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APPLICATION NUMBER:

207999Orig1s000

PHARMACOLOGY REVIEW(S)

Comments on NDA207999 obeticholic acid

From A. Jacobs

Date : Nov 23, 2015

1. I concur that there are no pharm-tox related approval issues
2. I have conveyed other comments to the reviewer and she has addressed them as appropriate.

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/s/

ABIGAIL C JACOBS
02/18/2016

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	207999
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	SDN 02: June 29, 2015
	SDN 10: August 26, 2015
	SDN 18: September 21, 2015
	SDN 19: September 22, 2015
Product:	Obeticholic acid (OCA)
Indication:	Treatment of primary biliary cirrhosis (PBC) in combination with ursodeoxycholic acid (UDCA) in adults with an inadequate response to UDCA or as monotherapy in adults unable to tolerate UDCA
Applicant:	Intercept Pharmaceuticals, Inc.
Review Division:	Division of Gastroenterology and Inborn Errors Products (DGIEP)
Reviewer:	Tracy Behrsing, Ph.D.
Supervisor/Team Leader:	Sushanta Chakder, Ph.D.
Division Director:	Donna Griebel, M.D.
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Reference ID: 3870157

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1 Executive Summary

1.1 Introduction

Obeticholic acid (OCA) is a modified bile acid and farnesoid X receptor (FXR) agonist. Intercept Pharmaceuticals, Inc. seeks to market OCA for the treatment of primary biliary cirrhosis (PBC) in combination with ursodeoxycholic acid (UDCA) in adults with an inadequate response to UDCA or as monotherapy in adults unable to tolerate UDCA.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical safety package for OCA included pharmacology, pharmacokinetics/ ADME/toxicokinetics, single-dose and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproductive and development toxicology, and special toxicity studies.

OCA is a FXR agonist, with EC50 values ~100-fold lower than the natural FXR agonist chenodeoxycholic acid (CDCA). Like endogenous bile acids, OCA is conjugated with the amino acids taurine and glycine. With the exception of the rabbit, OCA is primarily metabolized to the taurine conjugate with minimal or no metabolism to glyco-OCA in nonclinical species (mouse, rat, and dog). In contrast, both the taurine and glycine conjugates are major metabolites in humans, and exposures to the conjugates exceed those to the parent compound. Based on EC50 values, the glycine and taurine conjugates of OCA have potencies at FXR which are similar to the parent compound; and thus, these are considered to be active metabolites.

In repeat-dose oral toxicity studies in rodents and non-rodents, the hepatobiliary system was identified as the primary target system of toxicity. In the 26-week oral toxicity study in rats, treatment with OCA produced changes in clinical chemistry parameters (e.g., increases in ALT, AST, and ALP), increased liver weights, and bile duct hyperplasia with hepatocellular hypertrophy. Clinical signs such as yellow skin were observed in high dose animals (60 mg/kg/day). In the 9-month oral toxicity study in dogs, OCA produced clinical signs of toxicity that could be associated with liver function (yellow discoloration of the skin, mucous membranes, and eyes) and elevated ALT levels. While there were no microscopic changes in the liver in the 9-month toxicity study, histopathological changes were noted in the liver and gallbladder in a shorter duration study in dogs. Increased liver enzymes were also observed in humans at higher doses than 10 mg, proposed for the current indication (see the Clinical Review). Additional primary target organs in the 26-week toxicity study in rats were the large intestine (subacute inflammation) and bone marrow (increased cellularity).

Overall, the estimated systemic exposures to total OCA equivalents (i.e., OCA and its taurine and glycine conjugates) at the NOAELs in the 26-week and 9-month toxicity studies in rats and dogs, respectively, exceed those in humans at the maximum recommended human dose (MRHD) of 10 mg proposed for the current indication. The NOAEL from the 26-week toxicity study in rats (6 mg/kg/day) was estimated to produce systemic exposures approximately 2.3 times those in humans at the MRHD. The NOAEL from the 9-month repeat-dose toxicity study in dogs (15 mg/kg/day) was

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estimated to produce systemic exposures approximately 12 times those in humans at the MRHD.

In a 2-year oral carcinogenicity study in Crl:CD1 mice, there were no drug-related neoplastic findings at OCA doses up to 25 mg/kg/day. In an oral carcinogenicity study in Crl:CD(SD) rats of up to 2 years in duration, 20 mg/kg/day OCA caused an increase in the incidence of benign granulosa cell tumors in the ovaries and benign granular cell tumors in the cervix and vagina of female rats. There were no drug-related neoplastic findings in male rats at OCA doses up to 20 mg/kg/day.

OCA was not genotoxic in the Ames test, a human peripheral blood lymphocyte chromosomal aberration test, and a mouse micronucleus test. The glycine conjugate of obeticholic acid was also not genotoxic in an Ames test and human peripheral blood lymphocyte chromosome aberration test. The taurine conjugate of obeticholic acid was not genotoxic in an Ames test, and was negative in a human peripheral blood lymphocyte chromosomal aberration test in the presence of metabolic activation; whereas, the findings of the chromosomal aberration assay in the absence of metabolic activation were inconclusive.

In an oral fertility and early embryonic development study, treatment of male and female rats with up to 50 mg/kg/day OCA did not affect fertility or early embryonic development. The NOAELs for male and female systemic toxicity in this study were 50 and 25 mg/kg/day, respectively. In an embryofetal development study in rats, 75 mg/kg/day OCA caused decreased fetal body weights and increased numbers of early or late resorptions and nonviable fetuses. In maternal animals, this dose produced mortality, decreased body weight, body weight gain, and food consumption, and abortion. Therefore, the developmental toxicity observed at this dose may be secondary to maternal toxicity. The NOAEL for maternal toxicity and embryofetal development in this study was 25 mg/kg/day. In an embryofetal development study in rabbits, the NOAEL for maternal and developmental toxicity was 20 mg/kg/day OCA (the highest dose tested). Finally, in a pre- and postnatal development study in rats, there was no evidence of any adverse effect on pre- and postnatal development at oral doses of OCA up to 40 mg/kg/day (the highest dose tested).

1.3 Recommendations

1.3.1 Approvability

From a nonclinical standpoint, this product is approvable for indication proposed.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

In response to a July 29, 2015 Information Request and high-level labeling comments from FDA in the August 28, 2015 Filing Communication-Filing Review Issues Identified, the Applicant submitted proposed revised text for the label (SDN 18). Nonclinical

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review of the Applicant's proposed established pharmacologic class and sections 8.1, 8.2, 12.1, and 13.1 is provided below.

Established Pharmacologic Class:
Obeticholic acid is a first-in-class NME. The Applicant has proposed the term "Farnesoid X receptor agonist" as the established pharmacologic class for obeticholic acid. The proposed established pharmacologic class, which is based on the drug's mechanism of action, is acceptable.

Applicant's Proposed Version:

8.1 Pregnancy

Risk Summary

(b) (4)

Data

Animal Data

(b) (4)

Obeticholic acid exposure margins were calculated using systemic exposure (AUC) values of obeticholic acid plus obeticholic acid's active metabolite conjugates (tauro-obeticholic acid and glyco-obeticholic acid) in animals (at the indicated doses) and in humans at the MRHD of 10 mg.

Evaluation:

The Risk Summary section should be revised to state that there were no adverse effects at 25 mg/kg/day OCA in the rat embryo-fetal development study. In addition, adverse effects observed at 75 mg/kg/day in the embryo-fetal development study in rats should be included in the Animal Data section. The following recommended changes were developed in collaboration with the Maternal Health team.

Recommended Version:

Risk Summary

The limited available human data on the use of obeticholic acid during pregnancy are not sufficient to inform a drug-associated risk. In animal reproduction studies, no developmental abnormalities or fetal harm were observed in rats or rabbits at approximately 13 times and 6 times human exposures, respectively, at the maximum recommended human dose (MRHD) of 10 mg *[see Data]*.

The estimated background risks of major birth defects and miscarriage for the indicated population are unknown. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2%-4% and 15%-20%, respectively.

Data

Animal Data

In an embryo-fetal development study in rats, obeticholic acid was administered orally during the period of organogenesis at doses of 5, 25, and 75 mg/kg/day. At 25 mg/kg/day (a dose that produced systemic exposures approximately 13 times those in humans at the MRHD of 10 mg), there was no maternal or developmental toxicity. At 75 mg/kg/day (approximately 40 times the human exposure at the MRHD), decreased fetal body weights and increased numbers of early or late resorptions and nonviable fetuses were observed. In maternal animals, mortality, fetal loss, decreased body weight and food consumption as well as decreased body weight gain were observed at 75 mg/kg/day. Thus, the developmental toxicity observed at this dose may be secondary to maternal toxicity. In rabbits, obeticholic acid was administered orally during the period of organogenesis at doses of 3, 9, and 20 mg/kg/day. Obeticholic acid administered at doses up to 20 mg/kg/day (approximately 6 times the human exposure at the MRHD) was not teratogenic and did not produce any evidence of fetal harm.

In a pre- and postnatal development study, administration of obeticholic acid in rats during organogenesis through lactation at doses of 5, 25, and 40 mg/kg/day did not produce effects on pregnancy, parturition or postnatal development at any dose (the 40 mg/kg/day dose is approximately 21 times the human exposure at the MRHD).

Obeticholic acid exposure margins were calculated using systemic exposure (AUC) values of obeticholic acid plus obeticholic acid's active metabolite conjugates (tauro-

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obeticholic acid and glyco-obeticholic acid) in animals (at the indicated doses) and in humans at the MRHD of 10 mg.

Applicant's Proposed Version:

8.2 Lactation

Risk Summary

(b) (4)

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for TRADENAME and any potential adverse effects on the breastfed infant from TRADENAME or from the underlying maternal condition.

(b) (4)

Evaluation:

The following recommended changes were developed in collaboration with the Maternal Health team.

8.2 Lactation

Risk Summary

There is no information on the presence of obeticholic acid in human milk, the effects on the breast-fed infant or the effects on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for TRADENAME and any potential adverse effects on the breastfed infant from TRADENAME or from the underlying maternal condition.

Applicant's Proposed Version:

12.1 Mechanism of Action

(b) (4)

(b) (4)

Evaluation:

The text should be modified as recommended below.

Recommended Version:

12.1 Mechanism of Action

Obeticholic acid is an agonist for the farnesoid X receptor (FXR), a nuclear receptor expressed at in the liver and intestine. FXR is a key regulator of bile acid, inflammatory, fibrotic, and metabolic pathways. FXR activation decreases the intracellular hepatocyte concentrations of bile acids by suppressing *de novo* synthesis from cholesterol as well as by increased transport of bile acids out of the hepatocytes. These mechanisms limit the overall size of the circulating bile acid pool while promoting choleresis, thus reducing hepatic exposure to bile acids.

Applicant's Proposed Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Evaluation:

The text should be modified to reflect drug-related neoplasms observed in the rat carcinogenicity study.

Recommended Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenic potential of obeticholic acid was assessed in carcinogenicity studies of up to 2 years in duration in mice and rats. In mice, there were no drug-related neoplastic findings at doses up to 25 mg/kg/day obeticholic acid, a dose that produced systemic

exposures approximately 12 times those in humans at the MRHD of 10 mg. In rats, obeticholic acid was administered at doses of 2, 7, and 20 mg/kg/day. At 20 mg/kg/day (approximately 12 times the human exposure at the MRHD), obeticholic acid caused an increase in the incidence of benign granulosa cell tumors in the ovaries and benign granular cell tumors in the cervix and vagina of female rats. There were no drug-related neoplastic findings in male rats.

Obeticholic acid was not genotoxic in the Ames test, a human peripheral blood lymphocyte chromosomal aberration test, and a mouse micronucleus test. The glycine conjugate of obeticholic acid was also not genotoxic in an Ames test and human peripheral blood lymphocyte chromosome aberration test. The taurine conjugate of obeticholic acid was not genotoxic in an Ames test, and was negative in a human peripheral blood lymphocyte chromosomal aberration test in the presence of metabolic activation; the findings of the chromosomal aberration assay in the absence of metabolic activation were inconclusive.

Obeticholic acid, administered at oral doses of 5, 25, and 50 mg/kg/day to male rats for 28 days before mating and throughout the mating period, and to female rats from 14 days before mating through mating and until gestation day 7, did not alter male or female fertility or early embryonic development at any dose (the 50 mg/kg/day dose is approximately 13 times the human exposure at the MRHD).

2 Drug Information

2.1 Drug

Obeticholic acid

CAS Registry Number: 459789-99-2

Generic Name: Obeticholic acid (OCA)

Code Name: INT-747; DSP-1747; I 18; 6α-ethyl chenodeoxycholic acid (6-ECDCA); ATC code: A05AA04

Chemical Name: 3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oic acid

Molecular Formula/Molecular Weight: $C_{26}H_{44}O_4$, 420.6 (b) (4) g/mol

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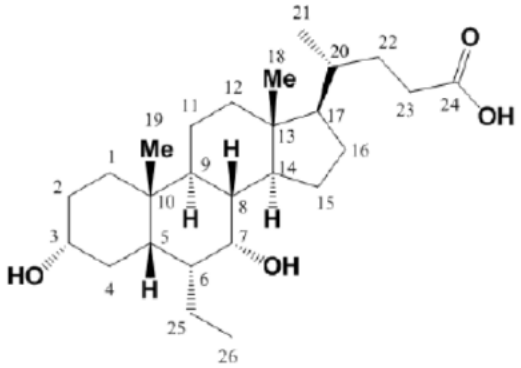
Structure or Biochemical Description:



Pharmacologic Class: Farnesoid X Receptor (FXR) agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 63307 (INT-747, Intercept Pharmaceuticals Inc.)
DMF (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4) DMF (b) (4) and DMF (b) (4)

2.3 Drug Formulation

The proposed drug product consists of obeticholic acid tablets available in 2 strengths (5 mg and 10 mg). The Applicant's table below presents the composition of the tablets.

Table 1: Quantitative Composition of OCA Tablet Formulations

Component	Amount Per Tablet (mg)	Function	Reference to Quality Standard
5 mg tablet			
Obeticholic acid[a]	5.0[a]	Active ingredient	(b) (4)
Microcrystalline cellulose	(b) (4)		NF/Ph. Eur.
Sodium starch glycolate			NF/Ph. Eur.
Magnesium stearate			NF/Ph. Eur.
Opadry II Yellow coating material[b]	(b) (4)	Coating material	(b) (4)
(b) (4)			USP/Ph. Eur.
Total weight	208.0		
10 mg tablet			
Obeticholic acid[a]	10.0[a]	Active ingredient	(b) (4)
Microcrystalline cellulose	(b) (4)		NF/Ph. Eur.
Sodium starch glycolate			NF/ Ph. Eur.
Magnesium stearate			NF/ Ph. Eur.

Table 1: Quantitative Composition of OCA Tablet Formulations (Continued)

Component	Amount Per Tablet (mg)	Function	Reference to Quality Standard
10 mg tablet			
Opadry II Yellow coating material[b]	(b) (4)	Coating material	(b) (4)
	(b) (4)		USP/Ph. Eur.
Total weight	208.0		

NF = National Formulary; Ph. Eur. = European Pharmacopeia

[a] OCA drug substance amount added assumes the drug substance content is 100%; actual amount added is adjusted based on the potency of the drug substance lot used; the amount of microcrystalline cellulose is correspondingly decreased.

[b] Refer to Module 3.2.P.4, Control of Excipients, for a summary of the components and composition of Opadry II Yellow (b) (4) Opadry II coating material is manufactured from NF compendial excipients or from colorant meeting compliance with Directive 2008/12/EC (formerly 95/45/EC) and Federal Food, Drug, and Cosmetic Act standards (b) (4)

2.4 Comments on Novel Excipients

With the exception of Opadry II Yellow (b) (4) all excipients are compendial, listed in the FDA Inactive Ingredients Database (IID), and used in approved oral drug products at levels greater than those in the drug product at the proposed maximum dose of 10 mg. Opadry II Yellow (b) (4) The ingredients contained in the Opadry coating material comply with USP or NF requirements and are summarized in the Applicant's table below. Although Opadry II Yellow (b) (4) is not listed in the IID, several other Opadry II Yellow coatings and the individual components of the coating are listed. Furthermore, the drug product reviewer indicated that there are no concerns for this coating material.

Table 2: Quantitative Formula for Opadry II Yellow (b) (4)

Components (Compendial Reference)	Opadry II Yellow (b) (4) (%w/w)	E Number[a]
Polyvinyl Alcohol – Part Hydrolyzed (USP, FCC, Ph. Eur.)	(b) (4)	E1203
Titanium Dioxide (USP, FCC, Ph. Eur.)		E171
Macrogol/PEG 3350 (NF, FCC, Ph. Eur. JECFA)		E1521
Talc (USP, FCC, Ph. Eur.)		E553b
Iron Oxide Yellow (NF)		E172

FCC = Food Chemicals Codex; JECFA = Joint Evaluation Committee on Food Additives; Ph. Eur. = European Pharmacopeia; USP = United States pharmacopeia.

[a] E number denotes compliance with Directive 2008/12/EC (formerly 95/45/EC).

2.5 Comments on Impurities/Degradants of Concern

Drug Substance:

Unspecified and Specified Impurities. The drug substance specification lists six impurities (Impurity (b) (4)), the structures of which are shown in the Applicant's figure below. Impurity (b) (4) is controlled as an unknown impurity.

Table 1: Summary of Known OCA Impurities

(b) (4)

Table 1: Summary of Known OCA Impurities (Continued)

(b) (4)

The proposed specification for Impurities (b) (4) Impurity (b) (4) and Impurity (b) (4) (NMT (b) (4) %) meets the ICH Q3A qualification threshold ((b) (4) % or (b) (4) mg/day, whichever is

lower) for drug substances with a maximum daily dose ≤ (b) (4) g/day. The specification for individual unknown impurities (including Impurity (b) (4) NMT (b) (4) %) meets the ICH Q3A identification threshold (0.1% or 1.0 mg/day, whichever is lower) for drug substances with a maximum daily dose ≤2 g/day.

The proposed specification for Impurity (b) (4) is NMT (b) (4) %. According to the submission, although the Applicant considers this impurity to be qualified at up to (b) (4) %, the proposed specification (b) (4) %) is lower based on experience indicating that the allowable level can be reduced. The level of Impurity (b) (4) in lots used in the nonclinical studies were < (b) (4) to < (b) (4) % (26 week rat toxicity study; 9 month dog study) and (b) (4) % (rat and rabbit developmental and reproductive toxicity studies). In Phase 2 capsule formulation clinical studies, levels of Impurity (b) (4) ranged from (b) (4) %.

Impurity (b) (4) has been identified as a (b) (4). The proposed specification limit of NMT (b) (4) % is justified by the qualification of Impurity (b) (4) at levels up to (b) (4) % in a rat 28-day toxicology study and up to (b) (4) % in clinical studies. In addition, INT-747 with (b) (4) % Impurity (b) (4) was negative in the in vitro bacterial reverse mutation assay and a human peripheral blood lymphocyte chromosome aberration test. (b) (4)

Elemental Impurities. The proposed drug substance specifications for overall (b) (4) are NMT (b) (4) ppm. Based on the maximum dose (10 mg/day OCA), the specification for (b) (4) ppm) results in a potential maximum intake of (b) (4) µg/day, which is far less than the oral permitted daily exposure (PDE) value for this inorganic ((b) (4) µg/day) (Q3D Elemental Impurities Guidance for Industry, September 2015). The proposed specification for overall (b) (4) ppm) results in a potential maximum intake of (b) (4) µg (b) (4) at the maximum dose ((b) (4) mg/OCA). This potential intake is below the lowest oral PDE value among the 24 elemental impurities addressed in the guidance (b) (4) as well as the oral PDE for (b) (4) µg/day). Thus, the proposed specifications are acceptable.

Residual Solvents. The proposed drug substance specifications for (b) (4) ppm) are within the recommended concentration limits in ICH Q3C.

Potential Genotoxic Impurities. According to the submission, the Applicant conducted a genotoxic impurity assessment on reagents, solvents, process intermediates, and known process impurities starting at (b) (4) through the manufacture of the drug substance which included (b) (4). The Applicant's assessment identified the following compounds of concern which are introduced in the OCA process and have genotoxic potential: 1) (b) (4) and 2) (b) (4). In addition, (b) (4) hese compounds are discussed below.

In representative lots of (b) (4) was detected at levels ranging from (b) (4) ppm. At the proposed maximum OCA dose (10 mg/day), the threshold of toxicological concern (TTC)-based limit (which equates to a maximum daily intake of (b) (4) µg/day acetaldehyde) is (b) (4) ppm. Thus, the detected levels ((b) (4) ppm) are (b) (4)-fold lower than (b) (4) ppm.

Although (b) (4) was identified as a potential genotoxic impurity based on in silico structure-activity relationship [(Q)SAR; DEREK Nexus and MultiCase], (b) (4) was shown to be negative when tested in the in vitro bacterial reverse mutation assay (Study No. (b) (4) 12-474 reviewed in Section 7.4). Thus, in accordance with the ICH M7 Guidance, this compound should be treated as a non-mutagenic impurity.

(b) (4) is an ICH Q3C Class 1 solvent and carcinogen, with an ICH limit o (b) (4) ppm. Based on the ICH limit, the limit for (b) (4) in OCA was set at (b) (4)% of this value ((b) (4) ppm). In five OCA development lots, (b) (4) was present at <(b) (4) ppm.

(b) (4) were detected at levels less than (b) (4) ppm, respectively, in six development lots of (b) (4) These detected levels (< (b) (4) and < (b) (4) ppm) are ~ (b) (4)-fold lower than (b) (4) ppm (the TTC-based limit). In addition, the detected levels are less than the limit of (b) (4) ppm which is (b) (4)% of the TTC-based limit.

(b) (4) is a known genotoxic substance and is used as a (b) (4) in the manufacture of (b) (4) According to the Applicant ((b) (4) is controlled by the specification of NMT (b) (4) ppm in (b) (4), which is less than the TTC-based limit of (b) (4) ppm. In addition, (b) (4) levels detected in ten batches of (b) (4) were low ((b) (4) ppm; (b) (4)-fold lower than (b) (4) ppm). Finally, the Applicant indicated that there are multiple (b) (4) within the (b) (4) which would mitigate risk to this potential impurity.

In response to an inquiry from the drug substance reviewer regarding the potential genotoxicity of an impurity (b) (4) observed in the starting material (b) (4) the CDER Chemical Informatics Group evaluated this impurity for bacterial mutagenicity using (quantitative) structure-activity relationship [(Q)]SAR] models [Derek Nexus 4.1.0 (DX), Leadscope Model Applier 2.0.3-1 (LMA), and CASE Ultra 1.6.00 (CU)]. (b) (4) was predicted to be negative for bacterial mutagenicity (b) (4) by all three software programs. Thus, in accordance with the ICH M7 Guidance, this should be treated as a non-mutagenic impurity.

Drug Product:

The proposed drug product specifications for individual unspecified impurities in 5 mg and 10 mg tablets are NMT (b) (4)% and NMT (b) (4)%, respectively, which meets the ICH Q3B(R2) identification threshold (b) (4)% or (b) (4) µg/day, whichever is lower) for drug products with a maximum daily dose of (b) (4) mg. The proposed specifications for Impurity (b) (4) (both degradation products) are NMT (b) (4)% and NMT (b) (4)%,

respectively. The specification for total impurities (including Impurity [(b) (4)]) is NMT [(b) (4)]%.

Impurity [(b) (4)] is a degradation product in both the drug substance and drug product. The proposed specification limit of NMT [(b) (4)]% is justified by the qualification of Impurity [(b) (4)] at levels up to [(b) (4)]% in a rat 28-day toxicology study and up to [(b) (4)]% in clinical studies. In addition, INT-747 with [(b) (4)]% Impurity [(b) (4)] was negative the in vitro bacterial reverse mutation assay and a human peripheral blood lymphocyte chromosome aberration test.

Impurity [(b) (4)] OCA impurity; see Applicant's figure below] is a degradation product primarily formed on accelerated stability testing. This impurity is produced by [(b) (4)] of OCA by [(b) (4)] that is an ingredient in the Opadry coating material. According to the Applicant, Impurity [(b) (4)] was not predicted to be mutagenic based on computational evaluation (assuming n=10) by Derek Nexus and Leadscope Model Applier (full computational assessment report provided in SDN 10). The proposed limit (NMT [(b) (4)]%) for Impurity [(b) (4)] (which was not qualified in nonclinical studies) meets the ICH Q3B qualification threshold [(b) (4)]% or [(b) (4)] µg total daily intake, whichever is lower) for drug products with a maximum daily dose of [(b) (4)] mg.

[(b) (4)]

The CDER Chemical Informatics Group evaluated [(b) (4)] of OCA for genetic toxicity using Derek Nexus 4.1.0 (DX), Leadscope Model Applier 2.0.3-1 (LMA), and MC4PC 2.4.1.4 (MC) or CASE Ultra 1.5.2.0 (CU). While the [(b) (4)] chain length can be up to [(b) (4)] repeating units, testing of such a large molecule is not feasible due to software restraints. Results from the Chemical Informatics evaluation showed that the overall software predictions for [(b) (4)] OCA impurity [(b) (4)] were negative for bacterial mutagenicity [(b) (4)] Although the [(b) (4)] OCA impurity [(b) (4)] was predicted to be positive by LMA for in vitro chromosomal aberrations, the alerting moiety also appears in the empirically negative API structure. Thus, the positive prediction was discounted in the overall expert prediction (i.e., negative for chromosome aberrations). With respect to the [(b) (4)] OCA impurity [(b) (4)], the positive prediction by LMA for in vitro chromosome aberrations was based on the same alerting moiety. However, expert review by the Chemical Informatics Group concluded that the [(b) (4)] impurity is not well represented by any of the model training sets and thus no overall expert call was made. The ICH M7 Guidance is for DNA reactive (mutagenic) impurities, and the guidance states that other

types of genotoxicants that are non-mutagenic typically have threshold mechanisms and usually do not pose carcinogenic risk at the level ordinarily present as impurities. Therefore, to limit a possible human cancer risk associated with the exposure to potentially mutagenic impurities, the bacterial mutagenicity assay is used to assess the mutagenic potential and the need for controls. Based on QSAR evaluation by the Applicant and Chemical Informatics group, Impurity (b) (4) is predicted to be negative in bacterial mutagenicity assays. Thus, Impurity (b) (4) should be treated as a non-mutagenic impurity and the proposed specification for Impurity (b) (4) in the drug product (NMT (b) (4) %) is acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication is for treatment of primary biliary cirrhosis (PBC) in combination with ursodeoxycholic acid (UDCA) in adults with an inadequate response to UDCA or as monotherapy in adults unable to tolerate UDCA. The recommended starting dose is 5 mg once daily and may be increased after 3 months, if tolerated, to 10 mg once daily to improve response.

2.7 Regulatory Background

On April 9, 2008, the Applicant's request for orphan designation of OCA for PBC was granted. OCA was also granted Fast Track designation on May 27, 2014. On November 18, 2014, the Division accepted the Applicant's request and plan for submitting portions of the proposed NDA as a rolling submission. Module 4 (Nonclinical Study Reports) and supporting nonclinical written and tabulated summaries under Module 2 were submitted on December 19, 2014 (SDN 01). Additional nonclinical study reports and revised nonclinical written and tabulated summaries were submitted under Modules 4 and 2, respectively, on June 26, 2015 (SND 02).

3 Studies Submitted

3.1 Studies Reviewed

STUDY	REPORT NUMBER
PHARMACOLOGY	
Primary Pharmacology	
Nuclear Receptor Selectivity of INT-747	NRS-001
In Vitro Pharmacology: High-Throughput Profile Study of INT-747	14862
Nuclear Receptor Selectivity of Glyco- and Tauro-Conjugated Derivatives of INT-747	NRS-003
AlphaScreen Assay hFXR Activity	02C_AlphaScreen-hFXR_001
HTR-FRET cAMP hTGR5 Activity	02C_HTR-FRET-cAMP-hTGR5_001
6α-Ethyl-Chenodeoxycholic Acid (6-ECDCA), a Potent and Selective FXR Agonist Endowed with Anticholestatic Activity	Pellicciari et al. 2002
Functional Characterization of Semisynthetic Bile Acid Derivative INT-767, a Dual Farnesoid X Receptor and TGR5 Agonist	Rizzo et al. 2010
Antifibrotic Activity of INT-747, A Novel FXR Activator, In Vitro and In	Albanis et al. 2005

Reference ID: 3870157

Experimental Liver Fibrosis and Cirrhosis	
Farnesoid X Receptor Antagonizes Nuclear Factor κB in Hepatic Inflammatory Response	Wang et al. 2008
Obeticholic Acid, a Farnesoid X Receptor Agonist, Improves Portal Hypertension by Two Distinct Pathways in Cirrhotic Rats	Verbeke et al. 2014
Hepatic Dimethylarginine-DiMethylaminohydrolase1 is Reduced in Cirrhosis and is a Target for Therapy in Portal Hypertension	Mookerjee et al. 2015
Secondary Pharmacology	
Farnesoid X Receptor Activation Inhibits Inflammation and Preserves the Intestinal Barrier in Inflammatory Bowel Disease	Gadaleta et al. 2011
Farnesoid X Receptor Ligands Inhibit Vascular Smooth Muscle Cell Inflammation and Migration	Li et al. 2007
FXR Activation Normalizes Insulin Sensitivity in Visceral Preadipocytes of a Rabbit Model of MetS	Maneschi et al. 2013
Farnesoid X Receptor Activation Improves Erectile Function in Animal Models of Metabolic Syndrome and Diabetes	Vignozzi et al. 2011
The Farnesoid X Receptor Modulates Renal Lipid Metabolism and Diet-Induced Renal Inflammation, Fibrosis, and Proteinuria	Wang et al. 2009
Diabetic Nephropathy is Accelerated by Farnesoid X Receptor Deficiency and Inhibited by Farnesoid X Receptor Activation in a Type 1 Diabetes Model	Wang et al. 2010
Safety Pharmacology	
In Vitro Human ERG Inhibition by INT-747	hERG-001
Effects of INT-747 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	070927.JPQ
A 4-Dose Crossover Oral Gavage Cardiovascular Safety Assessment Study with INT-747 in Beagle Dogs	7654-100
Neuropharmacological Profile (NPP) of INT-747 in Rats	0200RI42.001
Pulmonary Assessment of INT-747 in the Anesthetized Rat	1082RI42.001
A Gastrointestinal Propulsion Assay in Rats Administered INT-747	0239RI42.001
PHARMACOKINETICS/ADME/TOXICOKINETICS	
Absorption	
Pharmacokinetic Study of [^{14}C]DSP-1747 in Rats after Single Oral Administration	AE-6951-G
Distribution	
Plasma, Bile, and Liver INT-747 concentration and Its Major Metabolites After Oral Administration to Mice	RODA Mouse 2011
Biodistribution and Mass Balance of 6-ECDCA and Its Metabolites in Rats	6-ECDCA
Pharmacokinetic Study of [^{14}C]DSP-1747 in Rats after Single Oral Administration	AE-6951-G
[^{14}C]-INT-747: Tissue Distribution Studies in Partially Pigmented Rats to Support Human Dosimetry Calculation	ICP/01
A Protein Binding Study of INT-747, and Two Metabolites in Mouse, Rat, Dog, and Human Plasma In Vitro	(b) (4) 661035
Metabolism	
Analysis of Metabolites of [^{14}C]DSP-1747 in Male Rats	AE-6952-G
Pharmacokinetics and Metabolite Profiling Following a Single and Multi-dose Oral Gavage Administration of [^{14}C]OCA (Single Dose) and OCA (Multi-dose) to Male and Female Sprague-Dawley Rats	XBL15604
Determination of Relative Plasma Exposures of Obeticholic Acid (OCA) Metabolites in Rat Plasma and Human Plasma	XBL15617
Plasma, Bile, and Liver Concentration of INT-747 and Its Main Metabolites After Oral Administration to Rabbit	RODA Rabbit 2011
Metabolite Radioprofiling and Metabolite Identification of [^{14}C]OCA in Liver, Intestine, and Kidney S9 Fractions from Mice, Rats, Rabbits, Dogs, Monkeys, and Humans	XBL14830
Excretion	

Pharmacokinetic Study of [^{14}C]DSP-1747 in Rats after Single Oral Administration	AE-6951-G
Biliary Secretion and Metabolism of INT-747 in Bile-Fistula Rat After Duodenal (ID) and Femoral (IV) Administration	RODA Rat 2011
Toxicokinetics	
14-Day Oral (Gavage) Toxicokinetic Study of INT-747 in Mice	(b) (4) 661033
14-Day Oral (Gavage) Toxicokinetic Study of INT-747 in Sprague Dawley Rats	661032
An Oral (Gavage) Toxicokinetic Study of INT-747 and Metabolites in New Zealand White Rabbits	661078
INT-747: An Oral Comparative Crossover Toxicokinetic Study of Capsules and Tablets in Beagle Dogs	661039
TOXICOLOGY	
Single-Dose Toxicity	
Rat	
Oral	017166
Dog	
Oral	017259
Repeat-Dose Toxicity	
Mouse	
7-Day, Oral	(b) (4) 661023
91-Day, Oral	661025
Rat	
28-Day, Oral	017262
26-Week, Oral	019958
Dog	
7-Day, Oral	017260
28-Day, Oral	017261
9-Month, Oral	019959
GENOTOXICITY	
Ames test	AB05JZ.503BTL
In vitro mammalian chromosome aberration test	AB05JZ.341.BTL
Mammalian erythrocyte micronucleus test	AB05JZ.123M.BTL
Ames test, Metabolite UPF-832	(b) (4) 2-120
Ames test, Metabolite UPF-1443	2-121
Ames test, INT-747 with Impurity (b) (4)	12-102
Ames test, Impurity (b) (4)	2-474
In vitro mammalian chromosome aberration test, Metabolite UPF-832	2-122
In vitro mammalian chromosome aberration test, Metabolite UPF-1443	2-123
In vitro mammalian chromosome aberration test, INT-747 with Impurity (b) (4)	2-103
CARCINOGENICITY	
2-Year Carcinogenicity Study, Mouse, Oral	(b) (4) 661038
2-Year Carcinogenicity Study, Rat, Oral	661037
REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	
Rat	
Fertility and Early Embryonic Development, Oral	(b) (4) 661005
Embryofetal Development, Oral, Dose Range-Finding	661001
Embryofetal Development, Oral	661003
Pre and Postnatal Development, Oral	661049
Rabbit	
Embryofetal Development, Oral, Dose Range-Finding	(b) (4) 661002
Embryofetal Development Study, Oral	661004
SPECIAL TOXICOLOGY	
4-Week Repeated Dose Oral Toxicity Study of DSP-1747 with Impurity (b) (4) in Rats	P130020

3.2 Studies Not Reviewed

- The following analytical methods and validation reports were not reviewed: (b) (4) 661034, (b) (4) 661040, 017484-1, 017624-1, 017803-1, (b) (4) 661041, (b) (4) 661048, (b) (4) 661006, (b) (4) 661051, and (b) (4) 661077.
- Module 2.6.4 (Pharmacokinetics Written Summary) states that no studies of drug interaction have been conducted in animal cells in vitro or in animal models in vivo. Effects of OCA on cytochrome P450 enzymes are presented in Module 5 (Clinical Study Reports) and discussed in Module 2.7 (Clinical Summary). Study reports pertinent to pharmacokinetics using human biomaterials will be reviewed by the clinical pharmacology reviewer.

3.3 Previous Reviews Referenced

- IND 63307 Pharmacology review dated February 12, 2009 (C. Wu, Ph.D., DGP)
- IND 63307 Pharmacology review dated October 14, 2011 (C. Wu, Ph.D., DGIEP)
- IND (b) (4) Pharmacology review dated July 29, 2014 (P. Au, Ph.D., DGIEP)

4 Pharmacology

4.1 Primary Pharmacology

The Applicant submitted study reports, as well as a series of a publications pertaining to the primary pharmacology of OCA. Findings in the publications are briefly summarized herein, with an emphasis on OCA only (test compounds and methods not pertaining to OCA are not discussed).

Nuclear Receptor Selectivity of INT-747 (Study No. NRS-001)

The nuclear receptor selectivity of INT-747 was investigated in 13 nuclear receptor coactivator recruitment assays using time-resolved fluorescence resonance energy (TR-FRET) to detect ligand-dependent binding of the nuclear receptor ligand binding domain (LBD) to a transcriptional coactivator peptide. The following nuclear receptors were evaluated: constitutive androstane receptor (CAR), estrogen receptor α, FXR, glucocorticoid receptor, liver X receptor α, peroxisome proliferator-activated receptors (PPAR) α, β/δ, and ɣ, progesterone receptor, retinoic acid receptor α, retinoid x receptor (RXR) α, thyroid receptor β, and vitamin D receptor. In this study, INT-747 was shown to be a FXR agonist, with an EC50 of 44 nM. CDCA was 150-fold less potent (EC50 = 6.6 µM). INT-747 did not activate any of the other nuclear receptors tested. High concentrations of INT-747 inhibited the basal activation of CAR (EC50 = 58 µM), PPARα (EC50 = 430 µM), RXRα (EC50 = 220 µM).

In Vitro Pharmacology: High-Throughput Profile Study of INT-747 (Study No. 14862)

INT-747 was tested in *in vitro* radioligand binding assays conducted for 79 receptors, channels, and transporters. The concentration of INT-747 tested was 10 µM. In this study, INT-747 did not significantly affect the binding of radioligands to any of the receptors, channels, and transporters evaluated. Thus, no IC50 or Ki values were

calculated. The following weak to moderate effects on binding (defined as increasing or decreasing binding by 20 – 50%) were noted: Cl^- channel (GABA-gated; 30% inhibition), NE transporter (26% inhibition), TNF-α (21% inhibition), δ2 (20% inhibition), and CGRP (23% stimulation).

Nuclear Receptor Selectivity of Glyco- and Tauro-Conjugated Derivatives of INT-747 (Study No. NRS-003)

The nuclear receptor selectivity of glyco- and tauro-conjugated derivatives of INT-747 was investigated in 15 coactivator recruitment assays to evaluate the ability of the test compounds to stimulate ligand-dependent binding of nuclear receptor LBDs to a coactivator peptide. Alpha-Screen (Amplified Luminescent Proximity Homogeneous Assay) technology was used and the following nuclear receptors were evaluated: CAR, estrogen receptor, FXR, glucocorticoid receptor, liver X receptors α and β, PPAR α, β/ δ, and γ, pregnane X receptor, progesterone receptor, retinoic acid receptor α, RXR α, thyroid receptor β, and vitamin D receptor. In this study, the glyco- and tauro-conjugated derivatives of INT-747 were shown to activate FXR, with similar potency to INT-747. The EC50 values for glyco- and tauro-conjugated derivatives of INT-747 were 24 and 85 nM, respectively, compared to an EC50 of 45 nM for INT-747. At 100 µM, the glyco-derivative of INT-747 showed low activation of thyroid receptor β. However, the efficacy of the glyco-derivative at 100 µM relative to the reference compound (0.5 µM T3) was only 30%. The test compounds did not activate any of the other nuclear receptors tested.

AlphaScreen Assay hFXR Activity (Study No. 02C_AlphaScreen-hFXR_001)

The FXR AlphaScreen test was used to measure FXR activation by the following bile acid derivatives: UPF-2331, UPF-2334, and INT-747-3-O-Glucuronide (a metabolite of OCA). The test is based on detection of the interaction of the test compound with FXR LBD by amplified luminescent proximity homogenous assay (ALPHA technology). In this study, UPF-2331 activated hFXR with an EC50 of 0.7 µM. INT-747-3-O-Glucuronide activated FXR at micromolar concentration (EC50 = 6 µM), and UPF-2334 was not active at FXR. For reference, the EC50 for CDCA (control) was 10 µM.

HTR-FRET cAMP hTGR5 Activity (Study No. 02C_HTR-FRET-cAMP-hTGR5_001)

The ability of UPF-2331, UPF-2334, and INT-747-3-O-Glucuronide to stimulate G-Protein Coupled Receptor TGR5 (thereby inducing cAMP production) was evaluated. HTR-FRET was used to detect cAMP produced by NCI-H716 cells stimulated with GPCR agonists [control compound = lithocholic acid (LCA)]. In this study, UPF-2334 was not active, whereas UPF-2331 activated TGR5 at micromolar concentration (EC50 10 µM). The EC50 of INT-747-3-O-Glucuronide was 60 µM, compared to an EC50 of 9 µM for LCA. Thus, the INT-747-3-O-Glucuronide does not show significant activity at TGR5.

Reference ID: 3870157

6α-Ethyl-Chenodeoxycholic Acid (6-ECDCA), a Potent and Selective FXR Agonist Endowed with Anticholestatic Activity (Pellicciari et al. *J Med Chem*. 2002; 45(17): 3569-3572).

In this publication, a series of 6α-alkyl-substituted analogues of CDCA, including 6-ECDCA, were evaluated as potential FXR ligands. In a cell-free ligand sensing assay (which measured ligand-dependent recruitment of a steroid receptor co-activator 1 peptide to FXR by fluorescence resonance energy transfer), 6-ECDCA was shown to be a FXR agonist with an EC50 of 99 nM. This EC50 is 87-fold lower than CDCA (EC50 = 8.66 µM). In a reporter gene assay which employed the full length human FXR in HuH7 cells, 6-ECDCA was also an agonist (EC50 = 85 nM). When tested in a panel of nuclear receptor LBD-GAL4 chimeric receptors, 1 µM 6-ECDCA activated only the FXR(LBD)-GAL4 chimera with no significant activation of other receptors. In an animal model of cholestasis, 3 µmol/kg/min LCA administered via IV infusion to bile-duct cannulated male rats reduced bile flow and produced extensive liver cell necrosis. When 3 µmol/kg/min 6-ECDCA was co-infused with LCA, 6-ECDCA reversed LCA-induced bile flow impairment and protected against liver cell injury observed histologically. While 6-ECDCA protected against LCA-induced cholestasis, effects on bile flow diminished over time. CDCA (at doses up to 7 µmol/kg/min) was not effective in protecting against LCA-induced cholestasis.

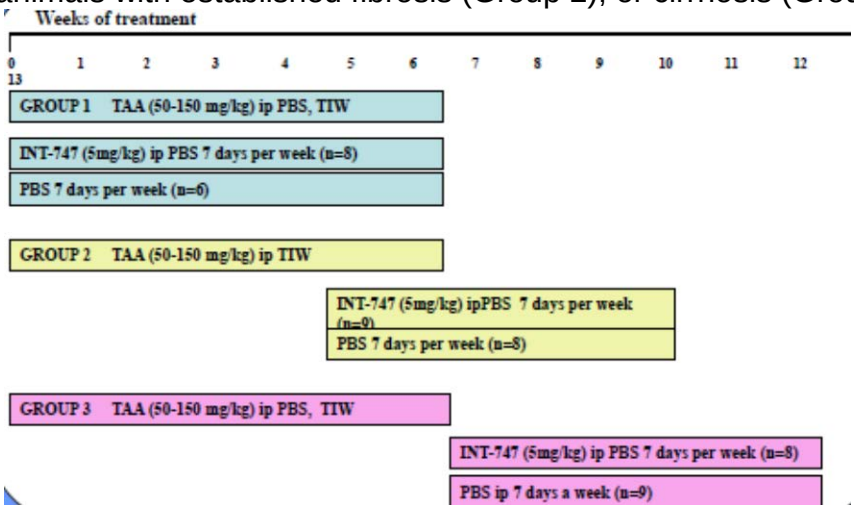
Functional Characterization of Semisynthetic Bile Acid Derivative INT-767, a Dual Farnesoid X Receptor and TGR5 Agonist (Rizzo et al. *Mol Pharmacol.* 2010; 78(4): 617-630).

Several semisynthetic bile acid derivatives, including INT-747, were characterized. Using the AlphaScreen coactivator recruitment assay, the EC50 for INT-747 at FXR was 100 nM, compared to 10 µM for CDCA. In transactivation assays using HepG2 cells transiently transfected with full-length hFXR and FXRE(IR1)-Luc, the EC50 values for INT-747 and CDCA were 30 nM and 8 µM, respectively. INT-747 demonstrated only weak agonist activity at TGR5 in cell-based assays. In FRET (cAMP induction) assays using NCI-H716 cells (enteroendocrine cells that constitutively express TGR5), the EC50 value for INT-747 was 20 µM, compared to 8 µM for LCA. In FRET (cAMP) TGR5-expressing HEK293 cells, the EC50 value for INT-747 was 0.5 µM, compared to 0.3 µM for LCA. In HepG2 cells, treatment with INT-747 modulated the expression of FXR target genes [↑ bile salt export pump (BSEP), organic solute transporter β (OSTβ), and small heterodimer partner (SHP) mRNA expression; ↓CYP7A1 mRNA expression]. INT-747 (1 µM) was more potent than CDCA (20 µM) for all target genes evaluated. INT-747 treatment did not significantly induce TGR5-dependent secretion of the active form of the incretin glucagonlike peptide-1 (GLP-1) in human intestinal cells NCI-H716. Finally, to evaluate in vitro cytotoxicity in HepG2 cells, cytotoxicity, necrosis, and apoptosis were evaluated by measuring ATP decrease, LDH release, and caspase-3 activation, respectively. INT-747 EC50 values for ATP decrease, LDH release, and caspase-3 activation were 230 µM, 210 µM, and 160 µM, respectively, compared to LCA EC50 values of 84 µM, 105 µM, and 65 µM, respectively.

Reference ID: 3870157

Antifibrotic Activity of INT-747, A Novel FXR Activator, *In Vitro* and In Experimental Liver Fibrosis and Cirrhosis (Albanis et al. *Hepatology*. 2005; 42(S1): Abstract 1040).

Antifibrotic activity of INT-747 was evaluated in vitro and in the thioacetamide (TAA) model of liver fibrosis in rats. Following treatment of serum-starved immortalized human hepatic stellate cells (HSCs; cell line LX-2) with 1 and 3 µM INT-747 for 1 h, INT-747 produced 70-90% decreases in the expression of fibrotic marker mRNAs (collagen I, α smooth muscle actin, TGFβ-1 receptor, MMP-2, TIMP-1, and TIMP-2), as compared to control cells. In the in vivo portion of the study, fibrosis or cirrhosis was induced in male Sprague-Dawley rats by intraperitoneal TAA injections, and animals were treated with INT-747 or vehicle according to the following study design (figure copied directly from the abstract). INT-747 was administered simultaneously with TAA (Group 1), or in animals with established fibrosis (Group 2), or cirrhosis (Group 3).



Control animals administered TAA and vehicle were cirrhotic in all three groups; while animals administered TAA plus INT-747 had mild fibrosis in Group 1, mild fibrosis with few septae in Group 2, and fibrosis with few bridging fibrous septae in Group 3. INT-747 also produced statistically significant decreases in portal pressure in all three groups, compared to respective control groups. Portal pressures of Group 1 INT-747 treated rats were 9.5±22.8 mm Hg, compared to 12.3±26.2 mm Hg in controls ($p<0.05$). Portal pressures of Group 2 INT-747 treated rats were 11.9±2.2 mm Hg, compared to 17.1±2.7 mm Hg in controls ($p<0.05$). Finally, portal pressures in Group 3 INT-747 treated rats 11.5±2.9 mm Hg, compared to 18.8±3.0 mm Hg in controls ($p<0.01$). Collagen morphometry showed that animals treated with INT-747 contained significantly less collagen in the liver, relative to controls. Livers of Group 1 INT-747 treated rats had 3.2+2.0% collagen, compared to 6.5+3.0% in controls ($p<0.001$; although reported as "+", it appears this should be "±"). Livers of Group 2 INT-747 treated rats had 2.2+3.8% collagen, compared to 2.8+4.6% in controls ($p=0.001$). Finally, livers of Group 3 INT-747 treated rats had 2.4+2.7% collagen, compared to 4.3+3.5% in controls ($p<0.001$). Finally, while serum transaminases were similar among all groups, there was a trend toward decreased fibrotic marker mRNA (e.g., collagen I) in the INT-747 treatment groups compared to controls. Thus, it was concluded that in this animal model, INT-747

inhibited the progression of fibrosis, and reversed established fibrosis and cirrhosis when administered post-injury.

Farnesoid X Receptor Antagonizes Nuclear Factor κB in Hepatic Inflammatory Response (Wang et al. *Hepatology*. 2008; 48(5): 1632-1643.

The potential for FXR activation to modulate NF-κB-mediated hepatic inflammation was investigated in this study. Expression of proinflammatory genes in Hep2G cells and primary hepatocytes from wild-type and FXR null mice was measured following exposure to constituents which activate the NF-κB pathway [12-O-tetradecanoyl-phorbol-13-acetate (TPA), lipopolysaccharide (LPS), or TNF-α]. Pretreatment of HepG2 cells with 3 µM 6ECDCA inhibited the induced expression of TNF-α, cyclo-oxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) (relative to control cells) in response to activation of the NF-κB pathway. Primary hepatocytes from FXR null mice were shown to be more sensitive to NF-κB activation than wild-type hepatocytes. In addition, pretreatment of wild-type hepatocytes with 6ECDCA inhibited the increased expression of iNOS and monocyte chemoattractant protein-1 (MCP-1) induced by LPS and TNF-α, respectively. However, inhibition of the induced expression of these genes did not occur in FXR null hepatocytes, suggesting that the inhibition of proinflammatory gene expression by 6ECDCA is mediated by FXR. Finally, 6ECDCA did not suppress the expression of antiapoptosis target genes induced by TNF-α in mouse primary hepatocytes.

Obeticholic Acid, a Farnesoid X Receptor Agonist, Improves Portal Hypertension by Two Distinct Pathways in Cirrhotic Rats (Verbeke et al. *Hepatology.* 2014; 59(6):2286-2298).

Effects of INT-747 on intrahepatic hemodynamic dysfunction and signaling pathways were evaluated in thioacetamide (TAA)-intoxicated and bile-duct-ligated (BDL) rat models of cirrhotic portal hypertension. In this study, two oral (gavage) doses of 30 mg/kg INT-747 were administered 24 and 4 h before *in vivo* hemodynamics and other parameters were assessed. Effects of INT-747 on total intrahepatic vascular resistance (IHVR) and intrahepatic vascular tone in an *in situ* liver perfusion system, and on hepatic stellate cell (HSC) contraction *in vitro* were also evaluated. In both animal models of cirrhosis, hepatic expression of FXR was decreased, portal pressure (PP) was increased, and mean arterial pressure (MAP) was decreased compared to healthy control animals. Treatment with INT-747 increased the mRNA expression of SHP, decreased PP, and lowered total IHVR without affecting MAP (compared to vehicle-treated cirrhotic animals). When studied in an *in situ* liver perfusion system, TAA- and BDL- cirrhotic rats showed a higher perfusion pressure (relative to controls) and there was impaired acetylcholine-induced vasorelaxation and methylxanthin-induced vasoconstriction. Following treatment with INT-747, the higher perfusion pressure was decreased and vasorelaxation capacity was improved, while response to methylxanthin was not affected. These effects were considered to be due to increased endothelial nitric oxide synthase (eNOS) activity, which was related to downregulation of Rho-kinase in the TAA model and upregulation of dimethylarginine dimethylaminohydrolase (DDAH)-2 in the BDL model. When studied *in vitro*, culture-activated primary HSCs were shown to express only low levels of FXR and SHP relative to liver tissue from

healthy animals. Treatment with 1-100 µM INT-747 produced a small decrease (10%) in HSC contraction capacity relative to vehicle alone.

Hepatic Dimethylarginine-Dimethylaminohydrolase1 is Reduced in Cirrhosis and is a Target for Therapy in Portal Hypertension (Mookerjee et al. *J Hepatol.* 2015; 62:325-331).

This publication characterized DDAH-1 in cirrhotic livers from humans and BDL rats. In addition, effects of obeticholic acid (OA) on levels of asymmetric-dimethylarginine [ADMA; an eNOS inhibitor], expression of DDAH-1(the key enzyme involved in hepatic ADMA metabolism), eNOS activity, plasma ALT, and PP were evaluated. In this study, the authors demonstrated that hepatic DDAH-1 is localized primarily in hepatocytes, and its expression is decreased in human and BDL rat cirrhotic livers. In BDL rats, there was reduced DDAH-1 expression, elevated hepatic ADMA levels, reduced eNOS activity, and elevated PP. Oral treatment of BDL rats with 5 mg/kg OA for five days produced increased hepatic DDAH-1 expression, reduced hepatic ADMA, improved eNOS activity, and reduced PP (compared to BDL rats treated with vehicle). Treatment with OA did not impact histological fibrosis however. Finally, when tested in an *in vitro* study, treatment of HepG2 cells with up to 1 µM OCA produced a dose-dependent increase in DDAH-1 gene expression.

4.2 Secondary Pharmacology

A series of studies in the published literature related to the secondary pharmacology of OCA were submitted. The findings reported in the publications specifically pertaining to OCA are briefly summarized herein (test compounds and methods not pertaining to OCA are not discussed).

Farnesoid X Receptor Activation Inhibits Inflammation and Preserves the Intestinal Barrier in Inflammatory Bowel Disease (Gadaleta et al. *Gut*. 2011; 60(4):463-472).

This study investigated the effects of FXR agonists (including INT-747) on intestinal inflammation and secretion of pro-inflammatory cytokines by human immune cells. Briefly, in vitro treatment of LPS-stimulated human peripheral blood mononuclear cells (PBMCs) and CD14 monocytes with up to 10 µM INT-747 decreased TNF-α secretion. Treatment with INT-747 also inhibited the differentiation of monocytes to dendritic cells. Ex vivo treatment of activated lymphocyte-enriched lamina propria mononuclear cells (LPMCs) from colonic or ileal biopsies of IBD patients with INT-747 produced a decrease in the secretion of INF-ɣ, IL-17, and TNF-α (compared with controls). When studied in mouse models of intestinal inflammation, 5 mg/kg/day INT-747 administered by oral gavage protected against dextran sodium sulfate (DSS)- and trinitrobenzenesulfonic acid (TNBS)-induced colitis. In one or both of these chemically-induced colitis models, INT-747 was reported to reduce body weight loss, rectal bleeding, colonic shortening, epithelial permeability, ulceration, inflammatory cell infiltration, and goblet cell loss. INT-747 also downregulated expression of pro-inflammatory genes while microbicidal genes were induced in the colon. In contrast to wild-type mice, INT-747 had no effects on colitis symptoms and histology in FXR null mice.

Farnesoid X Receptor Ligands Inhibit Vascular Smooth Muscle Cell Inflammation and Migration (Li et al. *Arterioscler Thomb Vasc Biol.* 2007; 27:2606-2611).
In this publication, the potential for synthetic FXR ligands (including 6ECDCA) to regulate inflammation and migration in vascular smooth muscle cells (VSMC) was evaluated. When tested in rat aortic smooth muscle cells (RASMC), 30 µM 6ECDCA induced expression of SHP, with RASMC death occurring at higher concentrations when tested at up to 30 µM. 6ECDCA also inhibited basal and IL-1-β-induced iNOS and COX-2 production. In a Matrigel cell migration assay, 6ECDCA inhibited RASMC migration basally and in the presence of IL-1β or stimulated by platelet-derived growth factor (PDGF)-BB. In human aortic vascular smooth muscle cells (HASMCs), 6ECDCA induced SHP expression, inhibited IL-1β-induced COX-2 expression, and inhibited PDGF-BB induced cell migration.

FXR Activation Normalizes Insulin Sensitivity in Visceral Preadipocytes of a Rabbit Model of MetS (Maneschi et al. *J Endocrinol.* 2013; 218(2): 215-231)
In this study using an animal model of metabolic syndrome (MetS), male New Zealand White rabbits were fed a high fat diet (HFD) and dosed concurrently with 10 mg/kg OCA by oral gavage five days/week for 12 weeks. Animals fed HFD and dosed with OCA had reduced fasting blood glucose levels, improved glucose intolerance, and reduced visceral adipose tissue (VAT) weight compared to animals fed HFD alone. In addition, treatment with OCA was reported to normalize the following changes observed in animals fed HFD relative to those fed control diet: increased adipocyte size, hypoxia, and increased expression of perilipin and cytosolic insulin-regulated glucose transporter GLUT4 in VAT. Treatment with OCA also reduced hepatic lipid accumulation and expression levels of markers of steatosis (PPARɣ) and inflammation (TNF-α) compared to animals fed HFD alone. In isolated rabbit preadipocytes from HFD rabbits, treatment with OCA was reported to ameliorate adipogenic differentiation and glucose uptake.

Farnesoid X Receptor Activation Improves Erectile Function in Animal Models of Metabolic Syndrome and Diabetes (Vignozzi et al. *J Sex Med.* 2011; 8(1): 57-77)
The effects of INT-747 on metabolic parameters and penile vascular function were tested in a rabbit model of HFD-induced MetS and a rat model of streptozotocin (STZ) induced type 1 diabetes. In rabbits fed HFD, concurrent dosing with INT-747 (10 mg/kg via oral gavage; 5 days/week for 12 weeks) was reported to normalize visceral adiposity and glucose intolerance relative to animals not treated with INT-747. Treatment with INT-747 also resulted in upregulated penile expression of FXR and SHP, and was reported to partially restore eNOS and DDAH-1 expression and improve impaired nitric oxide-dependent relaxation in precontracted penile strips. These effects were attributed to an upregulation of NO transmission and inhibition of RhoA/ROCK pathway. In STZ-induced diabetic rats, penile expression of FXR was not altered but treatment with INT-747 (10 mg/kg via oral gavage; 5 days/week for 8 weeks) produced an upregulation in FXR expression. INT-747 was also reported to improve penile erection induced by electrical stimulation of cavernous nerve and hypersensitivity to intracavernous injection of a ROCK-inhibitor, without improvements in hyperglycemia.

Testosterone and Farnesoid X Receptor Agonist INT-747 Counteract High Fat Diet-Induced Bladder Alterations in a Rabbit Model of Metabolic Syndrome (Morelli et al. *J Steroid Biochem Mol Biol.* 2012; 132: 80-92)

This study using a rabbit model of MetS investigated effects of INT-747 on HFD-induced bladder alterations. The authors showed that FXR mRNA was expressed in rabbit bladder and correlated with visceral adiposity. In HFD rabbits concurrently treated with INT-747 (10 mg/kg via oral gavage; 5 days a week for 12 weeks), INT-747 prevented HFD-induced hyperglycemia, glucose intolerance, and visceral adipose tissue accumulation, but did not affect other MetS parameters such as hypogonadism or dyslipidemia. While MetS rabbits developed bladder alterations including fibrosis, hypoxia, low-grade inflammation, and RhoA/ROCK hyperactivity, each of the HFD-induced bladder alterations were reduced by treatment with INT-747. Furthermore, in precontracted bladder strips from treated animals, INT-747 prevented the HFD-induced overactive relaxant response to a selective ROCK inhibitor. Following in vitro pretreatment with 10 µM INT-747, RhoA-mediated migration of isolated rabbit bladder smooth muscle cells was inhibited.

The Farnesoid X Receptor Modulates Renal Lipid Metabolism and Diet-Induced Renal Inflammation, Fibrosis, and Proteinuria (Wang et al*. Am J Physiol Renal Physiol.* 2009; 297: F1587-F1596)

This study evaluated the role of FXR activation in a mouse model of diet-induced nephropathy. The authors reported that the following effects were observed in DBA/2J mice fed a high-fat, high-cholesterol (Western Diet; WD): proteinuria, podocyte loss, mesangial expansion, renal lipid accumulation, and increased expression of proinflammatory factors, oxidative stress, and profibrotic growth factors. Concurrent treatment of these animals with 10 mg/kg/day INT-747 via oral gavage for 12 weeks was reported to ameliorate the development of albuminuria and renal structural changes (e.g., podocyte loss, mesangial expansion), promote antifibrotic effects, and modulate inflammation and oxidative stress. INT-747 was also reported to prevent renal triglyceride accumulation by inhibiting fatty acid synthesis pathways and stimulating the expression of genes that promote fatty acid oxidation and lipid hydrolysis. Treatment of $FXR^{-/-}$ mice with INT-747 for 1 week suggested that INT-747 effects on renal gene expression are mediated by the FXR receptor.

Diabetic Nephropathy is Accelerated by Farnesoid X Receptor Deficiency and Inhibited by Farnesoid X Receptor Activation in a Type 1 Diabetes Model (Wang et al*. Diabetes.* 2010; 59: 2916-2927)

The effects of INT-747 on the development of diabetic nephropathy was evaluated in a mouse model of STZ-induced type 1 diabetes. In this study, renal injury was shown to be accelerated in diabetic FXR knockout mice fed a high fat, high cholesterol Western Diet (WD) relative to their WD fed wild-type C57BL/6 counterparts. In diabetic DBA/2J mice fed WD after the onset of diabetes with or without 30 mg/kg INT-747 admixed in the diet for 8 weeks, INT-747 was reported to protect against diabetic nephropathy. The authors reported that compared to mice not administered INT-747, there were decreases in proteinuria and renal histopathology alterations, and modulation of renal lipid metabolism, macrophage infiltration, and renal expression of sterol regulatory

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element binding proteins (SREBPs), profibrotic growth factors, and oxidative stress enzymes.

4.3 Safety Pharmacology

In Vitro Human ERG (hERG) Inhibition by INT-747 (Study No. hERG-001)

INT-747 was tested in a high-throughput assay for the rapid determination of (human ether-a-go-go) hERG channel affinity. In this study, the Predictor™ Fluorescence Polarization assay was employed using CHO membrane fractions expressing hERG channel protein. This assay screens for the ability of a compound to compete with a high-affinity red fluorescent tracer for binding to hERG. Positive controls E-4031 and tamoxifen were shown to have IC50 values of 4 to 15 nM and 1.5 µM, respectively; whereas, INT-747 did not appear to bind/block the hERG potassium channel.

Effects of INT-747 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study No. 070927.JPQ)

The effects of INT-747 on the hERG channel current were evaluated using whole cell patch clamp technique. INT-747 was tested at target concentrations of 10, 100, and 300 µM in transfected HEK293 cells expressing hERG potassium channels. The positive control (terfenadine) was tested at 60 nM, and inhibited the hERG channel current by 83.9%. In this study, the target concentration of 300 µM INT-747 disrupted the cell/patch recordings and whole cell patch clamp recordings were not possible. At 8.31 and 82.8 µM (actual concentrations for the 10 and 100 µM target formulations), the mean percent inhibition of the hERG channel current was 5.1% and 10.1% (statistically significant from vehicle alone). Effects noted at the 82.8 µM (target 100 µM) concentration may be associated with an indirect effect of the test compound on the cell/patch recordings, since this concentration was 1/3rd of the 300 µM concentration where disruption of cell/patch recordings occurred. Furthermore, while statistically significant, inhibition at 8.3 µM (5.1%) was considered to represent variability rather than a treatment-related effect. Thus, INT-747 did not have clear treatment-related effects on hERG channel currents at concentrations ≤82.8 µM.

A 4-Dose Crossover Oral Gavage Cardiovascular Safety Assessment Study with INT-747 in Beagle Dogs (Study No. 7654-100)

In this study using telemeterized animals, four male beagle dogs were administered oral (gavage) doses of 2, 10, or 20 mg/kg INT-747, or vehicle (0.5% (w/v) carboxymethylcellulose in reverse osmosis water) in a crossover design. Animals were treated on Days 1, 4, 8, and 11. ECG leads were arranged in an approximate Lead II configuration. ECG, heart rate, and blood pressure measurements were recorded pre-dose, at dosing, and out to 24 h post-treatment. Quantitative evaluation of ECG measurements included RR interval, QT interval, and rate corrected QT (QTcf, according to Fridericia's method). The study showed that there were no INT-747-related changes in the heart rate, blood pressure, or qualitative or quantitative ECG parameters at up to 20 mg/kg. Transient differences in systolic, diastolic, and mean arterial pressure and heart rate which reached statistical significance at a subset of timepoints were considered to reflect normal variability given the small magnitude of the changes and findings preceding and following these timepoints. Thus, there were no

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treatment-related effects on cardiovascular parameters in male Beagle dogs following treatment with oral gavage doses of up to 20 mg/kg INT-747.

Neuropharmacological Profile (NPP) of INT-747 in Rats (Study No. 00200RI42.001)
Male Crl:CD®(SD)BR rats (n = 10/group) were administered a single oral (gavage) dose of 12, 40, or 120 mg/kg INT-747, or vehicle (0.5% carboxymethylcellulose in deionized water). Animals were observed for at 15, 30, and 45 min, and 1, 2, 3, 4, and 24 h post-dosing. Body temperatures were measured 1 h after dosing. Observations of the following parameters were recorded: seizures/convulsions, startle response, irritability, increased secretion, decreased grip strength, motor activity, abnormal posture, excretion, piloerection, pupil size, corneal reflex, awareness reaction, vocalization, decreased abdominal tone, body tremors, immobility, ataxia, stereotypy, decreased respiration, loss of righting, nociceptive (pain) response, and pinnal reflex. In this study, there were no treatment-related observations following treatment with up to 120 mg/kg INT-747. At 120 mg/kg, there was a small but statistically significant decrease in body temperature, as compared to controls. The mean body temperatures of animals in the control and 120 mg/kg INT-747 groups were 38.2±0.08 and 37.8±0.07 ºC, respectively. The decrease in body temperature (-0.4ºC, compared to controls) was not considered biologically relevant.

