CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208745Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

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

FROM: David B. Joseph
Lead Pharmacologist

DATE: December 2, 2016

SUBJECT: NDA 208,745 (SD # 1 dated January 29, 2016)

Sponsor: Synergy Pharmaceuticals Inc.

Drug Product: TRULANCE (plecanatide) tablets

Comments:

There are no nonclinical issues which preclude the approval of Trulance. I concur with the recommendations related to approvability, stated in the Pharmacology/Toxicology review by Dr. Yuk-Chow Ng. The final revisions to nonclinical information in labeling subsections 5.1, 8.1, 8.4, 12.1, and 13.1 include minor changes from the recommended version in Dr. Ng's review. The additional labeling revisions were developed through collaboration of the nonclinical team with other members of the review team.

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

cc:
NDA 208,745
DGIEP
DGIEP/PM
DGIEP/D. Joseph
DGIEP/Y-C. Ng
OND IO/A. Jacobs

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

/s/

DAVID B JOSEPH
12/02/2016

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:	208,745
Supporting document/s:	1
Applicant's letter date:	1/29/2016
CDER stamp date:	1/29/2016
Product:	Trulance (plecanatide) tablets
Indication:	Chronic idiopathic constipation
Applicant:	Synergy Pharmaceuticals, Inc
Review Division:	Gastroenterology and Inborn Errors Products
Reviewer:	Yuk-Chow Ng, PhD
Supervisor/Team Leader:	David B. Joseph, PhD
Division Director:	Donna Griebel, MD
Project Manager:	Maureen D. Dewey

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208,745 are owned by Synergy or are data for which Synergy has obtained a written right of reference. Any information or data necessary for approval of NDA 208,745 that Synergy does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208,745.

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1 Executive Summary

1.1 Introduction

Plecanatide (SP-304) is a synthetic hexadecapeptide that is designed to mimic the action of uroguanylin and guanylin, which are endogenous peptide agonists for the guanylate cyclase C (GC-C) receptor. These peptides are secreted in the GI tract and up-regulate intracellular production of cGMP (cyclic guanosine 3', 5'-monophosphate) in the intestinal epithelium. Elevated cGMP activates the cystic fibrosis transmembrane conductance regulator (CFTR), which leads to trans-epithelial efflux of chloride and bicarbonate from enterocytes lining the GI tract into the lumen of the gut, and secretion of water into the intestinal lumen. Increased secretion of water into the GI tract can loosen stools, stimulate bowel movements, and thus relieve constipation. The proposed indication for plecanatide is treatment of chronic idiopathic constipation.

1.2 Brief Discussion of Nonclinical Findings

Plecanatide is well tolerated in adult mice, rats, and monkeys at oral doses up to 3000-, 2000-, and 2000-times the recommended human dose (3 mg), respectively, on a mg/kg basis for an assumed human weight of 60 kg. It should be noted that plecanatide and its active metabolite are not measurable in human plasma following administration of the recommended dose, whereas limited systemic exposure to plecanatide was achieved in the nonclinical studies. Therefore, the animal to human dose ratios listed above are not indicative of relative exposure. Neonatal/juvenile mice have been shown to be particularly sensitive to plecanatide toxicity. Lethality of plecanatide was found to be highly age-dependent; the minimum lethal doses in PND (postnatal day) 7 and PND 14 mice were 0.5 and 10 mg/kg/day, respectively, as compared to the recommended human dose of 3 mg (0.05 mg/kg/day, based on a 60-kg bodyweight). There were no deaths at up to 300 mg/kg/day in juvenile mice administered plecanatide starting on PND 21. In the PND 7 and PND 14 mice, deaths occurred within the first or second day after dosing. Clinical signs included decreased motor activity and dehydration. No gross lesions were noted at necropsy. Treatment-related increases in the weight of intestinal contents were observed in juvenile mice following single oral doses of 1, 3, and 10 mg/kg/day plecanatide on PND 14, and to a lesser extent on PND 21. The increase in intestine weight after plecanatide administration appears to be consistent with the pharmacological action of plecanatide. The minimum lethal dose of 0.5 mg/kg/day at PND 7 is 10 times the recommended human dose (0.05 mg/kg/day) based on a 60-kg bodyweight. It is noted that the levels of expression of the receptor for heat-stable enterotoxin (i.e. GC-C) in the small and large intestine of children is age dependent; a greater number of receptors are present in infants, and the number decreases with increasing age. Thus, the data from neonatal/juvenile mice may have clinical relevance in infants or young pediatric patients.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical standpoint, there are no approvability issues.

1.3.2 Additional Non Clinical Recommendations

Recommendations for labeling changes are shown in the following section.

