have blood collected approximately 0.5, 3, 6, 12, and 24 hours

post-dose. Histopathologic examination will be performed on the following organs/tissues in the control and high-dose groups and main study animals that are found dead or sacrificed prematurely: adrenal, aorta, brain, cecum, cervix, colon, duodenum, epididymis, esophagus, eye, femur with bone marrow, Harderian gland, heart, ileum, jejunum, kidney, lesions, liver, lung, lymph nodes (mandibular, mesenteric), mammary gland, optic nerve, ovary, pancreas, pituitary gland, prostate, rectum, salivary gland (mandibular), sciatic nerve, seminal vesicle, skeletal muscle (thigh), skin/subcutis, spinal cord (cervical, thoracic, lumbar), spleen, stomach (glandular and non-glandular), testis, thymus, thyroid (with parathyroid), tongue, trachea, urinary bladder, uterus, vagina. The protocol states that hematoxylin and eosin-stained tissue sections will be prepared from all of the above organs and tissues.

Group ^a	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)		
	Male	Female				
Carcinogenicity Animals						
1 (Control)	60	60		(b) (4)		
2 (Low)	60	60				
3 (Mid)	60	60				
4 (High)	60	60				
Toxicokinetic Animals^b						
5 (Control)	22	22				
6 (Low)	58	58				
7 (Mid)	58	58				
8 (High)	58	58				

a Group 1 and 5 will receive control article only.

b Toxicokinetic animals included solely for the purpose of blood sample collections. Four animals/sex/group will serve as replacement animals to compensate for possible mortality.

Dose Selection: High dose selection was based on toxicity and toxicokinetic results from the 3-month toxicity study. Based on morbidity, mortality, and a reduction in body weight, the sponsor proposed a high dose of 400 mg/kg/day. The sponsor cited ICH *Guidance For Dose Selection FOR Carcinogenicity Studies Of Pharmaceuticals & Limit Dose SIC(R2)* to support the high dose selection. The sponsor stated that 5/9 total deaths (two 600 mg/kg/day males, and two 800 mg/kg/day males and one 800 mg/kg/day female) were attributed to test article (b) (4)

The low dose of 40 mg/kg/day is lower than the low dose of 50 mg/kg/day tested in the 3-month dose ranging study, therefore the low dose would not be expected to produce adverse effects. The sponsor stated that a mid-dose of (b) (4) mg/kg/dose was selected (b) (4)

In the 3-month oral toxicity study in mice, a total of nine toxicity (main phase) animals were found dead or sacrificed prematurely; four males and one female given 600 mg/kg/day NKTR-118, and two males and two females given 800 mg/kg/day NKTR-118. In the toxicokinetic groups, a total of 28 (13 males and 15 females) animals were found dead or sacrificed prematurely; 1/57 females given 50 mg/kg/day, 4/57 males and 4/57 females given 400 mg/kg/day, 4/57 males and 1/57 females given 600 mg/kg/day, and 5/57 males and 8/57 females

given 800 mg/kg/day. The summary of animals that died prior to scheduled sacrifice or were sacrificed prematurely in the main study groups is provided in the table below.