Pulmonary Assessment of INT-747 in the Anesthetized Rat (Study No. 1082RI42.001)
Male Crl:CD(SD)IBG BR rats (n = 4/group) were administered a single oral (gavage) dose of 12, 40, or 120 mg/kg INT-747, or vehicle (0.5% carboxymethylcellulose in deionized water). Respiratory rate and depth were determined prior to and 1 h post-dosing. Animals were then anesthetized and catheters were placed in the esophagus to measure esophageal pressure and in the trachea to facilitate spontaneous breathing. After 5 min of stabilization, airway resistance, dynamic lung compliance, respiratory rate, tidal volume, and minute volume were recorded or calculated and reported every 10 min for a minimum of 30 min. In this study, there were no treatment-related effects on respiratory rate and depth at 1 h post-dosing (relative to pretreatment) at any of the dose levels. In addition, there were no statistically significant differences in pulmonary parameters measured post-anesthesia at any dose level, relative to controls.

A Gastrointestinal Propulsion Assay in Rats Administered INT-747 (Study No. 0239RI42.001)
Male Crl:CD(SD)IBG BR rats (n = 10/group) were administered a single oral (gavage) dose of 12, 40, or 120 mg/kg INT-747, or vehicle (0.5% carboxymethylcellulose in deionized water). A 10% suspension of activated charcoal in 0.25% methylcellulose was then administered p.o. 45 min after dosing with INT-747. Thirty minutes after receiving the charcoal, rats were euthanized and the intestines were removed. The length of the intestine (pyloric spincter to caecum) and distance traveled by the charcoal as a fraction of that length was evaluated. In this study, there were no treatment-related on gastrointestinal propulsion at any dose level.

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5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Pharmacokinetic Study of [^{14}C]DSP-1747 in Rats after Single Oral Administration (Study No. AE-6951-G)

Methods: A series of experiments were conducted to investigate the pharmacokinetics of DSP-1747 labeled with ^{14}C; plasma concentrations of radioactivity; excretion of radioactivity in urine, feces, and bile; enterohepatic circulation of radioactivity; and distribution of radioactivity in tissues. Non-fasted male Crl:CD(SD) rats (generally n=3/group) were administered a single oral dose of 15 mg/kg. The vehicle for dosing formulation was 0.5% carboxymethyl cellulose sodium salt solution, and the dosing volume was 5 mL/kg. In an experiment to evaluate biliary excretion of radioactivity, the test compound was administered to bile duct cannulated animals. To evaluate enterohepatic circulation of radioactivity, bile obtained up to 24 h after oral dosing of bile duct cannulated rats was administered by intraduodenal injection to a separate group of rats.

Results:
Following oral dosing, radioactivity from the test compound was rapidly absorbed and concentrations in plasma demonstrated a biphasic profile. The C_{max} and T_{max} were 1401 ng eq/mL and 0.5 h, after administration, respectively, and half life was 19 h from 8-48h or 8-72 h after administration. Excretion of radioactivity in the feces and urine was 93.7% and 0.0% of dose up to 168 h after administration, respectively, and residual radioactivity in the carcass was 1.4% of dose at 168 h. When [^{14}C]DSP-1747 was administered orally to bile duct cannulated rats, excretion of radioactivity in the bile, feces, and urine was 87.2%, 11.9%, and 0.0% of dose, respectively, up to 48 h after administration. The absorption rate from the digestive tract in these animals (estimated by summing the excretion of radioactivity in the bile and urine) was 87.2%. When bile obtained after oral dosing of bile duct cannulated rats was administered by intraduodenal injection to a separate group of rats, the excretion of radioactivity in bile was shown to be 93.2% of the radioactivity injected up to 48 h following administration. This suggests that most of DPS-1747 or its metabolites excreted in the bile were reabsorbed. There was no excretion in urine, and excretion in feces was 7.7% of radioactivity injected at up to 48 h.

In an experiment to determine tissue concentrations of radioactivity, the radioactivity concentration in plasma reached its maximum at 4 h. Concentrations in the thymus, skeletal muscle, aorta, prostate, seminal vesicle, and stomach reached their maximum at 1 h; while, concentrations in tissues other than the mesenteric lymph node, small intestine, cecum, and large intestine reached their maxima at 4 h. Concentrations in the mesenteric lymph node, small intestine, and cecum were reached at 8 h and the maximum concentration in the large intestine was reached at 24 h. At 1 h after administration, the radioactivity concentration (excluding the digestive tract) was highest

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in the liver (10.79-times that in plasma). Tissue/plasma ratios and the percentage distribution of radioactivity in tissues are shown in the Applicant's tables below.

Table 9 The ratio of radioactivity concentration in tissue relative to the plasma radioactivity concentration after single oral administration of [^{14}C]DSP-1747 to non-fasted male rats (dose: 15 mg/kg)

	Tissue/Plasma ratio					
Tissue	1 h	4 h	8 h	24 h	72 h	168 h
Plasma	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 **	N.C.
Blood	0.66 ± 0.02	0.66 ± 0.04	0.66 ± 0.02	0.66 ± 0.06	0.79 **	N.C.
Blood cell	0.17 ± 0.01	0.16 ± 0.02	0.09 **	0.23 **	N.C.	N.C.
Whole brain	0.05 *	0.03 *	N.C.	N.C.	N.C.	N.C.
Pineal body	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Pituitary gland	N.C.	0.23 *	N.C.	N.C.	N.C.	N.C.
Eyeball	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0.03	N.C.	N.C.	N.C.
Tongue	0.38 ± 0.04	0.28 ± 0.07	0.27 ± 0.07	0.16 ± 0.03	N.C.	N.C.
Harderian gland	0.42 ± 0.09	0.24 ± 0.03	0.36 ± 0.10	0.23 ± 0.04	N.C.	N.C.
Thyroid gland	0.24 ± 0.01	0.28 **	N.C.	N.C.	N.C.	N.C.
Mandibular gland	0.29 ± 0.03	0.22 ± 0.02	0.21 ± 0.02	0.15 ± 0.02	N.C.	N.C.
Mandibular lymph node	0.32 ± 0.05	0.19 ± 0.02	0.27 ± 0.04	0.16 **	N.C.	N.C.
Trachea	0.34 ± 0.07	0.17 ± 0.03	N.C.	N.C.	N.C.	N.C.
Thymus	0.16 ± 0.02	0.09 ± 0.02	0.14 ± 0.03	N.C.	N.C.	N.C.
Heart	0.33 ± 0.05	0.26 ± 0.01	0.23 ± 0.03	0.20 ± 0.02	N.C.	N.C.
Lung	0.29 ± 0.03	0.26 ± 0.04	0.29 ± 0.07	0.24 ± 0.03	N.C.	N.C.
Liver	10.79 ± 0.36	14.01 ± 7.78	31.41 ± 12.68	31.43 ± 3.52	32.45 **	N.C.
Kidney	0.51 ± 0.08	0.48 ± 0.09	0.79 ± 0.23	0.72 ± 0.16	N.C.	N.C.

continued

Table 9 continued

	Tissue/Plasma ratio					
Tissue	1 h	4 h	8 h	24 h	72 h	168 h
Adrenal gland	0.28 ± 0.05	0.28 ± 0.10	0.26 **	0.44 *	N.C.	N.C.
Spleen	0.15 ± 0.02	0.12 ± 0.01	0.20 ± 0.11	0.21 **	N.C.	N.C.
Pancreas	0.29 ± 0.06	0.72 ± 0.39	1.34 ± 1.79	0.56 ± 0.20	1.67 *	N.C.
Fat	0.13 ± 0.02	0.11 ± 0.07	0.12 **	0.20 ± 0.05	N.C.	N.C.
Brown fat	0.26 ± 0.05	0.19 ± 0.03	0.23 ± 0.05	0.23 ± 0.03	N.C.	N.C.
Skeletal muscle	0.17 ± 0.01	0.09 ± 0.02	0.10 **	N.C.	N.C.	N.C.
Skin	0.35 ± 0.06	0.19 ± 0.03	0.30 ± 0.01	0.19 ± 0.03	N.C.	N.C.
Bone marrow	0.16 ± 0.01	0.15 ± 0.02	0.16 *	N.C.	N.C.	N.C.
Thigh bone	0.09 ± 0.01	0.09 ± 0.03	0.12 ± 0.01	0.24 **	N.C.	N.C.
Aorta	0.48 ± 0.29	0.30 ± 0.16	1.09 ± 1.16	0.49 *	N.C.	N.C.
Mesenteric lymph node	2.91 ± 1.11	4.10 ± 2.76	13.60 ± 3.56	13.03 ± 3.16	9.53 **	N.C.
Testis	0.21 ± 0.02	0.14 ± 0.04	0.22 ± 0.01	0.13 **	N.C.	N.C.
Epididymis	0.28 ± 0.03	0.23 ± 0.04	0.35 ± 0.04	0.25 ± 0.09	N.C.	N.C.
Prostate gland	0.25 ± 0.03	0.12 ± 0.02	0.20 ± 0.02	0.13 **	N.C.	N.C.
Seminal vesicle	0.24 ± 0.05	0.11 ± 0.03	0.26 ± 0.07	0.17 ± 0.06	N.C.	N.C.
Preputial gland	0.29 ± 0.03	0.22 ± 0.02	0.25 ± 0.03	0.22 **	N.C.	N.C.
Urinary bladder	0.55 ± 0.21	0.46 ± 0.06	0.80 ± 0.15	0.63 **	N.C.	N.C.
Stomach	33.00 ± 12.84	14.98 ± 9.59	11.36 ± 7.82	10.91 ± 7.68	1.01 **	N.C.

continued

Table 9 continued

	Tissue/Plasma ratio					
Tissue	1 h	4 h	8 h	24 h	72 h	168 h
Small intestine	39.94 ± 23.07	32.18 ± 22.59	146.34 ± 80.99	92.80 ± 26.87	107.69 **	N.C.
Cecum	0.50 ± 0.05	0.89 ± 0.24	37.57 ± 9.84	49.71 ± 16.65	107.57 **	N.C.
Large intestine	0.48 ± 0.12	0.47 ± 0.22	19.56 ± 11.52	39.59 ± 27.46	25.14 **	N.C.

N.C.: Not calculated
*: Data are expressed as the value of one animal.
**: Data are expressed as the mean values of two animals.

Table 10 Percentage distribution of radioactivity in tissues after single oral administration of [^{14}C]DSP-1747 to non-fasted male rats (dose: 15 mg/kg)

	Radioactivity contents (% of dose)					
Tissue	1 h	4 h	8 h	24 h	72 h	168 h
Blood	0.17 ± 0.02	0.34 ± 0.36	0.06 ± 0.03	0.03 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
Whole brain	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pineal body	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pituitary gland	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eyeball	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Harderian gland	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thyroid gland	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mandibular gland	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thymus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Heart	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lung	0.00 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Liver	1.93 ± 0.16	2.94 ± 1.12	1.48 ± 0.16	1.02 ± 0.26	0.19 ± 0.02	0.04 ± 0.02
Kidney	0.02 ± 0.00	0.03 ± 0.03	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Adrenal gland	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Spleen	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pancreas	0.00 ± 0.00	0.02 ± 0.02	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fat	0.03 ± 0.01	0.04 ± 0.03	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.01	0.01 ± 0.01
Skeletal muscle	0.28 ± 0.03	0.27 ± 0.25	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Skin	0.32 ± 0.09	0.33 ± 0.34	0.10 ± 0.05	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Testis	0.01 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

continued

Table 10 continued

	Radioactivity contents (% of dose)					
Tissue	1 h	4 h	8 h	24 h	72 h	168 h
Epididymis	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Prostate gland	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Seminal vesicle	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Preputial gland	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Urinary bladder	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stomach	0.54 ± 0.24	0.28 ± 0.09	0.05 ± 0.04	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
Small intestine	1.97 ± 1.04	1.65 ± 0.59	1.88 ± 0.19	0.78 ± 0.12	0.17 ± 0.07	0.04 ± 0.03
Cecum	0.01 ± 0.01	0.01 ± 0.02	0.09 ± 0.03	0.07 ± 0.03	0.04 ± 0.02	0.01 ± 0.01
Large intestine	0.01 ± 0.00	0.02 ± 0.03	0.08 ± 0.01	0.11 ± 0.06	0.01 ± 0.01	0.01 ± 0.01
Gastric contents	41.68 ± 9.65	14.67 ± 6.61	1.05 ± 0.66	0.23 ± 0.13	0.01 ± 0.01	0.02 ± 0.02
Small intestinal contents	44.80 ± 10.51	70.02 ± 11.89	74.13 ± 9.19	43.35 ± 8.08	7.75 ± 1.55	0.96 ± 0.51
Cecal contents	0.02 ± 0.01	1.47 ± 2.09	11.18 ± 5.02	7.50 ± 1.74	1.43 ± 0.23	0.21 ± 0.10
Large intestinal contents	0.01 ± 0.01	0.19 ± 0.30	4.81 ± 2.38	6.59 ± 2.97	0.18 ± 0.05	0.13 ± 0.04

Data are expressed as the mean values ± S.D. of three animals.
Blood, fat, skeletal muscle, and skin were assumed to be 6.4%, 5.0%, 40.0%, and 22.0% of body weight, respectively.

In another experiment in which rats were administered intraduodenal injections of bile obtained up to 24 h after dosing of bile duct cannulated rats, radioactivity concentrations

in portal plasma were 2272 and 769 ng eq./mL, respectively, and excretion of radioactivity in the bile was 4.9% and 26.5% of radioactivity injected at 0.5 and 4 h after injection, respectively. Radioactivity in the intestinal contents was 78.4% and 67.7% of radioactivity injected at 0.5 and 4 h, respectively.

Overall, this study showed that there was rapid absorption; distribution was primarily confined to the gastrointestinal tract and hepatobiliary system; the primary route of elimination is in the feces via biliary excretion; and enterohepatic recirculation of test compound-related material in the bile is extensive.

Distribution

Plasma, Bile, and Liver INT-747 Concentration and Its Major Metabolites after Oral Administration to Mice (Study No. RODA Mouse 2011)

Methods: INT-747 was administered to mice by oral gavage for seven days at 30 mg/kg. Following the final dose and 12 h fasting, blood, gallbladder, and liver were collected. Concentrations of INT-747, metabolite tauro-INT-747, and the endogenous bile acids [taurocholic (TCA) and tauromuricholic acids (TMCA)] were determined in plasma, bile, and liver using HPLC-ES-MS/MS analysis. For comparison, the plasma, biliary, and liver bile acid composition of an untreated control mouse were also determined.

Results: In this study, tauro-INT-747 and the endogenous bile acids were detected in plasma, with tauro-INT-747 being the predominant bile acid relative to TMCA and TCA. In liver, concentrations of tauro-INT-747 were less than, but approached those of TCA. Concentrations of INT-747 and TMCA were similar. In bile, tauro-INT-747 represented ~7% of total bile acids measured. Relative to the untreated control mouse, mice treated with INT-747 had decreased concentrations of TCA in the bile while TMCA levels were similar. Overall, this study showed that the INT-747 was detected in liver only. The drug was recovered in bile, plasma, and liver as a taurine conjugate (the only metabolite detected), and accumulation of tauro-INT-747 in the bile was less than 10% of the total bile acids. The following table and figure copied from the Applicant's study report illustrate the plasma, liver, and bile composition of mice treated with the test compound.

	Control		747			
Mean n=6	T-muCA	TCA	T-muCA	TCA	747	T-747
BILE (mmol/L)	17.29	27.78	20.49	14.02	*n.d.*	2.44
PLASMA (µmol/L)	0.53	0.36	0.41	0.26	*n.d.*	0.72
LIVER (nmol/g)	1.66	23.37	3.90	18.47	1.27	13.36

Table 2. Mean values of INT-747, Tauro-INT-747 and endogenous bile acids in bile, plasma and liver

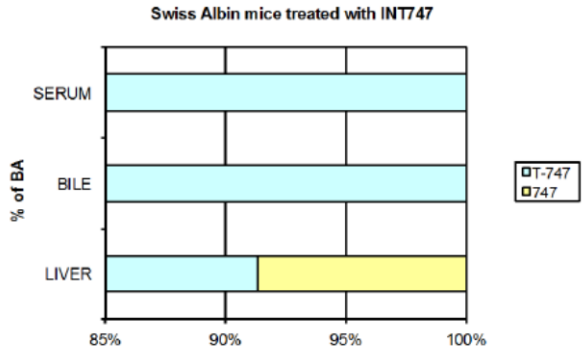


Figure 1. Relative percent of INT-747 and Tauro-INT-747 in plasma, liver and bile In mice treated with INT-747

Biodistribution and Mass Balance of 6-ECDCA and Its Metabolites in Rats (Study No. 6-ECDCA)

Methods: Fasted male Sprague Dawley rats were administered oral doses of 30 mg/kg 6-ECDCA or vehicle (carboxymethylcellulose 1% in water). The dosing volume was 300 µL. A subset of rats dosed with the test compound were sacrificed at timepoints ranging from 15 minutes to 8 h after dosing (n=4/timepoint). At termination, liver, kidney, blood, small intestine, colon, and fecal material in the small and large intestine were collected. In addition, two groups of rats (one control; one treated with the test compound; n=4/group) were placed in metabolic cages (0-24 h) for collection of urine and feces. After 24 h, the animals were sacrificed and liver, kidney, blood, small intestine, and colon were removed. 6-ECDCA and its tauro- and glyco-conjugate metabolites, as well as epimer 6-ethyl ursodeoxycholic acid (6-EUDCA; 7β epimer of ECDCA) and its tauro- and glyco-conjugates were quantified using a HPLC-ES-MS/MS method.

Results: 6-ECDCA was shown to be conjugated with taurine (and glycine to a lesser extent) in the liver. Approximately 96.5% of the dose administered was accounted for 24 h after-dosing, with distribution summarized in the Applicant's table copied below (numbers shown represent the percentage of dose administered). At 24 h, 37% of the administered dose was present in the small intestine (primarily as 6-ETCDCA). Approximately 27% of the dose was present in the colon as 6-ECDCA.

	6-ETCDCA	6-EGCDCA	6-EUDCA	6-ECDCA	6-ETUDCA	6-EGUDCA
PLASMA	0.05	0.01	0.01	0.04	0.01	0.00
LIVER	0.88	0.11	0.01	0.02	0.50	0.06
FAECES	0.05	0.03	0.22	10.48	0.00	0.00
SMALL INTESTINE CONTENT	37.70	0.91	0.55	1.51	13.77	0.33
COLON CONTENT	0.94	0.05	0.71	27.13	0.02	0.00
URINE	0.07	0.04	0.03	0.03	0.07	0.04
KIDNEY	0.02	0.01	0.00	0.01	0.01	0.00
SMALL INTESTINE TISSUE	0.03	0.00	0.01	0.01	0.03	0.00
COLON TISSUE	0.00	0.00	0.00	0.01	0.00	0.00

6-ETCDCA is expected to be deamidated to 6-ECDCA by intestinal bacteria, followed by partial reabsorption and partial excretion of 6-ECDCA in the stools. Furthermore, based on the detection of 6-ethyl LCA and 6-ethyl, 7-keto LCA in the stools of animals treated with 6-ECDCA, the test compound also appears to be 7-dehydroxylated by

intestinal bacteria to form 6-ethyl LCA. However, LCA was not increased in the treated group, suggesting that the 6-ethyl group is not removed by intestinal bacteria. The report postulates that the presence of the ethyl group in the 6 position on 6-ECDCA may sterically hinder the 7-oxidation and the 7-dehydroxylation pathways, which lead to the formation of 6-ethyl LCA and 6-ethyl, 7-keto LCA (less than 4% of the administered dose). While the test compound administered in this study contained 0.7% 6-EUDCA, the percentage of the dose recovered as 6-EUDCA and its taurine conjugate at 24 h indicate there is epimerization of the test compound at the 7-position. Less than 1% of 6-ECDCA was metabolized by glucuronidation/sulfation and excreted in urine.

[^{14}C]-INT-747: Tissue Distribution Studies in Partially Pigmented Rats to Support Human Dosimetry Calculation (Study No. ICP/01)

Methods: Male Lister Hooded rats (n=7) were administered a single oral (gavage) dose of 15 mg/kg [^{14}C]-INT-747 in 0.5% methocel in water. The dosing volume was 10 mL/kg. Rats were euthanized at timepoints out to 840 h after dosing, and frozen carcasses from out to 336 h were subjected to whole-body autoradiography. Following autoradiography, the eye was analyzed for total radioactivity.

Results: The highest radioactive concentrations were measured at 2 h after dosing, and were observed in the small intestine contents, stomach contents, small intestine mucosa, and liver. The majority of radioactivity was eliminated by 24 h after dosing, with the highest radioactive concentrations measured in the gastrointestinal tract. No affinity of drug-related material for melanin was identified based on concentrations in melanin-containing structures (skin, uveal tract).

A Protein Binding Study of INT-747, and Two Metabolites in Mouse, Rat, Dog, and Human Plasma *In Vitro* (Study No. (b) (4) -661035)

Methods: Using a Centrifree® ultrafiltration device, the *in vitro* protein binding of INT-747 and metabolites UPF-832 (the glycine conjugate of INT-747) and UPF-1443 (the taurine conjugate of INT-747) was determined in mouse, rat, dog, and human plasma. INT-747 was evaluated over a concentration range of 100 to 10,000 ng/mL and the metabolites were evaluated over a concentration range of 10 to 10,000 ng/mL. Samples were analyzed with an UPLC/MS/MS assay method. Control experiments were conducted at all concentrations and for all analytes using saline (rather than plasma) to determine non-specific binding to the ultrafiltration device.

Results: Significant non-specific binding to the ultrafiltration device occurred with all test substances. After correcting for non-specific binding, the actual concentration of INT-747 available for protein binding may have been as low as 3400, 226, and 17 ng/mL. Similarly, the actual concentrations of UPF-832 and UPF-1443 may have been as low as 9080, 73, and 5.6 ng/mL and 9300, 71, and 6.3 ng/mL, respectively. However, in the presence of plasma proteins, non-specific binding to the ultrafiltration device may be mitigated to some extent by the interaction of plasma proteins with the device. In this study, the mean binding of INT-747 to protein in mouse, rat, dog, and

Reference ID: 3870157

human plasma was >99.6%, >99.8%, >97.7%, and >99.1%, respectively. The mean binding of UPF-832 to protein in mouse, rat, dog, and human plasma was >98.8%, >98.4%, >98.5%, and >97.6%, respectively. Finally, the mean binding of UPF-1443 to protein in mouse, rat, dog, and human plasma was >99.3%, >97.5%, >97.1%, and >98.3%, respectively. Thus, this study indicated that INT-747 and metabolites UPF-832 and UPF -1443 are highly protein bound in plasma from all species evaluated.

Metabolism

Analysis of Metabolites of [^{14}C]DSP-1747 in Male Rats (Study No. AE-6952-G).

Methods: The metabolism of DSP-1747 in male rats was further evaluated using samples from Study AE-6951-G. Following single oral administration of 15 mg/kg DSP-1747 labeled with ^{14}C the test compound, samples of plasma, liver, small intestine, small intestine contents, and feces were collected from non-cannulated rats, and bile was collected from bile-duct cannulated animals. Samples were analyzed by HPLC and structures of metabolites were identified or estimated by LC-MS. In addition, following intraduodenal injection of bile collected from rats up to 24 h after oral administration to other bile-duct cannulated rats, samples of portal plasma, intestinal contents, and bile were collected and analyzed for metabolites.

Results: After oral dosing, the primarily components in the plasma were unchanged DSP-1747 (84.5% and 37.7% of sample radioactivity at 1 and 24 h, respectively) and DSR-99763 (taurine-conjugate; 11.4% and 50.0% of radioactivity at 1 and 24 h, respectively). An epimer of DSP-1747 accounted for 1.7% and 7.3% of the sample radioactivity at 1 and 24 h after dosing, respectively. In the liver, the taurine-conjugate accounted for 90.7% and 94.2% of sample radioactivity at 1 and 24 h, respectively; whereas, DSP-1747 was 1.5% at 1 h and undetected at 24 h. An epimer of the taurine-conjugate was identified at 3.5% and 2.7% at 1 and 24 h, respectively. In the small intestine, DSP-1747, the taurine conjugate, and an epimer of DSP-1747 accounted for 12.1%, 84.1%, and 1.5% of sample radioactivity, respectively, at 1 hr. At 24 h, DSP-1747 and the taurine-conjugate accounted for 1.9% and 96.9%, respectively. In the small intestine contents, DSP-1747, the taurine-conjugate, and an epimer of DSP-1747 accounted for 4.7%, 92.1%, and 2.4% of sample radioactivity, respectively, at 1 h. At 24 h, DSP-1747, the taurine-conjugate, and an unknown metabolite accounted for 2.1%, 95.0%, and 1.3% of radioactivity, respectively. In bile collected up to 48 h after administration, DSP-1747 was not detected; while the taurine-conjugate and an epimer of DSP-1747 accounted for 94.5% and 3.9% of sample radioactivity. In feces collected up to 96 h after dosing, DSP-1747 accounted for 73.0% of sample radioactivity. Dehydrogenated DSP-1747, an epimer of DSP-1747, and hydroxylated DSP-1747 accounted for 13.4%, 7.5%, and 2.9% of sample radioactivity, respectively.

Following intraduodenal injection of bile (in which which 94.4% of sample radioactivity was the taurine-conjugate), portal plasma collected at 0.5 h after administration showed DSP-1747, the taurine-conjugate, and an epimer of DSP-1747 accounting for 9.7%, 87.0%, and 1.4% of sample radioactivity, respectively. At 4 h after administration, DSP-

Reference ID: 3870157

1747 was the main component (64.1% of radioactivity), and the taurine-conjugate accounted for 27.6% of radioactivity. In the intestinal contents, DSP-1747, the taurine-conjugate, and unknown metabolites accounted for 20.0%, 75.8%, and 0.4-2.6% of sample radioactivity, respectively, at 0.5 h. At 4 h, the main component of the intestinal contents was DSP-1747 (73.0% of sample radioactivity). The taurine-conjugate accounted for 22.2% of sample radioactivity, and there were lesser amounts of unknown metabolites. Finally, in bile collected at 0.5 h, the taurine-conjugate and an epimer of DSP-1747 accounted for 97.4% and 1.7% of sample radioactivity. At 4 h, the taurine conjugate accounted for 94.3% of sample radioactivity, with lesser amounts of an epimer of DSP-1747 (2.8%) and two unknown metabolites. Other unidentified metabolites were observed in various samples but were present in low amounts (generally <1%).

Overall, this study showed that after oral administration in rats, DSP-1747 is conjugated with taurine and excreted into bile. After intraduodenal injection of a bile sample containing primarily the taurine-conjugate of DSP-1747, a portion of the taurine-conjugate was transformed to unconjugated DSP-1747 in the gastrointestinal tract, and the metabolite and unconjugated compound were re-absorbed.

Pharmacokinetics and Metabolite Profiling Following a Single and Multi-dose Oral Gavage Administration of [^{14}C]OCA (Single Dose) and OCA (Multi-dose) to Male and Female Sprague-Dawley Rats (Study No. XBL15604)

Methods: Two groups of Sprague-Dawley rats were administered [^{14}C]OCA by oral gavage. Group 1 rats (n=4/sex) were administered a single oral dose at 25 mg/kg, and urine, feces, and blood samples were collected up to 48 h after dosing. Group 2 rats (n=3/sex) were administered 25 mg/kg/day by oral gavage for 14 days and blood was collected for metabolite/identification in plasma following the dosing period. A control male animal (Group 3) was not dosed, and urine, feces, and a terminal blood sample were collected at 24 h. Analyses of metabolites in plasma, urine, and feces were conducted using radioprofiling and LC-MS/MS after single-dose administration (Group 1 animals). Analyses of samples after 14-days of dosing (Group 2) animals were conducted under Study XBL15617 (reviewed separately).

Results: PK parameters of total radioactivity in plasma following a single oral dose of [^{14}C]OCA are shown in the Applicant's table below. Half-life and t_{max} were similar between sexes, but exposures based on C_{max} and AUC values in males were 4.3-fold and ~2-fold higher, respectively, than in females.

Table S-1: PK Parameters of total radioactivity in male and female Sprague-Dawley rat plasma following a single oral dose of [^{14}C]OCA

Gender	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng eq./mL)	AUC_{0-48hr} (hr*ng eq./mL)	$AUC_{0-\infty}$ (hr*ng/mL)
Female	16.3	2	986	18,100	21,100
Male	19.9	2	4,220	39,800	44,200
C_{max} and AUC values are presented to 3 significant figures					

Mean radioactivity contributions of OCA and metabolites in plasma, urine, and feces are summarized in the Applicant's table below. Feces was the primary excretion route for OCA (64.6-66.3% of the dose recovered over the 0-48 h collection period). Urine was only a minor excretion route (0.03-0.18% of the dose recovered over the 0-48 h collection period). In plasma and feces, the parent compound was the major radioactive component. In plasma, four circulating metabolites [redacted] (b) (4), 6-EGCDCA, 6-ETCDCA, and M596/1) accounted for 1.72-26.8% of total radioactivity $AUC_{0\text{-}48h}$. Minor metabolites OCA 3-glucuronide, OCA 24-glucuronide, and OCA 7-dehydroxylation were detected by LC/MS/MS. In feces, metabolites [redacted] (b) (4) M436/1, M418/1, 6-EGCDCA, and 6-ETCDCA were identified (each <5% of the dose). In urine, OCA, and minor metabolites OCA 3-glucuronide and 6-ETCDCA were identified (≤0.1% of the dose in pooled 0-48 h urine).

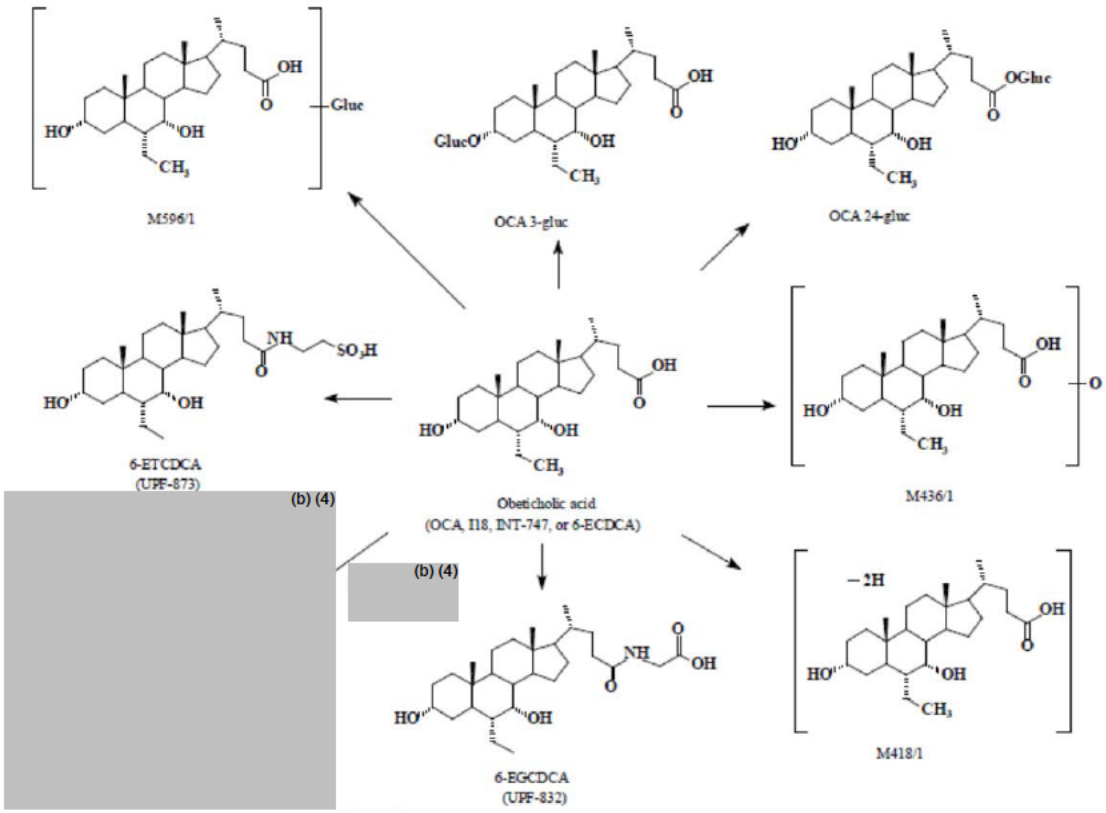
Table S-2 Mean radioactivity contributions of Obeticholic Acid and metabolites in rats following a single administration of [^{14}C]Obeticholic Acid

Compound Code	Modification	Male			Female		
		Plasma	Urine	Feces	Plasma	Urine	Feces
		$\%AUC_{0\text{-}t}$	%Dose	%Dose	$\%AUC_{0\text{-}t}$	%Dose	%Dose
TRA		100	0.03	66.3	100	0.18	64.6
Obeticholic Acid	Parent	57.9	<0.01	49.7	35.7	<0.01	49.9
3-Gluc OCA	Glucuronidation	MS	ND	ND	ND	<0.01	ND
[redacted] (b) (4)							
6-EGCDCA	Glycine conjugation	3.57	ND	0.37	4.79	ND	0.31
6-ETCDCA	Taurine conjugation	12.3	ND	1.44	26.8	0.10	1.37
M596/1	Glucuronidation	1.72	ND	ND	5.68	ND	ND
M436/1	Mono-oxidation	ND	ND	1.54	ND	ND	0.65
M418/1	dehydrogenation	ND	ND	2.31	ND	ND	1.57
	Total	91.8	<0.01	60.0	85.4	0.10	58.4

ND: non-detectable by radioprofiling. MS: non-detectable by radioprofiling, but was detected by LC/MS.

Metabolic pathways in rats were identified as the following: 1) amidation to form 6-EGCDCA and 6-ETCDCA, 2) mono-oxidation to form M436/1; 3) dehydrogenation to form M418/1, 4) glucuronidation to form M596/1, OCA 3-glucuronide, and OCA 24-glucuronide, and 5) reduction of M418/1 or [redacted] (b) (4) [redacted] (b) (4)
The Applicant's figure below summarizes the proposed metabolic pathways in rats.

Figure 10 Proposed major metabolic pathways of Obeticholic Acid in rats



M418/1 and M436/1 were detectable only in feces.

(b) (4)

Gluc = glucuronic acid

Determination of Relative Plasma Exposures of Obeticholic Acid (OCA) Metabolites in Rat Plasma to Human Plasma (Study No. XBL15617)

Methods: The purpose of this study was to investigate the relative exposure of OCA human metabolites in human and rat plasma. Briefly, rat and human AUC-pooled$_{(0\text{-}24h)}$ samples collected after 14-days of dosing with 25 mg/kg/day and 10 mg/day OCA, respectively, were prepared according to the Hamilton AUC pooling method. Rat plasma samples were obtained from XBL Study Number 15604, and human plasma samples were obtained from clinical study number 747-105. Each AUC-pooled sample was diluted with control plasma from the other species, and then the mixed samples were extracted and analyzed by LC-MS for OCA and its metabolites as identified in the in vivo human metabolism study number XBL14719. The peak area response of each compound to the internal standard ([d_5]-OCA) was used to estimate the exposure ratio of each component in rat relative to human. The peak area ratios of each compound to the internal standard were used to calculate the concentration of each compound in the AUC-pooled human plasma.

Results: The exposure ratios for each compound in rat-to-human plasma are summarized in the Applicant's table below. Each of the human metabolites investigated in this study were detected in rat plasma (one or both sexes). The mass spectrometer response ratios of OCA, 24-Gluc OCA, 6-ETCDCA, and (b) (4) (b) (4) were ≥2 in at least one gender; and therefore the results were considered adequate to demonstrate an actual exposure ratio of 1 or greater in the rat. In contrast, the exposure ratios for 3-Gluc OCA and 6-EGCDCA were <2-fold.

Table 2 Estimation of relative exposure of human OCA metabolites in rat plasma

	Relative exposure	
Species	Ratio (Male Rat/Human)	Ratio (Female Rat/Human)
Analytes	AUC $_{0\text{-}24\ hr}$ [a]	AUC $_{0\text{-}24\ hr}$ [b]
OCA	206.79	226.46
24-Gluc OCA	2.09	ND
3-Gluc OCA	ND	0.71
6-EGCDCA	0.06	0.19
6-ETCDCA	7.08	27.61
(b) (4)		

ND: Not detected.

[a] The exposure ratio of individual analyte in male rat plasma was calculated using human plasma sample (mixed with control male rat plasma).

[b] The exposure ratio of individual analyte in female rat plasma was calculated using human plasma sample (mixed with control plasma from female rats).

Refer to Appendix G for more detailed data.

As summarized in the Applicant's table below, 6-EGCDCA, 6-ETCDCA, and 3-Gluc OCA were identified as major components in human plasma. The relative exposure of the OCA 3-O-glucuronide based on total OCA and metabolites in the current study was ~20%. As shown in Table 3 above, the relative exposure ratio (female rat/human) for the 3-Gluc OCA metabolite was 0.71; whereas, OCA 3-O-glucuronide was not detected in male rat plasma.

Table 3 Summary of concentration and relative percent distribution of OCA and its major metabolites in $AUC_{0\text{-}24}$-pooled human plasma

Analytes	Concentration (ng/mL)	%Relative Distribution
6-ECDCA (OCA)	2.23	1.37
6-EGCDCA	55.4	33.9
6-ETCDCA	66.3	40.6
(b) (4)		
OCA 3-O-glucuronide	34.9	21.4
OCA 24-O-glucuronide	4.47*	2.74
Total [a]	39.4	100%

*Determined using an RF (response factor) of (b) (4) 0 ng/mL (for OCA 24-O-glucuronide) standards rather than the use of a calibration curve due to their low levels.

[a] No other major components were detectable in radiolabeled human metabolism study (Reference 4).

ND: Non-detectable.

Detailed data can be found in Appendix H.

Plasma, Bile, and Liver Concentration of INT-747 and Its Main Metabolites After Oral Administration to Rabbit (Study No. RODA Rabbit 2011)

Methods: This study evaluated the metabolism and biodistribution of INT-747 in the plasma, bile, and liver of rabbits. Rabbits were selected for use in this study since: 1) primary bile acids are extensively 7-dehydroxylated by intestinal bacteria to form

secondary bile acids in this species, and 2) rabbits preferentially conjugate bile acids with glycine (rather than taurine). Briefly, INT-747 was administered to New Zealand White rabbits by oral gavage for 3 months at 30 mg/kg. Following the final dose and 12 h fasting, blood, gallbladder, and liver were collected. Concentrations of INT-747, its primary metabolites, and endogenous bile acids were determined in plasma, bile, and liver by HPLC-ESI-MS/MS. For comparison, the plasma, biliary, and liver bile acid composition of an untreated control rabbit were also determined.

Results: The primary form of the test compound detected in plasma, bile, and liver was the glycine conjugate, with unconjugated INT-747 detected at in lower amounts. No other major metabolites resulting from a 7-dehydroxylation process, glucuronides, or other polar metabolites were identified. In bile and liver, the predominant endogenous bile acid was glycodeoxycholic acid; whereas, the predominant endogenous bile acid in plasma was deoxycholic acid. The absence of 6-ethyl lithocholic acid and glyco-6-ethyl lithocholic acid in bile, liver, and plasma was considered to confirm the metabolic stability of INT-747 toward intestinal bacteria responsible for bile acid 7-dehydroxylation. The following tables copied from the Applicant's study report illustrate the plasma, bile, and bile composition of rabbits treated with the test compound.

Table 1. Plasma bile acid composition in rabbits after chronic feeding with INT-747. Mean values ± standard deviation.

	INT-747									
	iso2OH	Giso2OH	DCA	GDCA	CA	GCA	LCA	GLCA	INT-747	G-INT-747
PLASMA (µM)	0.33	≤ 0.01	5.27	0.78	0.44	0.44	0.43	0.55	1.92	2.86
	0.45	≤ 0.01	7.17	0.81	0.27	≤ 0.01	0.61	0.40	2.47	2.66
	0.36	≤ 0.01	6.22	0.99	0.33	0.45	0.55	0.50	2.00	2.70
	0.40	≤ 0.01	6.78	1.10	0.40	0.39	0.45	0.46	2.10	1.98
Mean	0.39	≤ 0.01	6.36	0.92	0.36	0.43	0.51	0.48	2.12	2.55
SD	0.05		0.82	0.15	0.07	0.03	0.09	0.06	0.24	0.39
CV%	13.44		12.96	16.54	19.10	7.62	17.03	13.32	11.47	15.33

Table 3. Rabbit biliary bile acid composition after chronic feeding with INT-747. Mean values ± standard deviation.

	INT-747								
	iso2OH	Giso2OH	DCA	GDCA	CA	GCA	GLCA	INT-747	G-INT-747
BILE (µM)	≤ 0.01	3.16	0.27	254.60	0.31	73.26	49.69	0.09	71.81
	≤ 0.01	4.92	0.28	223.26	0.46	72.36	70.14	0.09	85.26
	≤ 0.01	4.91	0.40	181.88	0.39	49.82	69.61	0.10	92.04
	≤ 0.01	4.50	0.30	220.00	0.40	64.00	42.00	0.09	81.00
Mean	≤ 0.01	4.37	0.31	219.94	0.39	64.86	57.86	0.09	82.52
SD		0.83	0.06	29.78	0.06	10.86	14.23	0.01	8.47
CV%		19.03	19.09	13.54	15.55	16.74	19.35	5.81	10.26

Table 5. Liver bile acid composition in rabbits after chronic feeding with INT-747. Mean values ± standard deviation.

	INT-747									
	iso2OH	Giso2OH	DCA	GDCA	CA	GCA	LCA	GLCA	INT-747	G-INT-747
LIVER (nmol/g)	13.41	3.64	2.78	198.74	14.47	40.35	18.79	39.42	3.40	120.65
	15.86	3.57	3.02	196.61	18.64	40.79	25.39	42.34	3.47	168.20
	14.63	3.61	2.90	197.67	16.56	40.57	21.09	40.88	3.93	144.42
	14.00	4	3.50	200.01	16.80	41.00	20.98	41.56	4.45	145.78
Mean	14.47	3.70	3.05	198.26	16.62	40.68	21.56	41.05	3.81	144.76
SD	1.05	0.20	0.31	1.45	1.71	0.28	2.76	1.24	0.49	19.42
CV%	7.25	5.37	10.30	0.73	19.10	0.69	12.81	3.02	12.77	13.42

Metabolite Radioprofiling and Metabolite Identification of [^{14}C]OCA in Liver, Intestine, and Kidney S9 Fractions from Mice, Rats, Rabbits, Dogs, Monkeys, and Humans (Study No. XBL14830)

Methods: The purpose of this study was to determine the in vitro metabolite profiles of [^{14}C]OCA in liver, intestine, and kidney S9 fractions across multiple species, and characterize the prominent metabolites. 10 µM [^{14}C]OCA was separately incubated with liver, intestine, and kidney S9 fractions for 1 h with cofactors. Following termination of the incubations, the extracts were evaluated by radioprofiling and LC-MS/MS. Test systems used in the study are shown in the Applicant's table below.

Species	Gender		
	Liver S9	Intestine S9	Kidney S9
Mice (CD-1)	Mixed gender	Male	Male
Rats (Sprague Dawley)	Mixed gender	Male	Male
Rabbits (New Zealand)	Mixed gender	NA	NA
Dogs (Beagle)	Mixed gender	Male	Male
Monkeys (Cynomolgus)	Mixed gender	Male	Male
Humans	Mixed gender	Mixed gender	Mixed gender

NA: not available for rabbit intestine and kidney S9 fractions.

Results: The percent distribution of OCA and its metabolites following 1 h incubation is summarized in the Applicant's tables below. As shown, the percent of parent compound remaining after incubation with S9 was lowest in liver (63.2-88.7%) and highest in the kidney (86.9-96.9%). Nine metabolites were tentatively identified, with the primary metabolic pathways being conjugation with glycine and taurine, glucuronidation, epimerization, and mono-oxidation and glucuronidation. There were no unique OCA metabolites in human S9 fractions.

Table S-1 Summary of % distribution of OCA and its metabolites in incubations with human, mouse, rat, rabbit, dog, and monkey S9 fractions

Compound Code	Modification	% Distribution in Liver S9 Incubations					
		Human	Monkey	Rat	Mouse	Dog	Rabbit
OCA	Parent	65.7	73.6	63.2	84.9	81.1	88.7
6-EGCDCA	Glycine conjugate	0.65	ND	0.77	ND	5.12	ND
6-ETCDCA	Taurine conjugate	ND	3.51	1.75	ND	0.47	ND
OCA 3-gluc	Glucuronidation	3.61	1.74	20.0	ND	ND	2.86
(b) (4)							(b) (4)
M612/3	Mono-oxidation and Glucuronidation						
M612/1		1.38	1.43	ND	1.16	2.02	0.45
M612/2		16.7	5.40	0.41	5.79	1.69	0.74
M612/4		2.92	1.16	1.49	0.88	0.34	0.74
M612/5		1.84	6.13	ND	0.46	0.37	ND

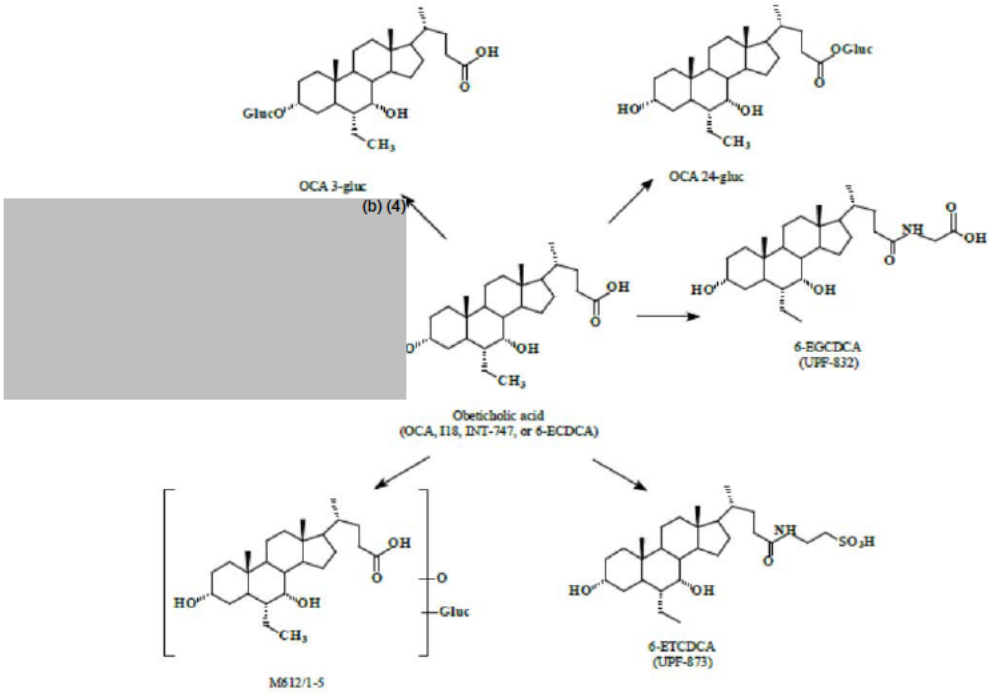
Compound Code	Modification	% Distribution in Intestine S9 Incubations				
		Human	Monkey	Rat	Mouse	Dog
OCA	Parent	80.1	94.8	95.0	91.4	85.8
6-EGCDCA	Glycine conjugate	0.48	0.38	ND	ND	5.04
6-ETCDCA	Taurine conjugate	2.25	ND	ND	ND	0.29
OCA 3-gluc	Glucuronidation	1.20	1.21	ND	ND	ND
(b) (4)						(b) (4)
M612/3	Mono-oxidation and Glucuronidation					
M612/1		0.52	0.54	ND	0.80	0.50
M612/2		5.97	ND	0.31	3.81	1.84
M612/4		1.08	0.67	0.73	0.44	0.65
M612/5		0.80	ND	0.24	ND	0.29

Compound Code	Modification	% Distribution in Kidney S9 Incubations				
		Human	Monkey	Rat	Mouse	Dog
OCA	Parent	90.5	89.7	94.1	86.9	96.9
6-EGCDCA	Glycine conjugate	ND	ND	ND	ND	ND
6-ETCDCA	Taurine conjugate	3.12	ND	ND	ND	ND
OCA 3-gluc	Glucuronidation	2.91	ND	ND	ND	ND
OCA 24-gluc [a]	Glucuronidation	ND	ND	ND	ND	ND
(b) (4)						(b) (4)
M612/3	Mono-oxidation and Glucuronidation					
M612/1		0.31	ND	1.38	3.15	0.33
M612/2		ND	0.61	ND	ND	ND
M612/4		0.66	0.81	0.69	0.87	0.77
M612/5		ND	0.61	ND	ND	ND

ND: Not detected by radioprofiling.

The proposed major metabolic pathways of OCA in human, mouse, rat, rabbit, dog, and monkey S9 fractions is shown in the Applicant's figure copied below.

Figure 7 Proposed major metabolic pathways of OCA in human, and mouse, rat, rabbit, dog, and monkey S9 fractions



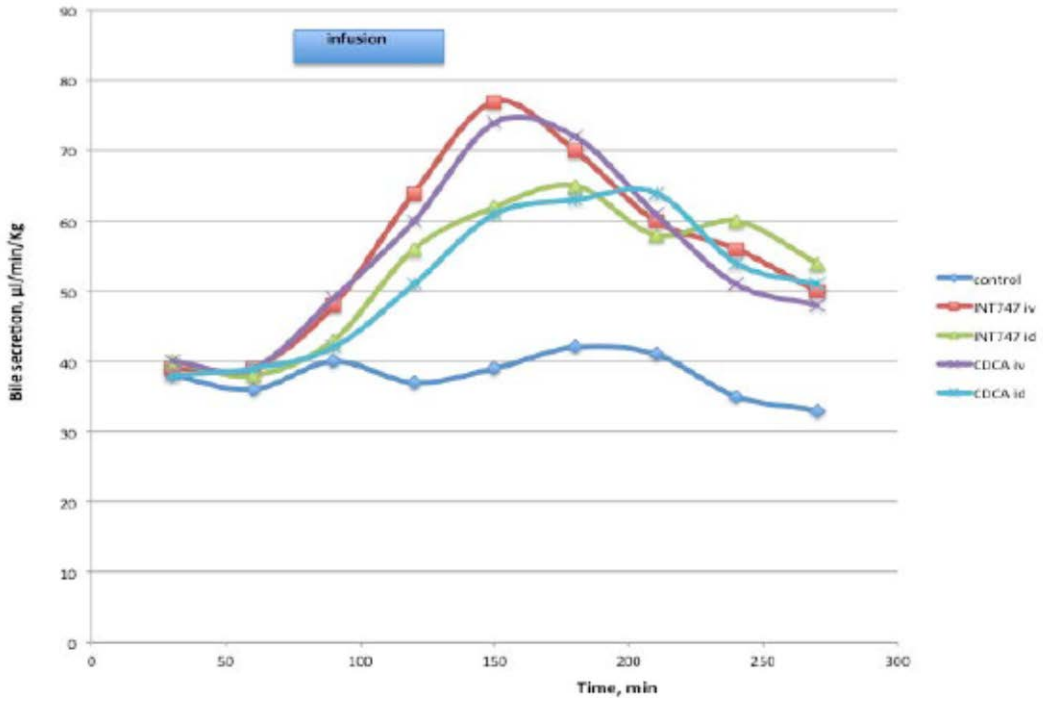
Gluc = glucuronic acid.

Excretion

Biliary Secretion and Metabolism of INT-747 in Bile-Fistula Rat After Duodenal (ID) and Femoral (IV) Administration (Study No. RODA Rat 2011).

Methods: Biliary secretion and metabolism of 1 µmol/min/kg INT-747 was evaluated in the bile-fistula rat model following duodenal infusion (ID) and intravenous infusion (IV) for 1 h (n=6 rats/group). Bile samples were collected before, during, and after the infusion over a time period of 4 h. The bile samples were weighed, bile secretion rate was calculated, and secretion of INT-747 and metabolites was determined. INT-747 and gluco- and tauro-conjugate metabolites of INT-747 were analyzed by HPLC-ES-MS/MS. The choleretic effect of INT-747 was evaluated relative to untreated bile fistula rats and CDCA at an equimolar dose. In addition, choleretic effects after ID and IV infusion were compared.

Results: Following IV infusion, INT-747 increased bile flow (maximum level ~80 µl/min/kg) to an extent similar to that seen following IV infusion of CDCA at equimolar dose. The increase in bile flow after duodenal infusion of INT-747 and CDCA was slightly lower than that induced by IV infusion of these compounds, but the maximum choleretic effect was of longer duration. The Applicant's figure below summarizes the effect of INT-747 and CDCA on bile secretion rat relative to control untreated bile fistula rats.



After IV infusion, INT-747 was secreted in the bile, primarily as the taurine-conjugate. The maximum secretion rate (0.92 µmol/kg/min) was achieved at the end of the infusion period and almost the entire administered dose was recovered in the bile. Only traces (<0.02%) of glycine conjugate was present, and the amount of INT-747 secreted in bile in the unconjugated form was very small (0.1-0.3%). After duodenal infusion, biliary secretion was similar to that after IV infusion, although the maximum biliary secretion rate (0.82 µmol/kg/min) was slightly lower after duodenal infusion and the maximum secretion rate occurred 60 mins after the end of infusion. The taurine-conjugate was the primary compound in bile, and only trace amounts (<0.2%) of INT-747 was conjugated with glycine and secreted as the glycine-conjugate in bile.

5.2 Toxicokinetics

A 14-Day Oral (Gavage) Toxicokinetic Study of INT-747 in Mice (Study No. (b) (4) 661033).

Methods: Crl:CD1(ICR) mice were administered oral (gavage) doses of 4, 12, and 40 mg/kg/day INT-747 (or vehicle, 0.5% carboxymethylcellulose in deionized water) once daily for 14 days. The dose volume was 10 mL/kg, and the groups consisted of 6 animals/sex (control) or 33 animals/sex (INT-747 treatment groups). Blood samples for TK evaluation were collected from controls at 2 h after dosing on days 0 and 13, and from the INT-747 treatment groups prior to dosing and out to 24 h after dosing on study days 0 and 13 and at 48 and 96 h after dosing on day 13. Plasma samples were analyzed for INT-747, UPF-832 (the glycine conjugate of INT-747), and UPF-1443 (the taurine conjugate of INT-747) concentrations using UPLC/MS/MS in the negative electrospray ionization (ESI-) mode.

Results: Three animals were found dead or sacrificed in extremis (1 high dose male, 1 low dose female, and 1 control female). Clinical signs primarily occurred in the high dose male euthanized on day 11 (e.g., impaired use of forelimbs, body cool to touch, pale extremities, and shallow respiration). Effects on body weight were limited to a small but statistically significant increase in body weight gain from day 0 to 7 in all INT-747 female treatment groups (compared to controls). Findings were limited to dark red areas on the lungs, dark red contents of the stomach, and red matting of the skin in the high dose male euthanized prematurely. TK analysis indicated that there was systemic exposure to INT-747 and UPF-1443. Metabolite UPF-832 was not detected. Exposure increased with increasing dose, and exposure to INT-747 was generally higher in females (relative to males) while exposure to UPF-1443 was similar in both sexes. Exposures to UPF-1443 were much greater than those of the parent compound in females. The half-life values were 1 to 2 h for INT-747, and 6 to 10 h on study day 0 and 12 to 23 h on study day 13 for UPF-1443. TK parameters are summarized in the Applicant's tables below.

Text Table 1. Summary of Toxicokinetic Parameters for INT-747

Dosage	AUC_{last} (ng•h/mL)		C_{max} (ng/mL)		T_{max} (h)	
	Day 0	Day 13	Day 0	Day 13	Day 0	Day 13
Males						
4 mg/kg/day	3.96	NC	15.8	0.0	0.5	NC
12 mg/kg/day	35.6	92.0	47.4	58.5	0.5	0.5
40 mg/kg/day	143	357	262	152	0.5	0.5
Females						
4 mg/kg/day	27.9	12.2	71.1	48.7	1	1
12 mg/kg/day	82.2	166	89.3	87.2	0.5	0.5
40 mg/kg/day	307	977	226	298	0.5	0.5

NC = Not Calculable

Text Table 2. Summary of Toxicokinetic Parameters for UPF-1443

Dosage	AUC_{last} (ng•h/mL)		C_{max} (ng/mL)		T_{max} (h)	
	Day 0	Day 13	Day 0	Day 13	Day 0	Day 13
Males						
4 mg/kg/day	1210	4807	80.5	234	8	8
12 mg/kg/day	4148	18097	313	637	8	2
40 mg/kg/day	12965	38708	945	1269	8	1
Females						
4 mg/kg/day	1558	4617	128	328	1	1
12 mg/kg/day	4329	20251	352	829	4	8
40 mg/kg/day	13743	62086	770	1960	8	8

A 14-Day Oral (Gavage) Toxicokinetic Study of INT-747 in Sprague Dawley Rats (Study No. (b) (4) 561032).

Methods: Crl:CD(SD) rats were administered oral (gavage) doses of 6, 15, and 60 mg/kg/day (or vehicle, 0.5% carboxymethylcellulose in deionized water) once daily for 14 days. The dose volume was 10 mL/kg, and the groups consisted of 3 animals/sex (control) or 9 animals/sex (INT-747 treatment groups). Blood samples for TK evaluation

were collected from controls at 2 h after dosing on days 0 and 13, and the INT-747 treatment groups prior to dosing and out to 24 h after dosing on study days 0 and 13 and at 48 and 96 h after dosing on day 13. Plasma samples were analyzed for INT-747, UPF-832 (the glycine conjugate of INT-747), and UPF-1443 (the taurine conjugate of INT-747) concentrations using UPLC/MS/MS in the negative electrospray ionization (ESI-) mode.

Results: There was an increased incidence of dried red material around the nose in INT-747 male treatment groups (relative to controls). From Day 0 to 13, body weight gain was reduced in the INT-747 treatment groups, but there was no dose-relationship since body weight gain was 67, 46, 54, and 44 g, respectively, for control, low-, mid-, and high dose males, respectively. In high dose females, body weight gain from Day 0 to 13 was also decreased relative to controls (36, 29, 21, 19 g, respectively for control, low-, mid-, and high dose females; statistically significant at the high dose only). TK analysis showed systemic exposure to INT-747, UPF-1443, and UPF-832, except for low dose females where UPF-832 was below the limit of quantitation on day 0. Exposure generally increased with increasing dose, and exposure to INT-747 (based on AUC) was typically higher for males versus females whereas exposure to UPF-1443 was generally higher in females than males. Exposures to INT-747 were typically higher than UPF-1443, other than in females on day 13. Exposure to UPF-832 was considered negligible relative to the parent compound (~0.1-1% of the AUC_{last} for INT-747). The half-life values for INT-747 were approximately 5 h on day 0 and 20 h on day 13. On day 13, the half-life value for UPF-1443 was approximately 20 h. Half-life was not reported for UPF-832 due to fluctuations in plasma concentration. Toxicokinetic parameters are summarized in the Applicant's tables below.

Text Table 1. Summary of Toxicokinetic Parameters for INT-747

Dosage	AUC_{last} (ng•h/mL)		C_{max} (ng/mL)		T_{max} (h)	
	Day 0	Day 13	Day 0	Day 13	Day 0	Day 13
Males						
6 mg/kg/day	3740	16,772	703	1317	0.5	0.5
15 mg/kg/day	8679	21,055	2876	3420	0.5	0.5
60 mg/kg/day	45,440	104,534	11,490	5890	1	0.5
Females						
6 mg/kg/day	1103	2973	522	601	0.5	0.5
15 mg/kg/day	3167	11,552	874	6953	0.5	0.5
60 mg/kg/day	31,476	77,257	11,337	6747	0.5	0.5

Text Table 2. Summary of Toxicokinetic Parameters for UPF-1443

Dosage	AUC_{last} (ng•h/mL)		C_{max} (ng/mL)		T_{max} (h)	
	Day 0	Day 13	Day 0	Day 13	Day 0	Day 13
Males						
6 mg/kg/day	862	4390	54.7	103	16	8
15 mg/kg/day	1499	15,150	93.9	407	16	16
60 mg/kg/day	5328	50,018	308	1883	16	8
Females						
6 mg/kg/day	1036	8880	51.0	237	24	4
15 mg/kg/day	1781	30,382	121	886	8	2
60 mg/kg/day	10,582	99,742	679	3603	16	8

Text Table 3. Summary of Toxicokinetic Parameters for UPF-832

Dosage	AUC_{last} (ng•h/mL)		C_{max} (ng/mL)		T_{max} (h)	
	Day 0	Day 13	Day 0	Day 13	Day 0	Day 13
Males						
6 mg/kg/day	59.9	27.2	5.60	4.03	4	24
15 mg/kg/day	92.4	24.5	12.1	0.930	2	0.5
60 mg/kg/day	439	147	39.7	4.65	8	8
Females						
6 mg/kg/day	0.00*	5.09	0.00*	0.790	NA*	4
15 mg/kg/day	30.2	100	4.66	3.87	8	8
60 mg/kg/day	116	476	7.83	20.0	16	2

NA = Not Applicable
* = Below lower limit of quantitation.

An Oral (Gavage) Toxicokinetic Study of INT-747 and Metabolites in New Zealand White Rabbits (Study No. (b) (4) -661078).

Methods: Pregnant female New Zealand White rabbits (n=4) were administered oral (gavage) doses of 20 mg/kg/day (or vehicle, 0.5% carboxymethylcellulose in deionized water) once daily from GD 7 through 20. The dosing volume was 5 mL/kg. Blood samples for TK evaluation were collected prior to dosing and out to 24 h after dosing on GD 20. Plasma samples were analyzed for INT-747, UPF-832 (the glycine conjugate of INT-747), and UPF-1443 (the taurine conjugate of INT-747) concentrations using UHPLC/MS/MS in the negative electrospray ionization (ESI-) mode.

Results: On scheduled termination on GD 21, all animals were determined to be gravid. TK analysis showed systemic exposure to INT-747 and UPF-832; whereas, exposure to UPF-1443 was detected in only a single animal. Exposures to UPF-832 were similar to the parent compound, while exposure to UPF-1443 was far lower than parent compound (metabolite/parent AUC ratio = 0.001). There was a high level of variability between animals, and half-life could not be determined. TK parameters are summarized in the Applicant's tables below.

Text Table 1. Toxicokinetic Parameters for INT-747, UPF-1443, and UPF-832 in Pregnant Rabbits at 20 mg/kg/day

Analyte	Subject	AUC_{last} (ng*h/mL)	Dose-Normalized AUC_{last}	C_{max} (ng/mL)	Dose-Normalized C_{max}	T_{max} (h)	T_{last} (h)
INT-747	Mean	23,500	1170	1800	90.2	0.9	24.0
	SD	15,000	752	832	41.6	0.8	0
UPF-1443	Mean	26.6	1.33	4.75	0.950†	4.0†	12.0†
	SD	53.2	2.66	9.50	NA	NA	NA
UPF-832	Mean	28,200	1410	2540	127	8.5	24.0
	SD	23,100	1150	2080	104	10.4	0

N = 4 except where noted by † where n = 1
Note: Units for dose-normalized AUC_{last} are (ng*h/mL)/(mg/kg); units for dose-normalized C_{max} are (ng/mL)/(mg/kg)
NA = Not Applicable

INT-747: An Oral Comparative Crossover Toxicokinetic Study of Capsules and Tablets in Beagle Dogs (Study No. (b) (4) 661039)

Methods:
The purpose of this study was to compare the TK profile of INT-747 when administered to dogs orally by tablet or capsule. Beagle dogs (n=2/sex/group) were administered INT-747 daily in the form of a gelatin capsule containing either a 25 mg capsule or tablet. The study used a crossover design in which groups 1 and 2 were dosed for 7 consecutive days with capsules or tablets, respectively. Following a 7-day washout period, groups 1 and 2 were dosed once daily for 7 days with tablets or capsules, respectively. Blood samples for TK evaluation were collected on study days 0, 6, 14, and 20 pre-dosing and out to 24 h after dosing. Concentrations of INT-747, UPF-832, and UPF-1443 in plasma were determined using ultra-high performance liquid chromatography tandem mass spectrometry method in the negative electrospray ionization mode.

Results:
Clinical observations included emesis and soft feces, both of which occurred in a limited number of animals on a subset of study days. Systemic exposure to INT-747 and the taurine conjugate of INT-747 were demonstrated; whereas, the glycine conjugate was detected in only 1 animal at a single timepoint. AUC_{last} values for INT-747 increased ~2-fold in females from the first to last day of capsule dosing. From the first to last day of tablet dosing, AUC_{last} values for INT-747 increased ~2-fold and 2.5-fold in males and females, respectively. For the taurine conjugate, AUC_{0-24h} values increased by less than 2-fold in both sexes from the first to last day of capsule or tablet dosing. In males, the INT-747 AUC_{last} value on Day 6/20 of dosing with tablets was 1.7-fold higher than the value for capsules. In females, the INT-747 AUC_{last} value on Day 6/20 of dosing with tablets was 0.7-fold the value for capsules. Dosing with both capsules and tablets produced higher exposures to INT-747 and the taurine conjugate in females (compared to males), and t_{max} ranged from 0.8-2.1 h and 6.5-9.5 h for INT-747 and the taurine

conjugate, respectively. It was concluded that there were no significant differences in the overall TK profiles of INT-747 when comparing the two different forms (capsule versus tablet).