1.3.3 Labeling

Established Pharmacologic Class (HIGHLIGHTS)

The Sponsor's proposed EPC (established pharmacologic class) text phrase in the Highlights of Prescribing Information is "guanylate cyclase-C agonist". Plecanatide is an analog of the endogenous human uroguanylin peptide, and both are guanylate cyclase-C agonists. The mechanism of action of plecanatide is identical to that of the approved drug LINZESS (linaclotide), an analog of guanylin. The EPC for linaclotide is "guanylate cyclase-C agonist". Therefore, the Sponsor's proposed EPC text phrase "guanylate cyclase-C agonist" for plecanatide is deemed appropriate.

The following subsections in the labeling should be revised as recommended.

Sponsor's Proposed Version:

5.1 (b) (4)

TRADENAME is contraindicated in (b) (4) 6 years of age. The safety and effectiveness of TRADENAME in (b) (4) patients (b) (4) 18 years of age have not been established. In young juvenile mice (b) (4) plecanatide increased fluid-secretion into the intestines as a consequence of stimulation of guanylate cyclase-C (GC-C) (b) (4) mortality in some within the first 24 hours apparently due to dehydration (b) (4)

Avoid the use of PLECANATIDE in (b) (4) patients 6 (b) (4) years of age. Although there were no deaths in older juvenile mice (b) (4), given the deaths in younger mice and the lack of clinical safety and efficacy data in pediatric patients, avoid the use of TRADENAME in (b) (4) patients 6 (b) (4) years of age *[see Contraindications (**Error! Reference source not found.**), Use in Specific Populations* (b) (4)

APPEARS THIS WAY ON ORIGINAL

Evaluation: The following revised version was developed in collaboration with the Medical and Pediatric teams.

Recommended Version:

5.1 Risk of Serious Dehydration in Pediatric Patients

TRADENAME is contraindicated in patients less than 6 years of age. The safety and effectiveness of TRADENAME in patients less than 18 years of age have not been established. In young juvenile mice (b) (4) plecanatide increased fluid secretion into the intestines as a consequence of stimulation of guanylate cyclase-C (GC-C) resulting in mortality in some mice within the first 24 hours, apparently due to dehydration. Due to increased intestinal expression of GC-C, patients less than 6 years of age may be more likely than patients 6 years of age and older to develop significant diarrhea and its potentially serious consequences.

Avoid the use of TRADENAME in patients 6 years to less than 18 years of age. Although there were no deaths in older juvenile mice (b) (4) given the deaths in younger mice and the lack of clinical safety and efficacy data in pediatric patients, avoid the use of TRADENAME in patients 6 years to less than 18 years of age *[see Contraindications (**Error! Reference source not found.**), Warnings and Precautions (5.2), Use in Specific Populations (8.4)]*. APPEARS THIS WAY ON ORIGINAL

Sponsor's Proposed Version:

8.1 Pregnancy

Risk Summary

(b) (4)

Data

Animal data

(b) (4)

(b) (4)

Limited systemic exposure to plecanatide was achieved in animals (area under the time-concentration curve [AUC_t] = 449 ng•h/mL (b) (4) rabbits given 250 mg/kg/day (b) (4) Plecanatide and its active metabolite are not measurable in human plasma following administration of the recommended clinical (b) (4) Therefore, animal and human doses should not be compared directly for exposure.

Evaluation: The following revised version was developed in collaboration with the Maternal Health team (Christos Mastroyannis and Tamara Johnson).

Recommended Version:

8.1 Pregnancy

Risk Summary

Plecanatide is negligibly absorbed systemically following oral administration *[see Clinical Pharmacology (12.3)]*, and is not expected to result in fetal exposure to the drug.

The available data on TRADENAME use in pregnant women are not sufficient to inform any drug-associated risk for major birth defects and miscarriage. In animal developmental studies, no effects on embryo-fetal development were observed with oral administration of plecanatide in mice and rabbits during organogenesis at doses much higher than the maximum recommended human dosage.

In the United States general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

Data

Animal Data

Pregnant mice and rabbits were administered plecanatide during the period of organogenesis. There was no evidence of harm to embryo-fetal development at oral doses up to 800 mg/kg/day in mice and 250 mg/kg/day in rabbits. Oral administration of up to 600 mg/kg/day in mice during organogenesis through lactation produced no developmental abnormalities or effects on growth, learning and memory, or fertility in the offspring through maturation.

The maximum recommended human dose is approximately 0.05 mg/kg/day, based on a 60-kg body weight. Limited systemic exposure to plecanatide was achieved in animals ([AUC_t] = 449 ng•h/mL in rabbits given 250 mg/kg/day during organogenesis). Plecanatide and its active metabolite are not measurable in human plasma following administration of the recommended clinical dosages. Therefore, animal and human doses should not be compared directly for evaluating relative exposure.