Dose	Sex	Day of Death	Clinical Symptoms	Gross Pathology (GP:)/ Histopathology (HP:)
600*	M	Moribund Day 46	Hypoactive, cold to touch, swollen abdomen, few feces, hunched posture, & general debilitation	<u>GP:</u> Distended stomach, duodenum, ileum, colon, cecum, & jejunum (contains gas). <u>HP:</u> Mixed infiltrate of liver and inflammation and necrosis of liver, lymphocytic depletion and necrosis of thymus, dilation of duodenum, ileum, colon, cecum, & jejunum, & myeloid hyperplasia of marrow (femur and sternum).
600*	M	Moribund Day 52	Swollen abdomen, audible breathing, & general debilitation	<u>GP:</u> Distended duodenum, ileum, colon, cecum, & jejunum (contains gas), & discolored (red/dark red) stomach mucosa. <u>HP:</u> Lymphocytic depletion and necrosis of thymus, mononuclear infiltrate of kidneys, & dilation of duodenum, ileum, colon, cecum, & jejunum.
600	M	Found dead (after dosing) Day 60	None	<u>GP:</u> None <u>HP:</u> Lymphocytic/macrophage infiltrate of liver, lymphocytic/macrophage infiltrate (alveolus) of lungs, & myeloid hyperplasia of marrow (femur and sternum).
600	M	Day 8 -death (b) (4)	None	<u>GP:</u> Perforated trachea <u>HP:</u> Lymphocytic infiltrate (peribronchial/perivascular) of lungs, & myeloid hyperplasia of marrow (femur & sternum).
600	F	Moribund Day 91	Few feces, thin appearance, rough haircoat, hunched posture, & general debilitation	<u>GP:</u> Large bilateral lymph node & discolored lobe (red/dark red) and raised area of liver. <u>HP:</u> Neutrophil infiltrate of trachea, extramedullary hematopoiesis of liver, extramedullary hematopoiesis of spleen, lymphocytic depletion/necrosis of spleen, lung inflammation, mandibular salivary gland atrophy, uterine atrophy, & myeloid hyperplasia of marrow (femur & sternum), & plasma cell hyperplasia and neutrophil infiltrate of lymph node (mandibular)
800*	M	Moribund Day 85	Few feces, thin appearance, rough haircoat, & general debilitation	<u>GP:</u> Large bilateral lymph node. <u>HP:</u> Extramedullary hematopoiesis of liver, necrosis and inflammation of liver, extramedullary hematopoiesis of spleen, macrophage infiltrate (alveolus) of lungs, chronic progressive nephropathy of kidneys, lymphocytic/ macrophage infiltrate of prostate, myeloid hyperplasia of marrow (femur & sternum), & plasma cell hyperplasia of lymph node (mandibular).
800*	M	Moribund Day 85	Rough haircoat, yellow haircoat, hunched posture, few feces, & general debilitation	<u>GP:</u> Large bilateral lymph node. <u>HP:</u> Extramedullary hematopoiesis of spleen, macrophage infiltrate (alveolus) of lungs, lymphocytic depletion and necrosis of thymus, salivary gland atrophy chronic progressive nephropathy of kidneys, myeloid hyperplasia of marrow (femur & sternum), & plasma cell hyperplasia of lymph node.
800*	F	Moribund Day 58	None	<u>GP:</u> None. <u>HP:</u> Lymphocytic/macrophage infiltrate of liver, lymphocytic infiltrate (peribronchial/perivascular) of lungs, uterine atrophy, & myeloid hyperplasia of marrow (femur & sternum).
800	F	Moribund Day 83 (death gavage related)	Hypoactive, limited use of right front paw, swollen cranial head & right shoulder	<u>GP:</u> Perforated esophagus (cranial), & mammary (multiple, clear, gelatinous regions) <u>HP:</u> Fibrosis/fibroplasia of esophagus, degeneration/necrosis of heart (myocardium), depletion of lymphocytes/necrosis of spleen, & lymphocytic infiltrate (peribronchial/perivascular) of

				lungs, thymic necrosis, interstitial infiltrate of kidney, atrophy of mandibular salivary gland, moderate skin inflammation, & periesophagus necrosis.
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*- Sponsor attributes these deaths to be test- article-related.

Other predominant clinical signs included few feces (more prevalent in males) in 1/20, 5/20, 4/20, and 8/20 animals 50, 400, 600, and 800 mg/kg/day groups respectively, and rough haircoat in males; two 400 mg/kg/day males, one 600 mg/kg/day male, and eight 800 mg/kg/day males.

In addition, there was a decrease in body weight from initial body weight measurements (32.9 ± 1.93 g) and final body weight measurements (31.6 ± 4.55 g) in male mice administered 800 mg/kg/day NKTR-118. A significant decrease (19%) of total body weight in the 800 mg/kg/day males occurred during the last two weeks of the dosing period. Subsequently, there was a 13% and 28% reduction in body weight gain in 400 and 600 mg/kg/day males, respectively. Females gained weight relative to controls in doses ≤ 600 mg/kg/day and there was no significant decrease of weight gain in 800 mg/kg/day females. Also, 800 mg/kg/day male and female mice had a significant decrease of 34% and 16% respectively, in mean food consumption values during the last week of the dosing phase. Hematological parameters showed increases in neutrophil counts in males and females dosed at ≥ 600 mg/kg/day. Clinical chemistry changes included a dose-dependent increase in glucose levels in both males and females, increased cholesterol levels in males and females dosed at ≥ 600 mg/kg/day, and dose-dependent decreases in alkaline phosphatase levels in females.

Gross pathology changes included enlarged lymph nodes in males (4/8) dosed with 800 mg/kg/day. There was a significant decrease in weight of the thymus and spleen in 800 mg/kg/day males, and a decrease of uterine weight in 800 mg/kg/day females. These were consistent with microscopic changes noted in the animals (discussed below). Significant microscopic findings included minimal to slight necrosis of the liver in 800 mg/kg/day males (3/8) and 400 mg/kg/day females (3/10; one female had moderate necrosis), and plasma cell hyperplasia of the lymph node in 800 mg/kg/day males (4/4) and females (2/2). Effects in spleen included, minimal to moderate extramedullary hematopoiesis of the spleen in males and females dosed with ≥ 600 mg/kg/day and necrosis in males (5/8) in the 800 mg/kg/day group. Thymic necrosis was noted in 5/6 males and 3/8 females in the 800 mg/kg/day group, and mild to moderate uterine atrophy was observed in 4/10, 7/10, 6/9, and 7/8 females in the 50, 400, 600, and 800 mg/kg/day groups, respectively. Other significant histopathology findings included mild to marked, myeloid hyperplasia of bone marrow (femur) in 2/10, 5/6, and 8/8 males in the 400, 600, and 800 mg/kg/day groups respectively, and 7/9 and 7/8 females in the 600 and 800 mg/kg/day groups, respectively. The most severe incidences (marked myeloid hyperplasia) were noted in 6/8 males and 3/7 females in the 800 mg/kg/day group. In addition, mild to marked, myeloid hyperplasia of bone marrow (sternum) occurred in 7/10, 5/6, and 6/7 males in the 400, 600, and 800 mg/kg/day groups respectively, and 6/10, 9/9, and 7/8 females in the 400, 600, and 800 mg/kg/day groups, respectively. The most severe incidences (marked myeloid hyperplasia) were noted in 5/7 males and 3/7 females in the 800 mg/kg/day group.