Text Table 1. Summary of Toxicokinetic Parameters for INT-747

Dosage	AUC_{last} (ng•h/mL)		C_{max} (ng/mL)		t_{max} (h)	
	Day 0/14	Day 6/20	Day 0/14	Day 6/20	Day 0/14	Day 6/20
Males						
25 mg/day Capsule	105	128	163	136	0.88	0.75
25 mg/day Tablet	99.6	214	120	135	1.0	1.4
Females						
25 mg/day Capsule	583	1050	361	409	2.1	1.9
25 mg/day Tablet	270	697	258	287	1.0	1.1

Text Table 2. Summary of Toxicokinetic Parameters for the Taurine Conjugate, the Major Metabolite of INT-747

Dosage	$AUC_{0-24\,h}$ (ng•h/mL)		C_{max} (ng/mL)		t_{max} (h)	
	Day 0/14	Day 6/20	Day 0/14	Day 6/20	Day 0/14	Day 6/20
Males						
25 mg/day Capsule	3890	5150	364	600	8.5	7.0
25 mg/day Tablet	3620	6320	361	652	8.5	9.5
Females						
25 mg/day Capsule	5100	9780	613	945	7.5	8.0
25 mg/day Tablet	6720	6980	547	687	6.5	7.5

Additional TK data are reviewed with the associated toxicity studies under Sections 6 (General Toxicology), 8 (Carcinogenicity), and 9 (Reproductive and Developmental Toxicology).

6 General Toxicology

6.1 Single-Dose Toxicity

Acute toxicity studies were conducted in rats (oral gavage) and dogs (oral capsule, oral suspension), and are briefly summarized below.

In Study No. 017166, Crl:CD®(SD)(IGS)BR rats (n=5/sex/group) were administered a single dose of 30, 100, and 300 mg/kg INT-747 or vehicle (0.5% carboxymethylcellulose) by oral gavage. No mortalities occurred and there were no gross lesions observed at necropsy on Day 14.

In a rising dose and acute oral toxicity study (Study No. 017259), INT-747 was tested in male and female Beagle dogs. In Phase 1, dogs (n=1/sex) were administered a single oral dose of 30 mg/kg by capsule. The dogs were subsequently given doses by capsule at 100, 300, 450, and 750 mg/kg, with 3 to 7 days between doses. A final dose of 750 mg/kg in 0.5% carboxymethylcellulose was administered by oral gavage due to vomiting that occurred with capsule dosing. In Phase 2, dogs (n=1/sex) were administered a single oral dose of 750 mg/kg by capsule. There were no mortalities in Phase 1 of the

study with doses up to 750 mg/kg. In Phase 1, doses ≥300 mg/kg produced emesis and weight loss, with diarrhea at higher doses. A decrease in the percent of reticulocytes and leukocytes was noted as the dose increased. In addition, there were elevated liver enzymes at ≥300 mg/kg, increased LDH and decreased uric acid levels at 750 mg/kg, and a trend towards decreasing blood glucose with increasing dose. At necropsy, the female had a distended gall bladder. In Phase 2, diarrhea and mucoid feces occurred. Elevated liver enzymes occurred in both sexes with elevations in the female persisting on Day 14 post-dosing.

6.2 Repeat-Dose Toxicity

Mouse

Study title: A 7-Day Oral (Gavage) Toxicity Study of INT-747 in Mice

Study no.:	(b) (4) -661023
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 4, 2010
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	INT-747, Lot no. 1106004P, 96.5%

Key Study Findings

In this dose-range finding study, INT-747 produced lethality or moribundity at ≥175 mg/kg/day (males) and 300 mg/kg/day (females). Clinical signs, body weight losses, and decreased food consumption (compared to controls) occurred in males at ≥175 mg/kg/day. Treatment-related changes in clinical chemistry parameters also occurred at ≥175 mg/kg/day (e.g., increased total bilirubin and liver enzymes, and decreased triglycerides, compared to controls). Finally, liver weights were increased in both sexes at ≥175 mg/kg/day (compared to controls), which correlated with macroscopic findings of pale liver (histopathological examination not conducted). The primary target organ was the liver.

Methods

Doses:	3, 50, 175, 300
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% carboxymethylcellulose (CMC) in deionized water
Species/Strain:	Crl:CD-1 mice
Number/Sex/Group:	5/sex/group
Age:	7 weeks
Weight:	Males: 26.3-33.8 g; Females: 20.6-25.1 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

Checks for mortality and moribundity were conducted twice daily. INT-747 produced mortality or moribund conditions at ≥175 mg/kg/day. At 175 mg/kg/day, a single male was found dead. At 300 mg/kg/day, four males died on study days 2 or 3 and a single female was euthanized in extremis on study day 3.

Clinical Signs

Clinical examinations were performed twice daily. Clinical signs occurred at ≥175 mg/kg/day in males and 300 mg/kg/day in females. Findings included hypoactivity, dermal atonia, decreased rate of respiration, bodies and extremities that were cool to the touch, partial closure of the eyes, and intermittent tremors.

Body Weights

Body weights were recorded weekly pretest, at randomization, and on study days 0, 3, 4, and 7. In males, there was mean body weight loss over days 4 to 7 and days 0 to 3 at 175 and 300 mg/kg/day, respectively.

Feed Consumption

Food consumption was recorded weekly pretest, at randomization, and on study days 0, 3, 4, and 7. Food consumption was decreased (compared to controls) at ≥175 mg/kg/day in males.

Hematology

Not conducted

Reference ID: 3870157

Clinical Chemistry

Blood samples collected at the scheduled necropsies on study days 3 and 7 were analyzed for standard clinical chemistry parameters. Samples from day 3 were analyzed only for high dose animals. However, samples from high dose males collected on day 3 were analyzed for only a limited number of parameters due to an insufficient amount of serum available. Samples from day 7 were limited to the 0, 3, 50, and 175 mg/kg/day dose groups.

At 175 mg/kg/day, total bilirubin, ALP, ALT, GGT, and SDH were increased and triglycerides were decreased (compared to controls) in both sexes. In males, urea nitrogen, creatinine, and AST were increased and cholesterol was decreased (compared to controls). In females, total protein was increased compared to controls. At 300 mg/kg/day, ALP, urea nitrogen, GGT, and SDH values were increased in males (compared to controls). In females, ALP, GGT, SDH, total protein, total bilirubin, ALT, and AST values were increased and A/G ratios and urea nitrogen values were decreased (compared to controls).

Urinalysis

Not conducted

Gross Pathology

At scheduled termination, all animals were necropsied including examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities including viscera. Treatment-related macroscopic findings in animals that were found dead, were euthanized in extremis, or at the scheduled necropsy were limited to pale liver. This occurred in both sexes at ≥175 mg/kg/day.

Organ Weights

Weights of the following organs were recorded at necropsy, and organ to final body weight and organ to brain weight ratios were calculated:

Adrenals	Liver (with drained gall bladder)
Brain	Spleen
Kidneys	Thymus
Heart	Thyroid with parathyroids*

Paired organs were weighed together. Designated organs (*) were weighed after fixation.

At ≥175 mg/kg/day, liver weights were increased as compared to controls. At necropsy on day 7, mean absolute liver weights in males and females from the 175 mg/kg/day dose group were 19 and 17.2% higher, respectively compared to controls. Mean liver weights relative to final body weight were increased by 26 and 18.7% in males and females, respectively, compared to controls. Mean liver weights relative to brain weight were increased by 21 and 19.8% in males and females, respectively, compared to controls.

Histopathology

Although a limited number of organs were collected and preserved for possible future analysis, they were not examined microscopically.

Special Evaluation

None

Toxicokinetics

Not conducted

Dosing Solution Analysis

Not conducted

Review of Study No. (b) (4) 661025 is incorporated below from the pharmacology review of IND 63307 dated October 14, 2011 (C. Wu, Ph.D., DGIEP). Subsequent to Dr. Wu's review, a revised final report was issued on July 20, 2011 and the revised report was submitted with the current NDA. Overall, the revised report contains no significant differences from the previously reviewed report which would impact the overall study conclusions. The primary revision in the revised report pertains to the incorporation of plasma analysis and TK data for the taurine and glycine conjugates of INT-747 (UPF-1443 and UPF-832, respectively), and TK parameters for these metabolites are summarized in the following tables copied directly from the revised report. As shown, exposure to UPF-1443 was greater than exposure to parent compound and metabolite/parent ratios decreased with increasing doses. Exposure to UPF-832 was minimal compared to INT-747 (metabolite/parent ratios ≤0.0066). For reference, Dr. Wu's October 2011 review copied below also contains a discussion of exposures to INT-747, UPF-1443, and UPF-832 based on information in the original study report which was reviewed as well as information contained in the Request for Special Protocol Assessment (SPA) (mouse carcinogenicity study protocol) submitted under IND 63307 in August 2011.

Table 9. UPF-1443 Toxicokinetic Parameters Following Oral Administration of INT-747 to Male Mice at 4, 12, 40, or 120/80 mg/kg/day

INT-747 Dosage (mg/kg/day)	AUC_{last} (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	Metabolite /Parent Ratio
Day 0				
4	1525	89.2	4	241
12	3840	225	4	86.3
40	13328	756	4	34.2
80*	14723	699	4	27.1
120*	28827	1813	2	10.0
Day 35				
4	2932	162	4	393
12	7386	395	4	184
40	25588	1146	0	91.4
80*	58030	4577	0.5	22.1
Day 90				
4	3171	193	2	1378
12	8778	473	2	89.9
40	26213	1429	2	51.7
80*	57003	3913	0.5	18.8

*Dosing at 120 mg/kg/day was discontinued from Day 3 through Day 7 and was then lowered to 80 mg/kg/day on Day 8. At 80 mg/kg/day, blood collected on Days 35 and 90 represented 28 and 83 consecutive days of dosing, respectively. Additional animals were added to the 80 mg/kg/day dose group such that animals bled on Day 8 received a single dose.

Table 10. UPF-1443 Toxicokinetic Parameters Following Oral Administration of INT-747 to Female Mice at 4, 12, 40, or 120/80 mg/kg/day

INT-747 Dosage (mg/kg/day)	AUC_{last} (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	Metabolite/ Parent Ratio
Day 0				
4	2113	139	4	149
12	5241	369	2	62.7
40	18326	1102	4	34.5
80*	27795	1519	4	8.72
120*	32435	1703	4	6.13
Day 35				
4	3040	190	2	43.4
12	11914	663	4	106
40	28693	1833	0.5	39.1
80*	63900	4417	2	18.7
Day 90				
4	2015	136	0.5	134
12	8366	487	0	55.9
40	33957	2403	0	20.4
80*	60998	3503	0.5	18.9

*Dosing at 120 mg/kg/day was discontinued from Day 3 through Day 7 and was then lowered to 80 mg/kg/day on Day 8. At 80 mg/kg/day, blood collected on Days 35 and 90 represented 28 and 83 consecutive days of dosing, respectively. Additional animals were added to the 80 mg/kg/day dose group such that animals bled on Day 8 received a single dose.

Table 12. UPF-832 Toxicokinetic Parameters Following Oral Administration of INT-747 to Male and Female Mice at 4, 12, 40, or 120/80 mg/kg/day

Study Day*/ INT-747 Dosage (mg/kg/day)	AUC_{last} (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	Metabolite/ Parent Ratio
Day 0‡				
120 (Males)	NR	0.360	24	0.0014
Day 8‡				
80 (Males)	NR	1.74	4	0.0035
Day 35				
80 (Males)	15.6	1.22	0.5	0.0066
80 (Females)	2.85	1.36	2	0.0009
Day 90				
80 (Males)#	6.03	1.85	0.5	0.0022
80 (Females)	NR	0.430	1	0.00004

AUC_{inf} and half-life are not presented because all values were considered approximations.
*Actual Study Day shown; due to dosing holiday and lowering of dose, Days 8, 35 and 90 correspond with administration of the 1st, 28th and 83rd doses at 80 mg/kg/day.
**Results not presented for 4 and 12 mg/kg/day dosages because all concentrations were below the LLOQ (1.00 or 5.00 ng/mL).
‡Following dosing, all concentrations for females were below the LLOQ; results not shown.
#One sample collected from a male 0.5 hours after dosing at 80 mg/kg/day on Day 90 was <LLOQ of 5.00 ng/mL and was excluded because other samples at the same time point had measurable concentrations between 1.00 and 5.00 ng/mL (and an LLOQ of 1.00 ng/mL).
NR = Not reported; concentrations measurable at <2 time points.

Study title: A 91-Day Oral (Gavage) Toxicity and Toxicokinetic Study of INT-747 in Mice

Study no.:	661025
Study report location:	Amendment #045, page 1-2370
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	March 29, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, Lot No. 43-440, 96.8% purity

Key Study Findings

This study was conducted to evaluate the toxicity and determine the toxicokinetics of INT-747 when administered daily by oral gavage to Crl:CD-1 mice for at least 13 weeks. Mice received daily oral dosing with INT-747 at 4, 12, 40, and 120/80 mg/kg/day. Due to extreme clinical signs, dosing for the 120 mg/kg/day group was discontinued on day 2. On day 8 dosing was resumed in this group at 80 mg/kg/day. In main toxicity study, a total of five 120/80 mg/kg/day group males were euthanized *in extremis* or found dead. In the TK group animals, 3 males and 1 female in the 120/80 mg/kg/day group were found dead. The hepatocellular change (necrosis) was associated with deaths in the 120/80 mg/kg/day group males and related serum chemistry changes (higher ALT, AST and Sorbitol Dehydrogenase (SDH) levels) and higher liver weights in the 120/80 mg/kg/day group males and females. Also, hepatocelluar necrosis was observed in the 4 mg/kg/day group males only. Therefore, the no-observed-adverse-effect level (NOAEL) could not be established for males. The NOAEL was 4 mg/kg/day for females, the lowest dose administered. Thus, the target organ for the test drug INT-747 was the liver.

Methods

Doses:	INT-747 was given at 0 (vehicle control), 4, 12, 40, and 120/80 mg/kg/day. Due to extreme clinical signs, dosing for the120 mg/kg/day group was discontinued on day 2. On day 8 dosing was resumed in this group at 80 mg/kg/day.
Frequency of dosing:	Once daily
Route of administration:	By gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% aqueous carboxymethylcellulose
Species/Strain:	Crl:CD-1 mice
Number/Sex/Group:	20 animals/sex/group (main study)
Age:	6 weeks
Weight:	24.6 to 34.7 g for males and 20.4 to 27.7 g for females
Satellite groups:	10 animals/sex/group for TK study
Unique study design:	none
Deviation from study protocol:	Not specified

Observations and Results

Mortality

In the toxicology group animals, three 120/80 mg/kg/day group males were euthanized *in extremis* on study days 21, 42, and 45, respectively. Two 120/80 mg/kg/day group males were found dead on study day 90. In the TK group animals, 3 males in the 120/80 mg/kg/day group were found dead on study days 34, 36 and 70, respectively, and one female in the 120/80 mg/kg/day group was found dead on study day 48.

Clinical Signs

Clinical observations of gasping and/or labored respiration were noted in 1 male and 2 females following 3 days (study days 0 through 2) of administration at 120 mg/kg/day (Group 5). These animals also displayed an unkempt appearance, dermal atonia, and/or extremities cool to the touch. As a result, all Group 5 animals received a dosing holiday from study days 3 through 7 with resumption of INT-747 administration on study day 8 at 80 mg/kg/day. Clinical observations related to INT-747 administration at 80 mg/kg/day (Group 5) were generally limited to the males that died or were euthanized *in extremis* on study days 21 through 90. These findings included hypoactivity, tremors (intermittent or continuous), dermal atonia, thin, extremities pale and/or cool to touch, body cool to touch, and/or abnormal respiration (decreased rate, labored, or shallow). In most cases, these clinical observations were observed on the day prior to or on the day of death.

Body Weights

During the period of INT-747 administration at 120 mg/kg/day and the subsequent dosing holiday (study week 0 to 1), Group 5 male no. 8081 and female no. 8283 had remarkable body weight losses (2.2 and 2.9 grams, respectively) when compared to the mean body weight gains in the control group males and females (1.1 and 0.2 grams, respectively). Consistent with the body weight losses, these animals were also noted with severe INT-747-related clinical observations.
During INT-747 administration to Group 5 at 80 mg/kg/day, the overall (cumulative for study weeks 1 to 13) mean body weight gains in both males and females were similar to the control group although individual animal body weights were highly variable. Mean body weights and body weight gains in the 4, 12, 40 mg/kg/day group males and females were unaffected by INT-747 administration. There were no definite trends, nor statistically significant differences for the INT-747-treated groups compared to the control group as shown in the Tables below.

Table 1 Male mouse body weight changes during 3 month study

Dose group (mg/kg/day)	Pre-dose (g)	Week 6 (g)	Week 13 (g)
0	25.2±1.48	34.2±2.36	36.7±3.20
4	25.0±1.19	33.1±2.17	36.9±2.79
12	25.2±1.23	32.8±2.60	34.2±2.35
40	24.8±1.65	33.1±2.25	35.4±2.54
120/80	25.1±1.48	33.2±2.88	35.5±3.62

Table 2 Female mouse body weight changes during 3 month study

Dose group (mg/kg/day)	Pre-dose (g)	Week 6 (g)	Week 13 (g)
0	24.1±1.49	27.5±1.39	29.9±2.41
4	24.4±1.65	27.3±2.04	28.8±2.39
12	24.0±1.50	27.8±1.97	29.2±1.49
40	23.9±1.39	26.5±1.29	28.2±1.61
120/80	23.9±1.60	27.4±1.79	29.4±2.52

Food Consumption

INT-747-related slightly lower food consumption was noted in the 120/80 mg/kg/day group males only. Statistically significantly lower mean food consumption was noted for the 120/80 mg/kg/day group males at weeks 2 to 3 and 8 to 9. For the week 2 to 3 interval, lower mean food consumption was likely attributable to the lower individual food consumption value for male no. 7801 that was euthanized *in extremis* on study day 21. There were no INT-747-related effects on food consumption in the 4, 12, and 40 mg/kg/day group males and females and the 120/80 mg/kg/day group females.

Ophthalmoscopy

Not performed.

Hematology

Hematology parameters were unaffected by INT-747 administration on Day 91 and Day 182.

Clinical Chemistry

INT-747-related higher alanine aminotransferase (ALT, 20-fold increased in males and 2-fold in females), aspartate aminotransferase (AST, 5-fold increased in males and 1.5-fold in females), sorbitol dehydrogenase (SDH, 4-fold increased in males) and alkaline phosphatase (ALP, 2-fold increased in males) levels were noted in 120/80 mg/kg/day group males and females compared to the control group. Additionally, INT-747-related higher albumin (5% increase) and lower cholesterol (50% decreased) levels were noted in 120/80 mg/kg/day group females only. Although a dose response was not always present, the magnitude of the difference in the 120/80 mg/kg/day group and the presence of correlating histologic changes suggests that the differences in these values were related to administration of INT-747.

Urinalysis

Not reported.

Gross Pathology

An INT-747-related gross finding of mottled liver was noted in a single 120/80 mg/kg/day group male. This animal (no. 7859) was euthanized *in extremis* on study day 45, and its gross finding of mottled liver correlated with severe hepatocellular coagulative necrosis observed microscopically. Clear fluid within the uterus was noted in 4 females from the 40 mg/kg/day group (2 at study week 5 and 2 at study week 13) and 4 females from the 120/80 mg/kg/day group (at study week 5). This change was also noted in one control group female at study week 13. This finding correlated microscopically with minimal to mild uterine luminal dilatation and an estrous cycle stage of late proestrus or early estrus.

Organ Weights

INT-747-related higher mean liver weights were noted in the 120/80 mg/kg/day group males (22% increase) and females (10% increase) when compared to the control group. Higher mean absolute and relative (to body and brain weights) liver weights were noted in the 120/80 mg/kg/day group males (20% increase) and females (13% increase) at the interim and primary necropsies (study weeks 5 and 13, respectively) when compared with

control group values. Most of the differences from the control group were statistically significant. There were no other INT-747-related effects on organ weights. However, statistically significantly higher mean absolute and relative (to body weight and brain weight) spleen weights were noted for the 12 mg/kg/day group females at the interim necropsy and lower absolute spleen weight and spleen weight relative to body weight were noted for the 40 and 120/80 mg/kg/day females at primary necropsy. All individual values were within the [redacted] (b) (4) historical control data reference range (version 3.1) and the direction of change was different between the interim and primary necropsies; therefore, the change was not considered to be related to administration of INT-747. Higher mean absolute thyroid/parathyroid weight and thyroid/parathyroid weight relative to brain weight were noted for the 4 mg/kg/day group females at the primary necropsy when compared with control group values. A dose response was not present; therefore, the differences were not considered to be related to administration of INT-747.

Histopathology

Adequate Battery

Yes

Peer Review

Yes

Histological Findings

All animals in the control and in high-dose groups (Groups 1 and 5), all animals that died on study prior to the final sacrifice, and all animals at the final sacrifice (Groups 1 through 5) were evaluated. The following tissues or organs were examined histologically from the above mentioned study animals: adrenals, aorta, bone with bone marrow smears, brain (cerebellum, cerebrum, midbrain, medulla), cecum, colon, duodenum, epididymides, esophagus, eyes, femur, gall bladder, heart, ileum, jejunum, kidneys, larynx, liver (section from 2 main lobes), lungs (section from two major lobes including bronchi), lymph nodes -Axillary-mandibular-mesenteric, optic nerves, ovaries (with oviduct), pancreas, Peyer's patches, pituitary, prostate, rectum, salivary glands – mandibular, sciatic nerves, seminal vesicles, skeletal muscle, skin with mammary glands (caudal area), small intestine, spinal cord (transverse and longitudinal sections at the cervical, thoracic and lumbar levels), spleen, sternum, stomach, testes, thymus, thyroid with parathyroids, tongue, trachea, ureters, urinary bladder, uterus with cervix , and vagina and gross lesions.

INT-747-related histopathology changes were noted in the liver in the 4, 12, 40 and 120/80 mg/kg/day group males and 12, 40 and 120/80 mg/kg/day group females and in the spleen and thymus in the 120/80 mg/kg/day group males only. Several histologic lesions were present in the liver of INT-747-treated males and females at the interim necropsy and primary necropsy (study week 13). Lesions were more severe in males than females at both time points, and an increased incidence of liver lesions (increased

mitoses, cytomegaly & karyomegaly, coagulative necrosis, neutrophil infiltration) was dose related in both males and females as shown in Table 1 below. All other histological findings were not considered to be test article related.

Table 1 Incidence of Selected Histopathologic Findings in Mice, Study Week 13 Primary Necropsy, including Unscheduled Deaths*

	Males					Females				
Dosage (mg/kg/day):	**0**	**4**	**12**	**40**	**120/80**	**0**	**4**	**12**	**40**	**120/80**
Liver	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Increased mitoses	0	0	2	1	7	0	0	0	1	6
Minimal	-	-	2	1	4	-	-	-	1	5
Mild	-	-	0	0	1	-	-	-	0	1
Moderate	-	-	0	0	2	-	-	-	0	0
Cytomegaly and karyomegaly	0	0	0	2	5	0	0	0	0	1
Minimal	-	-	-	2	1	-	-	-	-	1
Mild	-	-	-	0	4	-	-	-	-	0
Necrosis, coagulative	1	0	1	0	5	1	2	0	0	0
Minimal	1	-	1	-	1	1	2	-	-	-
Mild	0	-	0	-	0	0	0	-	-	-
Moderate	0	-	0	-	1	0	0	-	-	-
Severe	0	-	0	-	3	0	0	-	-	-
Necrosis, single cell	0	3	1	3	7	0	0	1	1	4
Minimal	-	2	1	3	2	-	-	1	1	4
Mild	-	1	0	0	4	-	-	0	0	0
Moderate	-	0	0	0	1	-	-	0	0	0
Infiltrate, neutrophil	0	3	1	3	9	0	0	1	1	4
Minimal	-	3	1	3	3	-	-	1	1	4
Mild	-	0	0	0	4	-	-	0	0	0
Moderate	-	0	0	0	2	-	-	0	0	0

*Animals in the high dose group received 120 mg/kg/day on Days 0-2, were given a dosing holiday on Days 3-7, and resumed dosing at 80 mg/kg/day on Day 8. Eighteen animals /sex were added to this group for TK evaluation on Day 8.

Special Evaluation

None

Toxicokinetics

As shown in Table 2 below, exposure to the taurine conjugate was 10-fold or more above exposure to parent INT-747 at all doses. Exposure to the glycine conjugate was minimal. Comparison of the AUCs at the various doses used in this study suggests that a modest accumulation of INT-747 and its taurine conjugate occurred over the first 35 days of

dosing in both males and females and thereafter remained steady through the 90-day time point. Total INT-747-related drug exposure appears to have reached a steady state between Day 0 and Day 35. Total INT-747-related drug exposure AUC_{last} (3,171, 8,856, 26,616 and 59,426 ng•hr/mL for males, and 2,015, 8,485, 35,287 and 63,570 ng•hr/mL for females) at the 90 day time point compared to total exposure in humans (5,436 ng•hr/mL) at the MRHD (25 mg), represents margins of exposure of 0.58x, 1.63x, 4.90x, and 10.93x in males and 0.37x, 1.56x, 6.49x, and 11.69x in females, at the 4, 12, 40, and 80 mg/kg/day dose levels, respectively. Since the parent and active conjugated metabolites are essentially confined to the intestine and hepatobiliary system and recirculated, exposure margins were calculated on an mg/kg basis. There were no remarkable differences between males and females in total INT-747-related drug exposure and total exposure was approximately linear with dose for both sexes. Doses of INT-747 equal to or greater than ~7 mg/kg/day represent a margin of exposure of at least 1.0.

Table 2 Plasma Toxicokinetics: Mean AUC Estimates of INT-747 and the Glycine and Taurine Conjugates on Days 0, 35, and 90 in CD-1 Mice

AUC_{last} (ng•hr/mL)	Day	Dose (mg/kg/d)				
		4	12	40	80	120
Males						
INT-747	0	NR	35.4	310	432*	2,295
INT-747-Glycine		NR	NR	NR	NR	NR
INT-747-Taurine		1,525	3,840	13,328	14,723	28,827
INT-747-Total Active Compounds		**1,525**	**3,875**	**13,638**	**15,155**	**31,122**
INT-747	35	NR	31.9	222	2,086	
INT-747-Glycine		NR	NR	NR	15.6	
INT-747-Taurine		2,932	7,386	25,588	58,030	
INT-747-Total Active Compounds		**2,932**	**7,418**	**25,810**	**60,132**	
INT-747	90	NR	77.7	403	2,417	
INT-747-Glycine		NR	NR	NR	6.03	
INT-747-Taurine		3,171	8,778	26,213	57,003	
INT-747-Total Active Compounds		**3,171**	**8,856**	**26,616**	**59,426**	
Females						
INT-747	0	NR	66.5	423	2,533	4,208
INT-747-Glycine		NR	NR	NR	NR	NR
INT-747-Taurine		2,113	5,241	18,326	27,795	32,435
INT-747-Total Active Compounds		**2,113**	**5,308**	**18,749**	**30,328**	**36,643**
INT-747	35	55.7	89.2	583	2,720	
INT-747-Glycine		NR	NR	NR	2.85	
INT-747-Taurine		3,040	11,914	28,693	63,900	
INT-747-Total Active Compounds		**3,096**	**12,003**	**29,276**	**66,623**	
INT-747	90	NR	119	1,321	2,572	
INT-747-Glycine		NR	NR	NR	NR	
INT-747-Taurine		2,015	8,366	33,957	60,998	
INT-747-Total Active Compounds		**2,015**	**8,485**	**35,278**	**63,570**	

NR = not reportable; *One 4-hour sample excluded as an outlier

Rat

Study title: A 28-Day Oral Toxicity Study in Sprague-Dawley Rats with INT-747

Study no.:	017262
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 10, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, Batch C, Lot 1204004/L, ≥92%

Key Study Findings

Treatment with 150 mg/kg/day INT-747 produced mortality/moribundity and clinical signs (e.g., soft or mucoid feces and decreased activity) in both sexes. Although there were no treatment-related effects on body weight, food consumption was increased (compared to controls) at ≥75 mg/kg/day in males during the treatment period, and at 150 mg/kg/day in recovery males and females. Changes in hematological parameters (e.g., increased RBCs and WBCs; decreased MCV, MCH, hemoglobin) occurred primarily at the high dose, while changes in clinical chemistry parameters occurred at all doses (e.g., increased chloride, phosphorous, uric acid). Several changes in clinical chemistry parameters (e.g., increased ALT, ALP) occurring primarily at 75 mg/kg/day were consistent with other effects on the liver such as increased organ weight. Microscopic changes included mononuclear cell infiltrates in the mucosa of the large intestine (all doses levels). Other primary target organs included the liver, mesenteric lymph node, and mesenteric fat. In high dose recovery animals, increased mononuclear cell infiltrates in the mucosa of the large intestine occurred at an increased incidence compared to controls. Based on findings at all dose levels (including mononuclear cell infiltrates in the mucosa of the large intestine), a NOAEL was not established in this study.

Reference ID: 3870157

Methods

Doses:	25, 75, 150 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% aqueous carboxy methylcellulose (CMC)
Species/Strain:	Crl:CD®(SD)(IGS)BR rats
Number/Sex/Group:	Main study: 10/sex/group, 5/sex recovery group (control and high dose groups only) TK study: 10/sex/group (except controls)
Age:	8 weeks
Weight:	Males: 191-262 g; Females: 164-201 g
Satellite groups:	Yes
Unique study design:	No
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

Observations and Results

Mortality

Viability checks were conducted twice daily. INT-747 produced mortality at 150 mg/kg/day. Three high dose males and 5 high dose females died. A single high dose female died during blood collection, and an additional high dose main study female was sacrificed in extremis during the recovery period on Day 29.

Clinical Signs

Main study animals were observed for clinical signs at 1 and 2 h post-dosing. Detailed clinical evaluations were conducted once weekly.

At 75 mg/kg/day, clinical signs were limited to a single incidence of soft feces in 1 female. At 150 mg/kg/day, clinical signs included soft or mucoid feces and colored material around the nose. In high dose animals that died prematurely, clinical signs such as decreased activity preceded death in both sexes. In one female, there were numerous additional abnormal clinical signs preceding death such as severe dehydration, rough coat, cold to the touch, and slight ataxia.

Body Weights

Body weights were recorded twice pretest, once weekly during the study, and at necropsy. There were no treatment-related effects on body weight.

Feed Consumption

Food consumption was measured weekly (main study groups only). In males, there were small (8%) but statistically significant increases in food consumption at 75

Reference ID: 3870157

mg/kg/day on Day 27 only and at 150 mg/kg/day on Days 13, 20, and 27. Increases in mean food consumption relative to body weight also occurred in males at these dose levels. Mean food consumption relative to body weight remained increased in high dose males (relative to controls) during the recovery period. In females, food consumption was increased by 11% at the high dose on Day 34.

Ophthalmoscopy

An ophthalmic examination (indirect) was conducted on main study animals pretest and during the last week of dosing. There were no treatment-related findings.

ECG

Not conducted

Hematology

Blood samples were collected from fasted animals prior to scheduled terminal necropsy (main study only). Samples were analyzed for standard hematological and coagulation parameters.

In high dose males, there were statistically significant changes in numerous parameters compared to controls (RBC +6%, MCV -4%, MCH -5%, platelets +26%). There were also statistically significant increases (compared to controls) in WBC (+53%) and absolute lymphocytes (+44%), monocytes (+159%), eosinophils (+100%), and leucocytes (+150%). At the end of the recovery period, several changes persisted (RBC +4%, MCV -8%, MCH -8%, compared to controls) at 150 mg/kg/day.

In females, there were statistically significant differences at all dose levels but changes occurred primarily at the high dose. Prothrombin time was decreased at all doses by 10 to 12%, compared to controls. MCHC was decreased by 6% at 75 and 150 mg/kg/day. HGB and MCH were decreased by 4% and 6%, respectively, at 150 mg/kg/day (compared to controls). Additional statistically significant changes (compared to controls) in high dose females included increased WBCs (+40%) and absolute neutrophils (+88%), lymphocytes (+31%), and large unclassified cells (+67%). While not statistically significant, the increase in percent neutrophils (+34% compared to controls) and decrease in percent lymphocytes (-6% compared to controls) were considered consistent with increased inflammation in the liver. Following the recovery period, MCH and MCHC were decreased by 4% and prothrombin time was increased by 14% at 150 mg/kg/day.

Clinical Chemistry

Samples collected at necropsy were evaluated for standard serum chemistry parameters.

Reference ID: 3870157

In males, chloride, phosphorous, and uric acid were increased at all dose levels (up to +5%, +24%, and +157%, respectively, compared to controls). At 75 mg/kg/day, there were statistically significant increases in ALT (+34%), ALP (+27%), and albumin (+5%). At 150 mg/kg/day, ALT, AST, ALP, and LDH were increased by 478%, 526%, 193%, and 603%, respectively, compared to controls. There were also increases in GGT (1 IU/L compared to 0 IU/L in controls), total protein (+6%), albumin (+8%), A/G ratio (+7%), and cholesterol (+31%). While not significant, other parameters such as total bilirubin and creatine kinase were increased compared to controls. In recovery males, cholesterol was increased by 55%, compared to controls.

In females, chloride was increased at all dose levels (up to +5%, compared to controls). LDH was also increased in all groups (+211%, +206%, and +91%, at 25, 75, and 150 mg/kg/day, respectively; only statistically significant at mid- and high-dose). At 75 mg/kg/day, statistically significant changes compared to controls included increased creatinine (+14%), ALP (+32%), and uric acid (+36%). At 150 mg/kg/day, there were the following statistically significant changes relative to controls: creatinine (+14%), AST (+46%), ALP (+40%), uric acid (+55%), sodium (+1%), calcium (+5%), albumin (+8%). At the end of the recovery period, cholesterol and triglycerides were increased by 68% and 123%, respectively, compared to controls.

Urinalysis

Not conducted

Gross Pathology

At terminal sacrifice following 28 days of dosing or the recovery period, full necropsies were performed on main study animals. At necropsy, yellow discoloration of tissues and distended bile duct were observed at 150 mg/kg/day. In addition, discoloration (red, black, or dark) was observed in numerous tissues of animals that died and this correlated with histopathological findings of congestion or hemorrhage.

Organ Weights

At scheduled necropsy, the following organs were weighed and organ/body weight and organ/brain weight ratios were calculated.

Adrenals	Pituitary (post fixation)
Brain	Spleen
Heart	Testes
Kidneys	Epididymides
Liver	Thymus
Lungs	Thyroid with parathyroids (post fixation)
Ovaries	Uterus

In males, there were statistically significant increases in absolute (+13%) and relative (+15%) liver weights at the high dose compared to controls. Absolute and relative pituitary weights were increased by up to +33%, compared to controls. Relative (to body weight) heart weights were increased by 10%. In recovery males, liver weights remained increased by up to 11%.

In females, statistically significant increases in absolute and relative liver weights occurred at all doses. Absolute liver weights were increased by 19%, 26%, and 34% at 25, 65, and 150 mg/kg/day, respectively. Absolute and relative weights of thyroid/parathyroid were decreased by up to 40% at 25 mg/kg/day and 48% at the higher doses. At the high dose, absolute and relative spleen weights were increased by up to 37% and relative thymus weights were increased by up to 32%. In recovery females, liver weights remained increased by up to 20% and absolute kidney weights were increased by 15%, compared to controls.

Histopathology

At necropsy, the following organs and tissues were collected and preserved.

Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymides
Tongue	Prostate
Esophagus	Seminal Vesicles
Stomach	Ovaries
Small Intestine	Uterus
Duodenum	Cervix
Jejunum	Vagina
Ileum	Endocrine
Large Intestine	Adrenals
Cecum	Pituitary
Colon	Thyroid/Parathyroids
Rectum	Skin/Musculoskeletal
Pancreas	Skin/Mammary Gland
Liver	Bone (femur)
Respiratory	Skeletal Muscle (thigh)
Trachea	Smooth muscle (urinary bladder)
Lungs (with mainstem bronchi)	Nervous/Special Sense
Lymphoid/Hematopoietic	Eyes with Optic Nerve
Bone Marrow (sternum)	Sciatic Nerve
Thymus	Brain (cerebrum, cerebellum and medulla/pons)
Spleen	Spinal Cord (cervical and thoracic)

Lymph Nodes	Other
Cervical	Mesenteric fat
Mesenteric	Gross Lesions

Tissues and organs listed above from all main study control and high dose animals and all animals that died prematurely were examined histopathologically. For Groups 2 and 3, all gross lesions as well as the following tissues were examined: (1) both sexes – cecum, colon, rectum, liver, mesenteric lymph node, salivary gland (mandibular), spleen, and thymus, (2) males only: pancreas, and (3) females: mesenteric fat.

Adequate Battery: Yes

Peer Review: No

Histological Findings

In animals that died prematurely, microscopic findings were present in mucosa of the small and/or large intestine (congestion/hemorrhage), liver (cholangiohepatitis, bile duct hyperplasia, hepatocyte degeneration/necrosis), mesenteric lymph node (sinus histiocytosis), and mesenteric fat (atrophy). Lesions observed in high dose females such as acute myofiber degeneration in the heart, lymphoid depletion in the spleen, atrophy and/or degeneration/necrosis of cortical lymphocytes in the thymus, and congestion/hemorrhage in the adrenals were considered to be likely secondary terminal event- or stress-related findings.

At terminal sacrifice, increased mononuclear cell infiltrate was observed in the cecal, colonic, and/or rectal mucosa at all dose levels. In high dose females and a single mid-dose male, there was sinus histiocytosis in the mesenteric lymph node. In mid- and high dose females, atrophy of the mesenteric fat was observed. Mucosal/submucosal edema was observed in the rectum or cecum of high dose males and females. In the salivary gland, there was degeneration/necrosis of the convoluted ducts in males (all dose levels) and females (control, low, and mid-dose females).

In high dose recovery animals, increased mononuclear cell infiltrates in the mucosa of the large intestine occurred at an increased incidence compared to controls.

Special Evaluation

None

Toxicokinetics

Blood samples for TK analysis were collected prior to dosing, and at nine timepoints (out to 24 h) after dosing on Day 0 and during Week 4. Plasma samples were analyzed using a validated method.

INT-747 was rapidly absorbed (Tmax range 0.25 – 2 h), and the half-life varied between dose levels (range 3.8 to 12.11 h on Day 27). On Day 0, AUC increased in a greater than dose proportional manner in males and females. Accumulation ratios ranged from 0.41 to 1.43. The following table copied from the Applicant's report summarizes toxicokinetic parameters (Groups 5, 6, and 7 were treated with 25, 50, and 100 mg/kg/day, respectively).

Reference ID: 3870157

Table PK-2: Data Summary

Dose Day	Sex	Group	R^2	Half-life (hr)	Tmax (hr)	Cmax (ng/mL)	AUCall (hr•ng/mL)	Dosage (mg/kg/day)	Dose Ratio	AUC ratio	Accum. Ratio	Cmax ratio
0	F	5	1.00	12.05	0.25	4,363.76	8,430.64	25	-	-	-	-
		6	0.96	7.61	0.5	14,118.36	38,528.75	75	3	4.57	-	-
		7	0.68	NR	2	63,267.92	196,355.46	150	6	23.29	-	-
	M	5	0.91	24.13	0.25	3,806.87	10,440.65	25	-	-	-	-
		6	0.99	9.47	0.5	8,372.03	29,432.33	75	3	2.82	-	-
		7	0.57	NR	1	13,717.76	107,619.52	150	6	10.31	-	-
27	F	5	1.00	8.04	0.25	5,885.89	8,572.77	25	-	-	1.02	1.35
		6	0.98	12.11	0.25	7,894.44	28,319.63	75	3	3.30	0.74	0.56
		7	0.90	3.8	0.25	12,196.82	80,564.24	150	6	9.40	0.41	0.19
	M	5	0.18	NR	0.25	4,590.16	13,804.51	25	-	-	1.32	1.21
		6	0.91	5.58	0.5	6,678.89	42,174.84	75	3	3.06	1.43	0.80
		7	0.58	NR	0.25	11,588.49	72,723.27	150	6	5.27	0.68	0.84

NR - not reported: half-life not reported when $R^2 < 0.85$

Note: AUC ratio is ratio of AUC of Group 6 or 7 to low the dose group (Group 5).
Accumulation Ratio is ratio of Day 27 AUC/Day 0 AUC for a given group
Cmax ratio is ratio of Cmax of group to low dose group (Group 5).

Dosing Solution Analysis

Dosing solution samples were evaluated for concentration and homogeneity during Weeks 1 and 3. The mean percentage of nominal concentrations ranged from 98.3 – 105.6% during Week 1 and 94.3 – 97.5% during Week 3, which met the ±10% criterion for this study.

Review of Study No. 019958 is incorporated below from the pharmacology review of IND 63307 dated February 12, 2009 (C. Wu, Ph.D., DGP). Although the February 2009 review copied below identifies the study report title as “Interim Report through Week 13”, this review actually includes study results through the 26-week terminal sacrifice. The correct study title is “A 26-Week Oral Toxicity Study in Sprague-Dawley Rats with INT-747 (Including Results from the 13-week Interim Report).” In addition, it is noted that in a second pharmacology review of the study under IND 63307 dated October 14, 2011 (C. Wu, Ph.D., DGIEP), the NOAEL was identified as 6 mg/kg/day for both males and females. In the NDA, the Applicant also identified the NOAEL for this study as 6 mg/kg/day for both sexes based on findings of increased liver weights and changes in albumin levels observed at 15 mg/kg/day.

Study Title: A 26-Week Oral Toxicity Study in Sprague-Dawley Rats with INT-747 (Interim Report through Week 13) (Study # 019958)

Study No.: 019958
Conducting laboratory and location: [redacted] (b) (4)
(b) (4)
Date of study initiation: 06-22-2006
GLP compliance: Yes
QA report: Yes (x) no ( )
Drug, Lot # and % purity: INT-747, Lot# 43-440. The purity by HPLC was 96.8%

METHODS:

Doses: 6, 15 and 60 mg/kg/day
Species/strain: Sprague-Dawley rats
Number/sex/group: 25 rats/sex/group
Route, formulation, volume: Doses were administered by gavage. The dosing formulation was prepared weekly by [redacted] (b) (4) The dosing preparation was prepared by suspending the test article in 0.5% aqueous carboxymethylcellulose (CMC) to achieve the desired concentrations and divided into seven containers for 7 days of dosing. The dose preparations were stirred for about 20 minutes prior to dosing. The concentration of the test article was such that a dosing volume of 10 mL/kg body weight delivered the intended dose level(s).
Satellite groups used for toxicokinetics or recovery: 12 rats/sex were used for toxicokinetics study
Age: 8½ weeks
Weight: Male rats were 212-288 g and female rats were 157-211 g.
Sampling times: Described in Observation and Times section

OBSERVATION AND TIMES:

Clinical signs: Each animal was observed once each morning and afternoon throughout the study for viability. In addition, animals in Groups 1-4 were observed at least once daily, beginning 4 days prior to dosing, for changes in appearance and behavior. Once dosing began, all rats in Groups 1-4 were observed daily at 1 and 2.5 hours following dosing for changes in appearance and behavior.
Animals in the toxicokinetics groups were not observed for clinical signs.
A detailed clinical examination was performed on all animals in Groups 1-4 prior to the initial dose and then weekly for the duration of the study.

Body weights: Body weights were measured on all animals twice pretest (including once for randomization) and once weekly throughout the study. In addition, fasted body weights were measured for the main study animals (Groups 1-4) prior to necropsy for calculation of organ/body weight ratios.

Food consumption: Food consumption was measured on all main study animals (Groups 1 to 4) weekly, beginning the week prior to dosing and continuing weekly throughout the study.

Ophthalmoscopy: An ophthalmic examination (indirect) was conducted on all main study animals prior to the first dose on Day -7 and during the last week of dosing prior to the scheduled interim/terminal necropsy (Day 87 or 180). The examination was conducted using indirect ophthalmoscopy preceded by mydriasis produced by topical application of 1 % Mydriacyl®.

EKG: Not performed

Clinical Pathology Evaluation: Blood samples for evaluation of hematology, coagulation and serum chemistry parameters were collected from the retro-orbital plexus while under CO_2/O_2 anesthesia from all surviving animals in Groups 1-4 within a week of their scheduled termination. The animals were fasted overnight prior to blood collection. Additionally, at the discretion of the Study Director, blood samples for evaluation of serum chemistry parameters were collected from some moribund animals prior to their termination.
Blood samples were handled and analyzed as described below.

Hematology: Approximately 1 mL of blood was collected in EDTA-containing tubes. The following parameters were evaluated:

Red blood cells (RBCs) (count and morphology)	Mean corpuscular hemoglobin (MCH)
White blood cells (WBCs) (total and differential)	Mean corpuscular hemoglobin concentration (MCHC)
Hemoglobin concentration	Mean corpuscular volume (MCV)
Hematocrit	Platelet counts
	Reticulocyte counts

Clinical chemistry: Approximately 1 mL of blood was collected in a tube without anticoagulant. The sample was allowed to clot and then was centrifuged to obtain serum. The detailed parameters were evaluated and showed in the RESULTS

Urinalysis: Urinalysis was performed on all animals scheduled for termination (interim or terminal sacrifice) during the last week of dosing. The following urinalysis parameters were measured:

Specific gravity	Ketones
Occult blood	Glucose
Protein	Nitrite
pH	Urobilinogen
Bilirubin	Volume
Leukocytes	Color
	Clarity

Gross pathology: A complete necropsy was conducted with tissue collection on all animals found dead or terminated during the study (both scheduled and unscheduled

terminations). All abnormalities were described completely and recorded. The necropsy included examination of:

. Carcass and muscular/skeletal system
. All external surfaces and orifices
. Cranial cavity and external surface of the brain
. Neck with associated organs and tissues
. Thoracic, abdominal and pelvic cavities with their associated organs and tissues

Organ weights: For all scheduled terminations, the following organs (when present) were weighed. Paired organs were weighed together. The pituitary and thyroids/parathyroids were weighed post fixation. Organ weights were not taken from animals found dead or euthanized in a moribund condition.

Adrenals	Pituitary (post fixation)
Brain	Testes
Heart	Epididymides
Kidneys	Ovaries
Liver	Thymus
Spleen	Thyroid with parathyroids (post fixation)

Histopathology: Peer review: yes (X), no ( )
For all animals from the control and high-dose groups (Groups 1 and 4) included in the interim or final termination and all animals that died or were euthanized in extremis during the study, the tissues listed in the table below were embedded in paraffin, sectioned and stained with hematoxylin and eosin. Target organs and gross lesions from Groups 2 and 3 were also prepared as above. The following organs and tissues were examined by light microscopy.

Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymides
Tongue	Prostate
Esophagus	Seminal Vesicles
Stomach	Ovaries
Small Intestine	Uterus
Duodenum	Cervix
Jejunum	Vagina
Ileum	Endocrine
Large Intestine	Adrenals
Cecum	Pituitary
Colon	Thyroid/Parathyroids
Rectum	Skin/Musculoskeletal
Pancreas	Skin
Liver	Mammary Gland (females)
Respiratory	Bone (femur)
Trachea	Skeletal Muscle (thigh)
Lungs (with mainstem bronchi)	Smooth Muscle (urinary bladder)
Lymphoid/Hematopoietic	Nervous/Special Sense
Bone Marrow (sternum)	Eyes with Optic Nerve
Thymus	Sciatic Nerve
Spleen	Brain (cerebrum, cerebellum and medulla/pons)
Lymph Nodes	Spinal Cord (cervical and thoracic)
Cervical	Other
Mesenteric	Mesenteric Fat
	Gross Lesions

Toxicokinetics: Blood was collected from the TK animals (Groups 6, 7 and 8) for toxicokinetic analysis. Some control samples (Group 5) were also taken. Approximately 0.5 mL of blood was collected in EDTA-containing tubes via the retro-orbital plexus while under CO_2/O_2 anesthesia. Three sets of TK animals per dose group comprising 4/sex/set were set aside for toxicokinetic sampling. One set was bled at the initial dose, a second set was bled at Week 13, and the third set was bled during the last week of the study. A total of four blood samples were taken from each animal. The actual sampling times were recorded. For a given set, the animals were terminated, without necropsy, following their last sample collection.

Sample Collection Time Points
(approximate times)

Initial Dose	Week 13	Last week of study	A, B*	C, D*
Pre-dose	Pre-dose	Pre-dose	X	
15 min	15 min	15 min		X
30 min	30 min	30 min	X	
1 hr	1 hr	1 hr		X
2 hr	2 hr	2 hr	X	
4 hr	4 hr	4 hr		X
8 hr	8 hr	8 hr	X	
24 hr	24 hr	24 hr		X

** At each of the 3 weeks of blood collection, there were 4 rats/sex/dose group (shown above as A, B, C and D) used for TK analyses. The 4 rats were bled according to this scheme.*

RESULTS:

Mortality

Six male and seven female mortalities (euthanized or found dead) occurred in Groups 2-4 (main study animals), plus five additional mortalities occurred among the high-dose toxicokinetic (TK) group animals during the 26 weeks of the study. Among males, all deaths except one moribund/euthanized (injured animal in Group 2) and one found dead in cage on the day of necropsy (Group 3) were Group 4 animals. The four mortalities in Group 4 males included two animals moribund on Days 145 and 172 and two animals found dead in cage on Days 55 and 174. In females, all seven mortalities were in Group 4: one animal was terminated after injury; two died after eye bleeding; two were found moribund on Days 62 and 174; and two were found dead in their cages on Days 51 and 140. All unscheduled deaths occurred between Days 34 and 182. Deaths or moribund states were preceded by decreased activity, hunched posture, colored material around the nose and/or eyes and yellow skin. Generally, the animals appeared clinically normal until about 1-4 days before death/morbidity.

Clinical Signs

Most animals appeared clinically normal during the study, with only minor abnormal clinical signs such as soft feces, scabs, and hair losses. As noted earlier, for animals that died or became moribund, clinical signs appeared within just a few days of death such as malocclusion, red nasal discharge and yellow discoloration of the skin.

Body Weights

Mean body weights increased weekly. For control and Group 4 male rats, the mean body weights were increased from 192±8.4 and 191±7.9 gram at pretest to 559±57.2 and 553±55.4 gram on Day 90, and to 654±84.2 and 611±75.5 gram on Day 181, respectively. In comparison, for control and Group 4 female rats, the mean body weights were increased from 162±7.2 and 161±9.1 gram at pretest to 283±24.1 and 277±18.7

gram on Day 90, and to 319±27.6 and 306±18.8 gram on Day 181, respectively. There were no significant differences between control and treatment groups with respect to body weights.

Food Consumption

Starting approximately the third week of dosing, males in Groups 3 and 4 consumed significantly more feed than controls. The mean food consumption of control rats was increased from 24 g/day to 26 g/day whereas it was increased from 24 g/day to 29 g/day in Group 4 rats at Day 27, then sustained at similar levels at Day 90 (47-50 g/day), but significantly increased to 45 g/day for Group 4 vs. 40 g/day for control on Day 181. As noted above, there was no corresponding increase in body weight with this increase in food consumption. In females, there were no significant differences between control and treatment groups with respect to food consumption throughout 181-day study.

Ophthalmoscopy

Two of the males scheduled for the interim necropsy had ocular abnormalities. One Group 1 (control) male had a diffuse keratitis of the cornea in one eye, and one Group 2 male had a focal corneal dystrophy of one eye. It was concluded that there were no test article-related effects on the eyes through Week 26.

Seven animals scheduled for the terminal necropsy at Week 26 had ocular abnormalities, Two Group 2 males, one Group 3 male, two Group 4 males and one Group 2 female developed chromodacryorrhea, one Group 3 male developed a pigmented area on both eyes.

Hematology

In Group 4 males on Day 91, RBC (8.69 vs. 8.12 M/µL), hemoglobin (15.3 vs. 14.3 g/dL) and hematocrit (46.4 vs. 43%) values were statistically different from controls ($p < 0.05$), and there appeared to be a trend for decreasing values for these parameters as doses increased. Although there was a possible trend for some of these values to decrease in females, there were no changes of statistical significance. One exception for females at Day 91 was that the absolute reticulocyte count was significantly lower in Group 4 than in controls (195.6 vs. 151.8 $x10^9$/L). There were no changes in red blood cells on Day 182 in either sex that were considered test article-related. This indicated that the changes that were seen on Day 91 were transient. On Day 91, platelet values for all test article-treated males were greater than controls (1299 K/µL, 1324 K/µL and 1536 K/µL of Group 2, 3 and 4 respectively vs. 1126 K/µL of Group1, $p < 0.05$), and there appeared to be a trend for platelets to increase with increasing dose, However, in females, the mean platelet values of the control and test article-treated groups were almost identical.
On Day 182, platelet values for Group 3 and 4 animals (both sexes) were greater than controls ($p < 0.01$) and there appeared to be a trend for platelets to increase with increasing dose (1172 K/µL of Group1 vs. 1256 K/µL, 1326 K/µL, 1486 K/µL of Group 2, 3 and 4 respectively for males, 1047 K/µL of Group 1 vs. 1116 K/µL, 1244 K/µL and

1350 K/µL of Group 2, 3 and 4 respectively for females. On Day 91, the white blood cell count (WBC) and absolute lymphocyte count (Abs Lymph) for the Group 4 males and females were numerically higher than controls, but not statistically significant. On Day 182, the absolute eosinophil count (Abs Eos) for Group 2 males (0.1 vs. 0.07 K/µL) and the WBC (7.79 vs. 11.12 K/µL) and Abs Lymph (6.29 vs. 8.06 K/µL) for the Group 4 females were statistically different when compared to the controls. The lower mean Abs Eos for the Group 2 males was considered to be the result of individual animal variation. Although not shown to be statistically significant, the WBC and Abs Lymph values on Days 91 and 182 for the Group 4 males were minimally higher when compared to the controls. The minimally higher WBC and Abs Lymph values observed in the Group 4 male and female rats on Days 91 and 182 were considered to be a test article related effect.

Clinical Chemistry

Parameters with values considered possibly related to treatment, based on being outside the historical range and/or demonstrating a trend with increased dosage were observed most often in Group 4 and included: increased ALT (3-fold increase seen in Group 4 males as compared to the control and 2-fold increase in Group 4 females on Day 91 and 2-fold increase in Group 4 males on Day 182, $P \leq 0.01$), increased AST (2-fold increase in Group 4 males as compared to control, and 5-fold increase in Group 4 females, $p \leq 0.01$, on Day 182), increased ALP (203% increase in Group 4 males with $p \leq 0.01$ and 135% in Group 4 females with $p \leq 0.05$ as compared to control on Day 91, while on Day 182, there was a 2.5 fold ALP incease in Group 4 males and a 8-fold ALP increase in Group 4 females $p \leq 0.01$), increased albumin on Day 91 (4.3 g/dL of Groups 3 and 4 males vs. 4.0 g/dL of controls with $p \leq 0.05$ in all cases except Group 4 males, $P \leq 0.01$, whereas 4.8 g/dL of Groups 3 and 4 females vs. 4.5 g/dL of controls with $p \leq 0.05$) and on Day 182 , a significant albumin increase was observed in Group 3 females (5.0 g/dL vs. 4.7 g/dL of controls) and an increased albumin in Group 4 males (4.4 g/dL vs. 4.0 g/dL of controls with $p \leq 0.01$), increased A/G of 1.5 vs. 1.4 of controls on Day 91 in Group 4 males with $p \leq 0.01$, but in Group 4 females with $p \leq 0.05$ and on Day 182, increase of A/G was seen in Group 4 males (1.4 vs, 1.3 of controls with $p \leq 0.01$), increased uric acid (1.6 and 1.3 mg/dL of Group 4 males and females respective vs. 0.9 mg/dL of controls with $P \leq 0.05$ on Day 91), and (1.6 mg/dL of Group 4 males vs. 0.7 mg/dL of controls with $p < 0.01$ and 1.4 mg/dL of Group 4 Female vs. 0.9 mg/dL of controls with $P \leq 0.05$ on Day 182), and increased LDH from 95 IU/L of controls to 146 IU/L and CK from 105 IU/L of controls to 215 IU/L (only significantly different in Group 4 females, $p \leq 0.05$) on Day 91, also CK increased in Group 4 males and females on Day 182 with $p \leq 0.05$. The increase in the parameters associated with liver function may correlate with the bile duct hyperplasia, hepatocellular hypertrophy and multifocal inflammation seen histologically in the liver of males and females of Group 4 (see Histopathology, below). Increased albumin and A/G ratio without associated increases in globulins may be secondary to test article-related inflammation of the liver and intestinal tract. Other statistically significant changes (e.g., high calcium in Group 4 females and high cholesterol in Group 3 males) were inconsistent between sexes and/or were within historical reference ranges for this age and strain of rat, and were not considered test article-related. Several parameters associated

with liver function were much greater than normal in Group 4 females on Day 182 as compared those on Day 91 including total bilirubin (T Bili), ALP, LDH, AST, suggesting a continuous injury of these animals.

Table 1 Mean Clinical Chemistry Data in Male Rats at Day 91

	BUN mg/dL	CREAT mg/dL	ALT IU/L	AST IU/L	ALP IU/L	LDH IU/L	T Bili mg/dL	GGT IU/L	ALB g/dL	A/G %	URIC ACID mg/dL
Group1 (0 mg)											
Mean	14	0.7	34	75	94	99	0.4	2	4.0	1.3	0.9
SD	1.9	0.09	6.9	4.0	12.7	42.7	0.10	0.7	0.17	0.08	0.26
Group2 (6 mg)											
Mean	13	0.7	29	71	99	110	0.4	2	4.2	1.3	1.1
SD	2.3	0.06	5.0	9.9	22.9	52.9	0.07	0.5	0.16	0.11	0.20
Group3 (15 mg)											
Mean	13	0.7	30	73	125	116	0.4	2	4.3*	1.4	1.0
SD	1.6	0.07	4.8	13.7	28.3	56.1	0.05	0.5	0.24	0.03	0.37
Group4 (60 mg)											
Mean	15	0.8	108**	150**	191**	127	0.4	1	4.3**	1.5**	1.6**
SD	1.4	0.00	64.3	57.9	47.4	51.0	0.07	0.5	0.21	0.07	0.33

Table 2 Mean Clinical Chemistry Data in Male Rats at Day 182

	BUN mg/dL	CREAT mg/dL	ALT IU/L	AST IU/L	ALP IU/L	LDH IU/L	T Bili mg/dL	GGT IU/L	ALB g/dL	A/G %	URIC ACID mg/dL
Group1 (0 mg)											
Mean	13	0.7	37	75	94	151	0.3	0	4.0	1.3	0.7
SD	1.9	0.09	6.9	4.0	12.7	108	0.10	0.7	0.17	0.08	0.22
Group2 (6 mg)											
Mean	14	0.7	33	84	100	178	0.3	0	4.0	1.3	1.1
SD	2.3	0.06	11.2	43	40.7	141	0.07	0.5	0.23	0.11	0.51
Group3 (15 mg)											
Mean	14	0.7	27	71	111	159	0.3	0	4.1	1.3	1.2*
SD	1.6	0.07	2.9	9.3	19.1	130	0.05	0.5	0.18	0.03	0.44
Group4											

(60 mg)											
Mean	16	0.8	80**	169*	196**	188	0.3	0	4.4**	1.4**	1.6**
SD	1.4	0.00	35.7	79.7	48.4	106	0.07	0.5	0.21	0.07	0.69

Each group contains 10 rats at doses of 0, 6, 15 and 60 mg/kg/day.
* Statistically significant deference from control group ($p < 0.05$)
** Statistically significant deference from control group ($p < 0.01$)

Table 3 Mean Clinical Chemistry Data in Female Rats at Day 91

	BUN mg/dL	CREAT mg/dL	ALT IU/L	AST IU/L	ALP IU/L	LDH IU/L	T Bili mg/dL	CK IU/L	ALB g/dL	A/G %	URIC ACID mg/dL
Group1 (0 mg)											
Mean	14	0.7	33	84	60	95	0.3	105	4.5	1.4	0.9
SD	2.5	0.11	17.9	24.1	20.9	74.0	0.09	38.1	0.24	0.11	0.16
Group2 (6 mg)											
Mean	14	0.8	25	65	52	76	0.3	105	**4.8**	1.4	0.9
SD	2.8	0.11	9.2	9.3	12.2	31.1	0.05	47.3	**0.22**	0.08	0.22
Group3 (15 mg)											
Mean	14	0.8	26	68	59	115	0.3	145	4.8*	1.4	1.1
SD	2.6	0.09	5.9	5.5	10.4	80.4	0.05	62.3	0.29	0.08	0.28
Group4 (60 mg)											
Mean	13	0.8	69**	123	81*	146*	0.4	215*	4.8*	1.5	1.3*
SD	1.9	0.09	49.0	65.1	47.4	119	0.09	121	0.29	0.10	0.47

Table 4 Mean Clinical Chemistry Data in Female Rats at Day 182

	BUN mg/dL	CREAT mg/dL	ALT IU/L	AST IU/L	ALP IU/L	LDH IU/L	T Bili mg/dL	CK IU/L	ALB g/dL	A/G %	URIC ACID mg/dL
Group1 (0 mg)											
Mean	15	0.7	45	105	35	219	0.3	123	4.5	1.4	0.9
SD	1.8	0.11	33.3	62.9	11.1	109.0	0.09	37.3	0.24	0.11	0.21
Group2 (6 mg)											
Mean	14	0.7	30	84	32	234	0.4	139	4.8	1.4	1.1
SD	1.9	0.11	23.5	56.6	12.8	211	0.05	79.3	0.36	0.08	0.33

Group3 (15 mg)											
Mean SD	15 3.2	0.7 0.09	22 5.6	59** 13.5	28 8.1	179 165	0.4 0.05	168 195	5.0* 0.29	1.4 0.08	1.0 0.15
Group4 (60 mg)											
Mean SD	15 2.0	0.7 0.09	65 39.0	540* 1327	268** 690	693 1104.3	1.4 3.62	246* 202	4.9 0.29	1.5 0.10	1.4* 0.57

Each group contains 10 rats at doses of 0, 6, 15 and 60 mg/kg/day.
* Statistically significant deference from control group ($p < 0.05$)
** Statistically significant deference from control group ($p < 0.01$)

Urinalysis

The only urinary parameter that was significantly different between control and test article-treated rats was urinary pH in males. The pH of Group 4 urine was slightly lower than controls, but in Groups 2 and 3, urinary pH was slightly higher than controls; urine pH changes were not observed in females. On Day 182, the pH of Group 4 females urine was higher (6.5 vs. 6.2 of Group 1).

Gross pathology (Groups 1-4)

Moribund Terminations

One Group 2 male (195066) was terminated for humane reasons at Day 47 due to a malocclusion. Necropsy findings in this male were malocclusion, discolored (red) cervical lymph nodes, discolored (black) cecum contents, sections of small intestine distended with gas, and colored material (black) surrounding the eyes and nose.

A Group 4 male (195160) was terminated on Day 145, with the following findings at necropsy: anogenital staining, eye discoloration with surrounding red deposit, yellow thymus, brown pancreas, yellow kidneys, white areas on mucosa of nonglandular stomach and yellow mesenteric fat.

Another Group 4 male (195173) was terminated on Day 172. The following findings at necropsy were reported: yellow body (whole), black staining around eyes and nose, mottled lungs (all lobes), small thymus, brown thymus, green kidneys (both), dilated pelvis of right kidney, stomach distended with accumulated fluid, ventral lower abdomen discolored brown and liver (all lobes) with a tan granulated surface,

One female in Group 4 (195180) was terminated moribund on Day 34 and had the following findings at necropsy: discolored (red) cervical lymph nodes, malocclusion, discolored stomach and cecum contents.

A second female in Group 4 (195198) was terminated moribund on Day 62 and had the following findings at necropsy: discolored (yellow) skin, paws, uterus, ovaries, and mesenteric fat, hard and/or discolored (black) contents of the gastrointestinal tract, mottled red glandular and nonglandular stomach and enlarged adrenals.

A third female in Group 4 (195193) was terminated on Day 174 with the following findings at necropsy: yellow hair coat and body, small, brown thymus, green kidneys (both), thickened and a tan liver with granulated surface.

Yellow discoloration of the skin and other tissues was considered test article-related, although there were no correlating microscopic changes. Black contents in the gastrointestinal tract of this animal may also correlate with gastric erosion.

Animals Found Dead

Three males and four females were found dead.

The following findings were seen in a Group 4 male (195174) found dead on Day 55: red material around the nose, anogenital staining, hair loss, normal and black material in the stomach and red lungs and sections of the gastrointestinal tract. The black material in the stomach is a sign of gastric bleeding, possibly related to ulceration.

The second male from Group 4 (195172), found dead on Day 174, had the following findings at necropsy: hair coat soiled and red surrounding the mouth, red cervical lymph nodes (all), salivary glands thymus and lungs (all lobes), red material surrounding the esophagus and trachea and red subcutis of the right caudal and dorsal thorax, red fluid in the pleural cavity and discolored yellow mesenteric fat adjacent to mesenteric lymph nodes. This animal likely died following esophagus injury during dosing (see Histopathology report). The yellow mesenteric fat was secondary to liver injury,

One male (195115) died after bleeding on Day 182. There were no gross lesions found at necropsy.

Two females from Group 4 (195184 and 195185) died after bleeding on Days 91 and 182, respectively. The necropsy findings in these animals were not related to the test article administration.

Two females (195196 and 195197) from Group 4 were found dead on Days 51 and 140 respectively, The necropsy findings reported in female animal 195196 were dark, yellow skin, stomach distended with food, jejunum distended with gas, enlarged adrenal glands, red material deposited around the nose and a distended fluid-filled uterus, The jejunum and uterus distention were probably related to the postmortem decomposition and vascular leakage, The accumulation of red material around the nose and enlarged adrenals were probably signs of prolonged stress. The skin discoloration was secondary to liver injury. The necropsy findings in female animal 195197 included hair loss, discolored hair coat, anogenital staining, yellow skin, small thymus, white area in the

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right kidney and red jejunum, duodenum and ileum. Red discoloration of the jejunum, ileum and duodenum was probably related to the postmortem decomposition and vascular leakage. The small thymus was a sign of prolonged stress or secondary to the prolonged stress. The white area in the kidney was probably an infarct secondary to the bacterial embolism. The skin discoloration was secondary to liver injury.