Sponsor's Proposed Version:

8.4 Pediatric Use

(b) (4)

TRADENAME is contraindicated in pediatric patients (b) (4) 6 years of age.

Evaluation: The following revised version was developed in collaboration with the Pediatric and Labeling Development teams.

Recommended Version:

8.4 Pediatric Use

TRADENAME is contraindicated in patients less than 6 years of age. Avoid use of TRADENAME in patients 6 years to less than 18 years of age *[see Contraindications (4), Warnings and Precautions (5.1)].* The safety and effectiveness of TRADENAME in patients less than 18 years of age have not been established.

In nonclinical studies, deaths occurred within 24 hours in young juvenile mice (human age equivalent of approximately 1 month to less than 2 years) following administration of one or two once daily oral doses of plecanatide, as described below in Juvenile Animal Toxicity Data. Because of increased intestinal expression of GC-C, patients less than 6 years of age may be more likely than patients 6 years of age and older to develop diarrhea and its potentially serious consequences. TRADENAME is contraindicated in patients less than 6 years of age. (b) (4) Given the deaths in young juvenile mice and the lack of clinical safety and efficacy data in pediatric patients, avoid the use of TRADENAME in patients 6 years to less than 18 years of age.

Juvenile Animal Toxicity Data

Single oral doses of plecanatide at 0.5 mg/kg and 10 mg/kg caused mortality in young juvenile mice on postnatal days (b) (4) 7 and 14, respectively (human age equivalent of approximately 1 month to less than 2 years). Treatment-related increases in the weight of intestinal contents were observed in juvenile mice following single doses of plecanatide on (b) (4) 14 (human age equivalent of approximately less than 2 years), (b) (4) consistent with increased fluid in the intestinal lumen. (b) (4) The (b) (4)

recommended human dose is approximately 0.05 mg/kg/day, based on a 60-kg body weight. Plecanatide and its active metabolite are not measurable in human plasma (b) (4) whereas systemic absorption was demonstrated in the juvenile animal toxicity studies. (b) (4), animal and human doses should not be compared directly for evaluating relative exposure.

Sponsor's Proposed Version:

12.1 Mechanism of Action

(b) (4) Both plecanatide and its active metabolite bind to GC-C and act locally on the luminal surface of the intestinal epithelium. Activation of GC-C results in an increase in both intracellular and extracellular concentrations of cyclic guanosine monophosphate (cGMP). Elevation of intracellular cGMP stimulates secretion of chloride and bicarbonate into the intestinal lumen, mainly through activation of the cystic fibrosis transmembrane conductance regulator ion channel (CFTR), resulting in increased intestinal fluid and accelerated transit. In animal models, plecanatide has been shown to increase fluid secretion into the gastrointestinal (GI) tract, accelerate intestinal transit, and cause changes in stool consistency. (b) (4) (b) (4)

Evaluation: The following revised version is recommended.

Recommended Version:

Plecanatide is a guanylate cyclase-C (GC-C) agonist. Both plecanatide and its active metabolite bind to GC-C and act locally on the luminal surface of the intestinal epithelium. Activation of GC-C results in an increase in both intracellular and extracellular concentrations of cyclic guanosine monophosphate (cGMP). Elevation of intracellular cGMP stimulates secretion of chloride and bicarbonate into the intestinal lumen, mainly through activation of the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel, resulting in increased intestinal fluid and accelerated transit. In animal models, plecanatide has been shown to increase fluid secretion into the gastrointestinal (GI) tract, accelerate intestinal transit, and cause changes in stool consistency. (b) (4)

Sponsor's Proposed Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

The carcinogenic potential of plecanatide was assessed in a 2-year carcinogenicity study in mice and (b) (4) rats. (b) (4)

(b) (4)

Mutagenesis

Plecanatide was not genotoxic in (b) (4) in vitro bacterial reverse mutation (Ames) assay (b) (4) in vitro mouse lymphoma (b) (4) assay (b) (4) (b) (4) in vivo mouse bone marrow micronucleus (b) (4)

Impairment of Fertility

(b) (4) Plecanatide had no effect on fertility (b) (4) or reproductive function at oral doses of up to 600 (b) (4)

Evaluation: The following revised version is recommended.

Recommended Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

The carcinogenic potential of plecanatide was assessed in 2-year carcinogenicity studies in mice and rats. Plecanatide was not tumorigenic in mice at oral doses up to 90 mg/kg/day or in rats at oral does up to 100 mg/kg/day. Limited systemic exposure to plecanatide was achieved at the tested dose levels in animals, whereas no detectable exposure occurred in humans. Therefore, animal and human doses should not be compared directly for evaluating relative exposure.

Mutagenesis

Plecanatide was not genotoxic in the *in vitro* bacterial reverse mutation (Ames) assay, *in vitro* mouse lymphoma mutation assay or the *in vivo* mouse bone marrow micronucleus assay.