Toxicokinetic (TK) analysis revealed that C_{max} values increased dose-proportionally on day 1 in males and females, however there was no clear dose-dependent trend observed on days 30 and 91. $AUC_{(0-24)}$ values appeared to be greater than dose-proportional at higher doses in both males

and females on day 1, and only in females on day 91. There was a high prevalence of inter-animal variability, thus making it difficult to accurately evaluate sex differences. However, there were a few sex-based trends in exposure level, which included an increase in drug exposure on days 1 and 30 in females administered 400 mg/kg/day NKTR-118 and day 91 in females administered 800 mg/kg/day NKTR-118. In males, there was an increase in drug exposure on days 30 and 91 in the 800 and 600 mg/kg/day groups respectively, when compared to females. Male mice had a 2.1 to 4.7-fold higher exposure on day 89 compared to day 1 and female mice had minimal accumulation from days 1 to 89.

Based on the toxicity, mortality, and body weight gain decreases ($\geq 10\%$) observed in the 13-week oral toxicity study in mice, the NOAEL (no observed adverse effect level) and MTD (maximum tolerated dose) in males appears to be greater than 50, but less than 400 mg/kg/day. Specifically, morbidity and mortality were observed in males at doses ≥ 600 mg/kg/day, and body weight gain decreases were observed in males dosed at ≥ 600 mg/kg/day. Microscopic examinations in male mice dosed with 800 mg/kg/day showed necrosis of the spleen, liver, and thymus. Based on toxicity and mortality, the NOAEL and MTD in females appears to be < 600 mg/kg/day. Test article-related morbidity was noted in females dosed at ≥ 600 mg/kg/day. Female mice had no significant changes in body weight gain. Lastly, microscopic examinations noted necrosis of the thymus in 800 mg/kg/day females.

3-Month Oral Gavage Toxicity and Toxicokinetic Study with NKTR-118 in Mice

Key Study Findings: Based on toxicity, mortality, and body weight gain decreases ($\geq 10\%$) observed in the 13-week oral toxicity study in mice, the MTD in males appears to be greater than 50, but less than 400 mg/kg/day, and in females the MTD appears to be < 600 mg/kg/day.

Study #: (b) (4) No. 7985-109 (LS-2007-056)

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: August 28, 2007 (report dated August 28, 2008)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: Lot# 149005; 98% pure

Methods: The test and control/vehicle articles were administered once daily by oral gavage for at least 90 days as described in the sponsor's table below. The animals were euthanized upon completion of the treatment periods.

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Mild to moderate, uterine atrophy was observed in females dosed with 50, 400, 600, and 800 mg/kg/day groups, respectively.

Based on the toxicity, mortality, and body weight gain decreases ($\geq 10\%$) observed in the 13-week oral toxicity study in mice, the NOAEL (no observed adverse effect level) and MTD in males appears to be greater than 50, but less than 400 mg/kg/day. Specifically, morbidity and mortality were observed in males at doses ≥ 600 mg/kg/day, and a decrease in body weight gain was observed in males dosed at ≥ 400 mg/kg/day. Microscopic examinations in male mice dosed with 800 mg/kg/day showed necrosis of the spleen, liver, and thymus. Based on toxicity and mortality, the NOAEL and MTD in females appear to be < 600 mg/kg/day. Test article-related morbidity was noted in females dosed at ≥ 600 mg/kg/day. There were no significant changes in body weight gain in female mice. Lastly, microscopic examinations showed necrosis of the thymus in 800 mg/kg/day females.

RECOMMENDATIONS:

1. The proposed dose levels and the sponsor's supporting rationale are unacceptable.
2. Dose selection for the proposed carcinogenicity study should be based on the MTD derived from the 13-week oral toxicity study in mice. The MTD should be based on toxicity, mortality/morbidity, and reduction in weight gain. The recommended dose levels for male and female mice are 0, 25, 75, and 200 mg/kg/day.

Niraj R. Mehta, Ph.D. Date
Pharmacologist, Division of Gastroenterology Products

Comment:

David B. Joseph, Ph.D. Date
Acting Pharmacology Team Leader,
Division of Gastroenterology Products

cc:

IND 78,781

DGP-180

DGP-180/CSO

DGP-180/Dr. Joseph

DGP-180/Dr. Mehta

R/D Init.: D. Joseph 10/13/09

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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
IND-78781	ORIG-1	NEKTAR THERAPEUTICS	NKTR 118

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/s/

NIRAJ R MEHTA
10/22/2009

DAVID B JOSEPH
10/27/2009