Scheduled Interim Necropsy

At the interim necropsy, 10 rats/sex/dose groups were terminated. However, due to mortalities, only 8 females were terminated at the interim necropsy in Group 4. The following minor gross abnormalities were noted: in Group 1 males, hair loss (1 male), and discolored/mottled thymus (2 males); in Group 2 males, discolored hair coat (1 male); in Group 3 males, discolored areas of the thymus (1 male); mottled thymus (1 male); in Group 4 males, mottled thymus (3 males); in Group 2 females, hair loss and enlarged uterus distended with fluid (1 female), discolored (red) cervical lymph node (1 female); in Group 3, enlarged uterus (both horns, 1 female), and an accessory spleen (1 female); in Group 4 females, a small thymus, hair loss, and discolored thymus each in 1 female. These observations were considered spontaneous, incidental findings with no relationship to test article administration.

Scheduled Final Necropsy

At the final necropsy, fifteen rats/sex/dose group were to be terminated. The number terminated was fewer than fifteen in most cases, due to the deaths/moribund sacrifices mentioned earlier.
Gross abnormalities of thymus such as small size, discoloration, gelatinous and mottled were noted in all groups, hence, were not considered related to the test article. Gross abnormalities in Group 1 males included red cervical lymph nodes, firm epididymis with a nodule, cyst in the kidney, and dilated kidney. In Group 2 males, red discoloration of the hair coat, black discoloration of the skin, lung adhesions, malocclusion and fluid in the pleural cavity were observed. Abnormalities in Group 3 males were red cervical lymph node(s) and malocclusion. In Group 4 males, black material around the left eye, enlarged preputial gland, tan liver, mallocclusion and a tan kidneys were reported. Gross abnormalities in Group 1 females included swollen ears, malocclusion and hair loss on the right forepaw. In Group 2 females, red areas on the adrenals, red and swollen ear(s), red cervical lymph nodes, distended fluid-filled uterus, small ovaries, missing upper teeth, hair loss on the dorsal forepaws and red lung were observed. In Group 3 females, red and/or swollen ear(s) and red lymph nodes were seen, In Group 4 females, a small adrenal gland, red haircoat around the eyes, yellow body and liver, distended gastrointestinal tract, hair loss on both forepaws and enlarged right horn on the uterus that contained clear fluid were reported, The yellow body and discoloration of the skin and internal organs were considered related to the test article. The necropsy incidences on Day 182 are summarized below:

Study	Group1	Group2	Group3	Group4	Group1	Group2	Group3	Group4
# examined	15	14	15	14	15	15	15	13
Species	10 Male Rats				10 Female Rats			
No gross lesion observed	9	6	8	5	7	8	10	5
Cervical lymph nodes-discolored, red	3		2	1		2		
Epididymis-firm	1							
Epididymis-nodule	1							
Eye-black colored material				1				
Kidney-cyst	1							
Kidney-dilated pelvis	1			1				
Kidney-colored, tan				1				
Hair coat-red discolored		1						1
Lungs-adhesion		1						
Pleural cavity-fluid		1		1				
Preputial gland-enlarged				1				
Ear-swollen					1	3	3	
Hair loss-forepaw					1	1		1
Adrenal gland-red area						1		
Lymph nodes-red							1	
Adrenal gland-small								1
Whole body-yellow color								2
Liver-yellow color								1
GI-distended								3
Uterus--distended								1

Organ Weights

A statistically significant difference in organ weights was seen in the livers of test article-treated males and females relative to controls on Day 91 as seen in Table 5. Increased absolute liver weights were seen in Group 3 males ($p \leq 0.05$) and Group 4 females ($p \leq 0.01$). When compared relative to body weight, the liver weights of both Groups 3 and 4 were significantly greater than controls ($p \leq 0.01$ for all except group 3 females. Although not statistically significant, the liver weights of Group 2 males appeared greater than controls, both as absolute and as relative to body weight. Liver weights were also greater when evaluated relative to brain weight, but only significantly different in females ($p \leq 0.05$ in Group 3 and $p \leq 0.01$ in Group 4). These differences were considered test article-related and correlate with bile duct hyperplasia, inflammation and hepatocellular hypertrophy in the Group 4 animals. The only other differences seen were in Group 2 males: increased testes and epididymides weights ($p \leq 0.05$). These differences were not considered related to test article. On Day 182, a statistically significant difference in organ weights was again seen in the livers of test article-treated females relative to controls as shown in Table 6. Increased absolute liver weights were seen in Group 3 ($p \leq 0.05$) and Group 4 ($p \leq 0.01$) females. Increased absolute spleen weights were also seen in Group 4 ($p \leq 0.05$) females. One statistically significant change in Group 4 males was thyroid weight (0.023 g of Group 4 vs. 0.034 g of Group 1, $p \leq 0.01$) on Day 182.

Table 5 Mean Organ Weights-Day 91

Study	Group1	Group2	Group3	Group4	Group1	Group2	Group3	Group4
Species		Male	Rats			Female	Rats	
Liver (g)	11.75	13.27	14.10*	13.80	6.59	6.50	7.15	8.29**
Testis (g)	3.55	3.95*	3.69	3.54				
Epididymides (g)	1.53	1.74*	1.51	1.47				

Table 6 Mean Organ Weights-Day 182

Study	Group1	Group2	Group3	Group4	Group1	Group2	Group3	Group4
Species		Male	Rats			Female	Rats	
Thyroid (g)	0.034	0.029	0.032	0.023**				
Liver (g)					6.92	7.62	8.60*	8.95**
Spleen (g)					0.481	0.511	0.549	0.587*

*Statistically significant difference from control group ($p \leq 0.05$)
**Statistically significant difference from control group ($p \leq 0.01$)

Histopathology

Overall, histopathology examinations have been conducted in adequate battery and been peer reviewed. 10 to 12 tissues were collected and examined from rats at control and Group 4 with high dose (60 mg/kg). At week 13, moderate or moderately-severe bile duct hyperplasia was present in the livers of three Group 4 rats that had been found dead or were sacrificed moribund. Together with the remaining rats of the Group 4, a total of incidences of bile duct hyperplasia in males and females were 8 out of 11 and 7 out of 12 specimen examined, respectively. Accompanying this finding were moderate diffuse subacute inflammation and mild/slight hypertrophy of the remaining hepatocytes in all three rats. The nature and extent of the findings in the livers of these three rats were contributed to their death or moribund condition. Additionally, in one Group 4 rat that was found dead, there was minimal bile duct hyperplasia. No bile duct hyperplasia in other groups of rats was found.

Minimal or mild/slight subacute inflammation of the large intestine (cecum, colon, and/or rectum) was present in the Group 2 rat that was sacrificed moribund and three of the Group 4 rats that were sacrificed moribund or found dead. Overall, inflammatory cell infiltrations observed in the large intestine appeared to be related to the treatment of INT-747. For instance, there were increased inflammation incidences in cecum from Group 2 to 4 rats in both male and female, and the same was true in colon. However, inflammation in rectum was present mainly in Group 4 rats.

For animals at the scheduled interim sacrifice, which received INT-747 daily for 13 consecutive weeks, test article-related findings were present in the liver, large intestine, and bone marrow. Tissues were initially examined for all animals in Groups 1 (negative control) and 4 (60 mg/kg/day) and when a potential test article-related finding was observed, the same tissue was examined from all animals in Groups 2 and 3. Minimal bile duct hyperplasia was present in the Group 4 males and females, but not in controls or in any animals in Groups 2 or 3. Minimal or mild/slight subacute inflammation of the large intestine (cecum, colon, and/or rectum) was of increased severity and/or incidence in all treated males (Groups 2-4) and Group 4 females compared to controls.

Minimal or mild/slight increased cellularity of the bone marrow (femur and/or sternum) was present in Group 4 males, but not any other groups (Group 4 females and animals in Groups 1 to 3) as seen in Table 7.

Table 7 Summary of Pathohistology Incidence (positive #/samples checked at week 13)

Study	Group1	Group2	Group3	Group4	Group1	Group2	Group3	Group4
# of tissues examined	15	14	14	11	15	15	15	10
Species	10	Male	Rats		10	Female	Rats	
Bone-Sternum (Cellularity				10				
Bone (femur) (Cellularity)				10				
Cecum								
Subacute inflammation		3	6	8	2	2	2	5
Colon								
Subacute inflammation		2	7	10	3	3	3	7
Rectum								
Subacute inflammation				4		1		2
Ileum (inflammation)				1				
Jejunum (inflammation)				1				
Kidneys	8/10	1/1		7/11	4/10			9/12
Bile Duct Hyperplasia Minimal				7				4
Lymph nodes, cervical		1		2		1		3
Lymph nodes, mesenteric	1			2				2
Ovaries								1
Spleen				1/11				2/12
Stomach					1/10			5/12

Tissues in all organs listed in table were examined for inflammatory cell infiltrations unless specified and stayed in blank as non-positive findings.

At week 26, slight/mild to moderately-severe oval cell/bile duct hyperplasia was present in the livers of five of six (5/6) 60 mg/kg rats that had been found dead or were sacrificed moribund. Accompanying this finding were minimal to moderate, diffuse, subacute inflammation in 4/5 of the 60 mg/kg rats, and minimal or slight/mild hypertrophy of the hepatocytes in 4/5 60 mg/kg rats. Additionally, there was minimal hematopoiesis and minimal mineralization in 3/5 and 1/5 of the 60 mg/kg rats, respectively. Minimal or slight/mild subacute inflammation of the large intestine (cecum, colon, and/or rectum) was present in 6/6 rats that had received 60 mg/kg INT-747 and were sacrificed moribund or found dead. This finding was characterized by the presence of increased numbers of mononuclear cells in the mucosa, increased prominence of the crypts, and/or increased prominence of goblet cells. Minimal increased cellularity of the bone marrow (femur and sternum) was present in the three 60 mg/kg females that was sacrificed moribund or found dead. This finding appeared to involve the white cell series. Minimal myocyte degeneration in the heart of one 60 mg/kg male and one 60 mg/kg female that were sacrificed moribund and one 60 mg/kg female that was found dead may have been secondary to the liver pathology in these rats. The nature and extent of the findings in the livers and large intestine of four rats were interpreted to have contributed to their death or

moribund condition. Subacute inflammation of the large intestine was not present in male controls and was of similar incidence and severity in control females and females that had received 15 mg/kg INT-747. Minimal increased cellularity of the bone marrow (femur and sternum) was present in 10/10 females that received 60 mg/kg INT -747, but was not present in males that received 60 mg/kg INT-747, rats that had received ≤15 mg/kg INT-747, or controls as shown in Table 8. This finding appeared to involve the white cell series. It contrasted with the increased cellularity of the marrow at the interim sacrifice in males, but not females that had received 60 mg/kg.

Table 8 Summary of Pathohistology Incidence (positive #/samples checked at week 26)

Study	Group1	Group2	Group3	Group4	Group1	Group2	Group3	Group4
# of tissues examined	15	14	14	11	15	15	15	10
Species	10 Male Rats				10 Female Rats			
Bone-Sternum (Cellularity								10
Bone (femur) (Cellularity)								10
Cecum								
Subacute inflammation		2	2	11	1		2	3
Colon								
Subacute inflammation		3	1	5		1	1	3
Rectum								
Subacute inflammation				3				
Bile Duct Hyperplasia								
Minimal				7				8
Mild								1
Moderate								1

Tissues in all organs listed in table were examined for inflammatory cell infiltrations unless specified and stayed in blank as non-positive findings.

Toxicokinetics

Due to deaths of some Group 8 (high dose) TK animals, the TK analysis could be conducted in only one female and one male at week 26. Plasma concentration values for predose samples for Day 0 were all below the LOQ (5 ng/mL), indicating there was no exposure of animals to the test article prior to dosing. The peak plasma concentrations of INT-747 were measured at 0.25-0.5 hr postdose on all assay days for all groups with one exception: the surviving Group 8 female had a Tmax of 2 hours.

The plasma terminal half-lives following the initial dose were similar among dose groups, ranging from 6 to 8 hr for Day 0, 7 to 21 hr for Week 13 and 6 to 12 hr for Week 26. There did not appear to be a gender difference at any of the dose levels or time points. The extent of systemic exposure of rats to INT-747, characterized by Cmax and AUC, increased with increasing INT-747 dose level over the dose range of 6 to 60 mg/kg/day on all TK sampling days. The increases in Cmax were greater than dose-proportional for males and females in the mid and high dose groups on Day 0 and in most cases, less than dose-proportional at Weeks 13 and 26. On the first day of dosing, the ratio of the AUCs (mid or high dose level compared to the low dose level) was greater than dose proportional in females, but near or below dose proportional in males. At Weeks 13 and 26, the AUCs were typically 1.5 to 2 times the expected value based on the dose ratio,

To determine if there was an accumulation of the dose over time, Cmax and AUC for a given dose level and gender were compared at Week 13 and Week 26 relative to the first day of dosing. At the low dose (6 mg/kg/day), Cmax was 2-3 times greater than Cmax on Day 0, and AUC was about 0.6 to 2.6 times greater than AUC on Day 0. At the mid dose (15 mg/kg/day), Cmax was 0.6 to 4.2 times greater than on Day 0, and AUC was 1.9 to 2.8 times greater than on Day 0. At the high dose level (60 mg/kg/day), Cmax was always less than the corresponding values at Day 0 (i.e., all ratios < 1), while AUC ranged from about the same (1.0) to 1.6 times greater than the corresponding AUCs on Day 0. These AUC data suggest that there was typically an accumulation of the dose in plasma, but any accumulation in plasma did not increase from Week 13 to Week 26, suggesting that steady state had been reached by Week 13. The TK data for different groups are shown in the table below.

Table 9: Summary of Toxicokinetic Results for Day 0, Week 13 and Week 26

Study Week	Group	Dose (mg/kg/day)	Sex	$t_{1/2}$ (hours)	T_{max} (hours)	C_{max} (ng/mL)	AUC_{all} (hr-ng/mL)
1 (Day 0)	6	6	M	6.49	0.25	423	5,080
			F	5.94	0.5	491	1,560
	7	15	M	7.51	0.25	1,339	6,479
			F	6.65*	0.25	3,418	8,571
	8	60	M	5.29	0.5	13,689	61,054
			F	7.63	0.5	20,037	43,462
13	6	6	M	8.74	0.5	1,147	5,892
			F	13.07	0.25	1,135	3,530
	7	15	M	13.67*	0.25	5,629	17,936
			F	7.32*	0.5	4,492	16,097
	8	60	M	21.31	0.25	7,364	118,676
			F	10.74*	0.25	8,709	68,067

Study Week	Group	Dose (mg/kg/day)	Sex	$t_{1/2}$ (hours)	T_{max} (hours)	C_{max} (ng/mL)	AUC_{all} (hr-ng/mL)
26	6	6	M	8.67*	0.25	1,142	3,120
			F	5.94*	0.25	1,406	4,074
	7	15	M	11.43*	0.5	2,504	16,659
			F	11.65	0.25	1,966	17,714
	8	60	M	NC	4	4,496	96,187
			F	NC	2	9,179	43,528

* Reported $t_{1/2}$ may not be reliable; $r^2 < 0.8$.
NC = insufficient number of data points for kinetics regression analysis.

SUMMARIES

The purpose of this study was to evaluate the potential oral toxicity of INT-747 when administered daily by gavage to Sprague-Dawley rats for 26 weeks. The animals received INT-747 at once daily dosages of 0, 6, 15, or 60 mg/kg/day. There were very few

abnormal clinical signs. Mortality of four males and four females that occurred at the 60 mg/kg/day dose level was considered to be test article related. The only abnormal clinical signs were seen at the high dose and included decreased activity, hunched posture, decreased feces and yellow skin. When these abnormal signs were seen, the animals became moribund within a few days. Abnormal clinical pathology values were mostly seen in the high dose group and were mostly observed among parameters associated with liver function. Liver weights were increased in both the 15 and 60 mg/kg/day groups. The abnormal clinical pathology and increased liver weights were considered adverse effects. Test article-related histopathology findings were bile duct hyperplasia in the liver with accompanying hepatocellular hypertrophy (Group 4 only) and increased cellularity in the bone marrow (femur and sternum; Group 4 only). Although most effects were seen at 60 mg/kg/day, there were some liver weight and large intestine subacute inflammation differences (relative to controls) at 15mg/kg/day in males. The no observable adverse effect level (NOAEL) was considered to be 6 mg/kg/day in males and 15 mg/kg/day in females. The target organs of toxicity are liver and large intestine.

Dog

Study title: A Seven-Day Repeat Dose Toxicity Study in Dogs with INT-747

Study no.:	017260
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	December 10, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, Lot# B, >93%

Key Study Findings

In this study, only a single animal/sex/group was used. Thus, this is not considered to be a valid toxicology study.

Clinical signs occurred primarily at ≥100 mg/kg/day, and were related to incidences of abnormal fecal consistency and vomiting. Treatment-related body weight losses also occurred at ≥100 mg/kg/day. Clinical chemistry changes primarily related to liver function (e.g., increased ALT) occurred at ≥50 mg/kg/day in both sexes. Gross findings at necropsy (discoloration of mucous membranes, intestinal tract, and/or bones) were observed at ≥ 50 mg/kg/day in females and the male treated with 200 mg/kg/day. At ≥100 mg/kg/day in females and the male treated with 200 mg/kg/day, the livers were tan in color. At 200 mg/kg/day, the gall bladders were thickened with mucoid bile. Absolute testes weights at ≥100 mg/kg/day (observed to be small macroscopically) were decreased by 30 to 33% (compared to the control) but there was a lack of dose-relationship as well as large variability in testes weights across the different dose groups.

Methods

Doses:	20, 50, 100, and 200 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Orally by capsule
Dose volume:	Not applicable
Formulation/Vehicle:	Gelatin capsules
Species/Strain:	Beagle dogs (Canis familiaris)
Number/Sex/Group:	1/sex/group
Age:	~5 months of age
Weight:	Males: 5.65-7.55 kg; Females: 4.40-5.55 kg
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

Observations and Results

Mortality

Viability checks were conducted twice daily. There was no mortality in this study.

Clinical Signs

Animals were observed for clinical signs on dosing days at 1 and 2 h post-dose, and once daily on days in which animals were not dosed. Clinical signs were related to incidences of abnormal fecal consistency and vomiting. At 20 mg/kg/day, soft feces were observed in the male animal. At 50 mg/kg/day, mucoid feces occurred on Day 5 in the male animal. At 100 mg/kg/day, there was diarrhea in the male, while the female vomited on numerous occasions and was observed to have soft, mucoid, or few to no feces during the treatment period. At 200 mg/kg/day, both sexes exhibited vomiting, soft feces, mucoid feces, few to no feces, and/or diarrhea.

Body Weights

Body weights were recorded prestudy, and on Days 0, 3, and 7. Most animals (including controls) lost ≤0.5 kg during the study. The extent of weight loss in females dosed with ≥100 mg/kg/day and the male dosed with 200 mg/kg/day was greater than that occurring at other doses and was likely associated with vomiting in these animals.

Feed Consumption

Not monitored

Ophthalmoscopy

Not conducted

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ECG

ECGs (I, II, III, aVR, aVL, aVF) were taken once pretest, and between 15 – 30 min after the first dose (Day 0) and last dose (Day 6). Parameters evaluated included heart rate, P wave, PR duration, QRS, QT segment, T wave, Bazett QTc and axis. There were no treatment-related findings.

Hematology

Blood samples were collected pretest, on Day 3, and prior to scheduled terminal necropsy for evaluation of standard hematological and coagulation parameters. Changes in hematological parameters (relative to pretest values) were noted in the male dosed with 200 mg/kg/day. In the high dose male, platelet, WBC, and percent neutrophils were increased by 37%, 142%, and 37%, respectively, on Day 7 relative to pretest values.

Clinical Chemistry

Samples were analyzed for standard clinical chemistry parameters.

Treatment-related changes in clinical chemistry parameters, including increased liver enzyme levels, occurred at ≥50 mg/kg/day on Days 3 and 7 in both sexes. On Day 7, ALT levels in males were 205, 772, and 4730 IU/L at 50, 100, and 200 mg/kg/day, respectively, compared to 25 IU/L in the control. At 100 and 200 mg/kg/day, AST was 93 and 1240 IU/L, respectively, compared to 22 IU/L in the male control. In the high dose male, BUN, ALP, LDH, total bilirubin, GGT, CK, triglycerides, uric acid, albumin, and A/G ratio were increased, while cholesterol was decreased, compared to the control. In females, ALT levels were 391, 6880, and 4110 IU/L, respectively, at 50, 100, and 200 mg/kg/day, compared to 21 IU/L in the control on Day 7. At 100 and 200 mg/kg/day, there were increases in AST, ALP, GGT, LDH, total bilirubin, and uric acid and decreases in glucose, compared to the control.

Urinalysis

Not conducted

Gross Pathology

At terminal sacrifice, a full necropsy was performed on all animals. At ≥50 mg/kg/day in females and 200 mg/kg/day in males, there was discoloration (yellow, red, or grey) of mucous membranes, intestinal tract, and/or bones. At ≥100 mg/kg/day in females and the male treated with 200 mg/kg/day, the livers were tan in color. At ≥100 mg/kg/day, the testes and epididymides were observed to be small. At 200 mg/kg/day, the prostate was small. In the high dose male and female, the gall bladders were thickened with mucoid bile.

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Organ Weights

Weights of the following organs were determined at terminal necropsy:

Adrenals	Pituitary
Brain	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Lungs	Uterus
Ovaries	

Organ weights relative to body weight and brain weight were also calculated.

At 200 mg/kg/day, absolute weights of the lungs, spleen, and thymus in the male were decreased by 76%, 70%, and 57%, respectively, compared to the control. Weights of lung, spleen, and thymus relative to body weight were also decreased at 200 mg/kg/day (although relative spleen weights were similar to the 20 mg/kg/day male). Absolute testes weights at 100 and 200 mg/kg/day (observed to be small macroscopically) were decreased by 33% and 30% (compared to the control). However, there was no dose relationship and the absolute testes weight in the male dosed with 20 mg/kg/day was greater than the control (3.77 g versus 1.84 g) indicating a large variability across the dose levels. Weights of testes relative to body weight at 100 and 200 mg/kg/day were similar to the control, while weights relative to brain weight were decreased.

In females, absolute spleen weights were decreased by 30% and 20% at 100 and 200 mg/kg/day, respectively, compared to the control. Weights of spleen relative to brain weight were also decreased, but weights relative to body weight were similar to the control. Absolute thymus weights were decreased at all doses (-64%, -42%, -67%, and -78% at 20, 50, 100, and 200 mg/kg/day, respectively, compared to the control.) Relative thymus weights were also decreased at all doses. There also were decreases in absolute and relative uterus and pituitary weights at all doses, but these were not necessarily dose-dependent.

Histopathology

Although various tissues and organs were collected and preserved at terminal sacrifice, microscopic evaluation of tissues/organs was not conducted.

Special Evaluation

None

Toxicokinetics

Blood samples for TK analysis were collected prior to dosing, and at eight timepoints (out to 24 h) after dosing on Days 0 and 6. Plasma samples were analyzed using a validated method.

INT-747 was rapidly absorbed with Cmax occurring approximately 1 to 2 h after dosing. Half-life varied and could not be calculated for some animals. Systemic exposure

(measured as AUC) increased with dose. The accumulation ratio ranged from 0.6 to 2.4, but was 25 in the female treated with 100 mg/kg/day. TK parameters are summarized in the Applicant's table below.

Table TK - 3: Summary of Toxicokinetic Results

Dose Day	Sex	Animal	Group	Rsq	Half-life (hr)	Tmax (hr)	Cmax (ng/mL)	AUCall (hr•ng/mL)	Dosage (mg/kg)	Dose Ratio	AUC ratio	Accum. Ratio	Cmax ratio
Day 0	Female	301466	Group 2	0.69	NR*	0.5	1,180	1,247	20	1.0	1.0		1.0
		301467	Group 3	0.77	NR*	1	5,925	8,977	50	2.5	7.2		5.0
		301468	Group 4	0.94	0.95	1	2,084	6,452	100	5.0	5.2		1.8
		301469	Group 5	0.85	11.17	2	21,895	59,123	200	10.0	47.4		18.6
	Male	301461	Group 2	0.81	NR*	1	2,207	2,620	20	1.0	1.0		1.0
		301462	Group 3	0.98	20.14	1	1,005	3,171	50	2.5	1.2		0.5
		301463	Group 4	0.41	NR*	1	8,466	13,064	100	5.0	5.0		3.8
		301464	Group 5	0.96	5.25	4	12,575	46,227	200	10.0	17.6		5.7
Day 6	Female	301466	Group 2	0.33	NR*	1	910	2,301	20	1.0	1.0	1.8	1.0
		301467	Group 3	0.92	8.47	1	4,721	7,375	50	2.5	3.2	0.8	5.2
		301468	Group 4	0.90	2.51	2	42,457	161,468	100	5.0	70.2	25.0	46.7
		301469	Group 5	0.94	4.96	2	15,235	55,488	200	10.0	24.1	0.9	16.7
	Male	301461	Group 2	1.00	2.42	2	665	1,559	20	1.0	1.0	0.6	1.0
		301462	Group 3	0.99	3.41	1	1,506	7,572	50	2.5	4.9	2.4	2.3
		301463	Group 4	0.99	7.12	2	3,750	16,056	100	5.0	10.3	1.2	5.6
		301464	Group 5	0.31	NR*	2	7,023	43,399	200	10.0	27.8	0.9	10.6

* NR = not reported due to poor correlation coefficient (<0.85)

Note: AUC ratio is ratio of AUC of group 3, 4 or 5 to the low dose group (Group 2).
Accumulation Ratio is ratio of Day 6 AUC/Day 0 AUC for a given group
Cmax ratio is ratio of Cmax of group to low dose group (Group 2).

Dosing Solution Analysis

Not conducted.

Study title: A 28-Day Oral Toxicity Study in Dogs with INT-747

Study no.: 017261
Study report location: EDR
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: January 10, 2005
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: INT-747, Batch C, Lot 1204004/L, ≥92%

Key Study Findings

Following treatment with up to 50 mg/kg/day INT-747, there were numerous treatment-related effects on clinical chemistry parameters, including increased AST, ALT, ALP, GGT, albumin, and total protein levels (compared to controls). In high dose males, there were also decreases in LDH (compared to controls). At 50 mg/kg/day, histopathological examination revealed treatment-related findings in the liver (yellow to brown globular pigment; hepatocellular degeneration and diffuse atrophy) and gallbladder (cystic hyperplasia and increased secretion). These findings correlated with macroscopic observation of small liver and distended and thickened contents of the gallbladder in a single female animal. In the kidney, moderate vacuolation of the tubular

epithelium was noted in a high dose female. There was evidence of partial recovery based upon clinical chemistry and histopathological evaluation. The NOAEL was considered to be 15 mg/kg/day in both sexes. The primary target organs were the liver and gallbladder.

Methods

Doses:	5, 15, and 50 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Orally by capsule
Dose volume:	Not applicable
Formulation/Vehicle:	Gelatin capsules
Species/Strain:	Beagle dogs
Number/Sex/Group:	Main study: 4/sex/group Recovery: 2/sex/group
Age:	5-6 months of age
Weight:	Males: 6.35-8.15 kg; Females: 5.50-7.35 kg
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

Observations and Results

Mortality

Viability checks were conducted twice daily. There were no mortalities.

Clinical Signs

During the dosing phase, animals were observed daily at 1 and 2 h post-dosing for clinical signs. During the recovery phase, animals were observed for clinical signs once daily.

The primary clinical observations were emesis, salivation, and soft/mucoid/decreased feces. These findings either occurred across treatment groups (including controls) or there was a lack of dose relationship, indicating that the observations are unlikely treatment-related. One exception was the higher incidence of emesis in high dose females relative to controls.

Body Weights

Body weights were recorded pretest, once per week during the dosing phase, and at necropsy. There were no treatment-related effects.

Reference ID: 3870157

Feed Consumption

Food consumption was estimated three times per week. There were no treatment-related effects.

Ophthalmoscopy

Ophthalmic examinations were conducted pretest and during the final week of dosing. There were no treatment-related effects.

ECG

ECGs (I, II, III, aVR, aVL, aVF) were taken once pretest, and between 60-75 min after dosing on Day 1, and during the final week of treatment. Parameters evaluated included heart rate, P wave, PR duration, QRS, QT segment, T wave, and axis. There were no treatment-related findings.

Hematology

Blood samples were collected pretest, on Day 15, and during the last week of the dosing phase. Samples were analyzed for the standard hematological and coagulation parameters.

While there were limited statistically significant differences between groups in various parameters, no treatment-related effects on hematological or coagulation parameters were identified based on a lack of dose response and/or comparison to pretest values.

Clinical Chemistry

Samples were analyzed for the standard clinical chemistry parameters.

At 50 mg/kg/day, there were treatment-related effects on AST, ALT, ALP, GGT, total protein, and albumin on Days 15 and/or 26, as summarized in the following tables (not statistically significant in all cases). In high dose males, there were also statistically significant decreases in LDH (compared to controls). At the end of the recovery period, there was evidence of partial recovery, although liver enzyme levels in some high dose animals remained elevated relative to controls (e.g., GGT).

Mean Clinical Chemistry Values in Males – Day 15

	AST (IU/L)	**ALT** (IU/L)	**ALP** (IU/L)	**GGT** (IU/L)	**LDH** (IU/L)	**Albumin** (g/dL)	**Total Protein** (g/dL)
Control	25	23	107	0	119	3.1	5.8
5 mg/kg/day	27	21	127	1	143	3.1	5.7
15 mg/kg/day	29	27	128	1	133	3.1	5.8
50 mg/kg/day	**50****	**719***	**211***	4	100	**3.3****	6.1

*Statistically significant difference from control group, p≤0.05
**Statistically significant difference from control group, p≤0.01

Mean Clinical Chemistry Values in Males – Day 26

	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	LDH (IU/L)	Albumin (g/dL)	Total Protein (g/dL)
Control	32	23	109	0	116	3.1	5.9
5 mg/kg/day	31	23	131	1	109	3.1	5.9
15 mg/kg/day	37	30	131	1	101	3.2	6.0
50 mg/kg/day	**45***	**646***	**216****	**3***	**48***	**3.4****	6.1

*Statistically significant difference from control group, p≤0.05
**Statistically significant difference from control group, p≤0.01

Mean Clinical Chemistry Values in Females – Day 15

	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	LDH (IU/L)	Albumin (g/dL)	Total Protein (g/dL)
Control	30	22	146	0	136	3.2	5.9
5 mg/kg/day	26	21	139	0	119	3.3	5.8
15 mg/kg/day	32	25	126	0	138	3.2	5.9
50 mg/kg/day	60	**574***	154	1	92	3.4	**6.1***

*Statistically significant difference from control group, p≤0.05

Mean Clinical Chemistry Values in Females – Day 26

	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	LDH (IU/L)	Albumin (g/dL)	Total Protein (g/dL)
Control	34	22	139	0	113	3.2	5.8
5 mg/kg/day	29	23	139	0	79	3.2	5.8
15 mg/kg/day	38	29	118	0	81	3.2	5.8
50 mg/kg/day	318	**1184***	254	3	71	3.4	**6.1***

*Statistically significant difference from control group, p≤0.05

Urinalysis

Not conducted

Gross Pathology

At terminal sacrifice, a full necropsy was performed on all animals. Necropsy included examination of the carcass and muscular/skeletal system; all external surfaces and orifices, cranial cavity and external surface of the brain; neck with associated organs/tissues; and thoracic, abdominal, and pelvic cavities with associated organs/tissues.

In males, there was a dose-dependent increase in the incidence of small prostate (1/4, 1/4, 2/4, and 3/4 animals in the control, low-, mid-, and high dose groups, respectively). Small testes and epididymides were each noted in 1/4 mid-dose males. Small thymus and discolored cervical lymph node were each noted in 1/4 high dose males. In recovery animals, small spleen was noted in 1/2 mid-dose males and small thyroid was noted in 1/2 high dose males.

In high dose females, gross findings were noted in 3/4 animals. One female had yellow discoloration of the entire body, thickened and distended gall bladder, and small liver and thymus. In another female, discolored cecum-colon junction, thickened AV valve of

the heart, and red pinpoint areas in the rectum were noted. Finally, the third high dose female had a discolored lobe of the lungs. A pituitary gland cyst was noted in 1/4 females at 15 mg/kg/day. At recovery, enlarged ovary and thickened stomach were noted in 1/2 high dose females each.

Organ Weights

Weights of the organs identified in the Applicant's table below were measured at necropsy. Relative organ/body weight and organ/brain weight ratios were calculated.

Adrenals	Brain
Epididymides	Heart
Kidneys	Liver
Lungs	Ovaries
Pituitary	Spleen
Testes	Thymus
Thyroids with parathyroids	Uterus

There was a statistically significant decrease in adrenal relative to brain weights in high dose females at the end of the dosing phase (0.0165 g/g, versus 0.0127 g/g in controls). However, there were no statistically significant changes in absolute weights or correlating gross or histopathological findings.

Histopathology

Organs and tissues identified in the Applicant's table below were collected and preserved at necropsy. Histopathology evaluation of organs/tissues from the control and high dose groups was conducted. In addition, histopathology evaluation of target organs (liver, kidneys, and gallbladder) and gross lesions were conducted for the low- and mid-dose groups and recovery animals.

Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymides
Tongue	Prostate
Esophagus	Ovaries
Stomach	Uterus
Small Intestine	Cervix
Duodenum	Vagina
Jejunum	Respiratory
Ileum	Trachea
Large Intestine	Lungs
Cecum	Lymphoid/Hematopoietic
Colon	Bone Marrow (sternum)
Rectum	Thymus
Pancreas	Spleen
Liver	Lymph Nodes
Gall Bladder	Retropharyngeal & Cervical
Endocrine	Mesenteric
Adrenals	Nervous/Special Sense
Pituitary	Eyes with Optic Nerve
Thyroid/Parathyroids	Sciatic Nerve
Skin/Musculoskeletal	Brain
Skin/Mammary Gland	Spinal Cord (cervical, thoracic & lumbar)
Bone (sternum)	Other
Smooth muscle (urinary bladder)	Mesenteric fat
Skeletal Muscle (*biceps femoris*)	Gross Lesions

Adequate Battery: Yes

Peer Review: No

Histological Findings

At 50 mg/kg/day, treatment-related findings were observed in the liver, gallbladder, and kidney (females only). Findings in the liver and gallbladder are summarized in following tables copied directly from the Applicant's study report.

Selected Microscopic Observations
Terminal Necropsy
Liver

	Group 1 M/F	Group 2 M/F	Group 3 M/F	Group 4 M/F
Organ/Diagnosis				
Liver (no. examined)	4/4	4/4	4/4	4/4
Pigment	0/0	0/0	0/0	3/2
Minimal	0/0	0/0	0/0	3/1
Slight	0/0	0/0	0/0	0/1
Schmorl stain positive, hepatocytes, and/or Kupffer cells	3/3	N/A	N/A	4/4
Minimal	2/2	N/A	N/A	4/3
Slight	1/1	N/A	N/A	0/1

Selected Microscopic Observations
Terminal Necropsy
Gallbladder

	Group 1 M/F	Group 2 M/F	Group 3 M/F	Group 4 M/F
Organ/Diagnosis				
Gallbladder (no. examined)	4/4	4/4	4/4	4/4
Cystic Hyperplasia	1/0	2/1	1/1	3/4
Minimal	0/0	1/0	1/0	0/0
Slight	1/0	1/1	0/1	2/3
Moderate	0/0	0/0	0/0	1/1
Increased Secretion	0/0	0/0	0/0	2/1
Slight	0/0	0/0	0/0	2/0
Moderate	0/0	0/0	0/0	0/1

In the liver of high dose animals, yellow to brown globular pigment was observed within centrilobular hepatocytes and/or Kupffer cells. Additional treatment-related findings in the liver of a single high dose female included mild diffuse centrilobular hepatocellular degeneration and moderate diffuse atrophy. Minimal peribiliary histiocytic infiltrates were observed in the liver of a single mid-dose male and one male and female at the high dose. In the gallbladder, 50 mg/kg/day INT-747 produced increased secretion and an increased incidence and/or severity of cystic hyperplasia. In the kidney, vacuolation of the tubular epithelium in a single high dose female was considered treatment-related due to the increased severity of this finding as compared to other groups. Immature reproductive organs (including prostate, epididymides, and/or testes) were noted in numerous control and INT-747 treated male dogs. These findings correlated with macroscopic findings (small) but were not considered treatment-related since there were similar incidences in control animals. In recovery animals, slight/mild cystic hyperplasia of the gallbladder was noted in 1/2 and 2/2 low- and high dose males, respectively, and 1/2 high dose females.

Special Evaluation

None

Toxicokinetics

Blood samples for TK analysis were collected prior to dosing, at five timepoints (out to 24 h) after dosing on Day 0, and one day during Week 4. Plasma samples were analyzed for INT-747 concentrations using a validated method.

Cmax was generally achieved at ~1 h after dosing, and half-life varied from 0.5 to 12 h, with an average $t_{1/2}$ of 3.5 h. Summary TK parameters for Days 0 and 28 are shown in the Applicant's tables below.

Table 2: Day 0 Summary

Dose Day	Group	Sex	Animal	R^2	Half-life (hr)	Tmax (hr)	Cmax (ng/mL)	AUCall (hr•ng/mL)	Dosage (mg/kg/Day)	Average Dose Ratio	Average AUC ratio
Day 0	2	F	301504	0.61	NR	0.5	393.3	937.54	5	-	-
			301505	0.51	NR	1	477.37	1285.8	5		
			301506	NR	NR	1	211.92	486.18	5		
			301507	NR	NR	8	33.46	334.61	5		
			301508	NR	NR	1	271.05	591.75	5		
			301509	0.87	2.48	1	200.87	836.09	5		
		M	301480	1	0.5	0.5	771.63	1055.74	5	-	-
			301481	NR	NR	1	271.71	553.6	5		
			301482	1	1.96	0.5	343.12	934.02	5		
			301483	0.98	1.58	1	231.61	711.4	5		
			301484	0.33	NR	0.5	1259.93	7139.92	5		
			301485	NR	NR	4	85.27	461.38	5		
	3	F	301510	0.72	NR	1	1151.76	3106.72	15	3	6.58
			301511	0.64	NR	4	644.37	3677.6	15		
			301512	NR	NR	1	1378.16	3098.26	15		
			301513	0.77	NR	1	4123.61	10069.37	15		
			301514	0.77	NR	1	1576.32	4848.22	15		
			301515	0.76	NR	1	1437.88	4609.45	15		
		M	301486	0.86	1.38	1	2129.2	4935.19	15	3	1.83
			301487	NR	NR	1	1189.44	2391.86	15		
			301488	0.53	NR	1	1421.27	3135.12	15		
			301489	0.33	NR	1	809.66	3100.34	15		
			301490	0.71	NR	1	950.58	2643.07	15		
			301491	NR	NR	1	1788.29	3656.99	15		
	4	F	301516	0.59	NR	1	11113.35	24147.59	50	10	21.17
			301517	0.94	3.01	4	1487.54	7642.04	50		
			301518	0.92	3.69	1	7163.1	14922.55	50		
			301519	0.99	3.26	4	2345.79	15505.22	50		
			301520	0.85	2.78	1	8188.9	23683.3	50		
			301521	0.64	NR	1	3705.95	8778.62	50		
		M	301492	0.88	4.04	1	9844.95	21585.29	50	10	7.32
			301493	0.98	4.85	1	7288.71	16600.26	50		
			301494	0.5	NR	1	5038.15	15211.89	50		
			301495	0.94	11.99	1	2407.8	13907.73	50		
			301496	0.89	2.39	1	1320.57	8354.44	50		
			301497	1	5.04	4	335.08	3813.35	50		

Note: AUC ratio is ratio of AUC of Group 3 or 4 to low the dose group (Group 2).

Table 3: Day 27 Summary

Dose Day	Group	Sex	Animal	R^2	Half-life (hr)	Tmax (hr)	Cmax (ng/mL)	AUCall (hr•ng/mL)	Dosage (mg/kg/ Day)	Average Dose Ratio	Average AUC ratio	Accum. Ratio	Cmax ratio
Day 27	2	F	301504	0.62	NR	1	347.47	1466.94	5	-	-	1.56	0.88
			301505	NR	NR	1	664.52	1556.82	5			1.21	1.39
			301506	NR	NR	1	293.78	681.76	5			1.40	1.39
			301507	NR	NR	4	235.62	824.68	5			2.46	7.04
			301508	0.5	NR	0.5	438.44	749.52	5			1.27	1.62
			301509	1	2.22	1	244.37	1122.7	5			1.34	1.22
		M	301480	0.5	NR	1	517.35	1162.23	5	-	-	1.10	0.67
			301481	0.52	NR	0.5	405.18	1212.67	5			2.19	1.49
			301482	0.99	1.54	0.5	304.37	871.08	5			0.93	0.89
			301483	NR	NR	1	328.31	746.04	5			1.05	1.42
			301484	0.9	9.55	0.5	488.98	1244.84	5			0.17	0.39
			301485	NR	NR	1	303.15	711.73	5			1.54	3.56
	3	F	301510	0.01	NR	1	987.64	3944.72	15	3	4.87	1.27	0.86
			301511	0.65	NR	1	1461.31	4177.58	15			1.14	2.27
			301512	0.81	NR	1	1605.28	3602.77	15			1.16	1.16
			301513	0.16	NR	1	5618.59	13521.81	15			1.34	1.36
			301514	NR	NR	1	1422.64	3172.61	15			0.65	0.90
			301515	0.94	3.65	1	626.77	2757.91	15			0.60	0.44
		M	301486	0.92	3.48	4	1137.18	5727.45	15	3	3.47	1.16	0.53
			301487	NR	NR	4	315.79	1174.93	15			0.49	0.27
			301488	0.99	0.52	0.5	948.73	2073.42	15			0.66	0.67
			301489	0.5	NR	0.5	933.08	2833.02	15			0.91	1.15
			301490	0.3	NR	1	1091.21	5745.58	15			2.17	1.15
			301491	0.94	3.72	4	505.06	3076.9	15			0.84	0.28
	4	F	301516	0.96	3.75	1	7524.56	17430.35	50	10	19.46	0.72	0.68
			301517	0.73	NR	1	11149.29	34678.38	50			4.54	7.50
			301518	0.98	3.66	1	3483.2	14294.56	50			0.96	0.49
			301519	NR	NR	4	3878.72	25901.88	50			1.67	1.65
			301520	1	0.92	1	5223.59	13792.53	50			0.58	0.64
			301521	0.94	3.89	1	7596.41	18478.44	50			2.10	2.05
		M	301492	0.84	NR	1	8610.04	16162.32	50	10	16.60	0.75	0.87
			301493	0.91	2.43	1	8379.78	22628.82	50			1.36	1.15
			301494	0.92	2.75	4	1735.5	7958.62	50			0.52	0.34
			301495	0.98	3.44	1	7547.01	18635.16	50			1.34	3.13
			301496	0.96	4.02	1	8406.01	18282.15	50			2.19	6.37
			301497	0.98	5.87	1	6032.19	15050.15	50			3.95	18.00

NR - not reported: R^2 value not reported, or half-life not reported when $R^2 < 0.85$

Note: AUC ratio is ratio of AUC of Group 3 or 4 to low the dose group (Group 2).
Accumulation Ratio is ratio of Day 27 AUC/Day 0 AUC for a given group
Cmax ratio is ratio of Cmax of group to low dose group (Group 2).

Dosing Solution Analysis

Solutions of the contents of INT-747 dose capsules were analyzed. Samples were within 100 ± 3% of nominal concentrations, and thus acceptable.

Review of Study No. 019959 is incorporated below from the pharmacology review of IND 63307 dated February 12, 2009 (C. Wu, Ph.D., DGP). Under the current NDA, the Applicant submitted an amended study report for Study No. 019959 dated December 10, 2008; whereas, Dr. Wu previously reviewed the original final report. The amended report incorporates a correction to the label on several toxicokinetic tables. However, there were no changes which would impact the overall study results or conclusions. The corrected TK summary tables are copied below from the Applicant's amended study report. As shown in the bold strikethrough and bold underlined text, the study week was corrected from week 26 to 39.

Study Week	Dose (mg/kg/day)	Sex	C_{max} (ng/mL)	AUC_{all} (hr-ng/mL)
1 (Day 0)	5	M	365	384
		F	275	405
	15	M	1,093	1,673
		F	1,312	2,065
	50	M	1,065	5,013
		F	3,026	7,072
13	5	M	474	798
		F	496	835
	15	M	1,160	2024
		F	1,578	3578
	30	M	1,051	3,212
		F	1,949	4,094
~~26~~ 39	5	M	506	615
		F	446	681
	15	M	1,381	1,734
		F	1,917	2,719
	30	M	1,906	3,781
		F	2,336	4,445

Study Week	Group	Dose (mg/kg/day)	Sex	$t_{1/2}$ (hours)	T_{max} (hours)	C_{max} (ng/mL)	AUC_{all} (hr-ng/mL)
1 (Day 0)	2	5	M	0.84	0.64	365	384
			F	2.21	0.79	275	405
	3	15	M	1.52	0.79	1,093	1,673
			F	0.81	1.71	1,312	2,065
	4	50	M	3.42	1.36	1,065	5,013
			F	2.64	1.36	3,026	7,072
13	2	5	M	1.24	0.71	474	798
			F	1.41	0.79	496	835
	3	15	M	1.46	0.71	1,160	2024
			F	2.42	0.86	1,578	3578
	4	30	M	1.84	0.86	1,051	3,212
			F	5.57	1.29	1,949	4,094
~~26~~ 39	2	5	M	0.59	0.63	506	615
			F	1.60	0.63	446	681
	3	15	M	1.24	0.50	1,381	1,734
			F	1.26	0.63	1,917	2,719
	4	30	M	1.19	0.75	1,906	3,781
			F	1.69	0.75	2,336	4,445

A 9-Month Oral Toxicity Study in Dogs with INT-747 (Including results from the 13-Week interim report)

Study No.: 019959
Conducting laboratory and location: (b) (4)
(b) (4)
Date of study initiation: 06-22-2006
GLP compliance: Yes
QA report: Yes (x) no ( )
Drug, Lot # and % purity: INT-747, Lot 43-440. The purity by HPLC was 96.8%

METHODS:

Doses: 5, 15 and 50/30 mg/kg/day
Species/strain: Beagle dogs
Number/sex/group: 3 dogs/sex/group for 13 weeks and remaining 4 dogs/sex/group for 9 months
Route, formulation, volume: Doses were administered in a single daily dose via capsule.

Satellite groups used for toxicokinetics or recovery: None

Age: 12-12½ months for females and 11½-12 months for males
Weight: Males were 5.30-8.85 kg and females were 5.55-8.15 kg.
Sampling times: See the details in Observation and Times

Details of the study design are provided below:
A total of 56 dogs were assigned to four groups of 7 dogs/sex/group as shown in the table below. Groups 2, 3 and 4 received capsules once daily to attain INT-747 dosages of 5, 15, and initially 50 mg/kg/day of body weight. Beginning on Day 29 through the end of the study, the dose for Group 4 was reduced to 30 mg/kg/day due to signs of toxicity. The control group received empty capsules (same number as the high dose group). The first day of dosing was designated as Day 0 of the study. The animals were dosed once daily through one day prior to each scheduled termination.

Dosing was suspended in some Group 4 dogs, which was a deviation to the protocol but was done at the request of the Veterinarian and Study Director and in consultation with the Sponsor, due to signs of toxicity. At the time, these animals, along with all the other Group 4 animals, were receiving INT-747 at 50 mg/kg/day, dosing for Group 4 females 302562 and 302567 was suspended on Day 20 and dosing for dogs 302556 (male) and 302563 (female) was suspended on Day 25. Dosing for these four animals was resumed on Day 35 at 30 mg/kg/day.

A subset of each group (3/sexlgroup) was terminated after 13 weeks (91 days) of treatment. The remaining 4/sex/group continued to be dosed for a total of at least 273 consecutive days from the beginning of treatment (i.e., approximately 9 months of treatment).

Table 1 Group Assignment and Dose Levels

Group No.	No. of Animals (M/F)	Test Article	Dose Level (mg/kg/day)	Termination Week (#M/#F) Interim	Final
1	7/7	Control	0	13 (3M/3F);	39 (4M/4F)
2	7/7	INT-747	5	13 (3M/3F);	39 (4M/4F)
3	7/7	INT-747	15	13 (3M/3F);	39 (4M/4F)
4	7/7	INT-747	50 / 30*	13 (3M/3F);	39 (4M/4F)

The dose for Group 4 was reduced to 30 mg/kg/day starting on Day 29 due to signs of toxicity.

OBSERVATION AND TIMES:

Clinical signs: Each animal was observed once each morning and afternoon throughout the study for viability. In addition, animals in Groups 1-4 were observed at least once daily, beginning 7 days prior to dosing, for changes in appearance and behavior. Once dosing began, all dogs were observed daily at 1 and 2.5 hours following dosing for changes in appearance and behavior. The animals received a detailed clinical evaluation once weekly.

Body weights: Body weights were measured 5 times pretest (including once for randomization) and once weekly throughout the study. The final body weight was used to calculate organ/body weight ratios.

Food consumption: Food consumption was measured daily from 1 week prior to dosing until termination.

Ophthalmoscopy: An ophthalmic examination (indirect) was conducted by the Staff Veterinarian on all main study animals prior to the first dose on Day 6 and during the last week of dosing prior to the scheduled interim/terminal necropsy (Day 86 or 180) and during the final week (Day 268) of the treatment phase for all remaining animals. The examination was conducted using indirect ophthalmoscopy preceded by mydriasis produced by topical application of 1 % Mydriacyl®.

EKG: EKGs (I, II, III, aVR, aVL, aVF) were taken from all animals prior to the first dose on Day 6. During the treatment phase, EKGs were conducted between 30 and 60 minutes after the first dose (Day 0), on Day 86 prior to the interim necropsy at 13 weeks (interim termination animals only) and during the final week (Day 268) of the treatment phase for the remaining animals.

Clinical Pathology Evaluation: Blood samples for evaluation of hematology, coagulation and serum chemistry parameters were collected from the jugular vein from all animals on Day -7 (prior to the first dose) and from the interim termination animals only on Day 85 (Week 13) and from the remaining animals on Day 176 (Week 26) and prior to necropsy on Day 268 prior to their necropsy. On the days of blood collection, the animals were not fed prior to blood collection. Blood samples were handled and analyzed as described below.

Hematology: Approximately 3 mL of blood was collected in EDTA-containing tubes. The following parameters were evaluated:

Red blood cells (RBCs) (count and morphology)	Mean corpuscular hemoglobin (MCH)
White blood cells (WBCs) (total and differential)	Mean corpuscular hemoglobin concentration (MCHC)
Hemoglobin concentration	Mean corpuscular volume (MCV)
Hematocrit	Platelet counts
	Reticulocyte counts

Clinical chemistry: Approximately 4 mL of blood was collected in a tube without anticoagulant. The sample was allowed to clot and then was centrifuged to obtain serum. The detailed parameters were evaluated and shown in the RESULTS section.

Urinalysis: Urinalysis was performed on all animals prior to the first dose and the interim termination animals during Week 13, and on remaining animals during Week 26 (Day 176) and the last week (Day 267) of the dosing phase. The following urinalysis parameters were recorded and evaluated for each dog while housed individually during an overnight water restriction.

Specific gravity	Ketones
Occult blood	Glucose
Protein	Nitrite
pH	Urobilinogen
Bilirubin	Volume
Leukocytes	Color
	Clarity

Gross pathology: Three dogs per sex per group were terminated by exsanguination while under deep anesthesia induced with sodium pentobarbital after 13 weeks of treatment and the remaining animals (4 dogs/sex/group) were terminated after 39 weeks (Day 273) of treatment. Food was withheld on the day of necropsy. A complete necropsy with tissue collection was conducted on all animals scheduled for termination. All abnormalities were described completely and recorded. The necropsy included examination of:

. Carcass and muscular/skeletal system
. All external surfaces and orifices
. Cranial cavity and external surface of the brain
. Neck with associated organs and tissues
. Thoracic, abdominal and pelvic cavities with their associated organs and tissues

Organ weights: The following organs (when present) were weighed for all animals at necropsy. Paired organs were weighed together unless gross abnormalities were present, in which case they were weighed separately.

Adrenals	Pituitary (post fixation)
Brain	Testes
Heart	Epididymides
Kidneys	Ovaries
Liver	Thymus
Spleen	Thyroid with parathyroids (post fixation)

Histopathology: Peer review: yes (X), no ( )

For all animals from the control and high-dose groups (Groups 1 and 4) included in the interim termination and all animals that died or were euthanized in extremis during the study, the tissues listed in the Table below were embedded in paraffin, sectioned and stained with hematoxylin and eosin. Target organs and gross lesions from Groups 2 and 3 were also prepared as above. The following organs and tissues were examined by light microscopy.

Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymides
Tongue	Prostate
Esophagus	Ovaries
Stomach	Uterus
Small Intestine	Cervix
Duodenum	Vagina
Jejunum	Respiratory
Ileum	Trachea
Large Intestine	Lungs
Cecum	Lymphoid/Hematopoietic
Colon	Bone Marrow (sternum)
Rectum	Thymus
Pancreas	Spleen
Liver	Lymph Nodes
Gall Bladder	Retropharyngeal & Cervical
Endocrine	Mesenteric
Adrenals	Nervous/Special Sense
Pituitary	Eyes with Optic Nerve
Thyroid/Parathyroids	Sciatic Nerve
Skin/Musculoskeletal	Brain
Skin (both)/ Mammary Gland (females only)	Spinal Cord (cervical, thoracic & lumbar)
Bone (sternum)	Other
Smooth muscle (urinary bladder)	Mesenteric fat
Skeletal Muscle (*biceps femoris*)	Gross Lesions

Toxicokinetics: Approximately 1 mL of blood was collected from all animals into EDTA-containing tubes prior to dosing, and at five additional time-points following dosing on Day 0 and on the day immediately prior to the interim termination. The actual sampling times were recorded. The samples were mixed immediately by inverting and then were centrifuged to obtain plasma. Samples from the control animals from Day 0 were discarded but samples from the control animals collected on other sampling days were held for potential analysis. The plasma was frozen (< -20 °C) and transferred to

(b) (4) Bioanalytical Department for analysis of INT-747 using a validated method ((b) (4) Document Number 017484-1). The peak plasma concentration, the time achieved and the half-life (Cmax, Tmax and t½) were calculated, if possible. The area under the plasma concentration-time curve (AUC) was also determined.

Sample Collection Time Points

Day 0	One Day during each of Weeks 13, 39*
Pre-dose	Pre-dose
30 min	30 min
1 hr	1 hr
4 hr	4 hr
8 hr	8 hr
24 hr	24 hr

RESULTS

Mortality

There was no mortality during this study.

Clinical signs

Minor abnormal signs such as vomiting and salivation were seen in most dose groups, and were not considered related to the test article. Abnormal feces were seen in all dose groups, but the incidence increased with increased dosage.

Four high dose animals, one male and three females, had clinical signs that were considered test article-related when dosed at 50 mg/kg/day. These abnormal signs, which started around Days 19 to 24, included an icteric appearance (yellow discoloration of skin, mucous membranes and eyes), decreased activity, dehydration, decreased or no appetite, and decreased production of feces. On Day 20, dosing in females 302562 and 302567 were stopped because they had yellow discoloration of the skin and mucous membranes, were dehydrated, had decreased activity, and were not eating. On Day 25, dosing in male 302556 and female 302563 was stopped, as both had stopped eating, and the male had yellow mucous membranes and was inactive. On Day 35, dosing of these four dogs resumed at 30 mg/kg/day as the signs of toxicity had reversed. The abnormal signs did not recur with daily dosing at 30 mg/kg.

Body Weights

All groups had similar changes in body weight through the first 90 days of the dosing period, although there was a difference between Group 3 and 4 males and controls with respect to weight gain during the first 3 weeks of dosing (Group 3 with 6.78±0.88 kg and Group 4 with 6.8±1.16 kg vs. Group 1 with 7.13±0.98 kg from 7.20-7.24 kg of all groups

at predose). There were no statistically significant differences in mean body weights between the control and treatment groups. Cumulative body weight gain was generally similar between control and treated animals throughout the study. The few statistically significant differences in cumulative body weight gain occurred sporadically during the study (Group 3 and 4 males were different from controls on Days 13 (0.1 kg of Group1 vs. -0.29 kg and -0.27 kg of Group 3 and 4, respectively) and 20 (-0.09 kg vs. -0.46 kg and -0.43 kg of Group 3 and 4, respectively), and Group 4 males were different on Day 209 (0.30 kg of Group 1 vs. -0.02 kg of Group 4) and Day 216 (0.43 kg of Group 1 vs. 0.05 kg of Group 4).

Food Consumption

The food consumption by single animals from all groups decreased by up to 50%, on occasion, during the predose and dose period. During the dosing period, food consumption briefly decreased (up to 50%) slightly more often in animals from Group 4 compared with the other groups. One Group 4 male (302556) stopped eating on Day 22-25. A Group 4 female (302562) had decreased food consumption starting on Day 0 and did not eat on several occasions between Days 15 and 21. Another Group 4 female (302567) had decreased food consumption starting on Day 1 and stopped eating on Day 18. A third female in Group 4 (302563) had decreased food consumption starting around Day 15 and stopped eating on Day 23. In all of these cases, the dogs were offered dry food with warm water and/or canned food to encourage an appetite. This, coupled with a "dosing holiday" (no dose for a period of time) and a reduced dosage that was implemented for Group 4 (decrease from 50 to 30 mg/kg/day), allowed the animals to resume their normal food consumption.

Ophthalmoscopy

All animals examined had ocular findings within normal limits.

EKG Exams

On Day 0, one Group 2 animal (302530) was found with a partial block with heart rate of 76 BPM, and seven animals (six of them male) were found with bradycardia (two in Group 1, three in Group 2, one in Group 3 and one in Group 4). Some of these findings were also seen pretest with around 60 BPM, and none was considered related to test article administration. On Day 86, three females had bradycardia, one in Group 1, animal 302522 with 57 BPM and two in Group 4 (the two high dose animals, 302562 with 51 BPM and 302563 with 60 BPM, were icteric and had been less active earlier in the study). On Day 177, one Group 1 male (302516) had bradycardia. On Day 268, one Group 1 male (302516), one Group 2 male (302547) and one Group 2 female (302540) had bradycardia. No other ECG changes including P wave, P-R duration, QRS, Q-T segment, T wave, QTc or Axis were found significantly.

Hematology

Although a few statistically significant differences of Hemoglobin, Hematocrit, Absolute Reticulocytes and Reticulocytes were seen between control and in the treatment group 2 only during the pretest period, and none was considered biologically significant.

Clinical Chemistry

There were no statistically significant differences between the control and treatment groups ($p > 0.05$) with respect to serum chemistry parameters assayed at pretest and during the study (Days 85, 176 and 267), with one exception: elevated creatine kinase (CK) in one Group 3 female on Day 176 resulted in a difference from controls (123 IU/L of control vs. 197 IU/L of Group 3).
In Group 4 males, ALT mean value (91 IU/L) on Day 85 was greater than other groups (33 IU/L) except for Group1 (169 IU/L) which was due to high individual variation in the control dogs. Particularly a control male (302515) with a high ALT value at pretest (208 IU/L) increased to a much higher value on Day 85 (438 IU/L). It is not appropriate to have control dog (s) whose ALT levels were significantly higher than the treatment groups, even at the pretest. Also it is not proper to have SD value (233) to be greater than mean value (169) of ALT in male dogs of control group. This implies very large unacceptable variations between test animals, which should be kept in mind when data were interpreted, also with abnormal ALP noticed in Group 1. If the unusual ALT value (169 IU/L) in the control male dogs at Day 85 is put aside, there would be a significantly higher level (nearly 3 folds) of ALT (91 IU/L) in Group 4 dogs than two treated groups (Group 2 and 3). This would indicate the hepatic toxicity in 3-month treated male dogs with a dose of 30 mg/kg/day (Table 2). This is further indicated by the continuous elevation of mean ALT with 157 and 151 IU/L on Days 167 and 276 respectively. The mean ALT level (144 IU/L) of female dogs at 30 mg/kg/day Groups is 6-fold higher than the controls (23 IU/L), which should be indicated as a sign of liver toxicity although mean ALT level dropped back to 52 IU/L on Day 167 and 71 IU/L on Day 276. Individually, one Group 4 female (302563) had a substantial elevation in ALT levels, where ALT increased from 28 IU/L at pretest to 285 on Day 85. Another female in Group 4 (302562) had a less dramatic increase, from 22 IU/L at pretest to 79 IU/L on Day 85. The three Group 4 animals with increased ALT values on Day 85 all showed signs of icterus earlier in the study (before the daily dosage was decreased), therefore it appears that the increases in ALT values in Group 4 may be related to the treatment.

Table 2 Mean Clinical Chemistry Data in Male Dogs at Day 85

	BUN mg/dL	CREAT mg/dL	ALT IU/L	AST IU/L	ALP IU/L	LDH IU/L	T Bili mg/dL	GGT IU/L	ALB g/dL	A/G %	TRIG md/dL
Group1 (0 mg)											
Mean	23	0.8	169	25	140	77	0.3	2	3.6	1.3	45
SD	2.5	0.12	233	5.2	81.7	1.5	0.10	3.2	0.12	0.13	4.6
Group2 (6 mg)											
Mean	24	0.7	33	26	75	67	0.2	1	3.4	1.1	37
SD	2.3	0.10	7.2	5	29.1	19.9	0.0	1.5	0.06	0.04	10.3

Group3 (15 mg)											
Mean	23	0.8	33	23	64	59	0.3	1	3.6	1.2	50
SD	4.7	0.1	2.9	2.5	31.2	11.8	0.12	0.6	0.31	0.08	14.6
Group4 (50 mg) >30 mg											
Mean	21	0.8	91	26	75	75	0.2	1	3.2	1.1	45
SD	3.1	0.06	45.6	3.6	37	15.1	0.0	0.0	0.12	0.11	8.0

Each group contains 3 dogs at dose of 0, 5, 15 and 30 mg/kg/day.
No statistically significant differences

Table 3 Mean Clinical Chemistry Data in Female Dogs at Day 85

	BUN mg/dL	CREAT mg/dL	ALT IU/L	AST IU/L	ALP IU/L	LDH IU/L	T Bili mg/dL	GGT IU/L	ALB g/dL	A/G %	TRIG md/dL
Group1 (0 mg)											
Mean	24	0.7	23	19	110	49	0.1	2	3.3	1.2	41
SD	0.6	0.2	4.0	4.0	24.8	15.6	0.06	1.5	0.0	0.18	22.2
Group2 (6 mg)											
Mean	21	0.6	26	22	125	43	0.2	1	3.1	1.1	41
SD	2.1	0.12	7.2	4.2	56.1	14.2	0.00	1.0	0.15	0.06	3.6
Group3 (15 mg)											
Mean	20	0.7	39	22	91	79	0.2	2	3.3	1.2	51
SD	4.0	0.06	5.9	1.7	49	24.6	0.15	1.2	0.12	0.07	22.9
Group4 (50 mg) >30 mg											
Mean	22	0.7	144	23	93	78	0.2	2	3.5	1.3	43
SD	1.7	0.06	122.5	6.7	59.8	20.8	0.1	2.1	0.3	0.09	18.8

Each group contains 3 dogs at doses of 0, 5, 15 and 30 mg/kg/day.
No statistically significant differences

Urinalysis

There were no statistically-significant differences between the control and treatment groups with respect to urinalysis parameters such as volume, pH and specific gravity assayed on throughout 9-month test.

Gross pathology

In two Group 4 females (302562 and 302563), the entire body was discolored yellow. (These are two of the three females in which the dosing was suspended for a time earlier in the study). Other than the finding of an icteric condition in these two dogs, the other findings were minor, and included a small gall bladder (one Group 1 male), a red area on a lobe of a lung (one Group 3 male), pituitary cyst(s) (three Group 1 males, one Group 4 male, two Group 1 females, and one Group 2 female), a small thymus (one Group 1 male), a discolored cecum (one Group 4 female), and a gall bladder with black particulate matter (one Group 2 female).
Necropsy findings were also minor at the final necropsy on Day 273. The findings included pituitary cysts, red area of the cecum, jejunum and/or duodenum, small prostate, small thyroid, gall bladder with black particulate matter; small black particulate matter in gall bladder (one Group 2 and one Group 4 female), a white discolored right caudal lobe of a lung (Group 3 female), white discolored pancreas (Group 3 female), pituitary cysts (one Group 1 female, two Group 1 males and two Group 2 males), red area of the duodenum (one Group 3 female and one Group 4 male), red area of the jejunum (one female and one male, Group 3), and red cecum (four animals, 1 male and 1 female from Group 1, one male and one female from Group 3).

Organ Weights

There were no differences in absolute or relative (to body or brain) mean organ weights between control and treated animals. The absolute and relative mean liver weights of Group 4 females were greater than controls (230±48.9 gram vs. 207±44.3 gram and 33.4±3.59 g/kg body weight vs. 29.5±5.08 g/kg body weight, respectively), but there was no trend and the difference was not statistically significant. On Day 273 in Group 2 females, the mean absolute and relative (to body and brain) liver weights were significantly lower than in control ($p < 0.05$). There were no statistical differences in Group 3 or 4 females, and no trend for increased liver weight with increased dose, There were no differences ($p > 0.05$) in other organ weights (absolute and relative) between control and treated animals on Day 273.