Impairment of Fertility

Plecanatide had no effect on fertility or reproductive function in male and female mice at oral doses of up to 600 mg/kg/day.

Sponsor's Proposed Version:

(b) (4)

Evaluation: This subsection should be removed.

2 Drug Information

2.1 Drug

CAS Registry Number – 467426-54-6

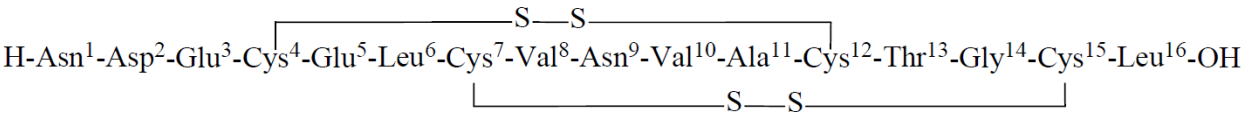
Generic Name - Plecanatide

Code Name – SP-304

Chemical Name -
L-Leucine, L-asparaginyl-L-α-aspartyl-L-α-glutamyl-L-cysteinyl-L-α-glutamyl-L-leucyl-L-cysteinyl-L-valyl-L-asparaginyl-L-valyl-L-alanyl-L-cysteinyl-L-threonylglycyl-L-cysteinyl-, cyclic (4→12),(7→15)-bis(disulfide)

Molecular Formula/Molecular Weight: $C_{65}H_{104}N_{18}O_{26}S_4$ /1682

Structure or Biochemical Description



Pharmacologic Class – guanylate cyclase-C agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 74,883 (Plecanatide for the treatment of chronic idiopathic constipation) Synergy Pharmaceuticals Inc., New York

2.3 Drug Formulation

Plecanatide tablets are an immediate release solid oral dosage form provided as a 3 mg (b) (4) dosage strength. The components and composition of plecanatide tablets, 3 (b) (4), are shown in the table below, taken from the Sponsor's submission.

Component	Quality Standard	Function	Dosage Strength (mg/tablet)	
			3	(b) (4)
Plecanatide[a]	In-house standard	Drug substance	3.0	
Microcrystalline cellulose[b]	USP-NF	(b) (4)	(b) (4)	
Magnesium stearate[c]	USP-NF			
(b) (4)	USP-NF			
Total (mg)				

2.4 Comments on Novel Excipients

There are no novel excipients used in the manufacture of plecanatide tablets. The excipients to be used in the plecanatide formulation appear to be safe. The FDA Inactive Ingredients Database confirms that all the excipients listed in the table above are present in approved oral formulations at levels (e.g., mg/tablet) that exceed the maximum daily dose (mg) in plecanatide tablets, based on the proposed maximum dose of (b) (4) plecanatide (b) (4)

2.5 Comments on Impurities/Degradants of Concern

In a pre-NDA meeting held on October 30, 2014, the CMC and nonclinical teams agreed that the reporting, identification, and qualification thresholds for potential impurities should be (b) (4)%, respectively, as proposed by the Sponsor. The Sponsor has reported and identified five drug product impurities, namely (b) (4) (b) (4) (b) (4) Based on a drug substance stress study, these 5 impurities were also identified as degradation products. All other drug product impurities are below the reporting threshold of (b) (4)%.

The specifications for (b) (4) and are therefore compliant with the qualification threshold. The specifications for (b) (4) are (b) (4)%, respectively. These limits exceed the qualification threshold, therefore the safety of (b) (4) were evaluated.

(b) (4)

The Sponsor qualified the (b) (4) by demonstrating the presence of substantial levels of these two impurities in the drug substance lots (b) (4) The table below taken from the Sponsor's submission shows the safety factors of these two impurities based on body surface area.

Table 45: Drug Product Impurity Specifications and Qualification Assessment

Specified Impurity	Specification	Maximum Total Daily Exposure for 6 mg Tablet (mg)	Specification above Qualification Threshold	Qualification Level[a] / DS Lot No.	Study Description/ Study No. / Duration of Use	Safety Factor (6 mg tablet)
(b) (4)			No	NA	NA	NA
			No	NA	NA	NA
			Yes	(b) (4)	26-week mouse / 1896-008 / Weeks 1-23	(b) (4)
					13-week rat / 1896-013 / Weeks 1-5	
					13-week rat / 1896-013 / Weeks 6-13	
					39-week monkey / 1896-009 / Weeks 1-24	
					39-week monkey / 1896-009 / Weeks 25-39	
			Yes		26-week mouse / 1896-008 / Weeks 1-23	
					13-week rat / 1896-013 / Weeks 1-5	
					13-week rat / 1896-013 / Weeks 6-13	
					39-week monkey / 1896-009 / Weeks 25-39	
			No	NA	NA	NA

DS = drug substance; NA = not applicable; NOAEL = no observed adverse effect level
[a] Qualification levels were calculated according to the following method: [(% area specified impurity / 100)*NOAEL dose (mg/kg/day)] / body surface area conversion factor (12.3 mouse, 6.2 rat or 3.1 monkey) * 60 kg human body weight.
[b] Safety margin = qualification level / maximum total daily exposure of impurity.