Histopathology

Overall, histopathology examinations have been conducted in adequate battery and been peer reviewed. Three tissues were collected and examined from dogs in all groups. For animals at the scheduled termination following 13 weeks (91 days) of dosing, there was only one gross finding that was interpreted as a test article (INT-747)-related effect. Specifically, whole body yellow discoloration was noted at necropsy for two females treated daily with INT-747 at 50/30 mg/kg; however, there were no microscopic findings in the tissues examined that correlated with this finding. These two females were among those in which dosing was discontinued for a period of days during the first month of the study due to clinical signs and symptoms, including yellow discoloration of mucous membranes in one female. The significance of this finding is unclear. No gross or microscopic findings, reported from the final necropsy were considered to be test article related. Microscopic findings by their nature and/or occurrence in test animals and

controls were considered incidental and consistent with spontaneous changes in this species.

Table 4 Summary of histopathology Incidence (positive #/samples checked at week 13)

Study	Group1	Group2	Group3	Group4	Group1	Group2	Group3	Group4
Species	3 Male Dogs				3 Female Dogs			
Adrenals		1/3			1/3			
Duodenum		2/3		1/3				1/3
Epididymis			1/3					
Esophagus		2/3						
Lymph nodes, cervical						1/3	1/3	
Rectum							1/3	
Sternum			1/3	1/3				
Stomach		1/3						

Tissues in all organs listed in table were examined for inflammatory cell infiltrations unless specified and stayed in blank as non-positive findings.

Table 5 Summary of histopathology Incidence (positive #/samples checked at 9-month)

Study	Group1	Group2	Group3	Group4	Group1	Group2	Group3	Group4
Species	3 Male Dogs				3 Female Dogs			
Adrenals		1/3				2/3		
Duodenum (crypt)		1/3			1/3	2/3		1/3
Epididymis			1/3					
Esophagus (subacute inflammation)		2/3						
Lymph nodes, cervical						1/3	1/3	
Testis (Bilateral tube hypospermatohenesis)	1			4				
Sternum hyperostosis			1/3	1/3				
Stomach minerization		1/3						

Tissues in all organs listed in table were examined for inflammatory cell infiltrations unless specified and stayed in blank as non-positive findings.

Toxicokinetics

Peak plasma concentrations were measured at 0.5-1.71 hr post-dose in all dose groups on the different sampling days. After reaching peak concentrations, INT-747 in plasma declined and terminal half-life values were observed between 0.59 and 5.57 hours. The terminal half-lives did not typically correlate with different sampling days, doses or genders.

Mean toxicokinetic parameters are presented in the table below by sex and sampling day:

Study Week	Group	Dose (mg/kg/day)	Sex	$t_{1/2}$ (hours)	T_{max} (hours)	C_{max} (ng/mL)	AUC_{all} (hr-ng/mL)
1 (Day 0)	2	5	M	0.84	0.64	365	384
			F	2.21	0.79	275	405
	3	15	M	1.52	0.79	1,093	1,673
			F	0.81	1.71	1,312	2,065
	4	50	M	3.42	1.36	1,065	5,013
			F	2.64	1.36	3,026	7,072
13	2	5	M	1.24	0.71	474	798
			F	1.41	0.79	496	835
	3	15	M	1.46	0.71	1,160	2024
			F	2.42	0.86	1,578	3578
	4	30	M	1.84	0.86	1,051	3,212
			F	5.57	1.29	1,949	4,094
26	2	5	M	0.59	0.63	508	615
			F	1.60	0.63	446	681
	3	15	M	1.24	0.60	1,381	1,734
			F	1.26	0.63	1,917	2,719
	4	30	M	1.19	0.75	1,906	3,781
			F	1.69	0.75	2,336	4,445

The extent of systemic exposure of dogs to INT-747, characterized by Cmax and AUC, generally increased with increasing INT-747 dose levels over the dose range of 5-30 mg/kg/day. (Based on AUC, the increase for the high dose was greater than dose-proportional on Day 0, when the dose was 50 mg/kg/day). Exposure was generally higher in females than in males. Over the dosing period the there was no significant accumulation of INT-747 systemically.

Summaries of the study:

The purpose of this study was to evaluate the potential oral toxicity of INT-747 when administered orally (capsule) to dogs for 9 months. Groups 2, 3 and 4 received capsules once daily (with the exceptions noted below) to attain INT-747 dosages of 5, 15, and initially 50 mg/kg of body weight. Beginning on Day 29 through the end of the study, the dose for Group 4 was reduced to 30 mg/kg/day due to signs of toxicity. The control group received empty capsules. There was no mortality during 9 months of dosing in this study. Abnormal feces were seen in all dose groups, but the incidence increased with increased dosage. Four high dose animals, one male and three females, had clinical signs that were considered test article-related when dosed at 50 mg/kg/day. These abnormal signs, which started around Days 19 to 24, included an icteric appearance (yellow discoloration of skin, mucous membranes and eyes), decreased activity, dehydration, decreased or no appetite, and decreased production of feces. Dosing was suspended in these four Group 4 dogs on either Day 20 or 25. On Day 35, dosing of these four dogs resumed at 30 mg/kg/day as the signs of toxicity had reversed. The abnormal signs did not recur with daily dosing at 30 mg/kg, with the exception that the skin appeared yellow at necropsy in two of these animals included in the interim sacrifice. There was a trend for ALT to

increase with increasing dose of INT-747 in both males and females on Day 85 and sustained on Days 167 and 276. This increase in ALT was considered biologically significant, since several of the animals with increased ALT also had abnormal clinical signs that could be associated with liver function. No microscopic findings correlated with the whole body yellow discoloration seen in these two high dose females. The absolute and relative mean liver weights of Group 4 females were greater than controls, but there was no trend and the difference was not statistically significant.
Toxicokinetics assessments revealed mean half-life was generally short, ranging from 0.8 to 5.1 hours. For a given dose group, half-life was similar at the initial dose and at Week 13. Mean plasma half-life appeared to increase with increasing dose in males; in females, any such increase was slight. The time to maximal plasma concentration (Tmax) was generally 0.5 or 1 hour at all dose levels and times, and for both sexes. Cmax and AUC increased with increasing dose at both Day 0 and Week 13 and week 26. There was no indication of any substantial accumulation of the test article in plasma between Day 0 and Week 13 and week 26. Overall, the no observable adverse effect level (NOAEL) was considered to be 15 mg/kg/day. The target organ of toxicity was the liver.

7 Genetic Toxicology

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

Review of Study No. AB05JZ.503BTL is incorporated below from the pharmacology review of IND 63307 dated October 14, 2011 (C. Wu, Ph.D., DGIEP).

Study title: Evaluation of INT-747 in Bacterial Reverse Mutation Assay

Study no.:	AB05JZ.503.BTL
Study report location:	Amendment #056, appendices p 1-90
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 24, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, UPF747/Batch B and 93% purity

Key Study Findings

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

Methods

Strains:	*Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and the *Escherichia coli* strain WP2*uvr*A
Concentrations in definitive study:	(15, 50, 150, 500, 1500, and 5000 µg/plate) of INT-747, both in the presence and absence of metabolic activation
Basis of concentration selection:	Initially a cytotoxicity study was performed with TA98, TA100, TA1535, TA1537, and WP2*uvr*A in the presence or absence of S9 activation using target concentrations of 1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/plate. No cytotoxicity of INT-747 was observed at concentrations up to 5000 µg/plate.
Negative control:	Solvent dimethyl sulfoxide (DMSO)
Positive control:	The positive control used for all strains in the presence of metabolic activation was 2-aminoanthracene (2AA). In the absence of metabolic activation, the following substances were used as positive controls: TA100- **2**-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF2), TA1535- sodium azide (NaN_3), WP2uvrA and TA98- **2**-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF2), and TA1537- 9-aminoacridine (9AA).
Formulation/Vehicle:	The solvent dimethyl sulfoxide (DMSO) was used as a vehicle for the test drug and also used for dissolving and diluting the test substance
Incubation & sampling time:	The tester strains were incubated with the test substance and positive or negative controls by the plate incorporation method. The plates were incubated at 37^0C for 48 hours before counting the revertant colonies.

Study Validity

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

Results

Incubation of the bacterial stains with INT-747 did not cause two-fold or greater increases in the number of revertants for any strain, either in the presence or absence of metabolic activation. The positive controls, on the other hand, showed two-fold or higher increases in the number of revertants when compared with the negative control. The number of revertant colonies for different strains in the absence or presence of metabolic activation is shown in the Table below. Thus, INT-747 was not genotoxic in the bacterial reverse mutation assay (Ames test).

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	29 ± 8	129 ± 14	27 ± 1	6 ± 5	19 ± 2
15	27 ± 4	144 ± 5	23 ± 4	8 ± 2	25 ± 3
50	29 ± 2	114 ± 6	26 ± 7	7 ± 3	26 ± 6
150	27 ± 4	139 ± 18	25 ± 4	7 ± 1	16 ± 3
500	27 ± 3	139 ± 15	25 ± 5	5 ± 2	21 ± 1
1500	15 ± 6	102 ± 16	16 ± 1	3 ± 3	11 ± 2
5000	11 ± 2	45 ± 7	13 ± 5	0 ± 0	13 ± 2
Positive	163 ± 30	591 ± 16	456 ± 17	444 ± 41	136 ± 14

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	42 ± 7	144 ± 7	18 ± 4	10 ± 3	23 ± 6
15	37 ± 8	158 ± 23	20 ± 6	7 ± 2	20 ± 4
50	40 ± 5	144 ± 28	24 ± 4	11 ± 3	20 ± 1
150	43 ± 11	159 ± 14	25 ± 6	12 ± 1	23 ± 3
500	38 ± 3	155 ± 12	23 ± 6	6 ± 3	17 ± 2
1500	19 ± 3	106 ± 19	24 ± 5	8 ± 4	15 ± 5
5000	9 ± 3	51 ± 6	13 ± 2	2 ± 1	13 ± 5
Positive	1497 ± 254	2152 ± 48	311 ± 10	290 ± 52	842 ± 26

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

7.2 *In Vitro* Assays in Mammalian Cells

Review of Study No. AB05JZ.341.BTL is incorporated below from the pharmacology review of IND 63307 dated October 14, 2011 (C. Wu, Ph.D., DGIEP).

Study title: *In Vitro* Mammalian Chromosome Aberration Test

Study no.:	AB05JZ.341.BTL
Study report location:	Amendment #056, appendices p 1-90
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 25, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, UPF747/Batch B, 93% purity

Key Study Findings

INT-747 was negative for the induction of structural and numerical chromosomal aberrations in human peripheral lymphocytes in the presence or absence of metabolic activation.

Methods

Cell line:	Human peripheral lymphocytes
Concentrations in definitive study:	For the 4-hour non-activated portion of the study, 10, 40, and 60 µg/mL; and for the 4-hour activated portion and 20-hour non-activated portion, 1.25, 2.5, and 5 µg/mL.
Basis of concentration selection:	The sponsor stated that the doses were selected on the basis of the substantial toxicity (at least 50% reduction in mitotic index relative to the solvent control) which was observed at concentrations ≥ 42 µg/mL in the non-activated 4 hour exposure group. Substantial toxicity was observed at concentrations ≥ 4.2 µg/mL in both the S9 activated 4 hour and the non-activated 20 hour exposure groups.
Negative control:	Solvent dimethyl sulfoxide (DMSO)
Positive control:	Mitomycin C (MMC) was used as the positive control substance in the short-term treatment without metabolic activation and the continuous treatment. In the short-term treatment with metabolic activation, cyclophosphamide (CP) was used as the positive control substance.
Formulation/Vehicle:	DMSO was used as a vehicle for the drug.
Incubation & sampling time:	Cells were treated with INT-747 for 4 hours in the presence or absence of S9 mix, or were continuously treated for 19 hours in the absence of S9 mix.

Study Validity

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

Results

In the minus S9 4-hour portion of the study, at the highest test concentration evaluated microscopically for chromosome aberrations, 60 µg/mL, mitotic inhibition was 55%, relative to the solvent control. The concentrations selected for analysis of chromosome aberrations were 10, 40 and 60 µg/mL. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any concentration ($p>0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) group was statistically significant (19.0%) as shown in the table below.

In the 4-hour treatment assay, in the presence of S9, at the highest test concentration evaluated for chromosome aberrations, 5 µg/mL, mitotic inhibition was 54%, relative to the solvent control. The concentrations selected for analysis of chromosome aberrations were 1.25, 2.5 and 5 µg/mL. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any concentration ($p>0.05$, Fisher's exact test). The percentage of structurally damaged cells in the CP (positive control) group was statistically significant (14.0%).

In the non-activated 20 hour exposure study, at the highest test concentration (5 µg/mL) evaluated microscopically for chromosome aberrations, mitotic inhibition was 55%, relative to the solvent control. The concentrations selected for analysis of chromosome aberrations were 1.25, 2.5 and 5 µg/mL. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any concentration ($p>0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) group was statistically significant (14.0%).

Thus, INT-747 was negative for the induction of structural and numerical chromosome aberrations in the non-activated and S9 activated test systems in the *in vitro* mammalian chromosome aberration test using human peripheral lymphocytes.

Reference ID: 3870157

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Ethanol	-S9	4	7.1	200	200	0.000	±0.000	0.5	0.0
INT-747									
10	-S9	4	6.7	200	200	0.000	±0.000	0.5	0.0
40	-S9	4	3.6	200	200	0.000	±0.000	1.5	0.0
60	-S9	4	3.2	200	200	0.000	±0.000	0.5	0.0
MMC, 0.6	-S9	4	3.7	200	100	0.250	±0.575	1.5	19.0**
Ethanol	+S9	4	7.8	200	200	0.000	±0.000	0.5	0.0
INT-747									
1.25	+S9	4	4.9	200	200	0.000	±0.000	0.5	0.0
2.5	+S9	4	5.0	200	200	0.000	±0.000	0.5	0.0
5	+S9	4	3.6	200	200	0.000	±0.000	1.5	0.0
CP, 20	+S9	4	3.1	200	100	0.140	±0.349	0.5	14.0**
Ethanol	-S9	20	8.6	200	200	0.000	±0.000	0.5	0.0
INT-747									
1.25	-S9	20	5.8	200	200	0.005	±0.071	0.5	0.5
2.5	-S9	20	5.4	200	200	0.000	±0.000	0.5	0.0
5	-S9	20	3.9	200	200	0.000	±0.000	0.5	0.0
MMC, 0.3	-S9	20	2.8	200	100	0.160	±0.420	0.0	14.0**

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

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

Review of Study No. AB05JZ.123M.BTL is incorporated below from the pharmacology review of IND 63307 dated October 14, 2011 (C. Wu, Ph.D., DGIEP).

Study title: Evaluation of INT-747 in the Mammalian Erythrocyte Micronucleus Test

Study no:	AB05JZ.123M.BTL
Study report location:	Amendment #056, appendices p 1-72
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 27, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, lot #: C, 92% purity

Key Study Findings

INT-747 did not result in an increase of micronucleus frequency in male or female mice. No dose-related effect was observed. Therefore, INT-747 was negative for induction of micronucleus in this test system under the experimental conditions used.

Methods

Doses in definitive study:	5, 10 or 20 mg/kg/day were administered intraperitoneally
Frequency of dosing:	Single dosing
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	1% carboxymethylcellulose
Species/Strain:	Male and female ICR mice approximately 6 to 8 weeks old
Number/Sex/Group:	5
Satellite groups:	None
Basis of dose selection:	The sponsor stated that the doses were selected on the basis of the earlier oral toxicity study, and the high dose caused piloerection and 40% of mice died at 40 mg/kg. Therefore, the highest dose was fixed at 20 mg/kg/day.
Negative control:	Vehicle control solution
Positive control:	Cyclophosphamide monohydrate (CP, 50 mg/kg)

Study Validity

The study was valid as the vehicle control group had less than 0.5% micronucleated PCEs and the positive control group had a statistically significantly higher mean percent MNPCEs than the vehicle control group at 1% level and an increase in MPCE occurred in a dose-dependent manner in the treated animals compared to control animals, which are within the fluctuation range of their historical data (Mean ± 3 S.D). One thousand erythrocytes (ERYs) per rat (500 ERYs per person) were observed by two persons and the number of PCEs was recorded.

Results

In the definitive micronucleus study, groups of 5 to 15 male mice were exposed to INT-747 at 5, 10, or 20 mg/kg, the negative (vehicle) or positive control article. No mortality was observed in any group in the micronucleus study. Lethargy and piloerection were observed in mice at all INT-747 doses tested. All animals treated with the control articles appeared normal following dose administration.
The incidence of micronucleated polychromatic erythrocytes per 10000 polychromatic erythrocytes scored (2000 PCEs/animal) and the proportion of polychromatic

erythrocytes per total erythrocytes are summarized in the table below. Reductions, up to 24%, in the ratio of polychromatic erythrocytes to total erythrocytes were observed in all of the test article-treated groups relative to the respective vehicle controls. The number of micronucleated polychromatic erythrocytes per 10000 polychromatic erythrocytes in test article-treated groups was not statistically increased relative to the respective vehicle controls, regardless of dose level or bone marrow collection time ($p > 0.05$, Kastenbaum-Bowman Tables). In contrast, CP induced a significant increase in micronucleated polychromatic erythrocytes in male mice ($p \leq 0.05$).

Treatment (20 mL/kg)	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes: Number/1000 PCEs (Mean +/- SD)	Micronucleated Polychromatic Erythrocytes: Number/ PCEs Scored[1]
1% CMC	M	24	5	0.515 ± 0.06	---	0.6 ± 0.42	6 / 10000
INT-747 5 mg/kg	M	24	5	0.450 ± 0.03	-13	0.5 ± 0.35	5 / 10000
10 mg/kg	M	24	5	0.454 ± 0.04	-12	0.4 ± 0.22	4 / 10000
20 mg/kg	M	24	5	0.390 ± 0.07	-24	0.3 ± 0.27	3 / 10000
Cyclophosphamide 50 mg/kg	M	24	5	0.348 ± 0.05	-32	13.6 ± 3.31	*136 / 10000
1% CMC	M	48	5	0.526 ± 0.01	---	0.7 ± 0.27	7 / 10000
INT-747 20 mg/kg	M	48	5	0.462 ± 0.05	-12	0.3 ± 0.27	3 / 10000

[1]*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)

7.4 Other Genetic Toxicity Studies

Study title: UPF-832 Salmonella-E.Coli/Mammalian Microsome Reverse Mutation Assay

Study no.: (b) (4) 12-120
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: March 15, 2012
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: UPF-832 (Glyco-6α-ethyl-chenodeoxycholic acid), Lot# FV-188099, 98.82%

Key Study Findings

Under the conditions tested, UPF-832 was negative in the *in vitro* bacterial reverse mutation assay using the plate incorporation method both with and without metabolic activation.

Methods

Strains:	Salmonella typhimurium TA98, TA100, TA1535 and TA1537, and Escherichia coli WP2uvrA
Concentrations in definitive study:	TA100, TA1535, and TA1537: 25, 50, 100, 250, 500, 1000, and 2500 µg/plate with and without metabolic activation; TA98 and WP2uvrA: 100, 250, 500, 1000, 2500, and 5000 µg/plate with and without metabolic activation
Basis of concentration selection:	In an initial trial conducted using 25 to 5000 µg/plate, cytotoxicity (reduction in background lawn) occurred at ≥1000 µg/plate in TA100, TA1535, and TA1537 without metabolic activation; at ≥2500 µg/plate in TA100 and TA1535 with metabolic activation; and at 5000 µg/plate in TA1537 with metabolic activation.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	The positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. The positive controls for testing in the absence of metabolic activation were as follows: ICR-191 acridine for TA1537; 2-nitrofluorene for TA98; sodium azide for TA100 and TA1535; and 4-nitroquinoline-N-oxide for WP2uvrA.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The tester strains were incubated with the test compound and positive or negative controls by the plate incorporation method. Commercially purchased S9 mixture (7.5% v/v) was used for metabolic activation. The plates were incubated at 37°C for 2 days.

Study Validity

The assay was considered valid based on comparison of the vehicle and positive control plates relative to revertant count ranges found in historical data from the conducting laboratory. The test article was considered positive if it induced a concentration-dependent increase in revertants, with increases at least 2 times the

Reference ID: 3870157

vehicle control background frequency for TA100 and 3 times the vehicle control frequency for TA1537, TA98, TA1535, and WP2uvrA. The increases should occur in at least 2 more successive concentrations or should be repeatable at a single concentration. In this study, positive control mean values were at least 3-fold greater than vehicle control means and comparable to historical data. Means of vehicle control data were also comparable to historical data. Thus, the assay was considered valid.

Results

In the confirmatory study, cytotoxicity occurred at ≥1000 µg/plate in TA1535 and at 2500 µg in TA1537 and TA100 without metabolic activation. With metabolic activation, cytotoxicity occurred at 2500 µg/plate in TA1535. Precipitates were not observed at any concentration regardless of metabolic activation. Incubation with UPF-832 did not produce a treatment-related increase in the number of revertants for any strain, either in the presence or absence of metabolic activation. Results of the initial and confirmatory assays are shown in the Applicant's tables below.

Table 2. Initial Assay: Summary Results of Plate Incorporation Experiment for UPF-832 in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay

REVERTANT COLONIES PER PLATE—Mean (SD) [a]

Treatment Group	µg/plate	TA1537 [b]	TA98 [b]	TA100 [b]	TA1535 [b]	WP2*uvrA* [b]
WITHOUT ACTIVATION						
DMSO	50 µL	3 (1)	6 (2)	84 (16)	8 (1)	9 (4)
ICR	0.5	257 (10)				
2NF	2.5		535 (29)			
SA	1.0			364 (19)	210 (61)	
NQNO	2.0					1127 (48)
UPF-832	25	2 (1)	13 (8)	84 (17)	10 (2)	13 (2)
	50	4 (3)	9 (3)	72 (10)	6 (2)	8 (1)
	100	2 (2)	9 (2)	83 (14)	8 (1)	15 (6)
	250	3 (3)	10 (3)	80 (6)	9 (4)	11 (8)
	500	3 (3)	13 (4)	82 (6)	8 (2)	13 (5)
	1000	-- [e] --	9 (1)	-- [e] --	-- [e] --	20 (2)
	2500	-- [e] --	8 (3)	-- [e] --	-- [e] --	14 (2)
	5000	-- [e] --	6 (3)	-- [e] --	-- [e] --	12 (5)
Treatment Group	**µg/plate**	**TA1537 [c]**	**TA98 [b]**	**TA100 [b]**	**TA1535 [b]**	**WP2*uvrA* [b]**
WITH ACTIVATION						
DMSO	50 µL	5 (0)	7 (5)	74 (11)	8 (2)	12 (2)
2AA	2.5	61 (18)	486 (106)	246 (95)	64 (21)	
2AA	10.0					365 (44)
UPF-832	25	6 (2)	5 (5)	76 (18)	7 (7)	6 (2)
	50	3 (0)	2 (1)	75 (5)	9 (2)	16 (5)
	100	4 (1)	2 (2)	90 (7)	8 (3)	12 (3)
	250	4 (1)	4 (1)	81 (12)	8 (4)	12 (5)
	500	4 (2)	12 (15)	72 (3)	6 (5)	16 (8)
	1000	4 (1)	4 (2)	86 [d] (15)	9 [d] (3)	18 (8)
	2500	4 [d] (2)	5 (2)	-- [e] --	-- [e] --	13 (7)
	5000	-- [e] --	6 (4)	-- [e] --	-- [e] --	9 (4)

2AA – 2-Aminoanthracene; 2NF – 2-Nitrofluorene; ICR – ICR-191 Acridine; NQNO – 4-nitroquinoline-N-oxide; SA – Sodium azide; SD – standard deviation; DMSO - Dimethylsulfoxide
a: Calculated from triplicate plates
b: Assay Date: 20-Mar-2012
c: Assay Date: 16-May-2012
d: Slightly reduced background lawn
e: Cytotoxicity: Reduction in background lawn, plates not counted

Table 3. Confirmatory Assay: Summary Results of the Plate Incorporation Experiment for UPF-832 in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay

REVERTANT COLONIES PER PLATE—Mean (SD) [a]

Treatment Group	µg/plate	TA1537	TA98	TA100	TA1535	WP2*uvrA*
WITHOUT ACTIVATION						
DMSO	50 µL	4 (0)	10 (3)	72 (15)	6 [d] (1)	42 (9)
ICR	0.5	244 (18)				
2NF	2.5		587 (68)			
SA	1.0			358 (17)	281 (17)	
NQNO	2.0					1417 (42)
UPF-832	25	4 (3)		69 (21)	4 (2)	
	50	3 (1)		81 (17)	5 (2)	
	100	4 (1)	10 (2)	58 (1)	9 (4)	47 (5)
	250	4 (1)	11 (5)	57 (5)	12 (0)	41 (10)
	500	4 (1)	11 (6)	61 (9)	9 (1)	39 (6)
	1000	4 [b] (1)	11 (3)	63 [b] (8)	-- [c] --	43 (7)
	2500	-- [c] --	9 (2)	-- [c] --	-- [c] --	46 (6)
	5000		8 [b] (1)			27 (5)
WITH ACTIVATION						
DMSO	50 µL	4 (3)	11 (6)	92 (15)	6 (1)	45 (7)
2AA	2.5	45 (11)	1355 (350)	748 (126)	75 (2)	
2AA	10.0					275 (57)
UPF-832	25	5 (3)		75 (9)	5 (1)	
	50	6 (2)		72 (5)	7 (3)	
	100	5 (2)	12 (4)	67 (6)	7 (2)	31 (5)
	250	6 (2)	11 (2)	72 (2)	7 (2)	35 (6)
	500	3 (2)	12 (5)	66 (18)	4 (1)	41 (2)
	1000	4 (3)	14 (5)	65 (15)	8 (5)	44 (9)
	2500	3 [b] (2)	7 (2)	46 [b] (15)	-- [c] --	41 (15)
	5000		8 (4)			44 (2)

2AA – 2-Aminoanthracene; 2NF – 2-Nitrofluorene; ICR – ICR-191 Acridine; NQNO – 4-nitroquinoline-N-oxide; SA – Sodium azide; SD – standard deviation; DMSO - Dimethylsulfoxide
a: Calculated from triplicate plates
b: Slightly reduced background lawn
c: Cytotoxicity: Reduced background lawn, plates not counted
d: Contaminated plate not counted, calculated from duplicate plates

Study title: UPF-1443 Salmonella-E.Coli/Mammalian Microsome Reverse Mutation Assay

Study no.:	(b) (4) 12-121
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 15, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	UPF-1443 (Tauro-6α-ethyl-chenodeoxycholic acid), Lot#'s FV-188100 and BC-229040, 99.38% and 100%

Key Study Findings

Under the conditions tested, UPF-1443 was negative in the *in vitro* bacterial reverse mutation assay using the pre-incubation method both with and without metabolic activation.

Methods

Strains:	Salmonella typhimurium TA98, TA100, TA1535 and TA1537, and Escherichia coli WP2uvrA
Concentrations in definitive study:	TA100 and TA1537: 25, 50, 100, 250, 500, 1000, 2500, and 5000 µg/plate without metabolic activation; 50, 100, 250, 500, 1000, 2500, and 5000 µg/plate with metabolic activation; TA98, TA1535, and WP2uvrA: 50, 100, 250, 500, 1000, 2500, and 5000 µg/plate with and without metabolic activation
Basis of concentration selection:	In an initial trial conducted using the plate incorporation method at 25 to 5000 µg/plate, cytotoxicity (reduction in background lawn) occurred at ≥1000 µg/plate in TA100 and TA1537 without metabolic activation; at ≥2500 µg/plate in TA100 with metabolic activation and TA1535 without metabolic activation; and at 5000 µg/plate in TA1537 with metabolic activation.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	The positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. The positive controls for testing in the absence of metabolic activation were as follows: ICR-191 acridine for TA1537; 2-nitrofluorene for TA98; sodium azide for TA100 and TA1535; and 4-nitroquinoline-N-oxide for WP2uvrA.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	In the confirmatory assays, the preincubation method was used. Tester strains were first incubated with the test compound and positive or negative controls in the presence or absence of metabolic activation at 37ºC for 20 min with shaking. After plating with agar, the plates were incubated at 37°C for 2 days. Commercially purchased S9 mixture (7.5% v/v) was used for metabolic activation.

Reference ID: 3870157

Study Validity

The assay was considered valid based on comparison of the vehicle and positive control plates relative to revertant count ranges found in historical data from the conducting laboratory. The test article was considered positive if it induced a concentration-dependent increase in revertants, with increases at least 2 times the vehicle control background frequency for TA100 and 3 times the vehicle control frequency for TA1537, TA98, TA1535, and WP2uvrA. The increases should occur in at least 2 more successive concentrations or should be repeatable at a single concentration. In this study, positive control mean values were at least 3-fold greater than vehicle control means and comparable to historical data and means of vehicle control data were comparable to historical data, with one exception. Because the mean numbers of revertant colonies in the vehicle and positive controls for WP2uvrA without metabolic activation were less than historical controls, a repeat confirmatory assay was conducted.

Results

In the confirmatory assay, cytotoxicity occurred only in strain TA100 at 5000 µg/plate in the presence of metabolic activation, and there were no observations of precipitation. In the confirmatory and repeat confirmatory assays (WP2uvrA only), there were no treatment-related increases in the mean number of revertants in any treatment group with or without metabolic activation. Because the highest concentration tested in strains TA100 and TA1537 without metabolic activation was 2500 µg/plate, a second repeat confirmatory assay was conducted in these strains without metabolic activation to test up to 5000 µg/plate UPF-1443. In the second repeat confirmatory assay, the test compound was also negative at all concentrations. Data from the initial, confirmatory and repeat confirmatory, and second repeat confirmatory assays are shown in the Applicant’s tables below.

Reference ID: 3870157

Table 2. Initial Assay: Summary Results of Plate Incorporation Experiment for UPF-1443 (Batch No. FV-188100) in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay

REVERTANT COLONIES PER PLATE—Mean (SD) [a]

Treatment Group	µg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
WITHOUT ACTIVATION											
DMSO	50 µL	4	(1)	15	(6)	101	(14)	10	(4)	16	(3)
ICR	0.5	301	(56)								
2NF	2.5			508	(43)						
SA	1.0					335	(180)	371	(28)		
NQNO	2.0									1715	(97)
UPF-1443	25	5	(3)	13	(1)	103	(12)	12	(2)	20	(4)
	50	2	(2)	15	(4)	121	(9)	8	(1)	14	(4)
	100	5	(2)	14	(0)	119	(9)	9	(4)	15	(3)
	250	5	(2)	14	(6)	96	(2)	8	(1)	17	(4)
	500	6	(3)	7	(4)	93	(11)	7	(2)	14	(3)
	1000	-- [b]	--	15	(4)	-- [b]	--	10	(3)	15	(5)
	2500	-- [b]	--	10	(2)	-- [b]	--	-- [b]	--	15	(5)
	5000	-- [b]	--	8	(5)	-- [b]	--	-- [b]	--	17	(1)
WITH ACTIVATION											
DMSO	50 µL	3	(2)	18	(5)	106	(8)	11	(7)	16	(6)
2AA	2.5	124	(16)	1336	(64)	1901	(45)	159	(28)		
2AA	10.0									427	(19)
UPF-1443	25	7	(3)	18	(2)	78	(30)	10	(2)	13	(0)
	50	2	(1)	14	(2)	78	(12)	6	(3)	16	(5)
	100	5	(2)	14	(2)	84	(21)	8	(4)	14	(2)
	250	6	(3)	18	(8)	92	(6)	9	(3)	17	(3)
	500	6	(4)	20	(7)	111	(2)	7	(4)	22	(5)
	1000	5	(2)	18	(6)	104	(7)	10	(4)	14	(3)
	2500	4	(2)	16	(14)	-- [b]	--	7	(2)	21	(5)
	5000	-- [b]	--	15	(4)	-- [b]	--	8	(2)	17	(5)

2AA – 2-Aminoanthracene; 2NF – 2-Nitrofluorene; ICR – ICR-191 Acridine; NQNO – 4-nitroquinoline-N-oxide; SA – Sodium azide; SD – standard deviation; DMSO - Dimethylsulfoxide

a: Calculated from triplicate plates

b: Cytotoxicity: Reduced background lawn, plates not counted

Table 3. Confirmatory and Repeat Confirmatory Assays: Summary Results of the Preincubation Experiment for UPF-1443 (Batch No. FV-188100) in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay

REVERTANT COLONIES PER PLATE—Mean (SD) [a]

Treatment Group	µg/plate	TA1537 [b]	TA98 [b]	TA100 [b]	TA1535 [b]	WP2*uvrA* [c]
WITHOUT ACTIVATION						
DMSO	50 µL	3 (3)	16 (1)	69 (19)	5 (2)	26 (3)
ICR	0.5	527 (31)				
2NF	2.5		333 (81)			
SA	1.0			372 (16)	314 (39)	
NQNO	2.0					840 (87)
UPF-1443	25	6 (3)		77 (26)		
	50	3 (2)	11 (2)	79 (9)	4 (3)	27 (7)
	100	4 (3)	12 (5)	79 (6)	4 (1)	25 (8)
	250	5 (3)	14 (1)	69 (18)	5 (3)	28 (3)
	500	3 (2)	13 (6)	56 (19)	3 (1)	26 (6)
	1000	2 (1)	13 (5)	49 (7)	4 (3)	22 (3)
	2500	5 (2)	9 (4)	45 (2)	6 (4)	25 (6)
	5000		8 (4)		6 (5)	15 (6)
Treatment Group	**µg/plate**	**TA1537 [b]**	**TA98 [b]**	**TA100 [b]**	**TA1535 [b]**	**WP2*uvrA* [b]**
WITH ACTIVATION						
DMSO	50 µL	3 (1)	8 (3)	59 (11)	7 (2)	8 (3)
2AA	2.5	212 (10)	1192 (124)	1684 (51)	193 (32)	
2AA	10.0					139 (32)
UPF-1443	50	3 (2)	10 (3)	67 (7)	6 (4)	8 (3)
	100	3 (1)	19 (7)	79 (21)	7 (2)	8 (3)
	250	3 (3)	16 (4)	71 (7)	7 (2)	6 (3)
	500	4 (1)	16 (2)	72 (8)	7 (3)	7 (3)
	1000	6 (2)	16 (2)	68 (8)	7 (3)	10 (2)
	2500	5 (2)	9 (4)	51 (4)	7 (3)	7 (1)
	5000	5 (1)	5 (1)	17 [d,e] (7)	6 (1)	8 (3)

2AA – 2-Aminoanthracene; 2NF – 2-Nitrofluorene; ICR – ICR-191 Acridine; NQNO – 4-nitroquinoline-N-oxide; SA – Sodium azide; SD – standard deviation; DMSO - Dimethylsulfoxide
a: Calculated from triplicate plates
b: Assay Date: 15-May-2012
c: Assay Date: 01-Jun-2012
d: Slightly reduced background lawn
e: Cytotoxicity: >50% reduction in mean number of revertant colonies

Table 4. Second Repeat Confirmatory Assay: Summary Results of the Preincubation Experiment for UPF-1443 (Batch No. BC-229040) in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay

REVERTANT COLONIES PER PLATE—Mean (SD) [a]

Treatment Group	µg/plate	TA1537	TA100
WITHOUT ACTIVATION			
DMSO	50 µL	3 (0)	73 (8)
ICR	0.5	414 (32)	
SA	1.0		474 (62)
UPF-1443	25	5 (2)	89 (16)
	50	4 (2)	82 (11)
	100	3 (2)	89 (13)
	250	6 (3)	79 (11)
	500	4 (1)	70 (5)
	1000	5 (3)	85 (5)
	2500	2 (1)	62 (9)
	5000	3 (1)	42 (14)

ICR – ICR-191 Acridine; SA – Sodium azide;
SD – standard deviation; DMSO - Dimethylsulfoxide
a: Calculated from triplicate plates

Study title: INT-747 With Impurity (b) (4) almonella-E.Coli/Mammalian Microsome Reverse Mutation Assay

Study no.:	(b) (4) 12-102
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 27, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747 with Impurity (b) (4) Lot# Jo3601 K1, (b) (4) % for Impurity (b) (4)

Key Study Findings

Under the conditions tested, INT-747 with (b) (4) % Impurity (b) (4) was negative in the *in vitro* bacterial reverse mutation assay using the plate incorporation method both with and without metabolic activation.

Methods

Strains:	Salmonella typhimurium TA98, TA100, TA1535 and TA1537, and Escherichia coli WP2uvrA
Concentrations in definitive study:	TA98, TA100, TA1535, and WP2uvrA: 25, 50, 100, 250, 500, 1000, and 2500 µg/plate with and without metabolic activation; TA1537: 25, 50, 100, 250, 500, 1000, and 2500 µg/plate with metabolic activation, and 5, 10, 25, 50, 100, 250, 500, and 1000 µg/plate without metabolic activation
Basis of concentration selection:	In initial trials, precipitates were observed in all strains with and without metabolic activation at ≥2500 µg/plate. Cytotoxicity (reduction in background lawn) occurred at ≥250 µg/plate in TA1537 without metabolic activation; at ≥1000 µg/plate in TA100 and TA1535 with and without metabolic activation; and at ≥2500 and 5000 µg/plate in TA1537 without and with metabolic activation, respectively.
Negative control:	Ethanol
Positive control:	The positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. The positive controls for testing in the absence of metabolic activation were as follows: ICR-191 acridine for TA1537; 2-nitrofluorene for TA98; sodium azide for TA100 and TA1535; and 4-nitroquinoline-N-oxide for WP2uvrA.
Formulation/Vehicle:	Ethanol
Incubation & sampling time:	The tester strains were incubated with the test compound and positive or negative controls by the plate incorporation method. Commercially purchased S9 mixture (7.5% v/v) was used for metabolic activation. The plates were incubated at 37°C for 2 days.

Study Validity

The assay was considered valid based on comparison of the vehicle and positive control plates relative to revertant count ranges found in historical data from the conducting laboratory. The test article was considered positive if it induced a concentration-dependent increase in revertants, with increases at least 2 times the vehicle control background frequency for TA100 and 3 times the vehicle control frequency for TA1537, TA98, TA1535, and WP2uvrA. The increases should occur in at least 2 more successive concentrations or should be repeatable at a single

Reference ID: 3870157

concentration. In the initial assay and the first repeat initial assay, the positive control for TA1537 with metabolic activation was below the historical control range. Therefore, a second repeat initial assay was conducted for TA1537 in the presence of metabolic activation. In the confirmatory assay, positive control mean values were at least 3-fold greater than vehicle control means and comparable to historical data. Means of vehicle control data were also comparable to historical data. Thus, the assay was considered valid.

Results

In the confirmatory study, cytotoxicity occurred at ≥1000 µg/plate in TA100, TA1535, and TA1537 without metabolic activation and at 2500 µg in TA100 with metabolic activation. Precipitates were observed at 2500 µg/plate in all strains regardless of metabolic activation. Incubation with INT-747 with (b) (4)% Impurity (b) (4) did not produce a treatment-related increase in the number of revertants for any strain, either in the presence or absence of metabolic activation. The Applicant's tables below summarize the results of the initial and confirmatory assays.

Table 2. Initial and First and Second Repeat Initial Assays: Summary Results of Plate Incorporation Experiment for INT-747 With Impurity (b) (4) in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay

REVERTANT COLONIES PER PLATE—Mean (SD)[a]

Treatment Group	µg/plate	TA1537 [c]		TA98 [b]		TA100 [b]		TA1535 [b]		WP2*uvrA* [b]	
WITHOUT ACTIVATION											
Ethanol	50 µL	4	(1)	12	(1)	109	(11)	9	(2)	15	(3)
ICR	0.5	255	(28)								
2NF	2.5			554	(60)						
SA	1.0					466	(27)	334	(4)		
NQNO	2.0									1184	(60)
INT-747 With Impurity (b) (4)	(b) (4)										
Treatment Group	**µg/plate**	**TA1537 [d]**		**TA98 [b]**		**TA100 [b]**		**TA1535 [b]**		**WP2*uvrA* [b]**	
WITH ACTIVATION											
Ethanol	50 µL	4	(1)	8	(4)	106	(7)	9	(3)	14	(2)
2AA	2.5	106	(9)	1200	(45)	623	(185)	68	(18)		
2AA	10.0									370	(59)
INT-747 With Impurity (b) (4)	(b) (4)										

2AA: 2-Aminoanthracene
2NF: 2-Nitrofluorene
ICR: ICR-191 Acridine
NQNO: 4-nitroquinoline-N-oxide
SA: Sodium azide
SD: standard deviation
[a] Calculated from triplicate plates
[b] Assay Date: 27-Mar-2012
[c] Assay Date: 08-May-2012
[d] Assay Date: 01-Jun-2012
(b) (4)

Table 3. Confirmatory Assay: Summary Results of the Plate Incorporation Experiment for INT-747 With Impurity (b) (4) in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay

REVERTANT COLONIES PER PLATE—Mean (SD)[a]

Treatment Group	µg/plate	TA1537 [b]		TA98 [c]		TA100 [b]		TA1535 [b]		WP2uvrA [b]	
WITHOUT ACTIVATION											
Ethanol	50 µL	5	(2)	14	(2)	90	(19)	8	(1)	43	(11)
ICR	0.5	179	(14)								
2NF	2.5			476	(82)						
SA	1.0					617	(82)	353	(30)		
NQNO	2.0									1417	(62)
(b) (4)											

Treatment Group	µg/plate	TA1537 [b]		TA98 [c]		TA100 [b]		TA1535 [b]		WP2uvrA [b]	
WITH ACTIVATION											
Ethanol	50 µL	2	(1)	10	(3)	104	(28)	6	(3)	49	(5)
2AA	2.5	45	(8)	824	(59)	370	(17)	66	(6)		
2AA	10.0									275	(43)
INT-747 With Impurity (b) (4)	(b) (4)										

2AA: 2-Aminoanthracene
2NF: 2-Nitrofluorene
ICR: ICR-191 Acridine
NQNO: 4-nitroquinoline-N-oxide
SA: Sodium azide
SD: standard deviation
[a] Calculated from triplicate plates
[b] Assay Date: 08-May-2012
[c] Assay Date: 09-May-2012
[d] Slightly reduced background lawn
(b) (4)

Study title: (b) (4) Salmonella-E.Coli/Mammalian Microsome Reverse Mutation Assay (Report Amendment No. 1)

Study no.:	(b) (4) 12-474
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 11, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	I18-5, Lot# 2011051001, 97.6%

Key Study Findings

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

Methods

Strains:	Salmonella typhimurium TA98, TA100, TA1535 and TA1537, and Escherichia coli WP2uvrA
Concentrations in definitive study:	TA98, TA1537, and WP2uvrA: 100, 250, 500, 1000, 2500, and 5000 µg/plate with and without metabolic activation; TA100 and TA1535: 25, 50, 100, 250, 500, 1000, and 2500 µg/plate with and without metabolic activation
Basis of concentration selection:	In an initial trial with 25-5000 µg/plate, cytotoxicity (reduction in background lawn) occurred at ≥1000 µg/plate in TA100 and TA1537 with and without metabolic activation. Precipitates were observed at 5000 µg/plate in all strains with and without metabolic activation.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	The positive control for all strains in the presence of metabolic activation was 2-aminoanthracene. The positive controls for testing in the absence of metabolic activation were as follows: ICR-191 acridine for TA1537; 2-nitrofluorene for TA98; sodium azide for TA100 and TA1535; and 4-nitroquinoline-N-oxide for WP2uvrA.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The tester strains were incubated with the test compound and positive or negative controls by the plate incorporation method. Commercially purchased S9 mixture (7.5% v/v) was used for metabolic activation. The plates were incubated at 37°C for 2 days.

Study Validity

The assay was considered valid based on comparison of the vehicle and positive control plates relative to revertant count ranges found in historical data from the conducting laboratory. The test article was considered positive if it induced a concentration-dependent increase in revertants, with increases at least 2 times the vehicle control background frequency for TA100 and 3 times the vehicle control frequency for TA1537, TA98, TA1535, and WP2uvrA. The increases should occur in at least 2 more successive concentrations or should be repeatable at a single concentration. In this study, positive control mean values were at least 3-fold greater than vehicle control means and comparable to historical data. Means of vehicle control data were also comparable to historical data. Thus, the assay was considered valid.

Reference ID: 3870157

Results

In the confirmatory assay, cytotoxicity occurred in strains TA100 and TA1535 at 2500 µg/plate in the absence of metabolic activation. Precipitates were observed at ≥2500 µg/plate in all strains, regardless of metabolic activation. There were no treatment-related increases in the mean number of revertants in any treatment group with or without metabolic activation. Data from the initial and confirmatory assays are summarized in the Applicant's tables below.

Table 1. Initial Assay: Summary Results of Plate Incorporation Experiment for (b) (4) **in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay**

		REVERTANT COLONIES PER PLATE—Mean (SD)[a]									
Treatment Group	µg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
		WITHOUT ACTIVATION									
DMSO	100 µL	6	(5)	18	(3)	81	(6)	9	(1)	13	(1)
ICR	0.5	147	(19)								
2NF	2.5			525	(56)						
SA	1.0					884	(159)	391	(28)		
NQNO	2.0									1325	(91)
(b) (4)											
		WITH ACTIVATION									
DMSO	100 µL	4	(0)	30	(3)	103	(9)	9	(3)	10	(2)
2AA	2.5	142	(28)	1556	(198)	1687	(358)	73	(7)		
2AA	10.0									277	(48)
(b) (4)											

2AA: 2-Aminoanthracene
2NF: 2-Nitrofluorene
ICR: ICR-191 Acridine
NQNO: 4-nitroquinoline-N-oxide
SA: Sodium azide
SD: standard deviation
DMSO: Dimethylsulfoxide
[a] Calculated from triplicate plates

(b) (4)

Table 2. Confirmatory Assay: Summary Results of the Plate Incorporation Experiment for (b) (4) in the *Salmonella-E. coli*/Mammalian Microsome Reverse Mutation Assay

Treatment Group	µg/plate	REVERTANT COLONIES PER PLATE—Mean (SD)[a] TA1537		TA98		TA100		TA1535		WP2*uvrA*	
WITHOUT ACTIVATION											
DMSO	100 µL	4	(1)	26	(1)	84	(8)	5	(2)	12	(4)
ICR	0.5	206	(15)								
2NF	2.5			624	(80)						
SA	1.0					471	(4)	391	(23)		
NQNO	2.0									923	(39)
(b) (4)											
WITH ACTIVATION											
DMSO	100 µL	4	(1)	23	(8)	99	(9)	7	(3)	11	(2)
2AA	2.5	92	(12)	1303	(239)	807	(104)	106	(9)		
2AA	10.0									313	(12)
(b) (4)											

2AA: 2-Aminoanthracene
2NF: 2-Nitrofluorene
ICR: ICR-191 Acridine
DMSO: Dimethylsulfoxide;
NQNO: 4-nitroquinoline-N-oxide
SA: Sodium azide
SD: standard deviation
[a] Calculated from triplicate plates
(b) (4)

Study title: UPF-832 In Vitro Chromosome Aberration Test in Cultured Human Peripheral Blood Lymphocytes

Study no.:	(b) (4) 12-122
Study report location:	FDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 15, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	UPF-832 (Glyco-6α-ethyl-chenodeoxycholic acid), Lot# FV-188099, 98.82%

Key Study Findings

Under the conditions tested, UPF-832 was negative for induction of structural and numerical chromosomal aberrations in human peripheral blood lymphocytes in the presence or absence of metabolic activation.

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	3 h treatment without S9: 10, 50, 100, 150, 175, 200, 225, 250, 275, 300, and 350 µg/mL; 3 h treatment with S9: 10, 50, 100, 125, 150, 175, 200, 250, and 300 µg/mL; 22 h treatment without S9: 10, 50, 100, 150, 175, 200, 225, 250, 275, and 300 µg/mL; Repeat 22 h treatment without S9: 10, 100, 125, 150, 160, 170, 180, 190, 200, 225, and 250 µg/mL
Basis of concentration selection:	In a range-finding cytotoxicity assay, concentrations up to 5000 µg/mL were tested. Hemolysis occurred at ≥313 µg/mL. Cytotoxicity (reduction in mitotic index) was 73% and 100% at 313 and 625 µg/mL in the 3 h treatment without S9, 39% and 100% at 156 and 313 µg/mL in the 22 h treatment without S9, and 52% and 100% at 156 and 313 µg/mL in the 3 h treatment with S9.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	Cyclophosphamide (CP) and Mitomycin C (MMC) in the presence and absence of metabolic activation, respectively.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Cells were treated with UPF-832 for 3 h with and without metabolic activation, and 22 h without metabolic activation. Colcemid was added for the final 2 h to arrest cells in metaphase. All cultures were harvested at ~ 22 h. Aroclor 1254-induced rat liver S9 fraction was purchased commercially. The concentration of S9 homogenate in culture was 1.5%. Structural chromosomal aberrations were analyzed from a total of 200 metaphase cells (100 from each culture) or ≥30 aberrant cells from each concentration or controls. Numerical aberrations were evaluated from 400 cells per concentration (200 from each culture).

Reference ID: 3870157

Study Validity

Vehicle and positive control results were compared to historical data ranges. In this study, data from the controls were comparable to the historical control range, except that the percent of aberrant cells in the positive control for the 3 h treatment without S9 exceeded the historical control range. This was not considered to have negatively impacted the validity of the study. Positive control results were statistically significant, compared to relevant vehicle controls. The criterion for a test article to be considered positive for inducing chromosomal aberrations was a significant increase in the numbers of cells with aberrations. If a significant increase occurred at ≥1 dose levels, a dose-response should be observed.

Results

In the 3 h treatment assay without metabolic activation, concentrations evaluated for chromosomal aberrations were 200, 250, and 275 µg/mL. In the 22 h treatment without metabolic activation, concentrations analyzed were 10, 125, and 150 µg/mL. Finally, in the 3 h treatment assay with metabolic activation, concentrations evaluated for chromosomal aberrations were 125, 175, and 200 µg/mL. Under all three assay conditions, there were no statistically significant increases in either structural or numerical aberrations in the UPF-832 treatment groups, relative to vehicle controls. Summary results are presented in the Applicant's tables copied below.

Table 2. Cytotoxicity and Aberration Summary: 3-Hour Incubation without Metabolic Activation

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
DMSO (1%)	0	0.5	0.0	0.0	0.5
MMC 0.8 µg/mL	53	90.9 *	57.6 *	0.0	0.8
UPF-832					
200 µg/mL	3	0.5	0.0	0.0	0.5
250 µg/mL	36	0.5	0.5	0.0	1.0
275 µg/mL	64	2.5	0.0	0.0	1.3

Endo = Endoreduplicated cells DMSO = Dimethylsulfoxide
Abs = Aberrations MMC = Mitomycin C
Percent Aberrant cells: * $p \leq 0.01$ using Fisher's Exact Test

Table 3. Cytotoxicity and Aberration Summary: 22-Hour Incubation without Metabolic Activation

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
DMSO (1%)	0	1.0	0.0	0.0	0.5
MMC 0.3 µg/mL	60	60.0 *	40.0 *	0.0	0.0
UPF-832					
10 µg/mL	7	1.0	0.0	0.0	0.0
125 µg/mL	28	1.0	0.0	0.0	0.0
150 µg/mL	49	0.0	0.0	0.0	0.3

Endo = Endoreduplicated cells DMSO = Dimethylsulfoxide
Abs = Aberrations MMC = Mitomycin C
Percent Aberrant cells: * $p \leq 0.01$ using Fisher's Exact Test

Table 4. Cytotoxicity and Aberration Summary: 3-Hour Incubation with Metabolic Activation

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
DMSO (1%)	0	0.0	0.0	0.3	0.3
CP 20 µg/mL	0	30.0 *	6.0 *	0.0	0.5
UPF-832					
125 µg/mL	1	1.5	0.0	0.0	0.0
175 µg/mL	38	2.0	0.0	0.0	0.3
200 µg/mL	63	2.0	0.5	0.0	0.5

Endo = Endoreduplicated cells DMSO = Dimethylsulfoxide
Abs = Aberrations CP = Cyclophosphamide
Percent Aberrant cells: * $p \leq 0.01$ using Fisher's Exact Test

Study title: UPF-1443 In Vitro Chromosome Aberration Test in Cultured Human Peripheral Blood Lymphocytes

Study no.:	(b) (4) 12-123
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 15, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	UPF-1443 (Tauro-6α-ethyl-chenodeoxycholic acid), Lot# FV-188100, 99.38%

Key Study Findings

Under the conditions tested, UPF-1443 was negative for induction of structural and numerical chromosomal aberrations in human peripheral blood lymphocytes in the presence of metabolic activation with 3 h incubation and absence of metabolic activation with 24 h incubation. According to the specified study criteria, there was an equivocal response based upon a statistically significant increase in the mean percent of chromosomal aberrations at the highest concentration analyzed (compared to vehicle control) following 3 h incubation without metabolic activation.

Methods

Cell line:	Human peripheral blood lymphocytes
Concentrations in definitive study:	3 h treatment with and without S9: 100, 200, 250, 300, 350, 400, 450, 500, 550, 600, and 650 µg/mL; 22 h treatment without S9: 10, 100, 150, 200, 225, 250, 275, 300, and 400 µg/mL
Basis of concentration selection:	In a range-finding cytotoxicity assay, concentrations up to 5000 µg/mL were tested. Precipitates were observed at ≥2500 µg/mL in the 3 h treatment without S9 and at 5000 µg/mL in the 3 h treatment with S9. Hemolysis occurred at ≥313 µg/mL in the 22 h treatment and ≥625 µg/mL in the 3 h treatments regardless of metabolic activation. Cytotoxicity (reduction in mitotic index) was 2% and 100% at 313 and ≥625 µg/mL, respectively, in the 3 h treatment without S9; 58% and 100% at 313 and ≥625 µg/mL, respectively, in the 22 h treatment without S9; and 10% and ≥96% 100% at 313 and ≥625 µg/mL, respectively, in the 3 h treatment with S9.
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	Cyclophosphamide (CP) and Mitomycin C (MMC) in the presence and absence of metabolic activation, respectively.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Cells were treated with UPF-1443 for 3 h with and without metabolic activation, and 22 h without metabolic activation. Colcemid was added for the final 2 h to arrest cells in metaphase. All cultures were harvested at ~ 22 h. Aroclor 1254-induced rat liver S9 fraction was purchased commercially. The concentration of S9 homogenate in culture was 1.5%. Structural chromosomal

Reference ID: 3870157

aberrations were analyzed from a total of 200 metaphase cells (100 from each culture) or ≥30 aberrant cells from each concentration or controls. Numerical aberrations were evaluated from 400 cells per concentration (200 from each culture).

Study Validity

Vehicle and positive control results were compared to historical data ranges. In this study, data from the controls were comparable to the historical control range, except that the percent of aberrant cells in the positive control for the 22 h treatment without S9 exceeded the historical control range. This was not considered to have negatively impact the validity of the study. Positive control results were statistically significant, compared to relevant vehicle controls. The criteria for considering the test compound positive for inducing chromosomal aberrations were 1) a significant increase ($p \leq 0.01$) in the numbers of cells with aberrations at ≥1 dose levels, or 2) a significant increase ($p \leq 0.05$) observed at ≥2 dose levels. If a significant increase occurred at ≥1 dose levels, a dose-response should be observed. If there was no statistically significant increase ($p \leq 0.05$) at any of the dose levels, the test compound is considered negative. If neither the positive or negative criteria are met, the results were considered equivocal, and biological relevance should be considered.

Results

In the 3 h treatment assay without metabolic activation, concentrations evaluated for chromosomal aberrations were 200, 250, and 300 µg/mL (results summarized in the Applicant's table below). In this assay, there was a statistically significant ($p \leq 0.05$) increase in the mean percent of cells with chromosomal aberrations (3.5%) at 300 µg/mL UPF-1443, compared to the vehicle control (0.0%). The mean percent of cells with aberrations was only slightly above the historical vehicle control range (0 to 3%). In the UPF-1443 treatment groups, the percent of cells with >1 aberrations was 0, compared to 52.8% in the concurrent positive control. Based on the criteria specified for this study, UPF-1443 was considered to be equivocal for inducing structural aberrations under these treatment conditions. The study report concluded that the statistically significant increase in aberrations observed at 300 µg/mL was biologically relevant since it exceeded the historical vehicle control range, and given that there was an increase (not statistically significant) in the 22 h incubation without metabolic activation at a concentration producing similar cytotoxicity.

Table 2. Cytotoxicity and Aberration Summary: 3-Hour Incubation without Metabolic Activation

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
DMSO (1%)	0	0.0	0.0	0.0	0.3
MMC 0.8 µg/mL	45	83.3 *	52.8 *	0.0	0.3
UPF-1443					
200 µg/mL	-3	0.5	0.0	0.0	0.0
250 µg/mL	32	0.0	0.0	0.0	0.5
300 µg/mL	47	3.5 **	0.0	0.0	0.0

Endo = Endoreduplicated cells DMSO = Dimethylsulfoxide
Abs = Aberrations MMC = Mitomycin C
Percent Aberrant cells * p≤0.01; **p≤0.05 using Fisher's Exact Test

In the 22 h treatment without metabolic activation, concentrations analyzed were 150, 200, and 250 µg/mL. In the 3 h treatment assay with metabolic activation, concentrations evaluated for chromosomal aberrations were 200, 250, and 300 µg/mL. In the 24 h incubation assay without metabolic activation and the 3 h treatment assay with metabolic activation, there were no statistically significant increases in either structural or numerical aberrations in the UPF-1443 treatment groups, relative to vehicle controls. Summary results are presented in the Applicant's tables copied below.

Table 3. Cytotoxicity and Aberration Summary: 22-Hour Incubation without Metabolic Activation

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
DMSO (1%)	0	1.0	0.0	0.0	0.0
MMC 0.4 µg/mL	52	100.0 *	76.7 *	0.0	0.0
UPF-1443					
150 µg/mL	2	1.5	0.0	0.0	0.0
200 µg/mL	22	0.5	0.0	0.0	0.8
250 µg/mL	48	3.0	0.0	0.0	0.0

Endo = Endoreduplicated cells DMSO = Dimethylsulfoxide
Abs = Aberrations MMC = Mitomycin C
Percent Aberrant cells: * $p \leq 0.01$ using Fisher's Exact Test

Table 4. Cytotoxicity and Aberration Summary: 3-Hour Incubation with Metabolic Activation

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
DMSO (1%)	0	0.0	0.0	0.0	0.0
CP 20 µg/mL	12	40.5 *	10.8 *	0.0	0.3
UPF-1443					
200 µg/mL	12	0.5	0.0	0.0	0.8
250 µg/mL	34	1.5	0.0	0.0	0.3
300 µg/mL	54	1.5	0.0	0.0	0.0

Endo = Endoreduplicated cells DMSO = Dimethylsulfoxide
Abs = Aberrations CP = Cyclophosphamide
Percent Aberrant cells: * $p \leq 0.01$ using Fisher's Exact Test

Study title: INT-747 with Impurity (b) (4) n Vitro Chromosome Aberration Test in Cultured Human Peripheral Blood Lymphocytes

Study no.: (b) (4) 12-103
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: February 27, 2012
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: INT-747 with Impurity (b) (4) Lot# Jo3601 K1, (b) (4) % for Impurity (b) (4)

Key Study Findings

Under the conditions tested, INT-747 with Impurity (b) (4) %) was negative for induction of structural and numerical chromosomal aberrations in human peripheral blood lymphocytes in the presence or absence of metabolic activation.

Methods

Cell line: Human peripheral blood lymphocytes
Concentrations in definitive study: 3 h treatment without S9: 1.0, 15, 30, 60, 75, 85, 100, 125, 150, and 200 µg/mL (Trial 1) and 1.0, 15, 30, 60, 70, 80, 90, 100, 125, and 150 µg/mL (Trial 2);
22 h treatment without S9: 1.0, 5.0, 15, 30, 45, 60, and 75 µg/mL;
3 h treatment with S9: 1.0, 5.0, 15, 30, 45, 60, 75, 100, and 150 µg/mL (Trial 1) and 1.0, 5.0, 10, 20, 30, 40, 50, 60, 70, and 100 µg/mL (Trial 2)
Basis of concentration selection: In a range-finding cytotoxicity assay,

	concentrations up to 1250 µg/mL were tested. The highest concentration tested was limited based on solubility in the culture media. Precipitates and/or hemolysis were observed at ≥313 µg/mL. Cytotoxicity (reduction in mitotic index) was 33% and 76% at 78.1 and ≥156 µg/mL, respectively, in the 3 h treatment without S9; 42% and 100% at 39.1 and ≥78.1 µg/mL, respectively, in the 22 h treatment without S9; and 57% and ≥76% at 39.1 and ≥78.1 µg/mL, respectively, in the 3 h treatment with S9.
Negative control:	Ethanol
Positive control:	Cyclophosphamide (CP) and Mitomycin C (MMC) in the presence and absence of metabolic activation, respectively.
Formulation/Vehicle:	Ethanol
Incubation & sampling time:	Cells were treated with INT-747 with Impurity (b) (4) for 3 h with and without metabolic activatio and 22 h without metabolic activation. Colcemid was added for the final 2 h to arrest cells in metaphase. All cultures were harvested at ~ 22 h. Aroclor 1254-induced rat liver S9 fraction was purchased commercially. The concentration of S9 homogenate in culture was 1.5%. Structural chromosomal aberrations were analyzed from a total of 200 metaphase cells (100 from each culture) or ≥30 aberrant cells from each concentration or controls. Numerical aberrations were evaluated from 400 cells per concentration (200 from each culture).

Study Validity

Vehicle and positive control results were compared to historical data ranges. In this study, data from the controls were comparable to the historical control range, except that the percent of aberrant cells in the positive control for the 3 h treatment with S9 in Trial 2 were inadequate. Thus, a second repeat assay (Trial 3) was conducted for the 3 h with S9 treatment. Positive control results were statistically significant, compared to relevant vehicle controls. The criterion for the test article to be considered positive for inducing chromosomal aberrations was a significant increase in the numbers of cells with aberrations. If a significant increase occurred at ≥1 dose levels, a dose-response should be observed.

Results

In the 3 h treatment assay without metabolic activation, concentrations evaluated for chromosomal aberrations were 60, 80, and 125 µg/mL. In the 22 h treatment without metabolic activation, concentrations analyzed were 15, 30, and 45 µg/mL. Finally, in the 3 h treatment assay with metabolic activation, concentrations evaluated for chromosomal aberrations were 10, 30, and 40 µg/mL. There were no treatment-related increases in the mean percent of cells with chromosomal aberrations in any treatment group with or without activation. Data are summarized in the Applicant's tables below.

Table 2. Cytotoxicity and Aberration Summary: 3-Hour Incubation without Metabolic Activation (Trial 2)

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
Ethanol (1%)	0	1.0	0.0	0.0	0.5
MMC 0.8 µg/mL	34	69.8 *	37.2 *	0.0	0.0
					(b) (4)

Endo = Endoreduplicated cells DMSO = Dimethylsulfoxide
Abs = Aberrations MMC = Mitomycin C
Percent Aberrant cells: * $p \leq 0.01$ using Fisher's Exact Test

Table 3. Cytotoxicity and Aberration Summary: 22-Hour Incubation without Metabolic Activation (Trial 1)

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
Ethanol (1%)	0	0.5	0.0	0.0	0.3
MMC 0.4 µg/mL	44	83.3 *	52.8 *	0.0	0.3
					(b) (4)

Endo = Endoreduplicated cells DMSO = Dimethylsulfoxide
Abs = Aberrations MMC = Mitomycin C
Percent Aberrant cells: * $p \leq 0.01$ using Fisher's Exact Test

Table 4. Cytotoxicity and Aberration Summary: 3-Hour Incubation with Metabolic Activation (Trial 3)

Treatment	% Mitotic Reduction	% Cells w/Abs	% Cells w/>1 Abs	% Endo Cells	% Polyploid Cells
Ethanol (1%)	0	0.5	0.0	0.0	0.5
CP 20 µg/mL	24	37.5 *	18.8 *	0.0	0.5
(b) (4)					

Endo = Endoreduplicated cells DMSO = Dimethylsulfoxide
Abs = Aberrations CP = Cyclophosphamide
Percent Aberrant cells: * $p \leq 0.01$ using Fisher's Exact Test

8 Carcinogenicity

Study title: A 24-Month Oral (Gavage) Carcinogenicity Study of INT-747 in Rats

Study no.:	(b) (4) 661037
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 14, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, Lot #s T11-001 and T10/002; Purity 97.7% and 97.9% for Lots T11-001 and T10/002, respectively
CAC concurrence:	Yes (ECAC meeting minutes re: dose selection dated October 6, 2011, Attachment-1; Recommendations re: early termination procedures sent to the Applicant in an information request dated August 22, 2013 under IND 63307)

Key Study Findings

- In males, there were no statistically significant, treatment-related effects on survival. However, due to the observed high mortality rate, all surviving males were terminated during Week 100/101. There was an increased incidence of chronic progressive nephropathy as the cause of death or euthanasia (in extremis) in high dose males (relative to controls).

- In females, FDA pairwise comparisons showed statistically significant decreases in survival rates in the mid-dose group compared to the pooled controls and control group 2.
- There were no drug-related neoplastic findings in male rats. In females, there were drug-related increases in the incidences of benign granulosa cell tumors in the ovaries and benign granular cell tumors in the cervix and vagina at 20 mg/kg/day INT-747. (ECAC meeting minutes dated September 10, 2015, Attachment-2)

Adequacy of Carcinogenicity Study

The carcinogenicity study conduct was acceptable. The dose selection was based on recommendations from the Executive CAC (October 6, 2011). Based on mortalities observed at 60 mg/kg/day in the 26-week toxicity study, the Division and CDER Executive Carcinogenicity Assessment Committee (ECAC) recommended a high dose of 20 mg/kg/day (1/3rd the lethal dose). During the conduct of the study, the Division provided recommendations and information to the Applicant regarding early termination procedures which were in concurrence with the ECAC (IND 63307, T. Chakraborti, Ph.D., 08/21/2013). Subsequently, the Applicant submitted a notification of early termination of the male rats from all groups as per the Divisions' previous August 2013 recommendations. In IND 63307 nonclinical review dated 12/24/2013 (S. Chakder, Ph.D.), it was concluded that termination of the male rats from all groups after Week 100 was acceptable.