The safety factors shown in the Sponsor's table were calculated based on NOAEL and body surface area. However, similar to the parent compound plecanatide, (b) (4) are likely to have very little systemic exposure. Therefore, a dose multiple calculated on a mg/kg basis will be more appropriate. Based on a mg/kg comparison, the safety factors would have been greater than those shown in the above table.

In addition, considerations were given to address whether (b) (4) (b) (4) could be a mutagenic impurity, which would have a lower acceptable daily intake. It is noted that (b) (4) itself tested negative in the Ames assay (European Chemicals Agency registration-dossier), and therefore, (b) (4) appears unlikely to be a mutagenic impurity.

Taken together, the impurities known as (b) (4) are considered qualified at the proposed limits in the drug product.

2.6 Proposed Clinical Population and Dosing Regimen

Patients with chronic idiopathic constipation: 3 (b) (4) orally once daily.

2.7 Regulatory Background

IND 74,883 was submitted by Synergy Pharmaceuticals Inc. on 4/2/2008 for SP-304 (plecanatide) for the treatment of chronic constipation.

3 Studies Submitted

3.1 Studies Reviewed

THE FOLLOWING PHARMACOLOGY STUDIES WERE REVIEWED (starting on page 22)

Overview			**Test Articles: Plecanatide (SP-304); SP-338 (metabolite)**	
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**
Primary Pharmacodynamics				
SP-304: Stimulation of Intracellular cGMP Synthesis in T84 Cells	Human Colon Carcinoma T84 Cells	In Vitro	Synergy Pharma	SP-PH-001
Studies on SP-304 Thermostability, pH Dependency and Topoisomeric Stability	Human Colon Carcinoma T84 Cells	In Vitro	Synergy Pharma	SP-PH-004
Stimulation of Intracellular cGMP Synthesis in T84 Cells: SP-304 & SP-338, a Comparative Study	Human Colon Carcinoma T84 Cells	In Vitro	Synergy Pharma	SP-PH-008
Biological Activity of Potential Plecanatide Metabolites	Human Colon Carcinoma T84 Cells	In Vitro	Synergy Pharma	SP-PH-011
SP-304: Binding Affinity to the Guanylate Cyclase C Receptor	Human Colon Carcinoma T84 Cells	In Vitro	(b) (4)	SP-PH-003
SP-304:Synergistic Effects with Phosphodiesterase Inhibitors on cGMP Stimulation and Caspase-3 Activity in T84 Human Colon Carcinoma Cells	Human Colon Carcinoma T84 Cells	In Vitro	Synergy Pharma	SP-PH-002
Effect of pH on Plecanatide and Uroguanylin Mediated Activation of Guanylate Cyclase-C	Human Colon Carcinoma T84 Cells Mice Proximal Intestine and Colon Epithelial Cells and Tissues	In Vitro In Vitro / Ex Vivo	Synergy Pharma	SP-PH-020

Overview			Test Articles: Plecanatide (SP-304); SP-338 (metabolite)	
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**
Evaluation of the Bioactivity of Peptides that Target Receptor Guanylyl Cyclase C	Human Colon Carcinoma T84 Cells; HEK Cell Membranes; Wistar Rat Ileal Loop in Situ	In Vitro Injection into Loop	(b) (4)	SP-PH-010
Influence of Plecanatide (SP-304) and Its Biological Active Metabolite SP-338 on Intestinal Transit and Fluid Secretion in Ligated Duodenal Loops of Rats.	Female CD Rats	Oral gavage (intestinal transit) Injection into Duodenal Loop In Situ (fluid secretion)	Synergy Pharma, MB Research Laboratories Spinnerstown, PA	SP-PH-016
Influence of Plecanatide (SP-304) on Inflammation (TNBS) and Stress-induced Colorectal Hypersensitivity in Rats	TNBS-treated and Partially Restrained Male Wistar Rats	Oral gavage	(b) (4)	SP-PH-019
Assessment of SP-304 in DSS and TNBS Murine IBD Models	TNBS and DSS-treated BDF1 and BALB/c Male Mice	Oral gavage		06-119
Efficacy Analysis of SP-304 in a TNBS IBD Mouse Model	TNBS-treated BDF1 Male Mice	Oral gavage		06-169
Anti-inflammatory Activity of SP-304 in an Animal Model of Experimental Colitis in BALB/c Mice	Female BALB/c Mice	Oral gavage		SP-PH-005
Secondary Pharmacodynamics				
Effect of Plecanatide on Off-target Receptor and CYP450 Binding in Vitro	Ion Channel, Receptor and CYP Panel	In Vitro		AB20754

Overview			Test Articles: Plecanatide (SP-304); SP-338 (metabolite)	
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**
Effect of Plecanatide on Serotonin (5-HT) Receptor Binding and Pharmacodynamic Activity	Guinea Pig Brain Striatum Homogenates and Isolated Rat Esophagus	In Vitro/Ex Vivo	(b) (4)	AB23825
Safety Pharmacology				
Effect of Plecanatide on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	HEK Cells	In Vitro		120924.TZP
A Pharmacological Assessment of the Effect of SP-304 on the Cardiovascular System of the Cynomolgus Monkey Using Telemetry	Male Cynomolgus Monkeys	Oral gavage		692345
Compound SP-304: A Safety Pharmacology Study of the Central Nervous System Employing a Functional Observational Battery (FOB) Following Single Oral Administration in CD-1 Mice	Male and Female CD-1 Mice	Oral gavage		15056
Evaluation of Respiratory Function Following Oral Gavage Administration of SP-304 in Mice	Male CD-1 Mice	Oral gavage		1275MS58.001
Pharmacological Effects of SP-304 on the Gastrointestinal System of the Albino Mouse (Charcoal Propulsion Study)	Male CD-1 Mice	Oral gavage		692342

5-HT = 5-hydroxytryptamine; cGMP = cyclic guanosine monophosphate; CYP = cytochrome P450; DSS = dextran sulfate sodium; HEK = human embryonic kidney; hERG = human ether-à-go-go-related gene; IBD = irritable bowel disease; SP-338 = des-leucine[16] plecanatide; TNBS = trinitrobenzenesulfonic acid

THE FOLLOWING PHARMACOKINETIC STUDIES WERE REVIEWED (starting on page 43)

				Test Article: Plecanatide (SP-304)
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**
Absorption: Oral Bioavailability Studies in Mice and Monkeys				
Plecanatide: Oral Bioavailability and Formulation Comparison Study in Mice[a]	CD-1 Mice	Oral gavage, IV	(b) (4)	1896-020
Plecanatide: Oral Bioavailability and Formulation Comparison Study in Cynomolgus Monkeys[a]	Cynomolgus Monkey	Oral gavage, IV		1896-019
Absorption: Single-dose Toxicokinetic Studies in Mice and Monkeys				
Single-Dose Oral Toxicity and TK Study of Plecanatide[a]	CD-1 Mice	Oral gavage	(b) (4)	70474
Single Dose Oral Toxicity and TK Study of Plecanatide[a]	Cynomolgus Monkey	Oral gavage		30145
Absorption: Toxicokinetic Studies in Mice				
28-Day Oral Toxicity and TK Study of Plecanatide With 2-Week Recovery[a]	CD-1 Mice	Oral gavage	(b) (4)	VMF00007
13-Week Oral Toxicity and TK Study of Plecanatide[a]	CD-1 Mice	Oral gavage		1896-001
26-Week Oral Toxicity and TK Study of Plecanatide[a]	CD-1 Mice	Oral gavage		1896-008
Absorption: Toxicokinetic Studies in Rats				
13-Week Oral Toxicity and TK Study of Plecanatide[a]	Sprague Dawley Rat	Oral gavage	(b) (4)	1896-013

				Test Article: Plecanatide (SP-304)
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**
Absorption: Toxicokinetic Studies in Cynomolgus Monkeys				
14-Day Oral Dose Safety and TK Study of Plecanatide	Cynomolgus Monkey	Oral gavage	(b) (4)	30155
28-Day Oral Toxicity and TK Study of Plecanatide[a]	Cynomolgus Monkey	Oral gavage		VMF00009
13-Week Oral Toxicity and TK Study of Plecanatide[a]	Cynomolgus Monkey	Oral gavage		1896-002
39-Week Oral Toxicity and TK Study of Plecanatide[a]	Cynomolgus Monkey	Oral gavage		1896-009
Absorption: Toxicokinetic Studies in Pregnant Rabbits and Juvenile Mice				
Embryofetal Development Study of Plecanatide	Rabbit	Oral gavage	(b) (4)	20003036
Juvenile Animal Dose-ranging Toxicity Study of Plecanatide	Weanling Mice	Oral gavage		20049883
Distribution				
Binding of Plecanatide to Human Serum Proteins	Human HSA and AGP	In Vitro		RSN00008
P-gp and BCRP Transporter Substrate and Inhibition Study of Plecanatide	Caco-2 Cells	In Vitro		13SYNRP1A
P-gp and BCRP Transporter Substrate and Inhibition Study of SP-338	Caco-2 Cells	In Vitro		13SYNRP6A
Excretion of Radioactivity After Single Dose of ^{14}C-Plecanatide (Organ Distribution)	Rats	Oral gavage		1896-023