Appropriateness of Test Models

The Crl:CD(SD) rat is an acceptable test model for the carcinogenicity study.

Evaluation of Tumor Findings

FDA Statistical Analysis of Tumor Data: Tumor analyses consisted of trend analyses for dose response relationship in tumor incidence and pairwise comparisons of tumor incidences in individual treatment groups with combined control groups. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-k method. The tumor test was based on the pooled control groups, and tumor incidences were combined based on McConnell et al. (1986). All tumors presented as "#X TUMOR" or "#TUMOR, MULTIPLE" were combined for statistical analysis. In addition, tumor combinations evaluated by the FDA statistical reviewer included the following: 1) combined papilloma in ear, tail, skin, and paw (males only), and 2) and benign granular cell tumors in the cervix and vagina combined. For the adjustment of multiple testing of dose response relationship, common and rare tumors were tested at significance levels of 0.005 and 0.025, respectively. For multiple pairwise comparisons of treated groups with controls, test levels of 0.01 and 0.05 were used for common and rare tumors, respectively.

<u>Males:</u>
In males, there were no drug-related neoplastic findings.

Reference ID: 3870157

Females:

Benign Granulosa Cell Tumors in the Ovaries. There was an increasing trend in the incidence of benign granulosa cell tumors in the ovaries [p=0.0129 (FDA statistics review); Table 1]. Pairwise comparison also showed an increased incidence at the high dose as compared to combined controls (p=0.0309). Finally, the incidence of this tumor type in the ovaries (identified as rare in the study report) at the high dose (4.6%) exceeded the (b) (4) historical control range (up to 1.67%).

Benign Granular Cell Tumors in the Cervix and Vagina.
There was an increasing trend in the incidence of benign granular cell tumors in the cervix [p=0.0029 (FDA statistics review); Table 1]. Pairwise comparison showed an increased incidence at the high dose as compared to pooled controls (p=0.0030). In the vagina, there was also an increasing trend in the incidence of benign granular cell tumors [p=0.0064 (FDA statistical review)]. The p value for pairwise comparison of the incidence in high dose females versus pooled controls was 0.0309. Furthermore, trend analysis and pairwise comparison (high dose versus pooled controls) of granular cell tumors in the cervix and vagina combined showed this finding to be highly significant (p<0.001).

The following table summarizes the dose response relationship and pairwise comparisons of treated groups and pooled controls for the tumor findings in females discussed above.

Table 1

		Incidence				P-value (FDA statistical review)			
Organ Name	**Tumor Name**	**0 mg/kg/day (C) N=130**	**2 mg/kg/day (LD) N=65**	**7 mg/kg/day (MD) N=65**	**20 mg/kg/day (HD) N=65**	**Dose Response**	**C vs. LD**	**C vs. MD**	**C vs. HD**
Ovary	**Benign granulosa cell tumor**	0	1	1	3	**0.0129**	0.3396	0.3269	**0.0309**
Cervix	**Benign granular cell tumor**	0	2	2	5	**0.0029**	0.1139	0.1083	**0.0030**
Vagina	**Benign granular cell tumor**	0	0	0	3	**0.0064**	-	-	**0.0309**
Cervix/ Vagina Combined	**Benign granular cell tumor**	0	2	2	8	**<0.001**	0.1139	0.1083	**<0.001**

Methods

Doses:	0, 2, 7, 20 mg/kg/day (see Applicant's table below)
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral (Gavage)
Formulation/Vehicle:	0.5% carboxymethylcellulose (CMC) in deionized water
Basis of dose selection:	Maximum tolerated dose (MTD); Deaths occurred at 60 mg/kg/day in a 26-week toxicity and TK study in rats (Study No. (b) (4) 019958).
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	Toxicology: 65/sex/group (Groups 1-5) Satellite: 5/sex (Group 2A); 8/sex (Groups 3A, 4A, and 5A)
Age:	~7 weeks of age at initiation of dosing
Animal housing:	Individual
Paradigm for dietary restriction:	No diet restriction
Dual control employed:	Yes (2 identical vehicle control groups)
Interim sacrifice:	No
Satellite groups:	Yes (for TK analysis)
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

The following tables copied from the Applicant's study report show the study group assignments.

Toxicology Groups (b) (4) 561037M and (b) (4) 561037F)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals Males [a]	Number of Animals Females [b]
1	Vehicle [c]	0	10	65	65
2	Vehicle [c]	0	10	65	65
3	INT-747	2	10	65	65
4	INT-747	7	10	65	65
5	INT-747	20	10	65	65

[a] = All surviving animals were euthanized following a minimum of 704 consecutive days of dose administration.
[b] = All surviving animals were euthanized following a minimum of 728 days of dose administration.
[c] = 0.5% CMC, medium viscosity, in deionized water.

Toxicokinetic Groups (b) (4) 561037C and (b) (4) 561037D)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals [a] Males	Number of Animals [a] Females
2A	Vehicle [b]	0	10	5	5
3A	INT-747	2	10	8	8
4A	INT-747	7	10	8	8
5A	INT-747	20	10	8	8

[a] = All animals were euthanized following the final TK blood collection on Study Day 370.
[b] = 0.5% CMC, medium viscosity, in deionized water.

Observations and Results

Mortality

All animals were checked twice daily for signs of morbidity and mortality during the treatment period.

Results: The Applicant's table below summarizes the survival in males and females. In females, there was a statistically significant decreasing trend in survival rates when compared with the pooled control group (p=0.0298) and control group 2 (p=0.0414). However, survival in each treated female group (41-54%) was within the testing facility historical control range (31-70%; mean 43%) and the survival rate in control group 2 (60%) approached historical control maximum values.

Text Table 7. Survivability of Males and Females to Terminal Euthanasia

Dosage (mg/kg/day)	0	0	2	7	20
Males [a]	**65**	**65**	**65**	**65**	**65**
Number survival	20	18	17	16	15
Number of animals found dead or euthanized in extremis	45	45	48	49	50
Accidental deaths	0	2	0	0	0
% survival [b]	31%	29%	26%	25%	23%
Females [a]	**65**	**65**	**65**	**65**	**65**
Number survival	38	39	35	26	29
Number of animals found dead or euthanized in extremis	25	26	30	37	34
Accidental deaths	2	0	0	2	2
% survival [b]	60%	60%	54%	41%	46%

[a] = Number of animals per dose group.
[b] = % survival was corrected based on the number of accidental deaths.

Independent analysis of survival data by the FDA statistical reviewer (F. Zhou) showed that respective survival rates in the control 1, control 2, low dose, mid dose, and high dose groups were 31%, 28%, 28%, 26%, and 25% in males and 58%, 60%, 55%, 42%, and 46% in females. The FDA statistical reviewer's survival analysis determined that there was not a statistically significant dose response relationship in mortality across combined control and treated groups in either sex. The pairwise comparisons showed a statistically significant increased mortality in mid-dose females compared to the pooled controls (p=0.0487) and compared to control group 2 (p=0.0363). Results of the intercurrent mortality comparison for male and female rats are shown below in Tables 3A and 3B, respectively (copied from the statistical review from F. Zhou).

Reference ID: 3870157

Table 3A: Intercurrent Mortality Comparison – Male Rats

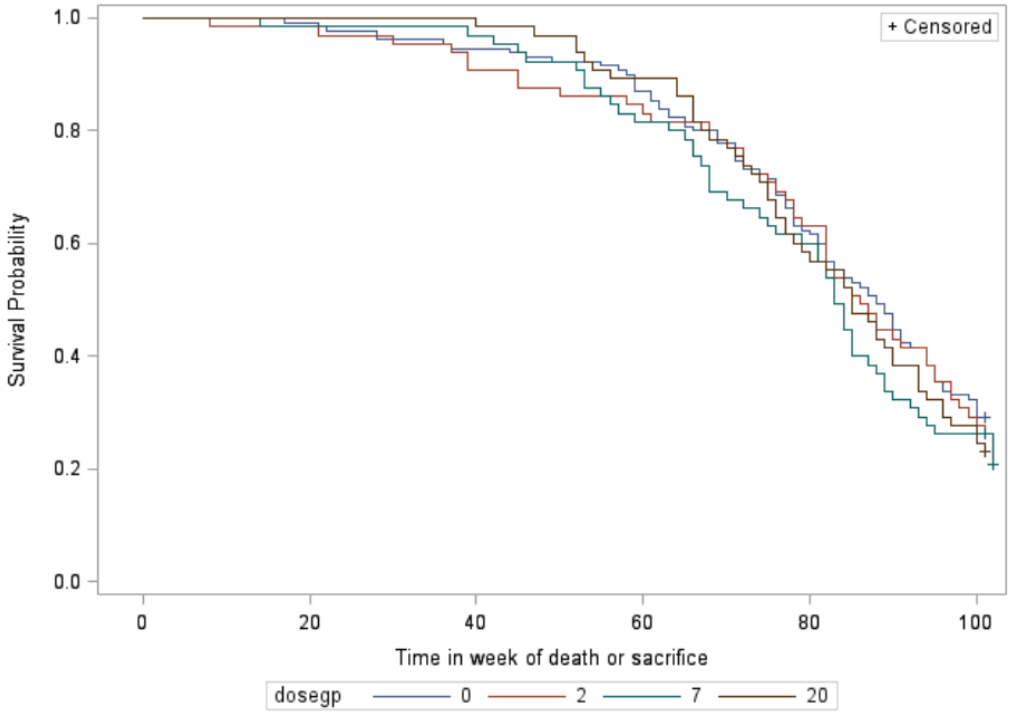
Test	Statistic	P-Value (Pooled Controls)	P-Value (Control-1)	P-Value (Control-2)
Dose-Response	Likelihood Ratio	0.4597	0.3545	0.8408
Homogeneity	Log-Rank	0.7587	0.5331	0.9596

Table 3B: Intercurrent Mortality Comparison – Female Rats

Test	Statistic	P-Value (Pooled Controls)	P-Value (Control-1)	P-Value (Control-2)
Dose-Response	Likelihood Ratio	0.0594	0.1706	0.0660
Homogeneity	Log-Rank	0.1310	0.3554	0.1250

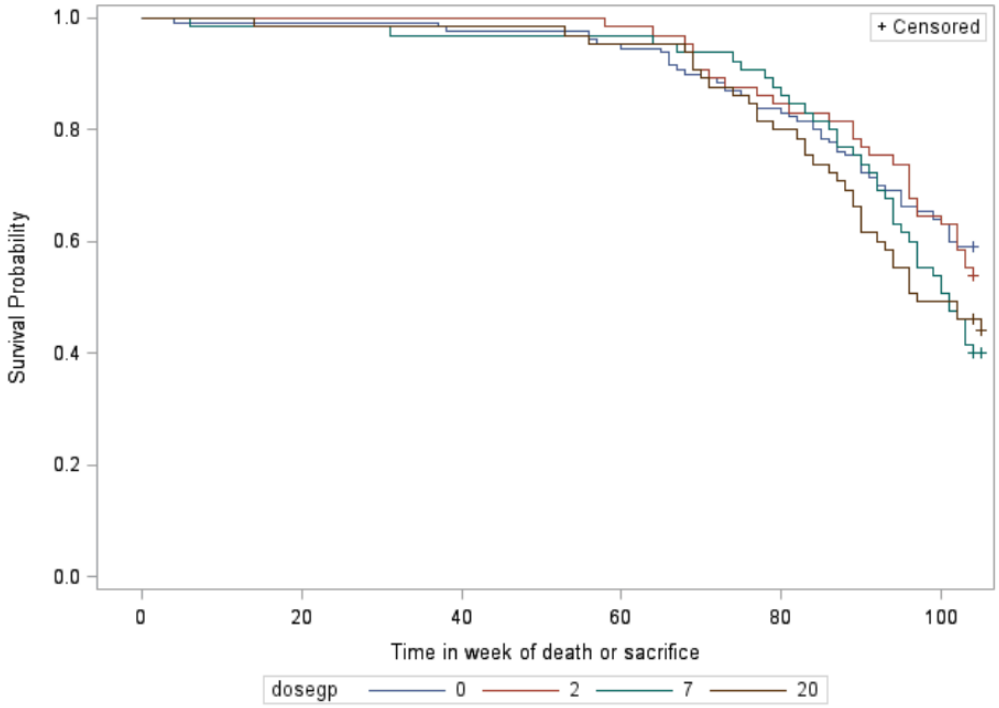
The Kaplan-Meier curves for survival rates of all treatment groups (from the FDA statistical review) are shown below in Figures 1A and 1B for male and female rats, respectively.

Figure 1A: Kaplan-Meier Survival Functions for Male Rats



Note: dose group should be 0, 2, 7, or 20-mg/kg/day

Figure 1B: Kaplan-Meier Survival Functions for Female Rats



Note: dose group should be 0, 2, 7, or 20-mg/kg/day

Due to the observed high mortality rate, all surviving males were terminated during Week 100/101. There was an increased incidence of chronic progressive nephropathy (CPN) as the cause of death or euthanasia (in extremis) in high dose males (14/65 animals, compared to 3/65 and 1/65 in control groups 1 and 2, respectively). In females, there was an increased incidence of benign pituitary adenomas as the cause of death or euthanasia (in extremis) in INT-747 treated females (21/65, 23/65, and 20/65 low-, mid-, and high-dose animals, respectively, compared to 14/65 animals each in control groups 1 and 2). However, this was not dose-dependent.

Clinical Signs

Toxicology animals were observed for clinical signs once daily, and detailed examinations were conducted once weekly. Toxicology animals were palpated for masses weekly, and the time of onset, location, size, appearance, and progression of palpable masses were recorded.

Results: There were no treatment-related findings.

Body Weights

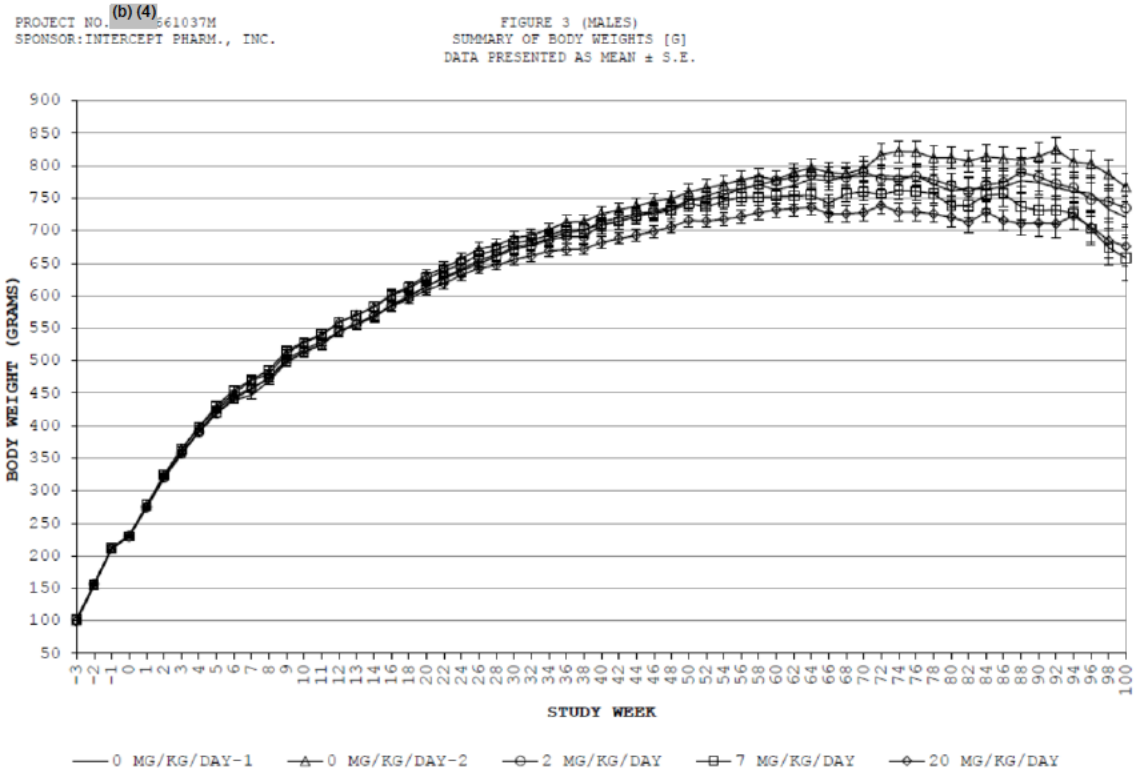
Body weights were recorded weekly beginning during acclimation through Week 14, and approximately every two weeks thereafter.

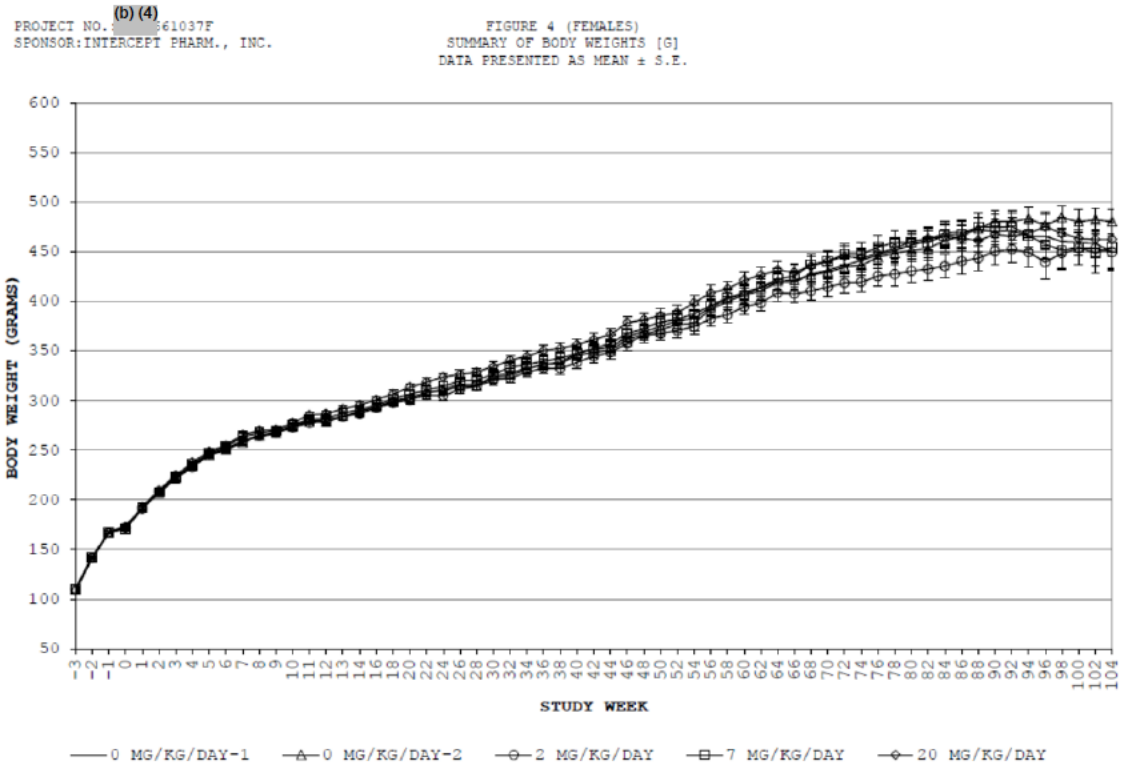
Results: In males, treatment-related effects on body weights occurred at ≥7 mg/kg/day. At the high dose, statistically significant decreases in body weight and body weight gain

(compared to control) were observed beginning during Week 36. Statistically significant decreases in body weight and body weight gain (compared to controls) occurred in males dosed with 7 mg/kg/day beginning during Week 72. At Week 100, body weights at 7 and 20 mg/kg/day were 9% and 6% lower, respectively, than the control group 1 weights and 14% and 12% lower, respectively, than control group 2 weights.

In females, there were no adverse treatment-related effects on body weight in any of the INT-747 treatment groups, relative to controls. At a subset of timepoints and study intervals, there were statistically significant increases in body weight gain which occurred primarily at 7 and 20 mg/kg/day (relative to control). However, there were no statistically significant changes in body weight.

The figures below show mean body weights in males and females over the course of the study period (Applicant's Figures 3 and 4).





Feed Consumption

Food consumption was recorded weekly beginning during acclimation through Week 14, and approximately every two weeks thereafter.

Results: In males, treatment-related increases in food consumption (relative to controls) occurred at ≥7 mg/kg/day during the first 53 weeks of the study. In females dosed at ≥7 mg/kg/day, food consumption was initially lower than controls, and then higher than controls beginning at Week 6.

Clinical Pathology

Blood samples for possible diagnosis of hematopoietic disorders were collected and blood smears were prepared for all animals euthanized in extremis (when possible) and all surviving rats at scheduled necropsy (toxicology groups only).

Results: Blood samples and smears were not examined.

Gross Pathology

Male toxicology animals were sacrificed during Study Week 100/101. Female toxicology animals were sacrificed during Study Week 104. Necropsies (including examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities including viscera) were conducted on all toxicology animals.

Results: No treatment-related gross pathological changes were identified in any group.

Histopathology

At necropsy, organs and tissues listed in the Applicant's table below were collected and preserved for histopathological examination (toxicology animals only).

Adrenals (2)	Ovaries (2) with oviducts [e]
Aorta	Pancreas
Bone with marrow	Peripheral nerve (sciatic)
Femur	Peyer's patches
Sternum	Pharynx
Bone marrow smear	Pituitary
(from femur) [a]	Prostate
Brain [b]	Preputial glands (2)
Cervix	Salivary glands (mandibular [2])
Clitoral glands (2)	Seminal vesicles (2)
Epididymides (2) [c]	Skeletal muscle
Eyes with optic nerves (2) [d]	(rectus femoris)
Gastrointestinal tract	Skin with mammary gland [f]
Esophagus	Spinal cord
Stomach	(cervical, thoracic, lumbar)
Duodenum	Spleen
Jejunum	Teeth
Ileum	Testes (2) [c]
Cecum	Thymus
Colon	Thyroid (with parathyroids [2]) [e]
Rectum	Tongue
Harderian glands (2)	Trachea
Heart	Urinary bladder
Kidneys (2)	Uterus
Larynx	Vagina
Liver (sections of 2 lobes)	Zymbal's glands (2)
Lungs (including bronchi, fixed by	All Gross lesions including masses
inflation with fixative)	(when possible)
Lymph nodes	
Axillary (2)	
Mandibular (2)	
Mesenteric	

[a] - These slides were not placed in formalin and the slides were not examined because they were not scientifically warranted. Bone marrow smears were not taken from animals found dead.
[b] - Histologic trimming of the brain was performed per (b) (4) SOP such that 7 cross (coronal) sections of the brain were taken.
[c] - Fixed in Bouin's solution.
[d] - Fixed in Davidson's solution.
[e] - Oviducts and parathyroids were examined microscopically when in the plane of section and in all cases where a gross lesion was present.
[f] - For females; a corresponding section of skin was taken from the same anatomical area for males.

Peer Review: A histopathology peer review was conducted.

Neoplastic:

Males: Analysis of tumor data by the FDA statistical reviewer showed a statistically significant positive trend ($p<0.025$) in the incidence of the following tumor types: benign papillomas in the paws and the combination of systemic benign hemangiomas and malignant hemangiosarcomas. The pairwise comparison also showed a statistically significant increased incidence of these tumor types in the high dose group compared to the pooled controls ($p<0.05$). These findings (evaluated as rare tumor types in the FDA statistical review based on the incidences in concurrent pooled controls) are summarized in the following table (adapted from the FDA statistical review) and

discussed below. The table also includes the incidence of all papillomas in ear, tail, skin, and paw combined for comparison.

Organ Name	Tumor Name	0 mg/kg/day C N=130	2 mg/kg/day LD N=65	7 mg/kg/day MD N=65	20 mg/kg/day HD N=65	P-Value			
						Dose Response	C vs. LD	C vs. MD	C vs. HD
PAWS	#B PAPILLOMA	0	0	0	3	0.0081*	.	.	0.0352*
SYSTEMIC TUMORS	#B HEMANGIOMA	1	0	2	3	0.0267	0.3279	0.2278	0.1026
	C_HEMANGIO_M+B	1	0	2	4	0.0077*	0.3279	0.2278	0.0393*
Multi/Organ**	C_PAPILLOMA_B	4	0	4	3	0.1675	0.7976	0.2026	0.4230

For dose response, *Indicted the significant at 0.005 or 0.025 alpha levels; for pairwise comparison, *Indicted the significant at 0.01 or 0.05 alpha levels.
**: Combined all papilloma tumors in organ of ear, tail, skin, and paw.

Papillomas. There was an increasing trend in the incidence of benign papillomas in paw, compared to the pooled control groups [p=0.0081 (FDA statistical review)]. Pairwise comparison also showed an increased incidence of benign papillomas in paw at the high dose compared to pooled controls (p=0.0352). The submitted tumor historical control data did not address paw. However, papillomas were also observed in skin and tail of control animals in this study. If the finding of papilloma in paw is evaluated as a common tumor type, the p-values do not meet statistical significance per CDER criteria. In addition, the combined incidence of all papillomas in ear, tail, skin, and paw were not statistically significant in analyses for dose-response or pairwise comparison between the treatment group and controls.

Hemangiomas and Hemangiosarcomas. There was an increasing trend in the incidence of combined hemangiomas/ hemangiosarcomas, compared to the pooled control groups [p=0.0077 (FDA statistical review)]. Pairwise comparison also showed an increased incidence of combined hemangiomas/ hemangiosarcomas at the high dose compared to pooled controls (p=0.0393). The FDA statistical analysis conclusion that there was an increased incidence of the combination hemangiomas/ hemangiosarcomas in males was based on an assumption that these are rare tumors given the incidence in concurrent pooled controls. However, when evaluated as a common tumor type (per the study report and given the occurrence of hemangioma in a control group 1 animal), the FDA dose response and pairwise comparison p values do not achieve statistical significance according to CDER criteria.

Hepatocellular Adenomas. A summary table of selected lesions in the liver of males is shown below.

	0 mg/kg/day (C Group 1)	0 mg/kg/day (C Group 2)	2 mg/kg/day (LD)	7 mg/kg/day (MD)	20 mg/kg/day (HD)
Benign hepatocellular adenoma	2/65 (3.1%)	0/65 (0%)	2/65 (3.1%)	3/65 (4.6%)	6/65 (9.2%)
Malignant hepatocellular carcinoma	0/65 (0%)	1/65 (1.5%)	0/65 (0%)	0/65 (0%)	0/65 (0%)
Combined hepatocellular adenomas and carcinomas	2/65 (3.1%)	1/65 (1.5%)	2/65 (3.1%)	3/65 (4.6%)	6/65 (9.2%)
Focus of cellular alteration					
Eosinophilic	5/65 (7.7%)	2/65 (3.1%)	3/65 (4.6%)	15/65 (23.1%)	8/65 (12.3%)
Basophilic	8/65 (12.3%)	6/65 (9.2%)	3/65 (4.6%)	4/65 (6.2%)	6/65 (9.2%)

Clear Cell	1/65 (1.5%)	5/65 (7.7%)	3/65 (4.6%)	10/65 (15.4%)	5/65 (7.7%)
Mixed	2/65 (3.1%)	1/65 (1.5%)	1/65 (1.5%)	2/65 (3.1%)	8/65 (12.3%)

While the incidences of benign hepatocellular adenomas and combined hepatocellular adenomas and carcinomas were numerically increased at the high dose (compared to controls), the FDA statistical analysis indicated that the dose response relationships and pairwise comparisons for these tumor types were not statistically significant according to CDER criteria for common tumors. Dose response p values for hepatocellular adenomas and combined hepatocellular adenomas and carcinomas were 0.0080 and 0.0153, respectively. The p values for pairwise comparison of the incidence of hepatocellular adenomas and combined hepatocellular adenomas and carcinomas in the high dose group compared to the pooled controls were 0.0164 and 0.0351, respectively.

Pheochromocytomas in the Adrenal Medulla. The FDA pairwise comparison showed a statistically significant increase in the incidence of malignant pheochromocytomas and the combination benign/malignant pheochromocytomas in the adrenal medulla at the low dose compared to the pooled controls (p=0.0419 and 0.0085, respectively). However, the dose response relationships for these tumor types were not statistically significant (the p values were 0.8376 and 0.3156 for malignant pheochromocytomas and the combination benign/malignant pheochromocytomas, respectively). A summary table of selected lesions in the adrenal medulla of males is shown below.

	0 mg/kg/day (C Group 1)	**0 mg/kg/day (C Group 2)**	**2 mg/kg/day (LD)**	**7 mg/kg/day (MD)**	**20 mg/kg/day (HD)**
Benign pheochromocytoma	6/65 (9.2%)	6/65 (9.2%)	11/65 (16.9%)	6/65 (9.2%)	10/65 (15.4%)
Malignant pheochromocytoma	1/65 (1.5%)	0/65 (0%)	4/65 (6.2%)	1/65 (1.5%)	0/65 (0%)
Combined benign/malignant pheochromocytomas	7/65 (10.8%)	6/65 (9.2%)	15/65 (23.1%)	7/65 (10.8%)	10/65 (15.4%)
Adrenal Medullary Hyperplasia	12 (18.5%)	11 (16.9%)	16 (24.6%)	19 (29.2%)	16 (24.6%)
Minimal	5/65	4/65	4/65	8/65	5/65
Mild	5/65	4/65	9/65	8/65	5/65
Moderate	2/65	3/65	3/65	3/65	6/65

Females: According to the FDA statistical review, there was a statistically significant positive trend (p<0.025) in the following tumor types: benign granular cell tumors in the cervix, vagina, and cervix and vagina combined; benign granulosa cell tumors in the ovaries; and malignant fibrosarcoma in skin, the combination fibroma and fibrosarcoma in skin, and malignant fibrosarcoma in combined skin/subcutis. The pairwise comparison also showed a statistically significant increased incidence of these tumor types in the high dose group compared to the pooled controls (p<0.05). These findings (evaluated as rare tumor types in the FDA statistical review based on the incidences in concurrent pooled controls) are summarized in the following table (adapted from the FDA statistical review) and discussed below.

Organ Name	Tumor Name	0 mg/kg/day C N=130	2 mg/kg/day LD N=65	7 mg/kg/day MD N=65	20 mg/kg/day HD N=65	P-Value			
						Dose Response	C vs. LD	C vs. MD	C vs. HD
CERVIX	#B GRANULAR CELL TUM	0	2	2	5	0.0029*	0.1139	0.1083	0 0030*
VAG NA	#B GRANULAR CELL TUM	0	0	0	3	0.0064*	.	.	0 0309*
CERVIX/ VAG NA	C_GRANULAR_CELL_B	0	2	2	8	<0.001*	0.1139	0.1083	<0.001*
OVARIES	#B GRANULOSA CELL TU	0	1	1	3	0.0129*	0 3396	0.3269	0 0309*
SKIN	#M FIBROSARCOMA	1	1	0	4	0.0105	0 5653	0.3269	0 0377
	C_F BROMA_M+B	1	2	0	4	0.0200	0 2663	0.3269	0 0377
SKIN/SUBCUTIS	C_F BROSARCOMA_M	1	2	1	4	0.0217	0 2719	0.5484	0 0377

For dose response, *Indicted the significant at 0.005 or 0.025 alpha levels; for pairwise comparison, *Indicted the significant at 0.01 or 0.05 alpha levels.

Benign Granulosa Cell Tumors in the Ovaries. There was an increasing trend in the incidence of benign granulosa cell tumors in the ovaries [p=0.0129 (FDA statistics review)]. Pairwise comparison also showed an increased incidence at the high dose as compared to combined controls (p=0.0309).

Benign Granular Cell Tumors in the Cervix and Vagina.

- Cervix. There was an increasing trend in the incidence of benign granular cell tumors in the cervix [p=0.0029 (FDA statistics review)]. Pairwise comparison also showed an increased incidence at the high dose as compared to pooled controls (p=0.0030).

- Vagina. In the vagina, there was also an increasing trend in the incidence of benign granulosa cell tumors [p=0.0064 (FDA statistical review)]. The p value for pairwise comparison of the incidence in high dose females versus pooled controls was 0.0309.

- Cervix and Vagina Combined. There was an increasing trend in the incidence of benign granular cell tumors in the cervix and vagina combined (p<0.001 (FDA statistical review)]. The p value for pairwise comparison of the combined incidence of this tumor type in the cervix and vagina of high dose females versus pooled controls was <0.001.

Skin and Combined Skin/Subcutis.

- Malignant Fibrosarcoma in Skin. There was an increasing trend in the incidence of malignant fibrosarcoma in skin, compared to controls (p=0.0105; FDA statistical review). The incidence of fibrosarcoma at the high dose also exceeded the incidence in pooled controls (p=0.0377). Although the study report identified this as a rare tumor type, fibrosarcoma occurred in a control group 2 animal. If this finding is evaluated as a common tumor type, the p-values do not meet statistical significance per CDER criteria.

- Combined Fibroma and Fibrosarcoma in Skin. There was an increasing trend in the incidence of the combination fibroma/fibrosarcoma (p=0.0200; FDA statistical review). The p-value for pairwise comparison of the high dose group relative to pooled controls was 0.0377. Although the FDA statistical review identified these as statistically significant findings based on assumption that they are rare tumor types, when evaluated as a common tumor type (per the study report and given the occurrence of fibrosarcoma in a control group 2 animal), the FDA trend analysis and pairwise comparison p values do not achieve statistical significance according to CDER criteria.

- Malignant Fibrosarcoma in Skin/Subcutis Combined. There was an increased incidence of malignant fibrosarcoma in the skin and subcutis combined (trend analysis p=0.0216; pairwise comparison of high dose versus pooled controls p=0.0377; FDA statistical review). As discussed above for fibrosarcoma in skin, the study report identified this as a rare tumor type although fibrosarcoma occurred in a control group 2 animal. If this finding is evaluated as a common tumor type, the p-values for skin and subcutis combined do not meet statistical significance per CDER criteria.

Non Neoplastic:

As summarized in the Applicant's table below, there were treatment-related non-neoplastic findings at ≥7 mg/kg/day INT-747. In the liver, there were dose-dependent increases in the incidence and/or severity of hepatocellular vacuolation. In the kidney, a dose-related increase in the incidence and/or severity of chronic progressive nephropathy was observed.

Text Table 14. Incidence of Selective Non-proliferative Lesions in Males and Females

	MALES					FEMALES				
Dosage (mg/kg/day):	0	0	2	7	20	0	0	2	7	20
Liver [a]	65	65	65	65	65	65	65	65	65	65
Vacuolation	11	13	14	23	33	16	15	8	18	32
Minimal	4	4	3	8	6	11	11	4	3	12
Mild	3	5	6	7	8	4	4	2	11	12
Moderate	3	4	5	8	16	1	0	2	3	7
Severe	1	0	0	0	3	0	0	0	1	1
Kidney [a]	65	65	65	65	65	65	65	65	65	65
Nephropathy, chronic progressive	52	48	51	56	60	26	28	32	39	51
Minimal	11	13	20	10	5	15	15	14	15	13
Mild	22	20	15	19	13	8	8	9	17	24
Moderate	8	8	7	13	28	1	4	7	5	11
Severe	11	7	9	14	14	2	1	2	2	3

[a] - Number of tissues examined from each group.

Toxicokinetics

Blood samples for TK analysis were collected from Group 2A at 2 h after dosing on Day 0, and at 3, 6, and 12 months. Samples were collected from Groups 3A, 4A, and 5A at 1, 6, 12, and 24 h after dosing on Day 0 and at 3 and 6 months. At 12 months, samples were collected from Groups 3A, 4A, and 5A at 1, 6, 12, 24, 36, and 48 h after dosing. Plasma samples were analyzed for INT-747, UPF-832 (the glycine conjugate of INT-747), and UPF-1443 (the taurine conjugate of INT-747) using an ultra-high performance

liquid chromatography tandem mass spectrometry (UHPLC/MS/MS) method in the negative electrospray ionization (ESI) mode.

Results: Systemic exposure to INT-747 and UPF-1443 was demonstrated at all dose levels, and there was accumulation of the parent compound and its taurine conjugate over the duration of the study. Exposures to UPF-832 were demonstrated only at higher doses, and were lower than UPF-1443. Exposures to INT-747 (based on AUC values) were higher in males than females; whereas, exposures to UPF-1443 (based on AUC values) were greater in females than males. Reportable terminal half-life values for INT-747 and UPF-1443 ranged from 6 to 9 h. TK for INT-747, UPF-1443, and UPF-832 are summarized in the Applicant's tables below.

Table TK1: Toxicokinetic Parameters for INT-747 in Rats following Oral Administration of INT-747 at 2, 7, and 20 mg/kg/day

Gender	Males			Females		
INT-747 Dosage (mg/kg/day):	2	7	20	2	7	20
Parameter (Units)			Study Day 0			
AUC_{last} (ng·h/mL)	1470	4750	20900	321	2890	10600
AUC_{0-24} (ng·h/mL)	1470	4750	20900	321	2890	10600
C_{max} (ng/mL)	283	823	5030	69.1	330	2480
T_{max} (h)	1	1	1	1	1	1
$T_{1/2}$ (h)	NR	NR	NR	NR	NR	NR
			Study Day 89			
AUC_{last} (ng·h/mL)	3020	10000	27100	458	3130	7040
AUC_{0-24} (ng·h/mL)	3020	10000	27100	646†	3130	7050
C_{max} (ng/mL)	297	984	2910	67.3	218	1000
T_{max} (h)	1	6	6	1	1	1
$T_{1/2}$ (h)	NR	NR	NR	NR	7.4	NR
Accumulation Ratio	2.1	2.1	1.3	2.0	1.1	0.67
			Study Day 179			
AUC_{last}(ng·h/mL)	2350	8700	45700	2050	2930	17500
AUC_{0-24} (ng·h/mL)	2350	8700	45700	2050	2930	17500
C_{max} (ng/mL)	326	806	3230	169	359	1720
T_{max} (h)	1	1	6	24	1	1
$T_{1/2}$ (h)	5.8	NR	NC	NC	8.6	NC
Accumulation Ratio	1.6	1.8	2.2	6.4	1.0	1.6
			Study Day 368			
AUC_{last} (ng·h/mL)	9300	17300	45500	1880	4460	21800
AUC_{0-24} (ng·h/mL)	5990	13600	30200	1610	2830	13100
C_{max} (ng/mL)	564	1240	2400	145	273	746
T_{max} (h)	6	6	1	6	1	1
$T_{1/2}$ (h)	NR	NR	NR	NR	NR	NR
Accumulation Ratio	4.1	2.9	1.4	5.0	0.98	1.2

Units for dose-normalized AUC_{last} are (ng·h/mL)/(mg/kg); units for dose-normalized C_{max} are (ng/mL)/(mg/kg).
NR = Not reportable, failed to meet acceptance criteria as indicated in Section 1.1, Definitions; NC = Not calculable.
†Value is considered to be an approximation, failed to meet acceptance criteria as indicated in Section 1.1, Definitions.

Table TK2: Toxicokinetic Parameters for INT-747 Taurine Conjugate (UPF-1443) in Rats following Oral Administration of INT-747 at 2, 7, and 20 mg/kg/day

Gender	Males			Females		
INT-747 Dosage (mg/kg/day):	2	7	20	2	7	20
Parameter (Units)			Study Day 0			
AUC_{last} (ng·h/mL)	323	869	2310	336	820	2600
AUC_{0-24} (ng·h/mL)	323	869	2310	336	820	2600
C_{max} (ng/mL)	19.0	61.4	162	20.8	58.0	179
T_{max} (h)	12	6	6	6	6	6
$T_{1/2}$ (h)	NC	7.6	6.3	NR	8.0	NR
Metabolite/Parent Ratio	0.18	0.15	0.09	0.83	0.23	0.20
			Study Day 89			
AUC_{last} (ng·h/mL)	567	1060	3320	699	2010	5910
AUC_{0-24} (ng·h/mL)	567	1060	3320	699	2020	5910
C_{max} (ng/mL)	27.1	58.6	183	43.3	99.1	346
T_{max} (h)	12	12	12	12	6	6
$T_{1/2}$ (h)	NC	NC	NC	NC	NR	NR
Accumulation Ratio	1.8	1.2	1.4	2.1	2.5	2.3
Metabolite/Parent Ratio	0.15	0.08	0.10	1.2	0.51	0.67
			Study Day 179			
AUC_{last} (ng·h/mL)	1320	3140	5140	1850	5300	11900
AUC_{0-24} (ng·h/mL)	1320	3140	5140	1850	5300	11900
C_{max} (ng/mL)	74.8	193	398	128	276	606
T_{max} (h)	1	12	6	1	12	12
$T_{1/2}$ (h)	NR	NC	8.5	NR	NC	NC
Accumulation Ratio	4.1	3.6	2.2	5.5	6.5	4.6
Metabolite/Parent Ratio	0.45	0.29	0.09	0.72	1.4	0.54
			Study Day 368			
AUC_{last} (ng·h/mL)	4910	11500	15800	5530	11600	29800
AUC_{0-24} (ng·h/mL)	2660	8770	13700	3100	7340	18000
C_{max} (ng/mL)	215	888	1200	215	434	917
T_{max} (h)	24	1	12	6	1	6
$T_{1/2}$ (h)	NR	NR	NR	NR	NR	NR
Accumulation Ratio	8.2	10	5.9	9.2	8.9	6.9
Metabolite/Parent Ratio	0.42	0.53	0.28	2.3	2.1	1.1

NR = Not reportable, failed to meet acceptance criteria as indicated in Section 1.1, Definitions; NC = Not calculable.

Table TK3: Toxicokinetic Parameters for INT-747 Glycine Conjugate (UPF-832) in Rats following Oral Administration of INT-747 at 2, 7, and 20 mg/kg/day

Gender	Males			Females		
INT-747 Dosage (mg/kg/day):	2	7	20	2	7	20
Parameter (Units)			Study Day 0			
AUC_{last} (ng·h/mL)	92.4	285	759	NA	NA	NA
AUC_{0-24} (ng·h/mL)	92.4	285	759	NA	NA	NA
C_{max} (ng/mL)	10.5	24.4	76.3	NA	NA	NA
T_{max} (h)	6	6	6	NA	NA	NA
$T_{1/2}$ (h)	NR	8.3	NR	NA	NA	NA
Metabolite/Parent Ratio	0.06	0.05	0.03	NA	NA	NA
			Study Day 89			
AUC_{last} (ng·h/mL)	NA	NA	NA	NA	NA	43.3
AUC_{0-24} (ng·h/mL)	NA	NA	NA	NA	NA	43.3
C_{max} (ng/mL)	NA	NA	NA	NA	NA	4.15
T_{max} (h)	NA	NA	NA	NA	NA	6
$T_{1/2}$ (h)	NA	NA	NA	NA	NA	NR
Accumulation Ratio	NA	NA	NA	NA	NA	NA
Metabolite/Parent Ratio	NA	NA	NA	NA	NA	0.005
			Study Day 179			
AUC_{last} (ng·h/mL)	NA	NA	74.3	NA	NA	88.9
AUC_{0-24} (ng·h/mL)	NA	NA	74.3	NA	NA	88.9
C_{max} (ng/mL)	NA	NA	7.65	NA	NA	3.84
T_{max} (h)	NA	NA	6	NA	NA	12
$T_{1/2}$ (h)	NA	NA	NR	NA	NA	NC
Accumulation Ratio	NA	NA	0.10	NA	NA	NA
Metabolite/Parent Ratio	NA	NA	0.001	NA	NA	0.004
			Study Day 368			
AUC_{last} (ng·h/mL)	NA	79.1	103	NA	38.0	255
AUC_{0-24} (ng·h/mL)	NA	71.4	86.3	NA	25.3	150
C_{max} (ng/mL)	NA	7.27	5.26	NA	2.02	8.87
T_{max} (h)	NA	1	6	NA	6	6
$T_{1/2}$ (h)	NA	NR	NR	NA	19	10
Accumulation Ratio	NA	0.25	0.11	NA	NA	NA
Metabolite/Parent Ratio	NA	0.004	0.002	NA	0.008	0.010

NR = Not reportable, failed to meet acceptance criteria as indicated in Section 1.1, Definitions; NC = Not calculable.
NA = Not applicable; inadequate data to assess parameters.

Dosing Solution Analysis

Samples were collected from the test article dosing solutions used for Weeks 0, 1, and 77 for homogeneity and concentration determination. The concentration of test article in the dosing solutions for all groups (including control) were determined during Weeks 1, 2, 3, 8, 12, and every 3 months thereafter (during Weeks 25, 36, 52, 65, 90, and 103). For assessment of stability, samples of the test article dosing solutions were collected during Week 0 and stored at room temperature for 24 and 48 h. Resuspension homogeneity and stability were also evaluated for the test article dosing solutions from Week 0 following refrigerated storage for 10 days.

Results: Samples from the mid- and high dose dosing solutions from Week 0 had relative standard deviation (RSD) values >10%, and did not meet SOP specifications for homogeneity. The concentration of the mid-dose dosing solution from Week 0 was 122% of target. After storage for 48 h at room temperature and 10 days refrigerated, the Group 4 dosing solution concentrations were <90% of the pre-storage values. Mean analyzed concentrations in the 48 h room temperature and 10 day refrigerated samples were 99.7% and 105% of target. None of the deviations were considered to impact the study given that the isolated incidents and the small magnitude of the deviations.

Study title: A 24-Month Oral (Gavage) Carcinogenicity Study of INT-747 in Mice

Study no.:	(b) (4) 661038
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 14, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, Lot # T11-001, Purity 97.7%
CAC concurrence:	Yes (ECAC meeting minutes re: dose selection dated October 6, 2011, Attachment-1)

Key Study Findings

- High dose females had a statistically significant decrease in morality, compared to controls.
- In males and females, there were no drug-related neoplastic findings at up to 25 mg/kg/day INT-747. (ECAC meeting minutes dated September 10, 2015, Attachment-2)

Adequacy of Carcinogenicity Study

The carcinogenicity study conduct was acceptable. The dose selection was based on recommendations from the Executive CAC (October 6, 2011). Based on the mortalities observed at 80 mg/kg/day in a 13-week toxicity study in CD-1 mice, the Division and CDER Executive Carcinogenicity Assessment Committee (ECAC) recommended a high dose of 25 mg/kg/day (about 1/3rd the lethal dose).

Appropriateness of Test Models

The Crl:CD1 mouse is an acceptable test model for the carcinogenicity study.

Evaluation of Tumor Findings

FDA Statistical Analysis of Tumor Findings: Tumor analyses consisted of trend analyses for dose response relationship in tumor incidence and pairwise comparisons of tumor incidences in individual treatment groups with combined control groups. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-k method. The tumor test was based on the pooled control groups, and tumor combinations were also evaluated as done by the Applicant based on McConnell et al. (1986). All tumors presented as "#X TUMOR" or "#TUMOR, MULTIPLE" were combined for statistical analysis. For the adjustment of multiple testing of dose response relationship, common and rare tumors were tested at significance levels of 0.005 and 0.025, respectively. For multiple pairwise comparisons of treated groups

Reference ID: 3870157

with controls, test levels of 0.01 and 0.05 were used for common and rare tumors, respectively.

In males and females, there were no drug-related neoplastic findings at up to 25 mg/kg/day INT-747.

Methods

Doses:	0, 4, 10, 25 mg/kg/day (see Applicant's table below)
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral (Gavage)
Formulation/Vehicle:	0.5% carboxymethylcellulose (CMC) in deionized water
Basis of dose selection:	Maximum tolerated dose (MTD); Deaths occurred at 80 mg/kg/day in a 13-week toxicity and TK study in mice (Study No (b) (4) 661025).
Species/Strain:	Crl:CD1 mice
Number/Sex/Group:	Toxicology: 65/sex/group (Groups 1-5) Satellite: 9/sex (Group 2A); 40/sex (Groups 3A, 4A, and 5A)
Age:	Approximately 6 weeks of age at initiation of dosing
Animal housing:	Individual
Paradigm for dietary restriction:	No diet restriction
Dual control employed:	Yes (2 identical vehicle control groups)
Interim sacrifice:	No
Satellite groups:	Yes (for TK analysis)
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

The following tables copied from the Applicant's study report show the study group assignments.

Toxicology Groups (b) (4) 661038M and (b) (4) 561038F)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals [a] Males	Females
1	Vehicle [b]	0	10	65	65
2	Vehicle [b]	0	10	65	65
3	INT-747	4	10	65	65
4	INT-747	10	10	65	65
5	INT-747	25	10	65	65

[a] = All animals were euthanized following a minimum of 728 days of dose administration.
[b] = 0.5% CMC, medium viscosity, in deionized water.

Toxicokinetic Groups (b) (4) 561038A and (b) (4) 561038B)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dose Volume (mL/kg)	Number of Animals [a] Males	Females
2A	Vehicle [b]	0	10	9	9
3A	INT-747	4	10	40	40
4A	INT-747	10	10	40	40
5A	INT-747	25	10	40	40

[a] = All animals were euthanized following blood collection.
[b] = 0.5% CMC, medium viscosity, in deionized water.

Observations and Results

Mortality

All animals were observed twice daily for signs of morbidity and mortality.

Results: The Applicant's table below summarizes survival in males and females. There was a higher survival rate in the high dose female group, compared to pooled controls (p=0.0098). The difference was statistically significant in the trend test (p=0.0095) for the pooled controls and control groups 1 (p=0.0240) and 2 individually (p=0.0036). The higher survival rate was considered to be treatment-related, and likely related to effects on body weight in the high dose female treatment group relative to controls. Mortalities occurring in the study did not appear to be related to treatment with INT-747.

Text Table 4. Survivability of Males and Females to Terminal Euthanasia

Dosage (mg/kg/day)	0	0	4	10	25
Males [a]	**65**	**65**	**65**	**65**	**65**
Number survival	21	29	25	21	25
Number of animals found dead or euthanized in extremis	44	35	39	43	40
Accidental deaths	0	1	1	1	0
% survival [b]	32%	45%	39%	33%	38%
Females [a]	**65**	**65**	**65**	**65**	**65**
Number survival	27	24	25	28	41
Number of animals found dead or euthanized in extremis	38	40	40	36	24
Accidental deaths	0	1	0	1	0
% survival [b]	42%	38%	38%	44%	63%

[a] = number of animals per dose group
[b] = % survival was corrected based on the number of accidental deaths

Independent analysis of survival data by the FDA statistical reviewer (F. Zhou) showed that respective survival rates in the control 1, control 2, low dose, mid dose, and high dose groups, respectively, at termination (Week 104) were 34%, 46%, 40%, 34%, and 38% in males and 42%, 37%, 38%, 43%, and 63% in females. High dose females had a statistically significant decrease in mortality when compared to the pooled controls. Results of the intercurrent mortality comparison for male and female mice are shown below in Tables 4A and 4B, respectively, (copied from the FDA statistical review).

Table 4A: Intercurrent Mortality Comparison – Male Mice

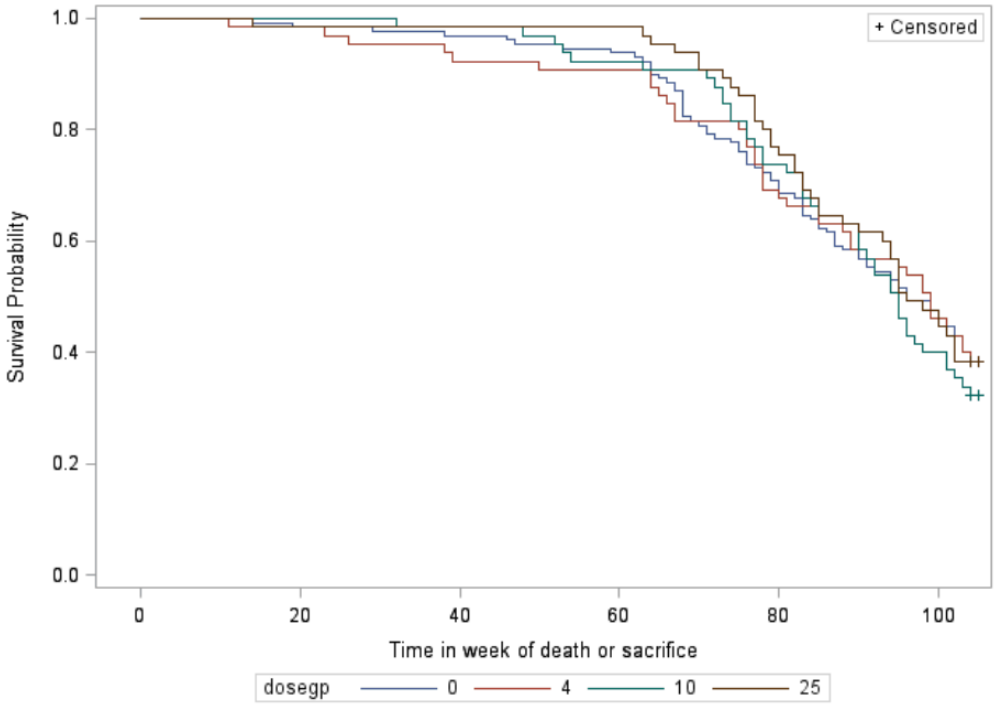
Test	Statistic	P-Value (Pooled Controls)	P-Value (Control-1)	P-Value (Control-2)
Dose-Response	Likelihood Ratio	0.8332	0.3703	0.7072
Homogeneity	Log-Rank	0.9026	0.6596	0.6026

Table 4B: Intercurrent Mortality Comparison – Female Mice

Test	Statistic	P-Value (Pooled Controls)	P-Value (Control-1)	P-Value (Control-2)
Dose-Response	Likelihood Ratio	0.0021	0.0064	0.0009
Homogeneity	Log-Rank	0.0174	0.0264	0.0136

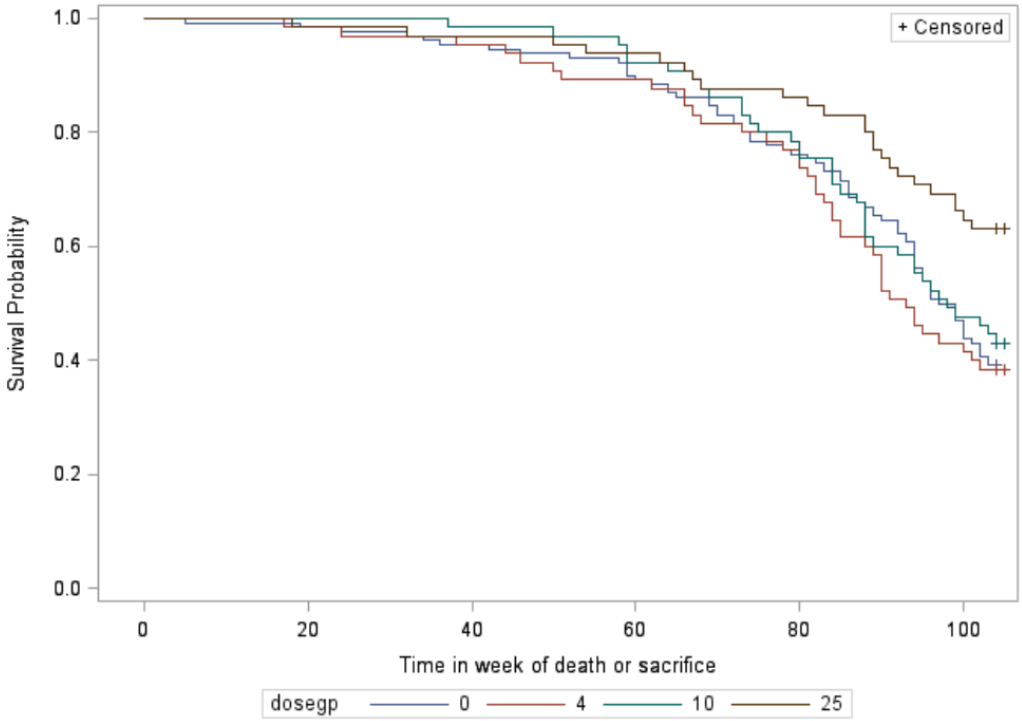
The Kaplan-Meier curves for survival rates of all treatment groups (from the FDA statistical review) are shown below in Figures 2A and 2B for male and female mice, respectively.

Figure 2A: Kaplan-Meier Survival Functions for Male Mice



Note: dose group should be 0, 4, 10, or 25-mg/kg/day

Figure 2B: Kaplan-Meier Survival Functions for Female Mice



Note: dose group should be 0, 4, 10, or 25-mg/kg/day

Clinical Signs

Toxicology animals were observed for clinical signs once daily at the time of dosing. Detailed examinations were conducted weekly (including examination for the presence of palpable masses). The time of onset, location, size, appearance, and progression of palpable masses were recorded.

Results: There were no treatment-related clinical observations.

Body Weights

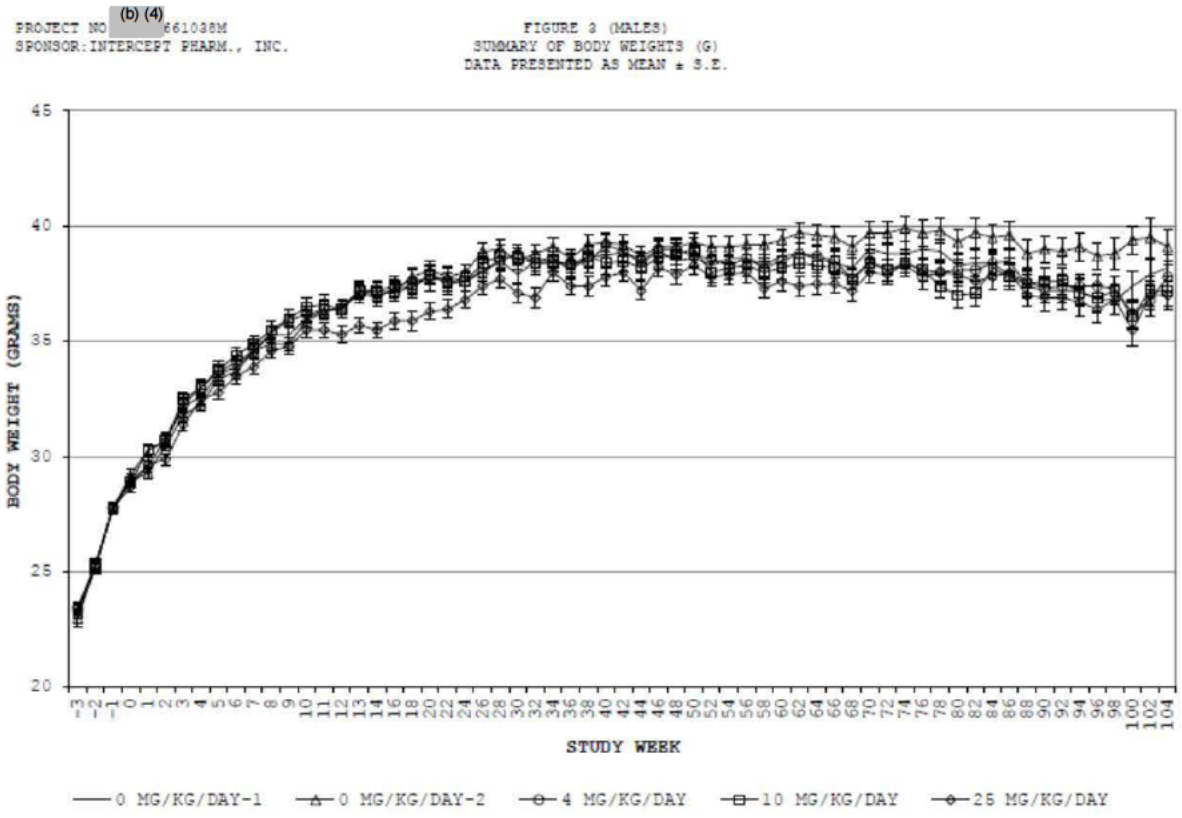
Body weights were recorded weekly beginning during the acclimation period through Week 14, and approximately every two weeks thereafter.

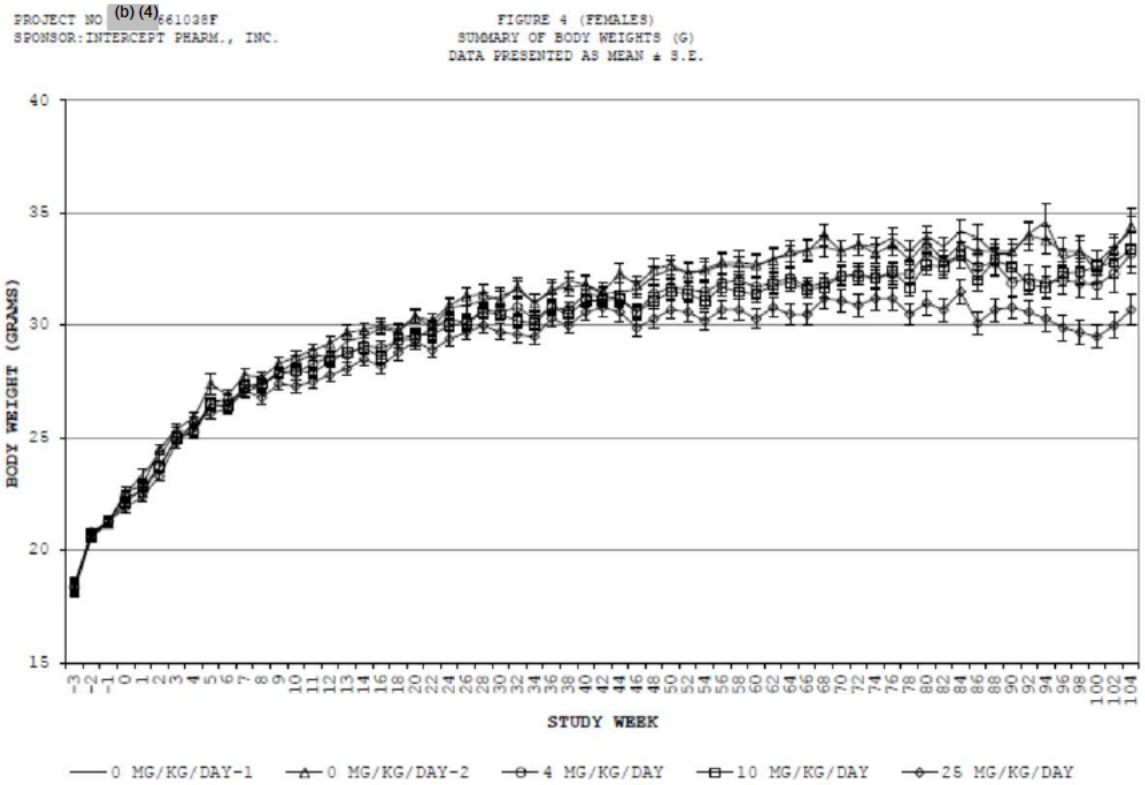
Results:
In males, there were no apparent effects on body weight or body weight gain. In high dose males, there was a small but statistically significant transient decrease in body weights (≤5% difference relative to controls) primarily from Weeks 13 through 18. At Week 104, body weights at 25 mg/kg/day were similar to controls.

In females, treatment with INT-747 produced lower body weights and body weight gains (compared to controls). While there were statistically significant differences at a subset of the timepoints at all dose levels, the effects primarily occurred in the 25 mg/kg/day dose group INT-747 beginning during Week 10. At Week 104, female body weights at 25 mg/kg/day were 10% and 11% lower than those of control groups 1 and 2,

respectively. Over the duration of the study, body weight gain in the 25 mg/kg/day female group was 25% lower than control groups 1 and 2.

The figures below show mean body weights in males and females over the course of the study period (Applicant's Figures 3 and 4).





Feed Consumption

Food consumption was recorded weekly during the acclimation period through Week 14, and approximately every two weeks thereafter.

Results:
In males, there were transient, statistically significant decreases in food consumption (compared to controls) which occurred primarily at the high dose during a subset of timepoints. Reductions in food consumption in INT-747 treated females (compared to controls) were of greater magnitude than those in males, and considered to be treatment-related. Although there were statistically significant differences at a subset of the timepoints at all dose levels, the effects occurred primarily in females at 10 and 25 mg/kg/day. During Weeks 103 to 104, mean food consumption at 10 and 25 mg/kg/day was 10% and 8% lower than that of the combined control groups. The decreases in food consumption in females at 25 mg/kg/day correlated with effects on body weight.

Clinical Pathology

Blood samples were collected for possible diagnosis of hematopoietic disorders from all animals euthanized in extremis (when possible) and all surviving rats at scheduled necropsy (toxicology groups only). Blood smears were prepared.

Results: Blood samples and smears were not evaluated.

Ophthalmic Examinations

Ophthalmic examinations were conducted on all toxicology animals during Weeks 46, 64, and 76.

Results: There were no treatment-related findings.

Gross Pathology

Complete necropsies (including examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities including viscera) were conducted on all toxicology animals.

Results:
At 25 mg/kg/day, there was a higher incidence of renal cysts [31/65(48%)] in males, as compared to control groups 1 and 2 [16/65(25%) and 20/65(31%), respectively).

Histopathology

The Applicant's table copied below lists the organs and tissues collected at necropsy and preserved for histopathological examination (toxicology animals only).