Test Article: Plecanatide (SP-304)				
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**
Metabolism: In Vitro and In Situ				
Analysis of Plecanatide Peptide Degradants	Simulated Intestinal Fluid	In Vitro	(b) (4)	(b) (4)
Analysis of Plecanatide Peptide Degradants	Simulated Intestinal Fluid	In Vitro		
Analysis of Plecanatide Peptide Degradants	Simulated Intestinal Fluid	In Vitro		
Analysis of Plecanatide Peptide Degradants	Simulated Intestinal Fluid	In Vitro		
Analysis of Plecanatide Peptide Degradants	Simulated Intestinal Fluid Simulate Gastric Fluid	In Vitro		
Analysis of Plecanatide Peptide Degradants	Simulated Intestinal Fluid	In Vitro	Synergy Pharmaceuticals Inc.	(b) (4)
Stability of Plecanatide in Surgically Ligated Rat Intestinal Loops	Sprague Dawley Rat	Direct injection into ligated intestinal loops - In Situ	(b) (4)	13SYNRP2
Sequence Assignment of Plecanatide Metabolites M5, M9, and M113	Sprague Dawley Rat	Direct injection into ligated intestinal loops - In Situ		13SYNRP2R1

Test Article: Plecanatide (SP-304)				
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study Number**
Stability of Plecanatide in Surgically Ligated Rat Intestinal Loops	Sprague Dawley Rat	Direct injection into ligated intestinal loops - In Situ	(b) (4)	14SYNRP2R3-A
Reanalysis of Plecanatide Metabolites in Rat Duodenum	Sprague Dawley Rat	Direct injection into ligated intestinal loops - In Situ		14SYNRP2R3-B
Metabolism: Cytochrome P50 Inhibition and Induction Studies				
Cytochrome P450 Metabolism Studies with Plecanatide	Human Liver Microsomes	In Vitro		13SYNRP1B
Cytochrome P450 Metabolism Studies with SP-338	Human Liver Microsomes	In Vitro		13SYNRP6B
Excretion				
Excretion of Radioactivity after Single Dose of ^{14}C-Plecanatide	Rats	Oral gavage		1896-023

AGP = alpha-1-acid glycoprotein; BCRP = breast cancer resistance protein; GLP = Good Laboratory Practice; HAS = human serum albumin; IV = intraven
P-gP = permeability glycoprotein; SP-338 = des-leucine^{16} plecanatide; TK = toxicokinetic
[a] GLP-compliant study

THE FOLLOWING TOXICOLOGY STUDIES WERE REVIEWED (starting on page 60)

						Test Article: Plecanatide (SP-304)	
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Doses[a] (mg/kg)**	**GLP Compliance**	**Testing Facility**	**Study Number**
Single-dose Toxicity	CD-1 Mouse	Oral	Single Dose	20, 200, 2000	Yes	(b) (4)	70474
Single-dose Toxicity	Cynomolgus Monkey	Oral	Single Dose	0.1, 25 250, 2000	Yes		30145
Single-dose Toxicity	Cynomolgus Monkey	Oral	Single Dose	0, 1, 10, 25, 50	No		30169
Repeat-dose Toxicity	CD-1 Mouse	Oral	5 days	1200	No		VMF00019
Repeat-dose Toxicity	CD-1 Mouse	Oral	7 days	20, 200, 2000	No		0722-07281
Repeat-dose Toxicity	CD-1 Mouse	Oral	28 days	0, 20, 200, 1000, 2000/12000	Yes		VMF00007
Repeat-dose Toxicity	CD-1 Mouse	Oral	13 Weeks	0, 20, 200, 800	Yes		1896-001
Repeat-dose Toxicity	CD-1 Mouse	Oral	26 Weeks	0, 20, 60, 150, 400	Yes		1896-008
Single- and Repeat-dose Toxicity	Sprague-Dawley Rat	Oral	Single-dose and 7 or 14 days repeat-dose	Single-dose: 2000 7 Days: 150, 500 14 Days: 0, 5, 50	No		018683