Adrenals (2)	Lymph nodes
Aorta	Axillary (2)
Bone with marrow	Mandibular (2)
Femur	Mesenteric
Sternum	Ovaries (2) with oviducts [e]
Bone marrow smear (from femur) [a]	Pancreas
Brain [b]	Peripheral nerve (sciatic)
Cervix	Peyer's patches
Clitoral glands (2)	Pharynx
Epididymides (2) [c]	Pituitary
Eyes with optic nerves (2) [d]	Preputial glands (2)
Gallbladder	Prostate
Gastrointestinal tract	Salivary glands [mandibular (2)]
Esophagus	Seminal vesicles (2)
Stomach	Skeletal muscle (rectus femoris)
Duodenum	Skin with mammary gland [f]
Jejunum	Spinal cord (cervical, mid-thoracic, lumbar)
Ileum	Spleen
Cecum	Teeth
Colon	Testes (2) [c]
Rectum	Thymus
Harderian glands (2)	Thyroid with parathyroids (2) [e]
Heart	Tongue
Kidneys (2)	Trachea
Larynx	Urinary bladder
Liver (sections of 2 lobes)	Uterus
Lungs (including bronchi, fixed by inflation with fixative)	Vagina
	Zymbal's gland (2)
	All gross lesions including masses (when possible)

[a] - These slides were not placed in formalin and were not examined because not scientifically warranted. Bone marrow smears were not taken from animals found dead.
[b] - Histologic trimming of the brain was performed per (b) (4) Standard Operating Procedure (SOP) such that a minimum of 5 cross (coronal) sections (possibly containing medulla oblongata) of the brain were taken.
[c] - Fixed in Bouins solution.
[d] - Fixed in Davidson's solution.
[e] - Oviducts and parathyroids were examined microscopically when in the plane of section and in all cases where a gross lesion was present.
[f] - For females; a corresponding section of skin was taken from the same anatomical area for males.

Microscopic examination of all tissues listed above was conducted.

Peer Review: A histopathology peer review was conducted.

Neoplastic:

Males:
In males, there were no statistically significant neoplastic findings at up to 25 mg/kg/day INT-747 based on either the Applicant's or FDA statistical reviewer's analysis.

Females:
In females, there was an increasing trend for hepatocellular carcinoma, compared to the pooled control groups [control 1/130; low dose 0/65; mid dose 1/65; high dose 4/65; p=0.0119 (FDA statistics review)]. While the pairwise comparison showed a numerically increased incidence of hepatocellular carcinomas at the high dose compared to the pooled controls, this did not meet statistical significance as per CDER criteria (p=0.0584; FDA statistics review). In addition, when evaluated as a common tumor (based on the occurrence of hepatocellular carcinoma in a control group 2 animal), the p-values for trend analysis and pairwise comparison (high dose versus pooled controls) do not meet CDER criteria for statistical significance.

Non Neoplastic:

In males, dosing at ≥10 mg/kg/day INT-747 produced an increased incidence and/or severity of non-neoplastic findings in the liver (centrilobular cytomegaly and karyomegaly) and Steno gland of the nasal cavity (ductal hyperplasia), compared to controls (see Applicant's tables below). In high dose males, treatment-related findings were also observed in the middle ear (suppurative exudate) and nasal cavity (suppurative exudate and degeneration of the olfactory epithelium).

In females, there was an increase in the incidence and/or severity of suppurative exudate in the middle ear and nasal cavity and degeneration of the olfactory epithelium at ≥4 mg/kg/day, compared to controls. While these effects did not follow a strict dose relationship (effects were greater in the low dose group, compared to the mid dose group), these were clearly treatment-related findings at the high dose. An increased incidence and severity of ductal hyperplasia in the Steno glands (nasal) occurred at ≥10 mg/kg/day. INT-747 produced centrilobular cytomegaly and karyomegaly in the liver of high dose females.

Reference ID: 3870157

Text Table 10. Incidence of Selected Histopathologic Findings; All Animals

	Males					Females				
Dosage (mg/kg/day):	**0**	**0**	**4**	**10**	**25**	**0**	**0**	**4**	**10**	**25**
Liver [a]	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**
Cytomegaly & karyomegaly, centrilobular										
All[b]	9	16	14	22	46	3	0	2	2	14
UD[c]	4	11	9	15	28	2	-	1	0	3
SN[d]	5	5	5	7	18	1	-	1	2	11
Middle ear [a]	**65**	**65**	**65**	**65**	**65**	**64**	**65**	**64**	**65**	**65**
Exudate, suppurative										
All[b]	2	2	5	4	13	3	3	7	4	12
UD[c]	2	2	5	4	11	2	3	5	4	6
SN[d]	0	0	0	0	2	1	0	2	0	6
Nasal cavity [a]	**65**	**60**	**62**	**62**	**58**	**61**	**62**	**64**	**64**	**65**
Exudate, suppurative										
All[b]	2	4	5	5	16	5	5	13	8	18
UD[c]	2	4	5	5	13	5	5	12	7	5
SN[d]	0	0	0	0	3	0	0	1	1	13
Degeneration, olfactory epithelium										
All[b]	1	1	0	1	9	0	1	3	1	9
UD[c]	1	1	-	1	5	-	1	3	0	2
SN[d]	0	0	-	0	4	-	0	0	1	7
Steno glands (nasal) [a]	**39**	**19**	**27**	**22**	**25**	**24**	**21**	**27**	**31**	**25**
Hyperplasia, ductal										
All[b]	16	8	11	15	20	7	6	5	21	19
UD[c]	9	7	8	12	18	7	5	4	12	7
SN[d]	7	1	3	3	2	0	1	1	9	12

[a] = Number of tissues examined from each group.
[b] = All= all intervals combined (UD and SN).
[c] = UD = unscheduled deaths.
[d] = SN = scheduled necropsy.

Text Table 11. Incidence of Selected Histopathologic Findings; Unscheduled Deaths

	Males					Females				
Dosage (mg/kg/day):	**0**	**0**	**4**	**10**	**25**	**0**	**0**	**4**	**10**	**25**
Liver [a,b]	**44**	**36**	**40**	**44**	**40**	**38**	**41**	**40**	**37**	**24**
Cytomegaly & karyomegaly, centrilobular	4	11	9	15	28	2	-	1	-	3
Minimal	4	11	8	11	17	2	-	1	-	3
Mild	0	0	1	2	9	0	-	0	-	0
Moderate	0	0	0	2	2	0	-	0	-	0
Middle ear [a]	**44**	**36**	**40**	**44**	**40**	**37**	**41**	**39**	**37**	**24**
Exudate, suppurative	2	2	5	4	11	2	3	5	4	6
Minimal	1	2	3	4	6	2	1	4	3	3
Mild	1	0	1	0	3	0	2	0	1	2
Moderate	0	0	1	0	2	0	0	1	0	1
Nasal cavity [a]	**44**	**36**	**40**	**43**	**40**	**38**	**40**	**40**	**37**	**24**
Exudate, suppurative	2	4	5	5	13	5	5	12	7	5
Minimal	1	3	5	3	8	5	4	10	7	3
Mild	1	1	0	2	4	0	1	1	0	1
Moderate	0	0	0	0	1	0	0	1	0	1
Degeneration, olfactory epithelium	1	1	0	1	5	0	1	3	0	2
Minimal	1	0	-	0	3	-	1	1	-	0
Mild	0	1	-	1	1	-	0	1	-	1
Moderate	0	0	-	0	0	-	0	1	-	0
Severe	0	0	-	0	1	-	0	0	-	1
Steno glands (nasal) [a]	**25**	**12**	**23**	**17**	**23**	**18**	**16**	**23**	**19**	**12**
Hyperplasia, ductal	9	7	8	12	18	7	5	4	12	7
Minimal	8	7	7	6	11	7	5	4	10	3
Mild	1	0	1	2	4	0	0	0	1	0
Moderate	0	0	0	3	0	0	0	0	1	3
Severe	0	0	0	1	3	0	0	0	0	1

[a] = Number of tissues examined from each group.
[b] = Number of animals examined from each group.

Text Table 12. Incidence of Selected Histopathologic Findings; Scheduled Necropsy

	Males					Females				
Dosage (mg/kg/day):	**0**	**0**	**4**	**10**	**25**	**0**	**0**	**4**	**10**	**25**
Liver [a,b]	**21**	**29**	**25**	**21**	**25**	**27**	**24**	**25**	**28**	**41**
Cytomegaly & karyomegaly, centrilobular	5	5	5	7	18	1	0	1	2	11
Minimal	5	4	5	6	14	1	-	1	2	11
Mild	0	0	0	0	4	0	-	0	0	0
Moderate	0	1	0	1	0	0	-	0	0	0
Middle ear [a]	**21**	**29**	**25**	**21**	**25**	**27**	**24**	**25**	**28**	**41**
Exudate, suppurative	0	0	0	0	2	1	0	2	0	6
Minimal	-	-	-	-	1	0	-	1	-	1
Mild	-	-	-	-	1	1	-	1	-	2
Moderate	-	-	-	-	0	0	-	0	-	3
Nasal cavity [a]	**21**	**24**	**22**	**19**	**18**	**23**	**22**	**24**	**27**	**41**
Exudate, suppurative	0	0	0	0	3	0	0	1	1	13
Minimal	-	-	-	-	2	-	-	0	1	8
Mild	-	-	-	-	1	-	-	1	0	4
Moderate	-	-	-	-	0	-	-	0	0	1
Degeneration, olfactory epithelium	0	0	0	0	4	0	0	0	1	7
Minimal	-	-	-	-	1	-	-	-	1	2
Mild	-	-	-	-	1	-	-	-	0	1
Moderate	-	-	-	-	0	-	-	-	0	1
Severe	-	-	-	-	2	-	-	-	0	3
Steno glands (nasal) [a]	**14**	**7**	**4**	**5**	**2**	**6**	**5**	**4**	**12**	**13**
Hyperplasia, ductal	7	1	3	3	2	0	1	1	9	12
Minimal	6	1	2	1	0	-	1	1	6	7
Mild	0	0	1	1	0	-	0	0	3	1
Moderate	1	0	0	1	0	-	0	0	0	2
Severe	0	0	0	0	2	-	0	0	0	2

[a] = Number of tissues examined from each group.
[b] = Number of animals examined from each group.

In addition to the findings summarized above, there was an increased incidence of renal cysts in high dose males [41/65(63%), compared to 25/65(38%) and 29/65(45%) in control groups 1 and 2, respectively) which correlated with macroscopic findings. For reference, the mean and maximum historical control incidences of cysts based on microscopic evaluation of the kidneys were 20% and 57%, respectively. There also was an increased incidence of reduced sperm in the lumen of the epididymides at 25 mg/kg/day [20/65(31%), compared to 8/65(12%) and 9/65(14%) in control groups 1 and 2, respectively). This finding was generally observed in a unilateral manner. Finally, in the livers of high dose females, the incidence of mononuclear cell infiltrates [28/65(43%)] and minimal to mild subacute inflammation [27/65(42%)] was increased as compared to controls. The overall incidence of mononuclear cell infiltrates and subacute inflammation in control groups 1 and 2 ranged from 25-31% and 23-28%, respectively.

Toxicokinetics

Blood samples for TK analysis were collected from Group 2A at 2 h after dosing on Day 0. Blood samples from Groups 3A, 4A, and 5A were collected at 1, 6, 12, 24, 36, and 48 h after dosing on Day 0 and at 12 months. Plasma samples were analyzed for INT-

747, UPF-1443 (the taurine conjugate of INT-747), and UPF-832 (the glycine conjugate of INT-747) using an ultra-high performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) method in the negative electrospray ionization mode (ESI).

Results: Systemic exposure to UPF-1443 was demonstrated at all dose levels, and there was accumulation of this metabolite over the duration of the study. Exposures to the parent compound (INT-747) were measured only at the mid- and high dose level on Day 363, and INT-747 exposures were much lower than UPF-1443 (metabolite:parent ratios ranged from 44.9 to 92.1 at the high dose). Concentrations of UPF-832 were only measurable in single samples from males administered 10 and 25 mg/kg/day and females dosed at 10 mg/kg/day on Day 363. Toxicokinetic parameters for INT-747 and UPF-1443 are summarized in the Applicant's tables below.

Table TK6: Toxicokinetic Parameters for INT-747 in Mice

Gender	Males			Females		
INT-747 Dosage (mg/kg/day):	4	10	25	4	10	25
Parameters (Units)						
Study Day 0						
AUC_{last} (ng·h/mL)	NA	NA	NA	NA	NA	NA
Dose Normalized AUC_{last}	NA	NA	NA	NA	NA	NA
C_{max} (ng/mL)	NA	NA	NA	NA	NA	NA
Dose Normalized C_{max}	NA	NA	NA	NA	NA	NA
T_{max} (h)	NA	NA	NA	NA	NA	NA
$T_{1/2}$ (h)	NA	NA	NA	NA	NA	NA
Study Day 363						
AUC_{last} (ng·h/mL)	NA	NA	649	NA	349	1300
Dose Normalized AUC_{last}	NA	NA	26.0	NA	34.9	52.0
AUC_{0-24} (ng·h/mL)	NA	NA	649	NA	349	991
C_{max} (ng/mL)	NA	NA	65.4	NA	40.8	143
Dose Normalized C_{max}	NA	NA	2.62	NA	4.08	5.72
T_{max} (h)	NA	NA	6	NA	1	1
$T_{1/2}$ (h)	NA	NA	NR	NA	7.8	NR

Units for dose-normalized AUC_{last} are (ng·h/mL)/(mg/kg); units for dose-normalized C_{max} are (ng/mL)/(mg/kg).
NA = Not applicable, data insufficient for toxicokinetic assessment.
NR = Not reportable, failed to meet acceptance criteria.

Table TK7: Toxicokinetic Parameters for INT-747 Taurine Conjugate (UPF-1443) in Mice

Gender	Males			Females		
INT-747 Dosage (mg/kg/day):	4	10	25	4	10	25
Parameters (Units)						
Study Day 0						
AUC_{last} (ng·h/mL)	2410	6630	13300	4260	8490	16300
Dose Normalized AUC_{last}	603	663	532	1065	849	652
AUC_{0-24} (ng·h/mL)	1690	4600	8680	3070	6840	12800
C_{max} (ng/mL)	132	265	624	207	527	761
Dose Normalized C_{max}	33.0	26.5	24.9	51.8	52.7	30.4
T_{max} (h)	6	12	6	6	1	6
Study Day 363						
AUC_{last} (ng·h/mL)	12900	33500	75000	14200	32700	73200
Dose Normalized AUC_{last}	3225	3350	3000	3550	3270	2928
AUC_{0-24} (ng·h/mL)	6850	17900	42700	9560	20800	45300
C_{max} (ng/mL)	376	926	2210	511	948	3230
Dose Normalized C_{max}	94.0	92.6	88.3	128	94.8	129
T_{max} (h)	12	6	6	12	6	6
Accumulation Ratio	5.4	5.1	5.6	3.3	3.9	4.5
Metabolite/Parent Ratio	NA	NA	92.1	NA	74.7	44.9

Units for dose-normalized AUC_{last} are (ng·h/mL)/(mg/kg); units for dose-normalized C_{max} are (ng/mL)/(mg/kg).
NA = Not applicable.

Dosing Solution Analysis

Samples were collected from the test article dosing solutions used during Week 0 for homogeneity and concentration determination. The concentration of test article in the dosing solutions for all groups (including controls) were determined during Weeks 1, 2, 3, 8, 17, and every 3 months thereafter (during Weeks 25, 36, 51, 65, 77, 90, and 103). For stability assessment, test article dosing solution samples were collected during Week 0 and stored at room temperature for 24 h. Samples of test article dosing solutions from Week 0 were also evaluated for resuspension homogeneity and stability following 10 days of refrigerated storage.

Results: Concentrations of INT-747 were within ±15% of target concentration, and thus met the criteria specified in the SOP. The dosing formulations were also homogenous, and met the acceptance criteria for stability at room temperature for 24 h and refrigerated for 10 days.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Study of Fertility and Early Embryonic Development to Implantation of INT-747 in Rats

Study no.:	(b) (4) 661005
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 6, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, Lot# 1106002P, 97.1%

Key Study Findings

Treatment with 50 mg/kg/day INT-747 produced moribund condition in a single female rat. In all other females and males at all dose levels, INT-747 did not affect fertility or reproductive performance. The NOAELs for male and female systemic toxicity were 50 and 25 mg/kg/day, respectively. The NOAEL for male and female reproductive toxicity and early embryonic toxicity was 50 mg/kg/day

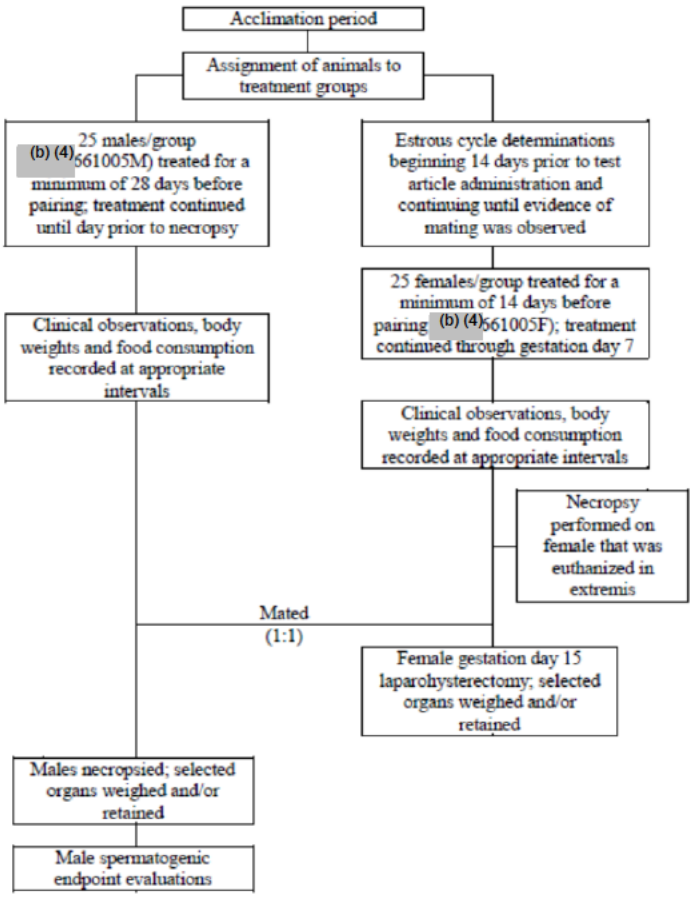
Methods

Doses:	5, 25, and 50 mg/kg/day
Frequency of dosing:	Daily. Male rats were dosed once daily for 28 days prior to mating, and throughout the mating period through 1 day prior to scheduled sacrifice (total dosing period of 58 to 59 days). Females received 14 daily doses prior to pairing, and were dosed through gestation day (GD) 7 (total dosing period of 22 to 26 days). A single female with no evidence of mating received 34 doses.
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% carboxymethylcellulose (CMC) in deionized water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	25/sex/group
Satellite groups:	None
Study design:	Shown below
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

The study design is shown in the Applicant's figure below.

Reference ID: 3870157

3. STUDY DESIGN



Observations and Results

Mortality

Animals were checked twice daily for signs of mortality and moribundity.

There were no deaths. However, a single female animal in the 50 mg/kg/day dose group was euthanized in extremis after a total of 34 doses (cause of moribundity undetermined). In this female, there was no evidence of mating and clinical signs included hunched posture, drooping eyelids, decreased defecation, yellow material on the urogenital, anogenital, and ventral abdominal areas, and red material on the forelimbs, nose, mouth, and eyes.

Clinical Signs

Clinical observations were recorded twice daily (at the time of dosing and 1 h post-dose).

Other than clinical signs (e.g., hunched posture, moribund condition) observed in the high dose female sacrificed in extremis, there were no treatment-related clinical signs.

Body Weight

Male body weights were recorded twice weekly. Female body weights were recorded twice weekly until evidence of copulation was observed, and then on GDs 0, 3, 6, 9, 12, and 15.

In males, there were no treatment-related effects on body weight. In females, there were statistically significant mean body weight losses and/or reduced mean body weight gain (compared to controls) during certain pre-mating study intervals. In addition, mean body weight gains were significantly decreased relative to controls over the entire pre-mating period (body weight gain from Day 14 – 25 was 13 g, 0 g, 1 g, and 3 g in the control, 5, 25, and 50 mg/kg/day dose groups, respectively). However, the differences were not dose-related and there were no statistically significant differences in mean body weights over the pre-mating period. During the gestation period, there were no treatment-related changes in body weight or body weight gain.

Feed Consumption

Food consumption was recorded twice weekly until pairing. Following mating, food consumption was measured twice weekly for males and at gestation intervals corresponding to body weights for females.

In males and females, there were no treatment-related effects on food consumption.

Toxicokinetics

Not conducted.

Dosing Solution Analysis

Dosing solution samples were collected for homogeneity determination during weeks 1 and 4. Samples were collected for stability and resuspension homogeneity following storage for 10 days. Samples for concentration analysis were collected during weeks 2, 5, and 8.

Following 10 days of storage of the dosing solution used for treatment of males during the first 7 days of dosing, dosing solutions for the mid- and high dose groups were 89.2 and 88.8% of the pre-storage concentrations. The post-storage concentration for the mid-dose was within the acceptable range (±15% of target), but the post-storage concentration for the high dose (82.5% of target) was outside the acceptable range. As a result, males were dosed at a concentration slightly lower than the target amount during the first 7 days of dosing. This was not considered to have a significant impact on the study since males were dosed for a total of 58-59 days.

Necropsy

Pregnant females were euthanized on GD15. Males were euthanized on day 15 following completion of mating. A complete necropsy was performed, including

Reference ID: 3870157

examination of external surfaces, all orifices, the cranial cavity, external surfaces of the brain and spinal cord, and the thoracic, abdominal, and pelvic cavities including viscera. The following tissues and organs were collected and preserved: cervix, coagulating glands, ovaries and oviducts, pituitary gland, prostate gland, seminal vesicles, testis with epididymis and vas deferens, uterus with vagina, and all gross lesions. The following organs were weighed and organ to final body weight and organ to brain weight ratios were calculated: brain, epididymides (total and cauda), ovaries, pituitary gland, and testes.

Other than yellow discoloration of the body noted for the single high dose female which was sacrificed prematurely, there were no treatment-related macroscopic findings. There were no treatment-related effects on organ weights.

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

Estrous Cycles
The estrous cycles of all females were determined for 14 days prior to initiation of dosing and until evidence of copulation was observed or termination (for females with no evidence of mating).

Mean estrous cycle lengths were similar in all treatment groups (range 4.1 to 4.2 days).

Breeding
Following 28 and 14 days of treatment for males and females, respectively, animals were paired on a 1:1 basis within each treatment group. If no evidence of copulation was observed after 10 days, then the female was mated with another male that had successfully mated for an additional 5 days. The following indices were calculated: male (female) mating index, male fertility index, male copulation index, female fertility index, and female conception index.

There were no treatment-related effects on male reproductive performance. The male mating index was 92%, 96%, 92%, and 96%, in the control, low-, mid-, and high dose groups, respectively. The fertility index was 92%, 92%, 92%, and 96%, respectively, for the control, low-, mid-, and high-dose groups, respectively. Finally, the copulation index was 100%, 95.8%, 100%, and 100%, respectively, for the control, low-, mid-, and high dose groups.

There were no statistically significant differences in female reproductive performance parameters compared to controls. A single low-dose female had evidence of mating, but was nongravid at necropsy. The high dose female which was sacrificed in extremis had no evidence of mating. The female mating index was 100, 100, 100, and 96%, respectively for the control, low-, mid-, and high dose groups. The fertility index was 100, 96, 100, and 96%, respectively, in the control, low-, mid-, and high dose groups. Finally, the conception index was 100, 96, 100, and 100%, respectively, in the control,

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low-, mid-, and high dose groups. The mean pre-coital intervals ranged from 2.6 – 3.8 days.

Spermatogenic Endpoint Evaluations

Sperm motility, sperm morphology, spermatid count, and sperm production rate were determined.

There were no statistically significant, test article-related effects on spermatogenic endpoints. Although not statistically significant, the percentage of sperm with normally shaped head separated from flagellum was slightly increased in high dose males (1.4%, compared to 0.3, 0.2, and 0.2% in control, low-, and mid-dose males, respectively). The mean percentage occurring in the high dose group (1.4%) was, however, within the range of historical control values (up to 1.8%). Furthermore, the mean percentage of normal sperm in this study was similar in all treatment groups (99.6, 99.8, 99.6, and 98.6% in control, low-, mid-, and high dose groups, respectively).

Uterine Examination

Pregnant females were euthanized on GD15. The following endpoints were assessed: evaluation of uteri without macroscopic evidence of implantation to identify early implantation loss, the number of corpora lutea, the number and location of all embryos, early resorptions, and the total number of implantation sites, and embryo viability. Group mean litter basis and proportional litter basis were calculated.

There were no statistically significant differences in preimplantation loss, postimplantation loss or on the numbers of corpora lutea, viable embryos, or implantation sites at up to 50 mg/kg/day, relative to controls. Summary data for these parameters are presented in the Applicant's tables below.

TABLE 31

PROJECT NO. (b) (4) 661005F ORAL FERT AND EARLY EMB DEV TO IMPLANTATION OF INT-747 IN RATS PAGE 1

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF EMBRYONIC DATA AT SCHEDULED NECROPSY

GROUP		VIABLE EMBRYOS	DEAD EMBRYOS	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	NO. OF GRAVID FEMALES
1	TOTAL	358	0	12	1	13	371	411	40	25
	MEAN	14.3	0.0	0.5	0.0	0.5	14.8	16.4	1.6	
	S.D.	3.11	0.00	0.59	0.20	0.65	3.09	2.00	2.71	
	S.E.	0.62	0.00	0.12	0.04	0.13	0.62	0.40	0.54	
2	TOTAL	340	0	23	1	24	364	416	52	24
	MEAN	14.2	0.0	1.0	0.0	1.0	15.2	17.3	2.2	
	S.D.	3.45	0.00	0.86	0.20	0.93	3.66	2.68	3.62	
	S.E.	0.70	0.00	0.18	0.04	0.19	0.75	0.55	0.74	
3	TOTAL	376	0	21	0	21	397	431	34	25
	MEAN	15.0	0.0	0.8	0.0	0.8	15.9	17.2	1.4	
	S.D.	3.78	0.00	0.85	0.00	0.85	3.38	2.42	2.60	
	S.E.	0.76	0.00	0.17	0.00	0.17	0.68	0.48	0.52	
4	TOTAL	363	0	23	0	23	386	405	19	24
	MEAN	15.1	0.0	1.0	0.0	1.0	16.1	16.9	0.8	
	S.D.	3.81	0.00	1.63	0.00	1.63	3.61	2.09	2.17	
	S.E.	0.78	0.00	0.33	0.00	0.33	0.74	0.43	0.44	

None significantly different from control group

MEAN NUMBER OF VIABLE EMBRYOS, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 5 MG/KG/DAY 3- 25 MG/KG/DAY 4- 50 MG/KG/DAY

TABLE 32

PROJECT NO. (b) (4) 661005F — ORAL FERT AND EARLY EMB DEV TO IMPLANTATION OF INT-747 IN RATS — PAGE 1

SPONSOR: INTERCEPT PHARM., INC. — SUMMARY OF EMBRYONIC DATA AT SCHEDULED NECROPSY [% PER LITTER]

GROUP:	0 MG/KG/DAY	5 MG/KG/DAY	25 MG/KG/DAY	50 MG/KG/DAY
CORPORA LUTEA				
MEAN	16.4	17.3	17.2	16.9
S.D.	2.00	2.68	2.42	2.09
S.E.	0.40	0.55	0.48	0.43
N	25	24	25	24
IMPLANTATION SITES				
MEAN	14.8	15.2	15.9	16.1
S.D.	3.09	3.66	3.38	3.61
S.E.	0.62	0.75	0.68	0.74
N	25	24	25	24
VIABLE EMBRYOS (%)				
MEAN	96.4	93.8	91.6	94.1
S.D.	4.72	5.57	19.64	9.60
S.E.	0.94	1.14	3.93	1.96
N	25	24	25	24
DEAD EMBRYOS (%)				
MEAN	0.0	0.0	0.0	0.0
S.D.	0.00	0.00	0.00	0.00
S.E.	0.00	0.00	0.00	0.00
N	25	24	25	24
EARLY RESORPTIONS (%)				
MEAN	3.4	6.0	8.4	5.9
S.D.	4.48	5.31	19.64	9.60
S.E.	0.90	1.08	3.93	1.96
N	25	24	25	24

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
CORPORA LUTEA AND IMPLANTATION SITES COMPARED USING DUNNETT'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

TABLE 32

PROJECT NO. (b) (4) 661005F — ORAL FERT AND EARLY EMB DEV TO IMPLANTATION OF INT-747 IN RATS — PAGE 2

SPONSOR: INTERCEPT PHARM., INC. — SUMMARY OF EMBRYONIC DATA AT SCHEDULED NECROPSY [% PER LITTER]

GROUP:	0 MG/KG/DAY	5 MG/KG/DAY	25 MG/KG/DAY	50 MG/KG/DAY
LATE RESORPTIONS (%)				
MEAN	0.2	0.2	0.0	0.0
S.D.	1.12	1.02	0.00	0.00
S.E.	0.22	0.21	0.00	0.00
N	25	24	25	24
TOTAL RESORPTIONS (%)				
MEAN	3.6	6.2	8.4	5.9
S.D.	4.72	5.57	19.64	9.60
S.E.	0.94	1.14	3.93	1.96
N	25	24	25	24
PRE-IMPLANTATION LOSS (%)				
MEAN	9.6	12.0	8.0	5.8
S.D.	16.37	21.37	16.72	17.83
S.E.	3.27	4.36	3.34	3.64
N	25	24	25	24
POST-IMPLANTATION LOSS (%)				
MEAN	3.6	6.2	8.4	5.9
S.D.	4.72	5.57	19.64	9.60
S.E.	0.94	1.14	3.93	1.96
N	25	24	25	24

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

9.2 Embryonic Fetal Development

Study title: A Dose Range-Finding Study of the Effects of INT-747 on Embryo/Fetal Development in Rats

Study no.:	(b) (4) 661001
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 16, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, Lot# 1106002P, 97.1%

Methods

Doses:	5, 25, 50, 100, and 150 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	0.5% carboxymethylcellulose in reverse osmosis-treated water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	8 females/group
Satellite groups:	None
Study design:	Pregnant females were dosed once daily from GD 6-17. Laparohysterectomies were performed on GD 20 and maternal and fetal parameters were evaluated.

Key Study Findings

In this dose range-finding study, INT-747 produced death in 3 and 2 females in the 100 and 150 mg/kg/day dose groups, respectively. Three and 6 females in the 100 and 150 mg/kg/day dose groups, respectively, were sacrificed in extremis. Clinical signs at these doses included piloerection, hunched posture, red and/or yellow material on various body surfaces, and decreased defecation and/or urination. At 100 mg/kg/day, hypothermia, hypoactivity, and paleness were observed, and one female in the 100 mg/kg/day aborted on GD 20. At 100 and 150 mg/kg/day, hair loss and scabbing were observed. Statistically significant decreases in body weight, body weight gain, and food consumption (compared to controls) occurred at ≥100 mg/kg/day. Mean body weight gains in the 50 mg/kg/day group were reduced by 18% (compared to controls) over the GD 6 to 18 interval, but this difference was not statistically significant. At ≥100 mg/kg/day, females had entirely resorbed litters, and one female at 100 mg/kg/day aborted 3 late resorptions and 1 early resorption and also had 1 and 3 late resorptions in the vagina and in utero, respectively. In the single litter in the 100 mg/kg/day dose group there were no resorptions. At 5, 25, and 50 mg/kg/day, there were no statistically significant treatment-related effects on the incidence of pre- or post-implantation loss, live litter size, sex ratios, or the numbers of corpora lutea and implantation sites. Small

Reference ID: 3870157

(but statistically significant) increases in mean fetal weights occurred at 50 mg/kg/day (+8%, compared to controls). There were no external malformations or variations at up to 100 mg/kg/day. Due to early group termination, fetuses from the 150 mg/kg/day dose group were not evaluated. In conclusion, based on significant maternal toxicity observed in this study, the MTD was considered to be less than 100 mg/kg/day. No developmental toxicity was observed at 50 mg/kg/day; and thus, the Applicant chose to pursue 5, 25, and 75 mg/kg/day for the definitive embryofetal development study in rats.

Study title: A Study of the Effects of INT-747 on Embryo/Fetal Development in Rats

Study no.:	(b) (4) 661003
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 3, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, Lot# 1106002P, 97.1%

Key Study Findings

Treatment with 75 mg/kg/day INT-747 produced mortality, decreased body weight, body weight gain, and food consumption, and abortion in maternal animals. Developmental toxicity occurring at 75 mg/kg/day included increased numbers of early or late resorptions and non-viable fetuses and decreased fetal body weights (compared to controls). At this dose level, there was also an increased incidence of skeletal variations. However, the observed skeletal variations were observed at a maternal toxic dose and the mean litter proportions for these findings were not statistically significant compared to controls. Based on the results of this study, the NOAEL for maternal toxicity and embryofetal development was considered to be 25 mg/kg/day.

Methods

Doses:	5, 25, and 75 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	0.5% carboxymethylcellulose in deionized water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	Main study: 25 females/group TK: 8 females/INT-747 group, 4 control females
Satellite groups:	Yes
Study design:	Pregnant females were dosed once daily from GD 6-17. Laparohysterectomies were performed on GD 20 and maternal and fetal parameters were evaluated.
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

Reference ID: 3870157

Observations and Results

Mortality

Animals were observed for moribundity and mortality twice daily.

At 75 mg/kg/day, treatment with INT-747 produced mortality in 2 females on GD 17 and 19. Death was preceded by reduced food consumption and body weight loss, and the observation of clinical signs as discussed below.

Clinical Signs

Clinical observations were recorded from GD 0-20 (main study animals) or GD 0-18 (TK animals). Observations for signs of toxicity were also recorded 1 h following dosing.

Clinical signs observed prior to death of 2 high dose animals included yellow material on the urogenital and anogenital areas and red material on the forelimbs and around the eyes and nose. One of the animals also exhibited additional clinical signs such as lacrimation, tremors, gasping, dilated pupils, yellow discoloration of the body, hypothermia, and red material under the cage pan on the day of death. Also at 75 mg/kg/day, two females aborted (one each on GD 19 and 20) following reduced food consumption and body weight losses. Finally, red material was observed around the nose of 6 females in the 75 mg/kg/day dose group.

Body Weight

Body weights of main study animals were determined on GDs 0, 6-18 (daily), and 20. For TK animals, body weights were recorded on GDs 0 and 6-17 (daily). Gravid uterine weight, net body weight (GD20 body weight exclusive of the weight of the uterus and contents), and net body weight change (GD 0-20 body weight change exclusive of the weight of the uterus and contents) were calculated at the scheduled laparohysterectomy.

There were statistically significant lower body weights at 75 mg/kg/day (up to -6.3%, compared to controls) from GD 16-18. Body weight gain in the high dose group during gestation was also significantly lower than controls over a subset of the time intervals evaluated in this study (-25%, compared to controls from GD 6-18). In addition, net body weight and net body weight gain at the high dose were 4% and 21% lower, respectively, than controls. There were no treatment-related effects on gravid uterine weight at any dose level.

Feed Consumption

Food consumption was determined for main study animals on GDs 0, 6-18 (daily), and 20.

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Statistically significant reductions in food consumption (relative to controls) occurred in the 75 mg/kg/day treatment group during the treatment period.

Toxicokinetics

Blood samples were collected from TK animals pre-dose and at 0.5, 1, 2, 4, 8, 12, and 24 h after dosing on GD 17, except for controls (samples collected at 1 h following dosing only). Plasma samples were analyzed for INT-747 concentrations.

The Applicant's table below summarizes TK parameters on GD 17. As shown below, AUC values increased in a greater than dose-proportional manner. At 25 mg/kg/day, Cmax was 7-fold higher than the value at 5 mg/kg/day, whereas Cmax values were similar at the 25 and 75 mg/kg/day dose levels.

Text Table 2. INT-747 Toxicokinetic Parameters on Gestation Day 17 following Oral Administration of INT-747 at 5, 25, or 75 mg/kg/day to Bred Rats

Oral INT-747 Dosage (mg/kg/day)	AUC_{0-24} (ng×h/mL)	C_{max} (ng/mL)	T_{max} (h)
5	3447	1170	0.5
25	16968	8288	0.5
75	68316	9659	1

Dosing Solution Analysis

Prior to initiation of dosing, samples for concentration and homogeneity analysis were collected from dosing solutions for each of the treatment groups. Following refrigerated storage for 10 days, samples were collected from the high dose group dosing solution for stability and resuspension homogeneity determinations.

Mean concentrations measured in the dosing solution samples were within the laboratory's standard operating procedures range (90 – 100% of target) and thus considered acceptable.

Necropsy

A gross necropsy was performed on females that died or aborted. The number and location of implantation sites, corpora lutea, and viable fetuses were recorded. At scheduled necropsy, macroscopic observations were recorded based on examination of the thoracic, abdominal, and pelvic cavities.

In animals that died prematurely or aborted, macroscopic findings included red or yellow matting of the anogenital, nasal, buccal and/or urogenital area, and two females had dark red contents in the vagina. Among the two animals found dead on GDs 17 and 19, there were 19 and 11 dead fetuses in the uterus of each respective animal and an early resorption in one animal. In the two females that aborted on GDs 19 and 20, there were 3 late resorptions in one animal and 13 dead fetuses and 5 late resorptions in the other.

No treatment-related macroscopic effects were observed in females at scheduled necropsy.

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

On GD 20, laparohysterectomies were performed on all surviving females. At necropsy, the number of corpora lutea on each ovary was recorded, and the number and location of all fetuses, early and late resorptions, and the total number of implantations in the uterus were recorded. Uteri with no macroscopic evidence of implantation were evaluated for early implantation loss. Postimplantation loss/litter was calculated on a group mean litter basis and proportional litter basis. Each fetus was sexed and weighed.

Treatment-related effects occurred at 75 mg/kg/day. At this dose, there were increases in the numbers of early or late resorptions and non-viable fetuses, compared to controls (see Necropsy above). In addition, there were decreases in fetal body weights at 75 mg/kg/day, as compared to controls. Although mean fetal body weights at terminal sacrifice were 3.6 g in each group, fetal body weights as low as 1.6 g to 2 g were measured in one litter from a high dose animal that survived to the scheduled necropsy. In addition, fetal body weights in the female that aborted on GD 20 were 1 to 1.3 g. No treatment-related effects occurred at 5 or 25 mg/kg/day. The following tables copied from the Applicant's report summarize the cesarean section data.

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF FETAL DATA AT SCHEDULED NECROPSY

GROUP		SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1	TOTAL	179	209	388	0	27	0	27	415	441	26	NA	25
	MEAN	7.2	8.4	15.5	0.0	1.1	0.0	1.1	16.6	17.6	1.0	3.6	
	S.D.	2.72	2.34	2.40	0.00	1.58	0.00	1.58	1.89	2.41	1.34	0.22	
	S.E.	0.54	0.47	0.48	0.00	0.32	0.00	0.32	0.38	0.48	0.27	0.04	
2	TOTAL	179	190	369	0	15	0	15	384	417	33	NA	24
	MEAN	7.5	7.9	15.4	0.0	0.6	0.0	0.6	16.0	17.4	1.4	3.6	
	S.D.	1.89	2.19	1.79	0.00	0.71	0.00	0.71	1.84	2.53	1.53	0.24	
	S.E.	0.39	0.45	0.37	0.00	0.15	0.00	0.15	0.38	0.52	0.31	0.05	
3	TOTAL	198	190	388	0	24	0	24	412	460	48	NA	25
	MEAN	7.9	7.6	15.5	0.0	1.0	0.0	1.0	16.5	18.4	1.9	3.6	
	S.D.	1.87	2.42	2.58	0.00	1.27	0.00	1.27	2.14	2.31	2.56	0.21	
	S.E.	0.37	0.48	0.52	0.00	0.25	0.00	0.25	0.43	0.46	0.51	0.04	
4	TOTAL	171	134	305	0	16	2	18	323	337	14	NA	20
	MEAN	8.6	6.7	15.3	0.0	0.8	0.1	0.9	16.2	16.9	0.7	3.6	
	S.D.	2.09	2.18	1.55	0.00	0.95	0.45	0.97	1.39	1.93	0.98	0.51	
	S.E.	0.47	0.49	0.35	0.00	0.21	0.10	0.22	0.31	0.43	0.22	0.11	

None significantly different from control group
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 5 MG/KG/DAY 3- 25 MG/KG/DAY 4- 75 MG/KG/DAY

Reference ID: 3870157

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF FETAL DATA AT SCHEDULED NECROPSY [% PER LITTER]

GROUP:	0 MG/KG/DAY	5 MG/KG/DAY	25 MG/KG/DAY	75 MG/KG/DAY
CORPORA LUTEA				
MEAN	17.6	17.4	18.4	16.9
S.D.	2.41	2.53	2.31	1.93
S.E.	0.48	0.52	0.46	0.43
N	25	24	25	20
IMPLANTATION SITES				
MEAN	16.6	16.0	16.5	16.2
S.D.	1.89	1.84	2.14	1.39
S.E.	0.38	0.38	0.43	0.31
N	25	24	25	20
VIABLE FETUSES (%)				
MEAN	93.5	96.2	93.7	94.4
S.D.	8.87	4.30	8.83	5.87
S.E.	1.77	0.88	1.77	1.31
N	25	24	25	20
DEAD FETUSES (%)				
MEAN	0.0	0.0	0.0	0.0
S.D.	0.00	0.00	0.00	0.00
S.E.	0.00	0.00	0.00	0.00
N	25	24	25	20
EARLY RESORPTIONS (%)				
MEAN	6.5	3.8	6.3	4.9
S.D.	8.87	4.31	8.83	5.61
S.E.	1.77	0.88	1.77	1.26
N	25	24	25	20

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
CORPORA LUTEA AND IMPLANTATION SITES COMPARED USING DUNNETT'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF FETAL DATA AT SCHEDULED NECROPSY [% PER LITTER]

GROUP:	0 MG/KG/DAY	5 MG/KG/DAY	25 MG/KG/DAY	75 MG/KG/DAY
LATE RESORPTIONS (%)				
MEAN	0.0	0.0	0.0	0.7
S.D.	0.00	0.00	0.00	3.20
S.E.	0.00	0.00	0.00	0.72
N	25	24	25	20
TOTAL RESORPTIONS (%)				
MEAN	6.5	3.8	6.3	5.6
S.D.	8.87	4.31	8.83	5.87
S.E.	1.77	0.88	1.77	1.31
N	25	24	25	20
PRE-IMPLANTATION LOSS (%)				
MEAN	5.4	7.3	9.7	3.8
S.D.	6.80	7.52	12.50	4.99
S.E.	1.36	1.54	2.50	1.12
N	25	24	25	20
POST-IMPLANTATION LOSS (%)				
MEAN	6.5	3.8	6.3	5.6
S.D.	8.87	4.31	8.83	5.87
S.E.	1.77	0.88	1.77	1.31
N	25	24	25	20
MALES (%)				
MEAN	45.7	48.7	51.8	56.2
S.D.	14.50	11.38	11.39	12.90
S.E.	2.90	2.32	2.28	2.88
N	25	24	25	20

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF FETAL DATA AT SCHEDULED NECROPSY [% PER LITTER]

GROUP:	0 MG/KG/DAY	5 MG/KG/DAY	25 MG/KG/DAY	75 MG/KG/DAY
FEMALES (%)				
MEAN	54.3	51.3	48.2	43.8
S.D.	14.50	11.38	11.39	12.90
S.E.	2.90	2.32	2.28	2.88
N	25	24	25	20
MALE FETAL WEIGHTS (g)				
MEAN	3.7	3.7	3.7	3.7
% DIFFERENCE		0.0	0.0	0.0
S.D.	0.29	0.28	0.21	0.53
S.E.	0.06	0.06	0.04	0.12
N	25	24	25	20
FEMALE FETAL WEIGHTS (g)				
MEAN	3.5	3.5	3.5	3.5
% DIFFERENCE		0.0	0.0	0.0
S.D.	0.23	0.24	0.24	0.50
S.E.	0.05	0.05	0.05	0.11
N	25	24	25	20
COMBINED FETAL WEIGHTS (g)				
MEAN	3.6	3.6	3.6	3.6
% DIFFERENCE		0.0	0.0	0.0
S.D.	0.22	0.24	0.21	0.51
S.E.	0.04	0.05	0.04	0.11
N	25	24	25	20

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
FETAL WEIGHTS COMPARED USING DUNNETT'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

Offspring (Malformations, Variations, etc.)

Each fetus was evaluated for external, visceral, and skeletal findings.

The numbers of fetuses (litters) examined morphologically were 388(25), 369(24), 388(25), and 305(20), respectively, in the control, low-, mid-, and high dose groups. Of these fetuses, malformations were observed in 1 fetus each from the control and low dose groups. The malformations were visceral and consisted of an absent kidney (single control animal) and hydrocephaly (single low dose animal). There were no external or skeletal malformations.

There were no external variations. Visceral variations (a major blood vessel variation and distended ureters in the 5 mg/kg/day group and retrocaval ureters in the 75 mg/kg/day group) occurred in single fetuses and were considered incidental. While not statistically significant compared to concurrent controls, an increased incidence of skeletal variations was noted at 75 mg/kg/day. At this dose, the mean litter proportions of reduced ossification of the vertebral arches (3.6%/litter), unco-ossified vertebral centra (1.1%/litter), and unossified pubis (4.2%/litter), ishium (4.2%/litter), entire sternum (4.2%/litter), and vertebral centra (4.0%/litter) were higher than concurrent controls (0% for all findings). The incidences of these findings also exceeded the WIL historical control data, which were 1.1%, 0.3%, 2.3%, 0.6%, 0.3%, and 0.3%, respectively. The increased incidences were primarily due to a single litter (discussed above) which was comprised of 2 late resorptions and 12 viable fetuses (each with skeletal variations). The fetal weights for this litter (1.6 to 2 g) were decreased relative to the group mean (3.6 g), and may be related to decreased maternal body weight gain and decreased food consumption in this dam. The Applicant did not consider skeletal development variations to be test article-related since the variations occurred primarily in a single litter, the differences were not statistically significant from the concurrent control group, and there were no corresponding skeletal malformations. Key findings from the fetal skeletal system exam are summarized in the table below:

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Fetal Variations				
Dosage (mg/kg/day)	0	5	25	75
Total examined[a]	388(25)	369(24)	388(25)	305(20)
Reduced ossification of vertebral arches	0(0)	2(2)	0(0)	9(2)
Unco-ossified vertebral centra	0(0)	0(0)	0(0)	3(1)
Unossified pubis	0(0)	0(0)	0(0)	10(1)
Unossified ischium	0(0)	0(0)	0(0)	10(1)
Unossified entire sternum	0(0)	0(0)	0(0)	10(1)
Unossified vertebral centra	0(0)	0(0)	0(0)	10(2)
a: ( ) = # litters				

Study title: A Dose Range-Finding Study of the Effects of INT-747 on Embryo/Fetal Development in Rabbits

Study no.: (b) (4) 661002
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: October 12, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: INT-747, Lot# 1106002P, 97.1%

Methods

Doses: 5, 25, 50, 100, and 150 mg/kg/day
Frequency of dosing: Once daily
Dose volume: 5 mL/kg
Route of administration: Oral (gavage)
Formulation/Vehicle: 0.5% carboxymethylcellulose in reverse-osmosis water
Species/Strain: New Zealand White rabbits
Number/Sex/Group: 6 females/group
Satellite groups: None
Study design: Pregnant females were dosed once daily from GD 7-20. Laparohysterectomies were performed on GD 29 and maternal and fetal parameters were evaluated. Blood samples were collected for TK evaluation on GD 20.

Key Study Findings

In this dose range-finding study, treatment-related mortality/moribundity occurred at ≥25 mg/kg/day INT-747. The number of animals found dead or sacrificed in extremis was 1, 1, 4, 6, and 6 females, respectively, at 0, 25, 50, 100, and 150 mg/kg/day INT-747. The

Reference ID: 3870157

death of a single female in the 25 mg/kg/day dose group was likely treatment-related based on reduced food consumption and body weight loss in this animal. At ≥50 mg/kg/day, there was an increased incidence of mortality or moribundity (compared to controls), which correlated with treatment-related body weight losses and decreased food consumption. Clinical signs related to excretion (e.g., decreased defecation) occurred at an increased incidence (compared to controls) primarily at ≥50 mg/kg/day. At necropsy, macroscopic findings including firm liver in 2 and 3 females at 50 and 100 mg/kg/day, respectively; distended gallbladder in 2 and 1 females each at 50 and 100 mg/kg/day, respectively; white areas on the gallbladder in 1 female at 100 mg/kg/day; edematous pyloric and/or fundic regions of the stomach in 3 females at 100 mg/kg/day; and brown matting around the urogenital area in 1 female each at 50 and 100 mg/kg/day and 4 females at 150 mg/kg/day were observed. Three of 4 females in the 50 mg/kg/day group euthanized in extremis had entirely resorbed litters. At 25 mg/kg/day, postimplantation loss occurred in 3 of 4 surviving gravid animals and the mean litter proportion of postimplantation loss (13.1%/litter) was increased compared to controls (5.0%/litter). In addition, the mean litter proportion of viable fetuses (86.9%/litter) was lower than controls (95.0%/litter). Mean fetal weight (38.8 g) at 25 mg/kg/day was lower than controls (44.5 g), but within the historical control range (38.0-43.0 g) for definitive studies. No external malformations or developmental variations were observed in any fetuses from the control, 5, 25, or 50 mg/kg/day groups. Based upon mortality/moribundity and effects on body weight and food consumption at ≥25 mg/kg/day and lower mean fetal weights at 25 mg/kg/day, the Applicant chose to pursue 3, 9, and 20 mg/kg/day for the definitive embryofetal development study in rabbits. TK results showed that there was systemic exposure to INT-747 following oral administration and AUC_{0-24} values increased in a more than dose-proportional manner. AUC_{0-24} values were 2512, 19344, and 143849 ng.h/mL at 5, 25, and 50 mg/kg/day, respectively.

Study title: A Study of the Effects of INT-747 on Embryo/Fetal Development in Rabbits

Study no.:	(b) (4) 661004
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 3, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, Lot# 1106002P, 97.1%

Key Study Findings

Following treatment with up to 20 mg/kg/day INT-747 from GD 7 through GD 20, there was no evidence of maternal toxicity or effects on embryofetal development. The NOAEL was considered to be 20 mg/kg/day.

Methods

Doses: 3, 9, and 20 mg/kg/day

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Frequency of dosing:	Once daily
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	0.5% carboxymethylcellulose in reverse-osmosis water
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	Main study: 22 females/group TK: 4 females/group
Satellite groups:	None
Study design:	Pregnant females were dosed once daily from GD 7-20. Laparohysterectomies were performed on GD 29 and maternal and fetal parameters were evaluated. Blood samples for plasma analysis were collected from TK animals on GD 20 out to 24 h after dosing, and TK animals were euthanized on GD 21.
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

Observations and Results

Mortality

Animals were observed for moribundity and mortality twice daily.

A single female in the 3 mg/kg/day TK group was sacrificed in extremis on GD 17 following body weight loss from GD 11-17. This animal had red material on the anogenital area, and veterinary examination findings included hypoactivity, pallor, and drainage from the vulva following palpation of the abdomen.

Clinical Signs

Clinical observations were recorded for main study animals from the day of receipt through GD 29 (prior to dosing from GD 7-20). Observations for signs of toxicity were also recorded 1 h following dosing. Observations from TK animals were recorded through GD 21.

A single female in the 9 mg/kg/day group aborted 3 late resorptions on GD 24, following a body weight loss of 112 g from GD 21-24. Red material on the anogenital area was observed on the day of abortion. In the absence of abortions at 20 mg/kg/day, this was not considered to be test-compound related.

Body Weight

Body weights of main study animals were determined on GDs 0, 4, 7-21 (daily), 24, and 29. For TK animals, body weights were recorded on GDs 0, 4, and 7-20 (daily). Gravid uterine weight, net body weight (GD29 body weight exclusive of the weight of the uterus

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and contents), and net body weight change (GD 0-29 body weight change exclusive of the weight of the uterus and contents) were calculated at the scheduled laparohysterectomy.

There were no statistically significant changes in mean maternal body weights and body weight gain, net body weights and net body weight gain, or gravid uterine weights at any dose, compared to controls.

Feed Consumption

Food consumption was determined for main study animals on GDs 4-29.

There were no treatment-related effects on food consumption at any dose.

Toxicokinetics

Blood samples were collected from the INT-747 TK groups on GD 20 pre-dose and out to 24 h post-dosing. Blood samples were collected from controls at 1 h after dosing. Plasma samples were analyzed for INT-747 concentration using a validated HPLC/MS/MS method.

TK analysis revealed systemic exposure to INT-747, with a dose-related increase in exposure that was proportional to the increase in dose. TK parameters are summarized in the Applicant's table below.

Text Table 3. Results of Toxicokinetic Analyses (Mean ± SD)

Dose (mg/kg/day)	AUC_{0-24} (ng·hr/mL)	C_{max} (ng/mL)	Tmax (hr)	Cmin (ng/mL)	Cavg (ng/mL)	% Fluctuation
3	1272 ± 189	136 ±31.4	1.0 ± 0.87	33.6 ± 7.95	53.0 ± 7.87	192 ± 54.6
9	3399 ± 849	269 ± 56.5	2.5 ± 1.0	80.0 ± 33.2	142 ± 35.4	137 ± 31.1
20	8552 ± 2160	790 ± 237	2.4 ± 1.9	194 ± 59.0	356 ± 90.0	165 ± 31.6

Dosing Solution Analysis

Dosing solution samples were collected for homogeneity determination. Dosing solution concentrations were 89.6-96.5% of nominal concentrations, and thus, were within the SOP range for suspensions (85-115%).

Necropsy

A gross necropsy was performed on the females that aborted during the study. The number and location of implantation sites, corpora lutea, and viable fetuses were recorded. At scheduled necropsy on GD 29, macroscopic observations were recorded based on examination of the thoracic, abdominal, and pelvic cavities.

As discussed under Clinical Signs, a single female in the 9 mg/kg/day group aborted 3 late resorptions on GD 24. There were 4 late resorptions noted in utero, with no

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apparent malformations. At the scheduled necropsy on GD 29, no treatment-related macroscopic findings were identified.

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

On GD 29, laparohysterectomies were performed on all surviving females. At necropsy, the number of corpora lutea on each ovary was recorded, and the number and location of all fetuses, early and late resorptions, and the total number of implantations in the uterus were recorded. Uteri with no macroscopic evidence of implantation were evaluated for early implantation loss. Postimplantation loss/litter was calculated on a group mean litter basis and proportional litter basis. Each fetus was sexed and weighed.

The only statistically significant difference from controls was reduced fetal weight (males and females combined) in the low dose group (38.9 g), compared to the control group (41.4 g). Based on a lack of effects at the mid- and high dose, this was not considered to be a treatment-related effect. While not statistically significant, the mean percent per litter early resorptions, late resorptions, and pre- and post-implantation losses were increased in the INT-747 treatment groups relative to controls. However, there was no dose-relationship and the values were within the range of historical control values for Covance Research Products, Inc. (rabbit supplier). The following tables copied from the Applicant's report summarize the cesarean section data.

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF FETAL DATA AT SCHEDULED NECROPSY

GROUP		SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1	TOTAL	104	94	198	0	4	2	6	204	210	6	NA	21
	MEAN	5.0	4.5	9.4	0.0	0.2	0.1	0.3	9.7	10.0	0.3	41.4	
	S.D.	1.75	1.60	0.98	0.00	0.40	0.30	0.46	1.10	1.18	0.46	4.18	
	S.E.	0.38	0.35	0.21	0.00	0.09	0.07	0.10	0.24	0.26	0.10	0.91	
2	TOTAL	81	121	202	0	11	6	17	219	234	15	NA	21
	MEAN	3.9	5.8	9.6	0.0	0.5	0.3	0.8	10.4	11.1	0.7	37.9*	
	S.D.	1.68	2.34	2.54	0.00	0.75	0.64	1.03	2.54	2.65	0.64	4.92	
	S.E.	0.37	0.51	0.55	0.00	0.16	0.14	0.22	0.55	0.58	0.14	1.07	
3	TOTAL	90	96	186	0	9	3	12	198	208	10	NA	21
	MEAN	4.3	4.6	8.9	0.0	0.4	0.1	0.6	9.4	9.9	0.5	42.1	
	S.D.	1.65	1.99	1.28	0.00	0.68	0.48	0.81	1.33	1.00	0.75	3.49	
	S.E.	0.36	0.43	0.28	0.00	0.15	0.10	0.18	0.29	0.22	0.16	0.76	
4	TOTAL	79	82	161	0	8	3	11	172	189	17	NA	18
	MEAN	4.4	4.6	8.9	0.0	0.4	0.2	0.6	9.6	10.5	0.9	41.6	
	S.D.	1.94	1.79	1.63	0.00	0.62	0.38	0.70	1.38	1.69	1.16	4.49	
	S.E.	0.46	0.42	0.38	0.00	0.15	0.09	0.16	0.33	0.40	0.27	1.06	

* = Significantly different from the control group at 0.05
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 3 MG/KG/DAY 3- 9 MG/KG/DAY 4- 20 MG/KG/DAY

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF FETAL DATA AT SCHEDULED NECROPSY [% PER LITTER]

GROUP:	0 MG/KG/DAY	3 MG/KG/DAY	9 MG/KG/DAY	20 MG/KG/DAY
CORPORA LUTEA				
MEAN	10.0	11.1	9.9	10.5
S.D.	1.18	2.65	1.00	1.69
S.E.	0.26	0.58	0.22	0.40
N	21	21	21	18
IMPLANTATION SITES				
MEAN	9.7	10.4	9.4	9.6
S.D.	1.10	2.54	1.33	1.38
S.E.	0.24	0.55	0.29	0.33
N	21	21	21	18
VIABLE FETUSES (%)				
MEAN	97.2	92.4	94.3	93.4
S.D.	4.51	10.33	8.03	7.77
S.E.	0.98	2.26	1.75	1.83
N	21	21	21	18
DEAD FETUSES (%)				
MEAN	0.0	0.0	0.0	0.0
S.D.	0.00	0.00	0.00	0.00
S.E.	0.00	0.00	0.00	0.00
N	21	21	21	18
EARLY RESORPTIONS (%)				
MEAN	1.8	4.8	4.3	4.8
S.D.	3.95	6.88	6.56	6.54
S.E.	0.86	1.50	1.43	1.54
N	21	21	21	18

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
CORPORA LUTEA AND IMPLANTATION SITES COMPARED USING DUNNETT'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF FETAL DATA AT SCHEDULED NECROPSY [% PER LITTER]

GROUP:	0 MG/KG/DAY	3 MG/KG/DAY	9 MG/KG/DAY	20 MG/KG/DAY
LATE RESORPTIONS (%)				
MEAN	0.9	2.8	1.5	1.8
S.D.	2.88	6.69	5.14	4.18
S.E.	0.63	1.46	1.12	0.99
N	21	21	21	18
TOTAL RESORPTIONS (%)				
MEAN	2.8	7.6	5.7	6.6
S.D.	4.51	10.33	8.03	7.77
S.E.	0.98	2.26	1.75	1.83
N	21	21	21	18
PRE-IMPLANTATION LOSS (%)				
MEAN	2.7	6.7	4.9	8.3
S.D.	4.45	6.09	7.77	9.28
S.E.	0.97	1.33	1.70	2.19
N	21	21	21	18
POST-IMPLANTATION LOSS (%)				
MEAN	2.8	7.6	5.7	6.6
S.D.	4.51	10.33	8.03	7.77
S.E.	0.98	2.26	1.75	1.83
N	21	21	21	18
MALES (%)				
MEAN	52.3	41.5	48.9	48.5
S.D.	17.14	16.63	19.81	19.93
S.E.	3.74	3.63	4.32	4.70
N	21	21	21	18

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF FETAL DATA AT SCHEDULED NECROPSY [% PER LITTER]

GROUP:	0 MG/KG/DAY	3 MG/KG/DAY	9 MG/KG/DAY	20 MG/KG/DAY
FEMALES (%)				
MEAN	47.7	58.5	51.1	51.5
S.D.	17.14	16.63	19.81	19.93
S.E.	3.74	3.63	4.32	4.70
N	21	21	21	18
MALE FETAL WEIGHTS (g)				
MEAN	41.6	38.3	42.2	41.8
% DIFFERENCE		-7.9	1.4	0.5
S.D.	4.61	5.70	3.64	5.48
S.E.	1.01	1.24	0.79	1.33
N	21	21	21	17
FEMALE FETAL WEIGHTS (g)				
MEAN	41.0	37.6	42.0	41.3
% DIFFERENCE		-8.3	2.4	0.7
S.D.	4.17	4.94	4.41	5.15
S.E.	0.91	1.08	0.96	1.21
N	21	21	21	18
COMBINED FETAL WEIGHTS (g)				
MEAN	41.4	37.9*	42.1	41.6
% DIFFERENCE		-8.5	1.7	0.5
S.D.	4.18	4.92	3.49	4.49
S.E.	0.91	1.07	0.76	1.06
N	21	21	21	18

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
FETAL WEIGHTS COMPARED USING DUNNETT'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
* = Significantly different from the control group at 0.05

Offspring (Malformations, Variations, etc.)

Each fetus was evaluated for external, visceral, and skeletal findings.

The numbers of fetuses (litters) examined morphologically were 198(21), 202(21), 186(21), and 161(18), respectively, in the control, low-, mid-, and high dose groups. The following tables copied from the study report summarize the percent per litter malformations and absolute numbers of fetuses and litters with malformations.

TOTAL MALFORMATIONS

PERCENT PER LITTER WITH EXTERNAL MALFORMATIONS	MEAN	1.1	0.0	0.0	0.6
	S.D.	3.56	0.00	0.00	2.36
	S.E.	0.78	0.00	0.00	0.56
PERCENT PER LITTER WITH SOFT TISSUE MALFORMATIONS	MEAN	2.6	0.0	2.6	2.9
	S.D.	4.71	0.00	9.54	4.82
	S.E.	1.03	0.00	2.08	1.14
PERCENT PER LITTER WITH SKELETAL MALFORMATIONS	MEAN	0.0	2.0	1.8	1.9
	S.D.	0.00	5.21	4.93	4.46
	S.E.	0.00	1.14	1.08	1.05
TOTAL PERCENT PER LITTER WITH MALFORMATIONS	MEAN	3.1	2.0	4.4	4.8
	S.D.	5.14	5.21	10.28	6.58
	S.E.	1.12	1.14	2.24	1.55

1- 0 MG/KG/DAY 2- 3 MG/KG/DAY 3- 9 MG/KG/DAY 4- 20 MG/KG/DAY
MODIFIED STATISTICS USED.
None significantly different from control group

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF FETUSES AND LITTERS WITH MALFORMATIONS [ABSOLUTE NO.]

DAY 29

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	198	202	186	161	21	21	21	18
ACEPHALY	0	0	0	1-A	0	0	0	1
GASTROSCHISIS	0	0	0	1-A	0	0	0	1
ECTRODACTYLY	0	0	0	1-A	0	0	0	1
CLAW ABSENT	0	0	0	1-A	0	0	0	1
CARPAL AND/OR TARSAL FLEXURE	0	0	0	1-A	0	0	0	1
NARROW PECTORAL REGION	1-B	0	0	0	1	0	0	0
LOCALIZED FETAL EDEMA	1-B	0	0	0	1	0	0	0
MICROPHTHALMIA AND/OR ANOPHTHALMIA	1	0	0	0	1	0	0	0
NUMBER EXAMINED VISCERALLY	198	202	186	161	21	21	21	18
LUNGS- LOBULAR AGENESIS	1	0	3	2	1	0	1	2
PANCREAS- ABSENT	0	0	0	1	0	0	0	1
MALPOSITIONED VESSEL	0	0	1	0	0	0	1	0
KIDNEY(S)- MALPOSITIONED	0	0	0	1	0	0	0	1
PERSISTENT TRUNCUS ARTERIOSUS	0	0	0	1	0	0	0	1
HEART AND/OR GREAT VESSEL ANOMALY	0	0	0	1-A	0	0	0	1
DIAPHRAGMATIC HERNIA	2	0	0	0	2	0	0	0
STENOTIC PULMONARY TRUNK	1	0	0	0	1	0	0	0
INTERVENTRICULAR SEPTAL DEFECT	1-B	0	0	0	1	0	0	0
NUMBER EXAMINED SKELETALLY	198	202	186	161	21	21	21	18
VERTEBRAL CENTRA ANOMALY	0	1	0	0	0	1	0	0
COSTAL CARTILAGE ANOMALY	0	1	0	0	0	1	0	0
RIB ANOMALY	0	1	1	0	0	1	1	0
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	0	0	2	3	0	0	2	3
SKULL ANOMALY	0	0	1	0	0	0	1	0

1- 0 MG/KG/DAY 2- 3 MG/KG/DAY 3- 9 MG/KG/DAY 4- 20 MG/KG/DAY

A = FETUS NO. 53525-06
B = FETUS NO. 53580-07

There were external malformations in 2(2) and 1(1) fetuses (litters) in the control and high dose groups, respectively. At 20 mg/kg/day, a single fetus had multiple external malformations including acephaly, gastroschisis, ectrodactyly and absent claw, and carpal flexure (mean litter proportion = 0.6%/litter for each finding, compared to the 0.0%/litter for each finding in controls). However, these findings occurred in only a single fetus and there were no statistically significant differences from the control group. No external developmental variations were noted in any group.

Visceral malformations were observed in 5(5), 0(0), 4(2), and 5(5) fetuses (litters) in the control, low-, mid-, and high dose groups, respectively. The mean litter proportion of lobular agenesis of the lungs, absent pancreas, malpositioned vessel, malpositioned kidney, persistent truncus arteriosus, and heart and/or great vessel anomaly exceeded concurrent control values as summarized in the Applicant's table below. However, the visceral malformation findings did not appear to be treatment-related based on the lack of statistically significant differences in the mean litter proportions relative to concurrent control groups; given that the values were within historical control ranges; a lack of dose-relationship; and/or since the findings occurred in fetuses with multiple malformations. Visceral variations such as accessory spleen, extra papillary muscle (heart), small or absent gallbladder, retrocaval ureter, and major blood vessel variations occurred in all groups (including controls) and did not appear to be treatment-related based on comparison among groups as well as historical control data.

Reference ID: 3870157

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF LITTER PROPORTIONS OF MALFORMATIONS
% PER LITTER DAY 29

DOSE GROUP:		1	2	3	4
NUMBER OF LITTERS EXAMINED VISCERALLY		21	21	21	18
LUNGS- LOBULAR AGENESIS	MEAN	0.6	0.0	2.0	1.2
	S.D.	2.73	0.00	9.35	3.42
	S.E.	0.60	0.00	2.04	0.81
PANCREAS- ABSENT	MEAN	0.0	0.0	0.0	0.6
	S.D.	0.00	0.00	0.00	2.36
	S.E.	0.00	0.00	0.00	0.56
MALPOSITIONED VESSEL	MEAN	0.0	0.0	0.5	0.0
	S.D.	0.00	0.00	2.42	0.00
	S.E.	0.00	0.00	0.53	0.00
KIDNEY(S)- MALPOSITIONED	MEAN	0.0	0.0	0.0	0.6
	S.D.	0.00	0.00	0.00	2.62
	S.E.	0.00	0.00	0.00	0.62
PERSISTENT TRUNCUS ARTERIOSUS	MEAN	0.0	0.0	0.0	0.6
	S.D.	0.00	0.00	0.00	2.62
	S.E.	0.00	0.00	0.00	0.62
HEART AND/OR GREAT VESSEL ANOMALY	MEAN	0.0	0.0	0.0	0.6
	S.D.	0.00	0.00	0.00	2.36
	S.E.	0.00	0.00	0.00	0.56
DIAPHRAGMATIC HERNIA	MEAN	1.0	0.0	0.0	0.0
	S.D.	3.01	0.00	0.00	0.00
	S.E.	0.66	0.00	0.00	0.00
STENOTIC PULMONARY TRUNK	MEAN	0.5	0.0	0.0	0.0
	S.D.	2.18	0.00	0.00	0.00
	S.E.	0.48	0.00	0.00	0.00

1- 0 MG/KG/DAY 2- 3 MG/KG/DAY 3- 9 MG/KG/DAY 4- 20 MG/KG/DAY
MODIFIED STATISTICS USED.
None significantly different from control group

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF LITTER PROPORTIONS OF MALFORMATIONS
% PER LITTER DAY 29

DOSE GROUP:		1	2	3	4
NUMBER OF LITTERS EXAMINED VISCERALLY		21	21	21	18
INTERVENTRICULAR SEPTAL DEFECT	MEAN	0.5	0.0	0.0	0.0
	S.D.	2.42	0.00	0.00	0.00
	S.E.	0.53	0.00	0.00	0.00

1- 0 MG/KG/DAY 2- 3 MG/KG/DAY 3- 9 MG/KG/DAY 4- 20 MG/KG/DAY
MODIFIED STATISTICS USED.
None significantly different from control group

There was an increased incidence of skeletal malformations at all dose levels, compared to controls [0(0), 3(3), 4(3), and 3(3) fetuses (litters) in the control, low-, mid-, and high dose levels, respectively]. The mean litter proportions of the skeletal malformations are summarized in the Applicant's table below. At the mid- and high dose, 2 and 3 fetuses, respectively, had vertebral anomalies either with or without associated rib anomalies. However, vertebral anomaly findings were not considered to be treatment-related as the mean litter proportion at the high dose was not statistically significantly different than concurrent controls, and the value was within the historical control range for Covance. Similarly, mean litter proportions for skeletal malformations at the low- and mid-dose were not considered treatment-related as they were not statistically significantly different from the control group, were within the range of historical controls, and/or the findings occurred in single fetuses.

SPONSOR:INTERCEPT PHARM., INC. SUMMARY OF LITTER PROPORTIONS OF MALFORMATIONS % PER LITTER DAY 29

DOSE GROUP:		1	2	3	4
NUMBER OF LITTERS EXAMINED SKELETALLY		21	21	21	18
VERTEBRAL CENTRA ANOMALY	MEAN	0.0	0.5	0.0	0.0
	S.D.	0.00	2.18	0.00	0.00
	S.E.	0.00	0.48	0.00	0.00
COSTAL CARTILAGE ANOMALY	MEAN	0.0	1.0	0.0	0.0
	S.D.	0.00	4.36	0.00	0.00
	S.E.	0.00	0.95	0.00	0.00
RIB ANOMALY	MEAN	0.0	0.5	0.5	0.0
	S.D.	0.00	2.42	2.18	0.00
	S.E.	0.00	0.53	0.48	0.00
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	MEAN	0.0	0.0	0.8	1.9
	S.D.	0.00	0.00	2.62	4.46
	S.E.	0.00	0.00	0.57	1.05
SKULL ANOMALY	MEAN	0.0	0.0	0.5	0.0
	S.D.	0.00	0.00	2.18	0.00
	S.E.	0.00	0.00	0.48	0.00

1- 0 MG/KG/DAY 2- 3 MG/KG/DAY 3- 9 MG/KG/DAY 4- 20 MG/KG/DAY
MODIFIED STATISTICS USED.
None significantly different from control group

Skeletal variations occurred in all groups (including controls), and consisted of findings such as 13th rudimentary rib(s), 13th full rib(s), sternebrae #5 and/or #6 unossified, hyoid arch(es) bent, 27 presacral vertebrae, and sternebrae with thread-like attachment. The mean litter proportion of 13th rudimentary ribs at 20 mg/kg/day (13.5%/litter) was significantly lower than the control value (28.4%/litter), but this decrease was not considered toxicologically relevant. Other skeletal developmental variations not considered treatment-related were noted in single fetuses, occurred to a similar extent in the control group, did not occur in a dose-related manner, and/or the mean litter proportions were within historical control range.

9.3 Prenatal and Postnatal Development

Study title: An Oral (Gavage) Study of the Effects of INT-747 on Pre- and Postnatal Development, Including Maternal Function in Rats

Study no.:	(b) (4) 661049
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 2, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	INT-747, Lot# T11-001, 97.7%

Key Study Findings

In F_0 animals, food consumption was increased at 25 and 40 mg/kg/day during the lactation period. At 40 mg/kg/day, the increase in food consumption correlated with a small but statistically significant increase in body weights. In F1 animals, the mean litter proportion of post-implantation loss at 25 mg/kg/day (7.5%/litter) and 40 mg/kg/day (10.1%/litter) exceeded that of concurrent controls (3.4%/litter) and the maximum historical control mean value (6.5%/litter), but the increase was not statistically significant. Furthermore, at 40 mg/kg/day, there was a single litter in which there were

Reference ID: 3870157

only 2 implantation sites which were early resorptions. Excluding this litter results in a mean litter proportion of post-implantation loss at the high dose which is within the historical control range. Overall, the NOAEL is considered to be 40 mg/kg/day.

Methods

Doses:	5, 25, and 40 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	0.5% carboxymethylcellulose (CMC) in deionized water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	Main Study: 25 females/group TK Study: 4 females/group
Satellite groups:	Yes (TK animals)
Study design:	Main Study: Pregnant females were dosed from GD 6 through lactation day (LD) 20, for a total of 36 to 38 doses. Females that failed to deliver were dosed through post-mating day 24, for a total of 18 doses. TK Study: Pregnant females were dosed from GD 6 - LD 10, for a total of 26 to 27 doses.
Deviation from study protocol:	Protocol deviations did not affect the quality or integrity of the study.

Observations and Results

F_o Dams:

Survival:
Mortality was observed twice daily. There were no unscheduled deaths.

Clinical Signs:
Animals were observed twice daily. There were no treatment-related clinical signs.

Body Weight:
Body weights were recorded on GDs 0, 6, 9, 12, 15, 18, and 20, and on LD 0, 1, 4, 7, 10, 14, 17, and 21 (main study) or 1, 4, 7, and 10 (TK study).

There were no treatment-related effects on body weight or body weight gain during gestation. During lactation, there was a small, but statistically significant increase in body weights at 40 mg/kg/day (+5%, compared to controls on LD 17). Over LD1-21, body weight gain was increased by 15% and 12% (compared to controls) at 25 and 40 mg/kg/day (not significant at either dose level), respectively.

Feed consumption:

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Food consumption was recorded on GDs 0, 6, 9, 12, 15, 18, and 20 and LD 1, 4, 7, 10, 14, 17, and 21 (Main Study animals only).

There were no treatment-related effects on food consumption during gestation. During lactation, there were statistically significant increases in food consumption during a subset of study intervals at 25 and 40 mg/kg/day (up to +9% and +15%, respectively, compared to controls). From LD 1 – 21, food consumption was significant increased (+10%, compared to controls) at 40 mg/kg/day.

Uterine content:

There were no treatment-related effects on the length of gestation or the parturition process, the number of pups born, the number of former implantation sites, or the number of unaccounted-for sites. Summary data for gestation length, number born, implantation sites, and unaccounted-for sites are presented in the Applicant's tables below.

TABLE S12 (F0)

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SPONSOR: INTERCEPT PHARM., INC. SUMMARY OF GESTATION LENGTHS [DAYS]

GROUP:	0 MG/KG/DAY	5 MG/KG/DAY	25 MG/KG/DAY	40 MG/KG/DAY
GESTATION LENGTH (DAYS)				
MEAN	21.8	21.8	22.0	22.0
S.D.	0.52	0.39	0.20	0.20
S.E.	0.10	0.08	0.04	0.04
N	25	23	24	25

MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

TABLE S16 (F0)

PROJECT NO. (b) (4)661049 ORAL STUDY OF EFFECTS OF INT-747 ON PRE- & POSTNATAL DEV IN RATS PAGE 1

SPONSOR: INTERCEPT PHARM., INC. SUMMARY OF IMPLANTATION SITES

GROUP:	0 MG/KG/DAY	5 MG/KG/DAY	25 MG/KG/DAY	40 MG/KG/DAY
IMPLANTATION SITES				
MEAN	15.2	14.5	15.1	15.4
S.D.	2.35	3.20	1.79	1.47
S.E.	0.48	0.68	0.37	0.31
N	24-A	22-A	23-A	22-A
NUMBER BORN				
MEAN	14.4	13.1	14.3	14.5
S.D.	2.28	3.27	1.99	1.53
S.E.	0.47	0.70	0.41	0.33
N	24	22	23	22
UNACCOUNTED SITES				
MEAN	0.8	1.4	0.8	0.9
S.D.	0.88	1.44	1.15	1.13
S.E.	0.18	0.31	0.24	0.24
N	24	22	23	22

MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group
NOTE: ONLY DAMS THAT HAVE DELIVERED ONE OR MORE PUPS ARE INCLUDED IN CALCULATION OF MEAN.
A = IMPLANTATION SITES NOT RECORDED FOR 1, 1, 1, AND 3 FEMALES IN THE CONTROL, 5, 25, AND 40 MG/KG/DAY GROUPS, RESPECTIVELY (SEE DEVIATIONS FROM THE PROTOCOL).

Necropsy observation:

Gross necropsies were performed on all surviving females with viable pups on LD 21, as well as females that did not deliver. For females that delivered, the numbers of former implantation sites was recorded. For females that failed to deliver, a pregnancy status was determined and the Applicant attempted to identify anatomic or pathologic findings that may have interfered with pregnancy.

At scheduled necropsy, a low incidence of clear fluid present in the uterus was observed (single female each at 25 and 40 mg/kg/day). This and other findings were considered incidental based on the infrequent occurrence and/or lack of dose response. At 5 and 25 mg/kg/day, respectively, two and one females failed to deliver and were determined to be nongravid.

Toxicokinetics:
Not conducted

Dosing Solution Analysis:
Samples for evaluation of homogeneity and concentration were collected from the first and last dosing solutions.

Dosing solution samples met the SOP range for suspensions (±15% of nominal concentrations) and were homogenous. Measured concentrations ranged from 90.3 to 93.3% of target.

F1 Generation:

Survival:
Beginning on PND 0, pups were sexed, examined, and the numbers of stillborn and live pups were recorded. Individual litters were examined daily for survival and all deaths were recorded. Any pups which died from PND 0 to 4 were necropsied. A detailed gross necropsy was performed on any pup found dead or euthanized in extremis after PND 4. Litters were culled to 8 pups/litter (4/sex if possible) on PND 4.

There was no treatment-related effect on liver litter size, sex ratio, or number of pups born. Postnatal survival between PND 0-1, 1-4, 4-7, 7-14, and 14-21, from birth to PND 4, and PND 4-21 was similar in all groups. At 25 and 40 mg/kg/day, 2 and 5 pups, respectively, were stillborn. During the pre- and postnatal development phase, the number of pups found dead or euthanized in extremis were 4, 4, 7, and 9, respectively, in the control, low-, mid-, and high dose groups. There were 6, 3, 1, and 3 pups missing in the control, low-, mid-, and high dose groups, respectively. No treatment-related deaths occurred in F1 adults.

Clinical signs:
Clinical signs were assessed daily. Detailed physical examination was performed on PND 1, 4, 7, 10,14, 17, and 21 (main study) or PND 1, 4, 7, and 10 (TK study).

No treatment-related clinical signs were identified in F1 pups or adults.

Body weight:
Body weights were recorded on PND 1, 4, 7, 10, 14, 17, and 21 (main study) or PND 1, 4, 7, and 10 (TK study). After weaning, F1 males and females were weighed weekly. Once evidence of mating was observed in F1 females, body weights were recorded on GDs 0, 6, 9, 12, and 15.

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During the postnatal period, there were no treatment-related effects on body weight or body weight gain in F1 pups.

In F1 generation females, there were small, but statistically significant, increases in body weight gain from PND 21 to 28 at 25 mg/kg/day and from PND 28 to 35 at 40 mg/kg/day. In addition, body weights were increased on PND 35 at 25 and 40 mg/kg/day and on PND 42 at 25 mg/kg/day. Based on the magnitude of the changes (generally <10% difference from controls), lack of dose-relationship, and lack of significant differences over the duration of the pre-mating period, this did not appear to be treatment-related. There were no statistically significant differences in body weights and body weight gain during gestation.

Feed consumption:
Not evaluated

Physical development:
Pups were sexed individually on PND 0, 4, 14, and 21 (main study) or PND 0 and 4 (TK study). Examination of animals (2/litter, if possible) for attainment of balanopreputial separation and vaginal patency was conducted beginning on PND 35 and 25, respectively.

There were no treatment-related effects on acquisition of balanopreputial separation or vaginal patency, or body weight at the age of attainment in F1 animals. The mean ages of attainment of balanopreputial separation were 44.9, 44.6, 45.0, 45.1 days in the control, low-, mid-, and high dose groups, respectively. The mean ages of attainment of vaginal patency were 33.0, 33.5, 32.9, 32.8 days in the control, low-, mid-, and high dose groups, respectively.

Neurological assessment:
One animal/sex/litter was evaluated for auditory startle response (PND 20 and 60), locomotor activity (PND 21 and 61), and learning and memory (beginning on PND 62). Another pup/sex/litter was evaluated for learning and memory beginning on PND 22.

There were no significant effects in the auditory startle test on PND 20 and 60 in any of the treatment groups, as compared to controls. INT-747 also did not have a significant effect on locomotor activity, or swimming ability and learning and memory as assessed by Biel maze swimming trials. Although the mean escape time and number of errors was significantly increased in females at the high dose (compared to controls) during a single trial at the PND 62 evaluation, the overall means for these parameters were similar across the trials. Thus, this was not considered to be related to the test compound.

Reproduction:
One F1 animal/sex/litter was selected for evaluation of developmental landmarks and reproductive performance (determination of estrous cycles and breeding). For

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breeding, F1 animals (at least 85 days old) were cohabitated on a 1:1 basis within each treatment groups. Females were necropsied on GD 15, and laparohysterectomies were performed on females with evidence of mating.

There were no statistically significant changes in male or female mating index, male or female fertility index, male copulation index, female conception index, estrous cycle length, number of times in estrus, or pre-coital interval (see Applicant's table below). In addition, there were no treatment-related effects on the number of males that failed to sire a litter. A single mid-dose female had no evidence of mating, and 1 female per group had evidence of mating but was determined to be nongravid.

Text Table 3. Results of F_1 Reproductive Performance

Parameter	Dosage Level (mg/kg/day) 0	5	25	40	(b) (4)HC[a] Mean (Range)
Male Mating Index (%)	100.0	100.0	95.8	100.0	95.8 (84.0-100.0)
Female Mating Index (%)	100.0	100.0	95.8	100.0	98.2 (92.0-100.0)
Male Fertility Index (%)	95.7	95.7	91.7	96.0	90.0 (60.0-100.0)
Female Fertility Index (%)	95.7	95.7	91.7	96.0	92.9 (60.0-100.0)
Male Copulation Index (%)	95.7	95.7	95.7	96.0	93.2 (71.4-100.0)
Female Conception Index (%)	95.7	95.7	95.7	96.0	92.9 (65.2-100.0)
Estrous Cycle Length (days)	4.1	4.2	4.2	4.7	4.3 (4.0-5.0)
Number of times in estrus	3.4	3.5	3.3	3.0	Not evaluated
Pre-Coital Interval (days)	2.7	3.3	2.9	2.6	3.3 (2.4-4.8)

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

The mean estrous cycle length was 4.7 days at 40 mg/kg/day, compared to 4.1 days in the control group. While a single female in the 40 mg/kg/day group had a 12-day estrous cycle, this animal mated within 3 days of the breeding period. Excluding this female, the mean estrous cycle length in this group was 4.4 days. Furthermore, the mean estrous cycle length at 40 mg/kg/day was within the range of historical control values (4-5 days).

Summaries of embryonic data at scheduled necropsy are presented in the Applicant's tables below. Although not statistically significant, the mean litter proportion of post-implantation loss at 25 mg/kg/day (7.5%/litter) and 40 mg/kg/day (10.1%/litter) exceeded that of concurrent controls (3.4%/litter) and the maximum mean historical control value (6.5%/litter). At 40 mg/kg/day, there was a single litter in which there were only 2 implantation sites that were early resorptions (thus resulting in 100% post-implantation loss). Excluding this litter, the mean litter proportion of post-implantation loss at 40 mg/kg/day was 6.2%/litter, which is within the historical control range. Given this, the lack of similar findings in other litters, and lack of statistical significance the Applicant concluded that the resorbed litter represented biological variation and was not related to the test compound. The Applicant did not address the observed increased mean litter proportion of early resorptions or post-implantation loss at 25 mg/kg/day. However, if the single litter in the 40 mg/kg/day group discussed by the Applicant is excluded, the mean litter proportion of post-implantation loss is less than that at the mid-dose indicating a lack of clear dose-relationship.

TABLE S47 (F2)

PROJECT NO. (b) (4) 661049D ORAL STUDY OF EFFECTS OF INT-747 ON PRE- & POSTNATAL DEV IN RATS PAGE 1

SPONSOR: INTERCEPT PHARM., INC. SUMMARY OF EMBRYONIC DATA AT SCHEDULED NECROPSY

GROUP	VIABLE EMBRYOS	DEAD EMBRYOS	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	NO. OF GRAVID FEMALES
1 TOTAL	330	0	11	0	11	341	374	33	22
MEAN	15.0	0.0	0.5	0.0	0.5	15.5	17.0	1.5	
S.D.	2.60	0.00	0.67	0.00	0.67	2.56	2.27	1.71	
S.E.	0.55	0.00	0.14	0.00	0.14	0.55	0.48	0.36	
2 TOTAL	341	0	11	0	11	352	377	25	22
MEAN	15.5	0.0	0.5	0.0	0.5	16.0	17.1	1.1	
S.D.	2.02	0.00	0.60	0.00	0.60	1.72	1.70	1.46	
S.E.	0.43	0.00	0.13	0.00	0.13	0.37	0.36	0.31	
3 TOTAL	324	0	25	0	25	349	363	14	22
MEAN	14.7	0.0	1.1	0.0	1.1	15.9	16.5	0.6	
S.D.	2.41	0.00	1.32	0.00	1.32	1.70	2.18	1.50	
S.E.	0.51	0.00	0.28	0.00	0.28	0.36	0.46	0.32	
4 TOTAL	330	0	25	0	25	355	405	50	24
MEAN	13.8	0.0	1.0	0.0	1.0	14.8	16.9	2.1	
S.D.	3.71	0.00	0.91	0.00	0.91	3.67	2.94	3.13	
S.E.	0.76	0.00	0.19	0.00	0.19	0.75	0.60	0.64	

MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group
MEAN NUMBER OF VIABLE EMBRYOS, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 5 MG/KG/DAY 3- 25 MG/KG/DAY 4- 40 MG/KG/DAY

TABLE S48 (F2)

PROJECT NO. (b) (4) 661049D ORAL STUDY OF EFFECTS OF INT-747 ON PRE- & POSTNATAL DEV IN RATS PAGE 1

SPONSOR: INTERCEPT PHARM., INC. SUMMARY OF EMBRYONIC DATA AT SCHEDULED NECROPSY [% PER LITTER]

GROUP:	0 MG/KG/DAY	5 MG/KG/DAY	25 MG/KG/DAY	40 MG/KG/DAY
CORPORA LUTEA				
MEAN	17.0	17.1	16.5	16.9
S.D.	2.27	1.70	2.18	2.94
S.E.	0.48	0.36	0.46	0.60
N	22	22	22	24
IMPLANTATION SITES				
MEAN	15.5	16.0	15.9	14.8
S.D.	2.56	1.72	1.70	3.67
S.E.	0.55	0.37	0.36	0.75
N	22	22	22	24
VIABLE EMBRYOS (%)				
MEAN	96.7	96.7	92.5	89.9
S.D.	4.53	3.97	9.26	19.89
S.E.	0.96	0.85	1.98	4.06
N	22	22	22	24
DEAD EMBRYOS (%)				
MEAN	0.0	0.0	0.0	0.0
S.D.	0.00	0.00	0.00	0.00
S.E.	0.00	0.00	0.00	0.00
N	22	22	22	24
EARLY RESORPTIONS (%)				
MEAN	3.4	3.3	7.5	10.1
S.D.	4.53	3.97	9.27	19.89
S.E.	0.97	0.85	1.98	4.06
N	22	22	22	24

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

TABLE S48 (F2)

PROJECT NO. (b) (4) 561049D ORAL STUDY OF EFFECTS OF INT-747 ON PRE- & POSTNATAL DEV IN RATS PAGE 2

SPONSOR: INTERCEPT PHARM., INC. SUMMARY OF EMBRYONIC DATA AT SCHEDULED NECROPSY [% PER LITTER]

GROUP:	0 MG/KG/DAY	5 MG/KG/DAY	25 MG/KG/DAY	40 MG/KG/DAY
LATE RESORPTIONS (%)				
MEAN	0.0	0.0	0.0	0.0
S.D.	0.00	0.00	0.00	0.00
S.E.	0.00	0.00	0.00	0.00
N	22	22	22	24
TOTAL RESORPTIONS (%)				
MEAN	3.4	3.3	7.5	10.1
S.D.	4.53	3.97	9.27	19.89
S.E.	0.97	0.85	1.98	4.06
N	22	22	22	24
PRE-IMPLANTATION LOSS (%)				
MEAN	8.8	6.4	3.4	12.9
S.D.	10.77	8.11	7.39	19.82
S.E.	2.30	1.73	1.58	4.05
N	22	22	22	24
POST-IMPLANTATION LOSS (%)				
MEAN	3.4	3.3	7.5	10.1
S.D.	4.53	3.97	9.27	19.89
S.E.	0.97	0.85	1.98	4.06
N	22	22	22	24

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
None significantly different from control group

Necropsy Evaluation:

Gross necropsies were performed on F1 pups not selected for the F1 generation on PND 21, and pups found dead or euthanized in extremis from PND 0 through the selection of the F1 generation. Evaluation of F1 females with evidence of mating were performed on GD 15. F1 males were necropsied following completion of the last day of F1 female necropsies.

At necropsy, there were no treatment-related findings.

Toxicokinetics:

Blood samples were collected from 4 pups/sex/group on PND 10 at ~3, 8, and 24 h following maternal dosing on LD 10. Concentrations of INT-747, UPF-832 (the glycine conjugate of INT-747), and UPF-1443 (the taurine conjugate of INT-747) in plasma were determined using LC/MS/MS.

Following oral administration of INT-747 to dams, plasma concentrations of INT-747 and its glycine conjugate were below the lower limits of quantitation (10 ng/mL and 1 ng/mL for INT-747 and UPF-832, respectively) with the exception of a single F1 female at 25 mg/kg/day in which UPF-832 was 1.06 ng/mL at 3 h after dosing. Exposure to the taurine conjugate of INT-747 was demonstrated in pups, and TK parameters for this metabolite are summarized in the Applicant's table below.

Text Table 2. TK Parameters for INT-747 Taurine Conjugate in F_1 Pups on PND 10

	Males			Females		
Dosage (mg/kg/day):	5	25	40	5	25	40
AUC_{last} (ng•hr/mL)	82.9	1550	1270	100	1760	1070
C_{max} (ng/mL)	4.88	134	60.4	4.99	183	63.7
T_{max} (hr)	3	3	8	8	3	3

10 Special Toxicology Studies

Review of Study No. P130020 is incorporated below from the pharmacology review of IND (b) (4) dated August 21, 2014 (P. Au, Ph.D., DGIEP).

Study title: Four-Week Repeated Dose Oral Toxicity Study of DSP-1747 with Impurity (b) (4) in Rats

Study no.:	P130020
Study report location:	Electronic submission (IND63307, 4.2.3.2), page 1-507
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 18, 2013
GLP compliance:	GLP per Japanese standards
QA statement:	Yes
Drug, lot #, and % purity:	DSP-1747 (obeticholic acid), lot T10/002, Purity: 100% DSR-119651, lot 121112, Purity: DSP-1747 95.34%; Impurity (b) (4)%

Key Study Findings

DSR-119651 (DSP-1747 spiked with approximately (b) (4)% of impurity (b) (4) was administered daily via oral gavage for 4 weeks to male and female rats at dose levels of 6 and 60 mg/kg/day to investigate its toxicity by comparing it with administration of DSP-1747. There were no mortalities, changes in body weight, food consumption, ophthalmoscopy and macroscopic pathology associated with administration of DSR-119651. Administration of 60mg/kg/day of DSR-119651 resulted in test article-related changes to hematology and serum chemistry parameters, organ weight, and microscopic pathology; however, similar changes were noted in rats administered with 60mg/kg/day of DSP-1747. The NOAEL for DSR-119651 and DSP-1747 was determined to be 6mg/kg/day in male and female rats. Based on the percentage of impurity (b) (4) in DSR-119651, the NOAEL for impurity (b) (4) was determined to be (b) (4) mg/kg/day in rats. The results of the study suggest no specific toxicity associated with impurity (b) (4) at a level up to (b) (4)% in the drug product.

Methods

Doses:	6 and 60 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% w/v% sodium carboxymethyl cellulose in water for injection
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	10 males and 10 females/group
Age:	6-week old
Weight:	Males: 115.8 to 133.9 g and Females: 90.4 to 105.0 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

Animals were observed twice daily for mortality

No deaths or moribundity occurred during study duration

Clinical Signs

All animals were observed twice daily during dosing period.

No test-article related clinical signs were observed.

Body Weights

All animals were weighted prior to dosing on Day 1 and weekly during dosing phase and prior to scheduled necropsy.

No test-article related changes were noted in body weight. The body weights for the different study groups are presented in the Tables below.

Sex: Male		Day(s) Relative to Start Date				
		1	8	15	22	28
Control	Mean	191.22	260.07	320.05	367.12	397.98
	SD	5.40	6.12	9.29	14.41	23.89
	N	10	10	10	10	10
DSP-1747 6 mg/kg	Mean	189.34	254.97	315.95	360.59	386.58
	SD	5.16	9.66	13.38	16.59	18.35
	N	10	10	10	10	10
DSP-1747 60 mg/kg	Mean	190.80	255.38	311.10	350.97	377.98
	SD	4.79	11.99	21.86	29.95	37.93
	N	10	10	10	10	10

Sex: Female		Day(s) Relative to Start Date				
		1	8	15	22	28
Control	Mean	141.39	169.18	191.30	206.98	216.92
	SD	5.94	8.33	15.05	16.10	18.69
	N	10	10	10	10	10
DSP-1747 6 mg/kg	Mean	141.20	174.39	197.54	217.36	225.13
	SD	4.40	6.39	11.49	18.69	18.20
	N	10	10	10	10	10
DSP-1747 60 mg/kg	Mean	139.56	168.22	189.95	204.59	214.40
	SD	4.98	6.43	9.09	12.53	14.38
	N	10	10	10	10	10

Feed Consumption

Food consumption was recorded on Days 1 and weekly during the dosing phase. The daily food consumption was calculated from the diet that was set and weighed 24hour prior to measurement.

No test article-related change to food consumption was observed.

Ophthalmoscopy

Ophthalmology examination was conducted on Day -1 and 28.

No test article-related abnormality was observed.

Hematology

Hematology, coagulation and clinical chemistry were evaluated in blood samples collected at necropsy (Day 28). The following parameters were evaluated:

Item	Method	
Leukocyte (White Blood Cell Count*)	Dual angle laser flow-cytometric measurement	
Erythrocyte (RBC, Red Blood Cell Count*)	Dual angle laser flow-cytometric measurement	
Hemoglobin* (HGB)	Modified cyanmethemoglobin method	
Hematocrit*	(RBC×MCV)/10	
Mean Corpuscular Volume (MCV*)	Dual angle laser flow-cytometric measurement	
Mean Corpuscular Hemoglobin (MCH*)	(HGB/RBC)×10	
Mean Corpuscular Hemoglobin Concentration (MCHC*)	(HGB/(RBC×MCV))×10^3	
Reticulocyte Count* (erythrocyte count × ratio)	Laser flow-cytometric measurement with RNA stain	
Platelets (Platelet Count*)	Dual angle laser flow-cytometric measurement	
Differential Leukocyte Count (leukocyte count × ratio) · Eosinophil* · Neutrophil* · Lymphocyte* · Basophil* · Monocyte* · LUC* (Large Unstained Cell)	Flow-cytometric measurement with peroxidase stain and dual angle laser flow-cytometric measurement	×10^3/μL
The above-mentioned 10 items were examined with a hematology system (ADVIA 120, Siemens Healthcare Diagnostics K.K.). One specimen for the reticulocyte count (Brecher's New Methylene Blue staining) and 1 specimen for the differential leukocyte count (May-Grünwald-Giemsa staining) were prepared and stored		
Prothrombin Time (PT*)	Scattered light detection method	sec
Activated Partial Thromboplastin Time (APTT*)	Scattered light detection method	sec
The above-mentioned 2 items were examined with an autocoagulometer (Sysmex CA-1500, Sysmex Corporation)		

Comparison of study groups administered with DSP-1747 and vehicle control showed a significantly low values for RBC (7.128 vs 7.471 x 10^6/μl), HGB (14.04 vs. 14.70 g/dL), and hematocrit (39.39 vs. 41.77 %) and a significantly high value for platelets (1278.5 vs. 1127.5 x 10^3/μl) in females receiving 60 mg/kg/day of DSP-1747. No change related to DSP-1747 was seen in males receiving 6 or 60 mg/kg/day or in females receiving 6 mg/kg/day.

Comparison of study groups administered with DSR-119651 and vehicle control showed a significantly low values for RBC (6.979 vs. 7.471 x 10^6/μl) and hematocrit (39.47 vs. 41.77 %) and a significantly high value for platelets (1245.2 vs. 1127.5 x 10^3/μl) in females receiving 60 mg/kg/day of DSR-119651.

While statistically significant changes in hematological parameters were noted in male and female rats administered with 60mg/kg/day of DSP-1747 or DSR-119651, the changes were small and do not appear to have any toxicological significance.

No change related to DSR-119651 was seen in males receiving 6 or 60 mg/kg/day or in females receiving 6 mg/kg/day.

No difference was noted between study groups administered with DSP-1747 and DSR-119651.

Clinical Chemistry

Serum samples were evaluated for the following parameters:

Item	Method	
Total Protein* (TP)	Biuret method	
Total Bilirubin* (TBil)	Vanadate oxidation method	
Asparate Aminotransferase (ASAT*)	UV-rate method	
Alanine Aminotransferase (ALAT*)	UV-rate method	
Gamma Glutamyltranspeptidase (γ-GTP, γGT*)	L-γ-Glutamyl-3-carboxy-4-nitroanilide reaction	
Lactate Dehydrogenase (LDH*)	UV-rate method	
Creatine Phosphokinase (CK, Creatine Kinase*)	UV-rate method	U/L
Alkaline Phosphatase (ALP*)	p-Nitrophenylphosphate reaction	U/L
Uric Acid* (UA)	Uricase•F-DAOS method	mg/dL
Total Cholesterol* (TC)	COD-HDAOS method	mg/dL
Triglyceride* (TG)	GPO-HDAOS, glycerol blanking method	mg/dL
Glucose* (Gluc)	Hexokinase•G-6-PDH method	mg/dL
Blood Urea Nitrogen (UN, Urea Nitrogen*)	Urease GIDH method	mg/dL
Creatinine* (CRE)	Creatininase•HMMPS method	mg/dL
Inorganic Phosphorus* (IP)	PNP•XOD method	mg/dL
Calcium (Ca*)	MXB method	mg/dL
The above-mentioned 16 items were examined with an auto-analyzer (7170, Hitachi Ltd.).		
Serum Protein Fractions (total protein × ratio)		
· α1 Globulin* · α2 Globulin* · β Globulin* · γ Globulin*	Cellulose acetate electrophoresis	g/dL
Albumin* (Alb)	Calculated from TP and albumin fraction of electrophoresis	g/dL
Albumin/Globulin ratio (A/G ratio*)	Calculated from fractions of serum protein fractions	
The above-mentioned 3 items were examined with an auto electrophoresis system (AES320, Beckman Coulter, Inc.).		
Sodium (Na*)	Ion selective electrode method	mmol/L
Potassium (K*)	Ion selective electrode method	mmol/L
Chloride (Cl*)	Ion selective electrode method	mmol/L
The above-mentioned 3 items were examined with an electrolyte analyzer (EA07, A&T Corporation).		

Comparison of study groups administered with DSP-1747 and vehicle control showed a statistically significant increase in the values for Alb (M: 2.963 vs. 2.586 g/dL, F: 3.139 vs. 2.787 g/dL), A/G ratio (M: 1.169 vs. 1.025 F: 1.329 vs. 1.111), and α2-globulin (M: 0.475 vs. 0.400 g/dL F: 0.404 vs. 0.368 g/dL) in males and females receiving 60 mg/kg/day of DSP-1747. Significantly high values for Total Protein (5.50 vs. 5.11 g/dL), Alanine Aminotransferase (25.0 vs. 20.9 U/L), Total Cholesterol (80.4 vs. 58.7 mg/dL), and UN (17.30 vs. 14.40 mg/dL) and a significantly low value for Triglyceride (28.4 vs. 45.8 mg/dL) were

noted in males receiving 60 mg/kg/day of DSP-1747. A significantly low value for α1-globulin (0.845 vs. 0.991 g/dL) and a significantly high value for Alkaline Phosphatase (288.1 vs. 215.0 U/L) were noted in females receiving 60 mg/kg/day of DSP-1747.

No change related to DSP-1747 was seen in males or females receiving 6 mg/kg/day.

Comparison of study groups administered with DSR-119651 and vehicle control showed a significantly high values for Alb (M: 2.959 vs. 2.586 g/dL, F: 3.228 vs. 2.787 g/dL), A/G ratio (M: 1.191 vs. 1.025, F: 1.347 vs. 1.111), and ALP (M: 549.0 vs. 426.2 U/L, F: 281.6 vs. 215.0 U/L) and a significantly low value for α1-globulin (M: 0.940 vs. 1.068 g/dL, F: 0.871 vs. 0.991 g/dL) in males and females receiving 60 mg/kg/day of DSR-119651. Significantly high values for TP (5.45 vs. 5.11 g/dL), α2-globulin (0.469 vs. 0.400 g/dL), ALAT (35.0 vs. 20.9 U/L), TC (85.0 vs. 58.7 mg/dL), and UN (16.54 vs. 14.40 mg/dL) and a significantly low value for TG (24.6 vs. 45.8 mg/dL) were noted in males receiving 60 mg/kg/day of DSR-119651.

No change related to DSR-119651 was seen in males or females receiving 6 mg/kg/day.

While statistically significant changes in serum chemistry parameters were noted in male and female rats administered with 60mg/kg/day of DSP-1747 or DSR-119651, the changes were small and do not appear to have any toxicological significance.

The toxicological findings appear similar in study groups administered with DSP-1747 and DSR-119651.

Gross Pathology

Animals were euthanized by exsanguination and all organs and tissues were immediately examined macroscopically.

No abnormality related to the test-article was noted.

Organ Weights

Designated organs (as indicated in Table below) from all animals were weighed at scheduled necropsy. The relative organ weights were calculated from the body weights.

A significantly high value for relative liver weight (M: 3.341 vs. 2.972 %, F: 3.535 vs. 2.920 %) was noted in males and females receiving 60 mg/kg/day of DSP-1747.

No change related to DSP-1747 was seen in males or females receiving 6 mg/kg/day.

A significantly high values for relative liver weights (M: 3.372 vs. 2.972 %, F: 3.583 vs. 2.92 %) were noted in males and females receiving 60 mg/kg/day of DSR-119651.

In addition, significantly high values for absolute and/or relative total adrenal weights (35.58 vs. 31.08 x 10^-3 %) were noted in females receiving 60 mg/kg/day of DSR-119651.

The changes in relative liver and adrenal weight were small and do not appear to have any toxicological significance. The changes in relative organ weight also did not correlate with histological findings.

Organs/Tissues	Collection (Laterality)	Organ weight measurement (Laterality)	Histopathology (Laterality)
Brain (cerebrum, cerebellum, medulla oblongata / pons)	• -	• -	• -
Pituitary	• -	• -	• -
Spinal cord (cervical and thoracic regions)	• -	- -	• -
Eyes	• Bilateral	- -	• Unilateral (Left)
Optic nerves	• Bilateral	- -	• Unilateral (Left)
Submaxillary lymph nodes	• Bilateral	- -	• Unilateral (Left)
Submaxillary glands	• Bilateral	- -	• Unilateral (Left)
Sublingual glands	• Bilateral	- -	• Unilateral (Left)
Thyroids	• Bilateral	• Bilateral [a]	• Unilateral (Left) [b]
Parathyroids	• Bilateral	Included in thyroid weight	• Unilateral (Left or right [c])
Thymus	• -	• -	• -
Trachea	• -	- -	• -
Lungs with bronchi	• -	• -	• -
Heart	• -	• -	• -
Tongue	• -	- -	• -
Esophagus	• -	- -	• -
Stomach	• -	- -	• -
Duodenum	• -	- -	• -
Jejunum	• -	- -	• -
Ileum with Peyer's patches	• -	- -	• -
Cecum	• -	- -	• -
Colon	• -	- -	• -
Rectum	• -	- -	• -
Mesenteric lymph node (including Mesenteric fat)	• -	- -	• -
Liver	• -	• -	• -
Pancreas	• -	- -	• -
Spleen	• -	• -	• -
Kidneys	• Bilateral	• Bilateral [a]	• Unilateral (Left)
Adrenals	• Bilateral	• Bilateral [a]	• Unilateral (Left)
Urinary bladder	• -	- -	• -
Testes	• Bilateral	• Bilateral [a]	• Unilateral (Left)
Epididymides	• Bilateral	• Bilateral [a]	• Unilateral (Left)
Prostate	• -	- -	• -
Seminal vesicle	• -	- -	• -
Ovaries	• Bilateral	• Bilateral [a]	• Unilateral (Left)
Uterus	• -	• -	• -
Vagina	• -	- -	• -

Femur	• Unilateral (Left)	- -	• Unilateral (Left)
Bone marrow of the femur	• Unilateral (Left)	- -	• Unilateral (Left)
Sternum	• -	- -	• -
Bone marrow of the sternum	• -	- -	• -
Skin (lower abdominal region)	• Unilateral (Left)	- -	• Unilateral (Left)
Mammary gland	• Unilateral (Left)	- -	• Unilateral (Left)
Aorta (thoracic region)	• -	- -	• -
Muscle biceps femoris	• Unilateral (Left)	- -	• Unilateral (Left)
Sciatic nerve	• Unilateral (Left)	- -	• Unilateral (Left)
Other organs/tissues with gross lesions	• -	- -	• -

•: Collected or examined.
a: Weights of bilateral organs were measured separately.
b: The thyroid was examined on right side for one animal (No. 289) due to defect of left thyroid.
c: The parathyroid was examined on either side; right side for four animals (Nos. 234, 270, 282, 289) and left side for the other animals.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings:

Designated organs and tissues (as indicated in Table above) were fixed in 10 vol% phosphate buffered formalin. The eyes, optic nerves, and Harderian glands were pre-fixed in Davidson's solution, and the testes and epididymides were pre-fixed in Bouin's solution, then these were preserved in the phosphate buffered formalin. The organ and tissue samples for all animals were embedded in paraffin, sectioned, stained with hematoxylin and eosin (HE), and examined microscopically.

In the colon and rectum, the following treatment-related changes were observed: diffuse inflammatory cell infiltration in the colon in 8 males and 10 females receiving 60 mg/kg/day of DSP-1747 and in 9 males and 8 females receiving 60 mg/kg/day of DSR-119651; and diffuse inflammatory cell infiltration in the rectum in 4 males and 3 females receiving 60 mg/kg/day of DSP-1747 and in 6 males and 1 female receiving 60 mg/kg/day of DSR-119651. Diffuse inflammatory cell infiltration in the colon and rectum was also sporadically observed in groups administered with vehicle control, 6 mg/kg/day of DSP-1747 or 6 mg/kg/day of DSR-119651 as indicated in the table below.

Removal Reason: Terminal scheduled sacrifice	Male					Female				
	Control	DSP-1747 6 mg/kg	DSP-1747 60 mg/kg	DSR-119651 6 mg/kg	DSR-119651 60 mg/kg	Control	DSP-1747 6 mg/kg	DSP-1747 60 mg/kg	DSR-119651 6 mg/kg	DSR-119651 60 mg/kg
Number of Animals	10	10	10	10	10	10	10	10	10	10
Cecum (Continued...)										
Cell infiltration; inflammatory, diffuse	4	5	4	2	6	2	1	4	2	3
... minimal	4	5	4	2	6	2	1	4	2	3
Colon										
Examined	10	10	10	10	10	10	10	10	10	10
No Visible Lesions	7	5	2	7	1	10	9	0	9	2
Cell infiltration; inflammatory, diffuse	3	5	8	3	9	0	1	10	1	8
... minimal	3	5	8	3	9	0	1	10	1	8
Rectum										
Examined	10	10	10	10	10	10	10	10	10	10
No Visible Lesions	10	7	6	8	4	10	10	7	10	9
Cell infiltration; inflammatory, diffuse	0	3	4	2	6	0	0	3	0	1
... minimal	0	3	4	2	6	0	0	3	0	1
Liver										
Examined	10	10	10	10	10	10	10	10	10	10
No Visible Lesions	6	8	8	6	9	1	8	7	7	6
Hypertrophy; hepatocyte, perilobular	0	0	1	0	0	0	0	0	0	2
... minimal	0	0	1	0	0	0	0	0	0	2
Hemorrhage; focal	1	0	0	0	0	0	0	0	0	0
... minimal	1	0	0	0	0	0	0	0	0	0
Necrosis; hepatocyte, focal	0	0	0	0	0	0	0	0	0	1

In the liver, perilobular hypertrophy of hepatocytes was observed in 1 male receiving 60 mg/kg/day of DSP-1747 and in 2 females receiving 60 mg/kg/day of DSR-119651.

No abnormality related to the test or reference substance was seen in either sex receiving 6 mg/kg/day.

Dosing Solution Analysis

DSP-1747 (nominal concentrations: 0.6 mg/mL and 6 mg/mL) were 0.575 mg/mL and 6.23 mg/mL.

DSR-119651: DSP-1747 (nominal concentrations: 0.6 mg/mL and 6 mg/mL) were 0.559 mg/mL and 6.40 mg/mL

Impurity (b) (4) (nominal concentrations: (b) (4) mg/mL and (b) (4) mg/mL) were (b) (4) mg/mL and (b) (4) mg/mL

11 Integrated Summary and Safety Evaluation

Obeticholic acid (OCA) is a modified bile acid and farnesoid X receptor (FXR) agonist that the Applicant is seeking to market for treatment of primary biliary cirrhosis (PBC). PBC is a rare, life-threatening liver disease characterized by cholestasis with progressive impairment of bile flow in the liver resulting in increased hepatocellular bile acid concentrations. Currently, the only drug approved to treat PBC is ursodeoxycholic acid (UDCA). However, the Applicant reports that up to 50% of UDCA-treated patients fail to respond or have suboptimal response to treatment. There is also a subset of PBC patients who are unable to tolerate UDCA. Thus, the proposed indication for OCA is for treatment of PBC in combination with UDCA in adults with an inadequate

response to UDCA or as monotherapy in adults unable to tolerate UDCA. The recommended starting dose for OCA is 5 mg/day. After 3 months, the dose may be increased to 10 mg/day [the maximum recommended human dose (MRHD)] to improve response. The Applicant has conducted pharmacology, pharmacokinetics/ ADME/toxicokinetics, single- and repeat dose general toxicology, genotoxicity, carcinogenicity, reproductive and developmental toxicology, and special toxicology studies in support of the NDA.

OCA is a FXR agonist, with EC50 values ~100-fold lower than the natural FXR agonist CDCA. Based on EC50 values, the glycine and taurine conjugates of OCA show similar activity at FXR, while the OCA-3-glucuronide metabolite has very low pharmacological activity (as compared to the parent compound). In safety pharmacology studies, OCA did not have any effects on neuropharmacologic profile, respiratory, cardiovascular, or gastrointestinal parameters. There were no treatment-related effects when tested in the hERG channel patch clamp assay.

OCA was shown to be rapidly absorbed in nonclinical species. Primary metabolic pathways of OCA in human, mouse, rat, rabbit, dog, and monkey S9 fractions included conjugation with glycine and taurine, glucuronidation, epimerization, and mono-oxidation and glucuronidation. OCA and its glycine and taurine conjugate metabolites exhibit high plasma protein binding (>98%), and tissue distribution studies indicate that distribution is primarily confined to the gastrointestinal tract and hepatobiliary system. The primary route of elimination is in the feces via biliary excretion, and OCA exhibits extensive enterohepatic recirculation (consistent with other bile acids).

Similar to endogenous bile acids, OCA is conjugated with the amino acids taurine and glycine. With the exception of the rabbit, OCA is primarily metabolized to the taurine conjugate with minimal or no metabolism to glyco-OCA in nonclinical species (mouse, rat, and dog). In contrast, both the taurine and glycine conjugates are major metabolites in humans, and exposures to the conjugates exceed those to the parent compound. Because OCA and the taurine and glycine conjugates have similar potencies at FXR and there are varying proportions of the glycine and taurine conjugates across species, comparison of exposure in nonclinical studies relative to human exposures was based on total systemic exposures of OCA plus tauro- and glyco-OCA. For exposure comparison, the Applicant estimated animal systemic exposures as total AUC exposures of OCA plus its conjugates, expressed as ng.h/mL of OCA ("total OCA equivalents"). For studies that measured OCA and its conjugates, the AUC for each conjugate (ng.h/mL) was converted to molar equivalents (µM.h) by dividing the conjugate's AUC value (ng.h/mL) by its molecular weight.[1] The total conjugate AUC (expressed as OCA equivalents) was then calculated by summing the molar equivalents for the taurine and glycine conjugates and multiplying by the molecular weight of OCA.[2] Finally, the "total OCA equivalent" AUC was calculated by summing the total conjugate AUC (ng.h/mL; expressed as OCA equivalents) with the OCA AUC (ng.h/mL). Since TK analysis was limited to OCA in many of the general

[1] Tauro-OCA molecular weight = 527.8 g/mole; Glyco-OCA molecular weight = 477.7 g/mole
[2] OCA molecular weight = 420.6 g/mole

Reference ID: 3870157

toxicology and reproductive and developmental toxicity studies, total OCA equivalents were estimated by the Applicant based upon metabolite:parent ratios determined from other studies in which both the parent compound and the conjugates were measured. For studies without TK analysis, exposures were estimated using AUC values from studies in which such analysis was conducted. Exposure ratios were calculated using the total OCA equivalent AUC_{0-24} value in healthy subjects at the MRHD of 10 mg (2972 ng.h/mL; Trial 747-105).

In repeat-dose oral toxicity studies in mice, rats, and dogs, respectively, the hepatobiliary system was identified as the primary target system of toxicity. In a 13-week toxicity study in mice, findings included clinical chemistry changes (e.g., increases in ALT, AST and sorbitol dehydrogenase), increased liver weights, and microscopic changes in the liver (increased mitoses, cytomegaly and karyomegaly, necrosis, and neutrophil infiltration). In a 26-week oral toxicity study in rats, OCA also produced clinical chemistry changes (e.g., increases in ALT, AST, and ALP), increased liver weights, and microscopic findings such as bile duct hyperplasia with hepatocellular hypertrophy. In addition, clinical signs such as yellow skin were observed in high dose animals (60 mg/kg/day OCA) prior to moribundity. Finally, in the 9-month oral toxicity study in dogs, OCA produced clinical signs of toxicity that could be associated with liver function (yellow discoloration of the skin, mucous membranes, and eyes) as well as elevated ALT levels. Although there were no microscopic changes in the liver in this study, there were histopathological findings in the liver and gallbladder in a 28-day toxicity study in dogs. For comparison, increased liver enzymes were also observed in humans at higher doses than 10 mg, proposed for the current indication (see the Clinical Review). Other primary target organs identified in the 26-week toxicity study in rats were the large intestine (subacute inflammation) and bone marrow (increased cellularity). With respect to safety evaluation, the estimated systemic exposures to total OCA equivalents at the NOAELs in the 26-week and 9-month toxicity studies in rats and dogs, respectively, are greater than those in humans at the MRHD of 10 mg. In the 26-week repeat-dose toxicity study in rats, the NOAEL was 6 mg/kg/day, a dose that was estimated to produce systemic exposures approximately 2.3 times those in humans at the MRHD.[3] The NOAEL dose from the 9-month repeat-dose toxicity study in dogs was 15 mg/kg/day, a dose that was estimated to produce systemic exposures approximately 12 times those in humans at the MRHD.[4]

Carcinogenic potential of OCA was assessed in oral carcinogenicity studies of up to 2 year in duration in mice and rats. In Crl:CD1 mice, there were no drug-related neoplastic findings at OCA doses up to 25 mg/kg/day, a dose estimated to produce systemic exposures approximately 12 times those in humans at the MRHD. In

[3] TK analysis in the 26-week toxicity study in rats (Study No. 019958) was limited to OCA. Thus, the Applicant estimated total OCA exposures at the NOAEL based on the total conjugate:OCA ratio from Day 179 in the 2-year carcinogenicity study in rats (Study No (b) (4) 661037). The total OCA equivalents AUC_{0-24} value at 6 mg/kg/day in the 26-week toxicity study was estimated to be 6982.0 ng.h/mL.

[4] TK analysis in the 9-month toxicity study in dogs (Study No. 019959) was limited to OCA. Thus, the Applicant estimated total OCA exposures at the NOAEL based on the total conjugate:OCA ratio from a 7-day TK study in dogs (Study No (b) (4) 661039). The total OCA equivalents AUC_{0-24} value at 15 mg/kg/day in the 9-month toxicity study was estimated to be 35791.6 ng.h/mL.

Reference ID: 3870157

Crl:CD(SD) rats, 20 mg/kg/day OCA (approximately 12 times the human exposure at the MRHD) caused an increase in the incidence of benign granulosa cell tumors in the ovaries and benign granular cell tumors in the cervix and vagina of female rats. There were no drug-related neoplastic findings in male rats at OCA doses up to 20 mg/kg/day.

OCA was not genotoxic in the Ames test, a human peripheral blood lymphocyte chromosomal aberration test, and a mouse micronucleus test. The glycine conjugate of obeticholic acid was also not genotoxic in an Ames test and human peripheral blood lymphocyte chromosome aberration test. The taurine conjugate of obeticholic acid was not genotoxic in an Ames test, and was negative in a human peripheral blood lymphocyte chromosomal aberration test in the presence of metabolic activation. When the taurine conjugate of OCA was test in the chromosomal aberration assay in the absence of metabolic activation, the findings were inconclusive.

In an oral fertility and early embryonic development study, treatment of male and female rats with up to 50 mg/kg/day OCA (approximately 13 times the human exposure at the MRHD) did not affect fertility or early embryonic development.[5] The NOAELs for male and female systemic toxicity in this study were 50 and 25 mg/kg/day, respectively. In an embryofetal development study in rats, 75 mg/kg/day OCA (approximately 40 times the human exposure at the MRHD) caused decreased fetal body weights and increased numbers of early or late resorptions and nonviable fetuses.[6] In maternal animals, this dose produced mortality, decreased body weight, body weight gain, and food consumption, and abortion. Therefore, the developmental toxicity observed at this dose may be secondary to maternal toxicity. The NOAEL for maternal toxicity and embryofetal development in this study was 25 mg/kg/day (approximately 13 times the human exposure at the MRHD). In an embryofetal development study in rabbits, the NOAEL for maternal and developmental toxicity was 20 mg/kg/day OCA (the highest dose tested; approximately 6 times the human exposure at the MRHD).[7] Finally, in a pre- and postnatal development study in rats, there was no evidence of any adverse

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[5] The fertility and early embryonic development study (Study No. (b) (4) 661005) did not include TK analysis. Thus, the Applicant estimated total OCA equivalents at the NOAEL using linear regression analysis of total OCA equivalent values from a 28-day rat toxicity study (Study No. 017262) at doses of 25, 75, and 150 mg/kg/day. Because TK analysis in Study No. 017262 was limited to OCA, total OCA exposures in this study were estimated based on the total conjugate:OCA ratios from a 14-day TK study in rats (Study No. (b) (4) 661032). The total OCA equivalents AUC_{0-24} value at 50 mg/kg/day in the fertility and early embryonic development study was estimated to be 37325.9 ng.h/mL.

[6] TK analysis in the definitive embryofetal development study in rats (Study No. (b) (4) 661003) was limited to OCA. Thus, the Applicant estimated total OCA equivalents at the NOAELs based on total conjugate:OCA ratios from a 14-day TK study in non-pregnant rats (Study No. (b) (4) 661032). The total OCA equivalents AUC_{0-24} values at 25 and 75 mg/kg/day in the embryofetal development study were estimated to be 37824.6 and 117944.2 ng.h/mL, respectively.

[7] TK analysis in the definitive embryofetal development study in rabbits (Study No. (b) (4) 661004) was limited to OCA. Thus, the Applicant estimated total OCA equivalents at the NOAEL based on total conjugate:OCA ratios from a TK study in pregnant rabbits (Study No (b) (4) 661078). The total OCA equivalents AUC_{0-24} value at 20 mg/kg/day in the embryofetal development study was estimated to be 17595.4 ng.h/mL, respectively.

effect on pre- and postnatal development at oral doses of OCA up to 40 mg/kg/day (the highest dose tested; approximately 21 times the human exposure at the MRHD).[8]

In regard to metabolite safety evaluation, glyco-OCA is a major metabolite in humans for which there is no or minimal exposure in nonclinical species other than the rabbit. When tested in *in vitro* genotoxicity studies, glyco-OCA was shown to be negative. There also were no treatment-related effects in the embryofetal development study in rabbits, a species for which there is preferential conjugation with glycine. Finally, although this Phase II metabolite was not tested separately in *in vivo* toxicology studies, the Applicant asserted that systemic exposure to this metabolite in nonclinical species would be unlikely following oral dosing with glyco-OCA since the conjugate would be de-conjugated in gut, absorbed as unconjugated OCA, and then preferentially re-conjugated to tauro-OCA in the liver. In addition to the glyco-OCA metabolite, 3-glucuronide OCA (estimated to represent >10% of total OCA-related exposure in humans) was identified as a disproportional human metabolite. The 3-glucuronide metabolite was shown to have very low pharmacological activity (based on ED50 values at FXR), as compared to the parent compound. In nonclinical species, 3-glucuronide OCA was formed as a minor metabolite in rats (females only). Finally, as discussed in FDA's Guidance for Industry Safety Testing of Drug Metabolites, Phase II conjugation reactions generally render a compound more water soluble and pharmacologically inactive, thereby eliminating the need for further evaluation.

In conclusion, the Applicant has submitted adequate nonclinical studies of OCA in support of the NDA. Thus, from a nonclinical perspective the NDA is approvable. Specific recommendations for the label are provided in Section 1.3.3 (Labeling).

12 Appendix/Attachments

[8] The pre- and postnatal development study in rats (Study No. (b) (4) 661049) did not include TK analysis. Thus, the Applicant estimated total OCA equivalents at the NOAEL using linear regression analysis of total OCA equivalent values from the definitive embryofetal development study (Study No. (b) (4) 661003) at doses of 5, 25, and 75 mg/kg/day. Because TK analysis in Study No. (b) (4) 661003 was limited to OCA, total OCA exposures in this study were estimated based on the total conjugate:OCA ratios from in a 14-day TK study in rats (Study No. (b) (4) 661032). The total OCA equivalents AUC_{0-24} value at 40 mg/kg/day in the pre- and postnatal development study was estimated to be 62386.8 ng.h/mL.

Reference ID: 3870157

Attachment-1

ECAC Meeting Minutes dated October 6, 2011

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: October 6, 2011

To: Pia Lindstrom, VP, Regulatory Affairs	**From:** Adele Seifried
Company: Intercept Pharmaceuticals Inc.	OND IO
Fax number: (858) 380-1111	**Fax number:** 301-796-9855
Phone number: (858) 652-6805	**Phone number:** 301-796-0535

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report - IND 63,307

Total no. of pages including cover: 4

Comments:

Document to be mailed: YES ☑ NO

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Executive CAC
Date of Meeting: October 4, 2011

Committee: Abby Jacobs, Ph.D., OND IO, Acting Chair
Paul Brown, Ph.D., OND IO, Member
Aisar Atrakchi, Ph.D., DPP, Alternate Member
Sushanta Chakder, Ph.D., DGIEP, Supervisor
Charles Wu, Ph.D., DGIEP Presenting Reviewer

Author of Minutes: Charles Wu, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format- Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008) and the associated Study Data Specifications document.

IND #: 63307
Submit date: August 29, 2011
Drug Name: INT-747
Sponsor: Intercept Pharmaceuticals

Background
INT-747, a modified bile acid and a farnesoid X receptor (FXR) agonist, is being developed for the treatment of primary biliary cirrhosis. The anticipated therapeutic dose of INT-747 for this indication is ≤ 25 mg/day. The sponsor submitted the rationale for the dose selection for a proposed 2-year oral gavage carcinogenicity study in SD rats and a 2-year oral gavage carcinogenicity study in CD-1 mice.

Rat Carcinogenicity Study Protocol and Dose Selection
The sponsor proposed to conduct a 2-year oral (gavage, 10 mL/kg) carcinogenicity study of INT-747 at 0 (vehicle), 0 (vehicle), 6, 15, and 25 mg/kg/day in Sprague-Dawley rats (n = 65/sex/dose). INT-747 will be administered once daily by gavage, in 0.5% (w/v) carboxymethylcellulose as the vehicle. A complete necropsy will be conducted on all animals (found dead, euthanized *in extremis*, and at scheduled necropsy) and a standard battery of tissues/organs will be processed for histopathological examinations with a peer review.

Based on deaths at the 60 mg/kg/day dose in both males and females in the 26-week studies in rats, the 25 mg/kg/day dose was proposed as the high dose for the 2-year rat carcinogenicity studies.

Mouse Carcinogenicity Study Protocol and Dose Selection
The sponsor proposed to conduct a 2-year oral (gavage, 10 mL/kg) carcinogenicity study of INT-747 at 0 (vehicle), 0 (vehicle), 10, 20, and 30 mg/kg/day in CD-1 mice (n = 65/sex/dose). INT-747 will be administered by oral gavage once daily, in 0.5% (w/v) carboxymethylcellulose as the vehicle. A complete necropsy will be conducted on all animals (found dead, euthanized *in extremis*, and at scheduled necropsy) and a standard battery of tissues/organs will be processed for histopathological examinations with a peer review.

In the 13-week oral repeat dose toxicity study in CD-1 mice, groups of animals were treated with 4, 12, 40 and 120 mg/kg/day. Due to extreme clinical signs observed at the 120 mg/kg/day dose, this dose was reduced to 80 mg/kg/day on Day 8 after a drug holiday from Day 2 through Day 7. There were deaths of male and female animals at the 120/80 mg/kg/day dose. Based on deaths in this dose group, 30 mg/kg/day was proposed as the high dose for the 2-year mouse carcinogenicity study.

Executive CAC Recommendations and Conclusions:

Rat:

- The Committee recommended doses of 2, 7, and 20 mg/kg/day by oral gavage for both males and females, based on deaths observed at 60 mg/kg/day in the 26-week toxicity study.

Mouse:

- The Committee recommended doses of 4, 10, and 25 mg/kg/day by oral gavage for both male and female mice, based on deaths observed at 80 mg/kg/day in the 13-week toxicity study.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, DGIEP
/S. Chakder, Ph.D., Supervisor, DGIEP
/C. Wu, Ph.D., Primary Reviewer, DGIEP
/ J. Benjamin, Project Manager, DGIEP
/A. Seifried, OND IO

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/s/

ABIGAIL C JACOBS
10/06/2011

Attachment-2

ECAC Meeting Minutes dated September 10, 2015

Executive CAC
Date of Meeting: September 8, 2015

Committee: Abby Jacobs, Ph.D., OND IO, Acting Chair
Paul Brown, Ph.D., OND IO, Member
Tim McGovern, Ph.D., ONDIO, Member
Sushanta Chakder, Ph.D., DGIEP, Pharm Tox Supervisor
Tracy Behrsing, Ph.D., DGIEP, Presenting Reviewer

Author of Draft: Tracy Behrsing, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA # 207999
Drug Name: INT-747
Sponsor: Intercept Pharmaceuticals, Inc.

Background
INT-747 is a modified bile acid and farnesoid X receptor (FXR) agonist under development for the treatment of primary biliary cirrhosis (PBC) in combination with ursodeoxycholic acid (UDCA) in adults with an inadequate response to UDCA or as monotherapy in adults unable to tolerate UDCA.

Rat Carcinogenicity Study
In the 24-month carcinogenicity study, Crl:CD(SD) rats (65/sex/group) were administered INT-747 by oral gavage at dose levels of 0, 2, 7, or 20 mg/kg/day. Animals in dual control groups received vehicle only [0.5% carboxymethylcellulose (CMC) in deionized water]. Dose selection was based on the maximum tolerated dose (MTD) as per the ECAC recommendations.

In males, there were no treatment-related effects on survival. However, due to the reduced group size in high dose males, all surviving males were terminated during Week 100/101. In females, pairwise comparisons showed statistically significant decreases in survival rates in the mid-dose group compared to pooled controls and control group 2.

No drug-related neoplastic findings were observed in male rats. In females, there was a statistically significant increase in the incidence of benign ovarian granulosa cell tumors at the high dose (3/65), as compared to the pooled control groups (0/130; p=0.0309). The incidence of this rare tumor type in the ovaries at the high dose (4.6%) exceeded the (b) (4) Research historical control range (up to 1.67%). In the cervix, vagina, and cervix and vagina combined, there was a statistically significant increase in the incidence of benign granular cell tumors at the high dose (5/65, 3/65, and 8/65, respectively), as compared to the pooled control groups (0/130; p=0.0030, p=0.0309, and p<0.001, respectively). The combined incidence of granular cell tumors in the cervix and vagina at the high dose (12.3%) approached the maximum (b) (4) Research historical control incidence for granular

cell lesions in the cervix and vagina combined (14.0%). Furthermore, trend analysis and pairwise comparison (high dose versus pooled controls) of granular cell tumors in the cervix and vagina combined showed this finding to be highly significant ($p<0.001$).

Mouse Carcinogenicity Study
In the 24-month carcinogenicity study, Crl:CD-1 mice (65/sex/group) were administered INT-747 by oral gavage at dose levels of 0, 4, 10, or 25 mg/kg/day. Mice in dual control groups received vehicle only (0.5% CMC in deionized water). Dose selection was based on the MTD as per the ECAC recommendations.

There were no treatment-related effects on survival in males. There was a statistically significant increase in the survival rate of high dose females, compared to pooled controls and control group 2.

There were no drug-related neoplastic findings in male or female mice.

Executive CAC Recommendations and Conclusions

Rat:

- The Committee concurred that the study was acceptable, noting prior concurrence with the protocol.
- The Committee concurred that benign granulosa cell tumors in the ovaries, and benign granular cell tumors in the cervix and vagina of females were drug related.

Mouse:

- The Committee concurred that the study was acceptable, noting prior concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in male or female mice.

Abby Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, DGIEP
/Sushanta Chakder, DGIEP
/Tracy Behrsing, DGIEP
/Anissa Davis, DGIEP
/ASeifried, OND IO

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ADELE S SEIFRIED
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SUSHANTA K CHAKDER
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