						Test Article: Plecanatide (SP-304)	
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Doses[a] (mg/kg)**	**GLP Compliance**	**Testing Facility**	**Study Number**
Repeat-dose Toxicity	Sprague-Dawley Rat	Oral	13 weeks	0, 30, 100, 300	Yes	(b) (4)	1896-013
Single- and Repeat-dose Toxicity	Cynomolgus Monkey	Oral	Single dose and 3 days repeat dose	Single dose: 0, 10, 100, 250, 500, 1000 3 days: 1, 10, 50, 100, 200, 1000	No		0722-07246
Repeat-dose Toxicity	Cynomolgus Monkey	Oral	14 days	100, 250	No		30155
Repeat-dose Toxicity	Cynomolgus Monkey	Oral	28 days	0, 1, 10, 75	Yes		VMF00009
Repeat-dose Toxicity	Cynomolgus Monkey	Oral	13 weeks	0, 1, 10, 100	Yes		1896-002
Repeat-dose Toxicity	Cynomolgus Monkey	Oral	39 weeks	0, 2, 10, 100	Yes		1896-009
Genotoxicity	E. coli: WP2 *uvr*A S.typhymurium: TA98, TA100, TA1535, and TA1537	In Vitro	48 to 72 hr	1.5, 5.0, 15, 50, 150, 500, 1500, 5000 µg per plate	Yes		AD27SJ.503.BTL

						Test Article: Plecanatide (SP-304)	
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Doses[a] (mg/kg)**	**GLP Compliance**	**Testing Facility**	**Study Number**
Genotoxicity	L5178Y/TK$^{+/-}$ Mouse Lymphoma Cells	In Vitro	24 hr exposure for nonactivated cultures; 4 hr exposure for Araclor 1254-induced rat liver S9-activated cultures	1000, 2000, 3000, 4000, 5000 µg/mL	Yes	(b) (4)	AD27SJ.704.BTL
Genotoxicity	ICR Mouse	Oral	24 hr 48 hr	500, 1000, 2000	Yes		AD27SJ.123.BTL
Carcinogenicity	CD-1 Mouse	Oral	2 years	0, 10, 30, 90	Yes		12-2324
Carcinogenicity	Sprague-Dawley Rat	Oral	104 weeks	0, 10, 30, 100	Yes		1896-011
Fertility and Early Embryonic Development	CD-1 Mouse	Oral	Males: ≥45 days (28 days prior to and during mating) Females: ≥26 days (14 days prior to mating to GD 7)	0, 20, 200, 600	Yes		20016090

						Test Article: Plecanatide (SP-304)	
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Doses[a] (mg/kg)**	**GLP Compliance**	**Testing Facility**	**Study Number**
Dose-ranging Embryo-Fetal Development	CD-1 Mouse	Oral	10 days (GD 6-15)	0, 20, 200, 1200	Yes	(b) (4)	VMF00028
Embryo-Fetal Development	CD-1 Mouse	Oral	10 days (GD 6-15)	0, 20, 200, 800	Yes		0020001133
Dose-ranging Embryo-Fetal Development	Dutch Belted Rabbit	Oral	7 days (GD 7-13)	100, 150, 250	No		VMF00029
Embryo-Fetal Development	Dutch Belted Rabbit	Oral	13 days (GD 7-19)	15, 75, 250	Yes		20003036
Pre-/Postnatal Development	CD-1 Mouse	Oral	GD 6 to LD 20; LD 22 for mice that did not deliver litter	0, 20, 200, 600	Yes		20053292
Juvenile Toxicity	CD-1 Mouse	Oral	Single dose	0, 0.01, 0.05, 0.1, 0.25	No		20034218
Juvenile Toxicity	CD-1 Mouse	Oral	Single dose	0, 1, 3, 10, 30, 50, 100, 200, 300	No		20039567
Juvenile Toxicity	CD-1 Mouse	Oral	Single dose	0, 1, 3, 10, 20 0, 25, 50, 100, 300	No		20046300

						Test Article: Plecanatide (SP-304)	
Type of Study	**Species and Strain**	**Method of Administration**	**Duration of Dosing**	**Doses[a] (mg/kg)**	**GLP Compliance**	**Testing Facility**	**Study Number**
Juvenile Toxicity	CD-1 Mouse	Oral	7 days	0, 0.05, 0.1, 0.5, 1.0	No	(b) (4)	20035794
Juvenile Toxicity	CD-1 Mouse	Oral	14 days	0, 1, 3, 10 0, 30, 100, 300	Yes		20049883
Juvenile Toxicity	CD-1 Mouse	Oral	14 or 13 weeks	0, 3[b], 10, 100, 300	Yes		20059246
Antigenicity	NZW Rabbit	SC Injection	105 days (6 doses)	0.5 mg (x2) 0.25 mg (x4) Plecanatide	No		0066-13
Antigenicity	NZW Rabbit	SC Injection	7 doses over 136 days	0.5 (x2), 0.25 (x2) KLH-Plecanatide	No		0066-13-01

GD = Gestation Day; GLP = Good Laboratory Practice; KLH = keyhole limpet hemocyanin; LD = Lactation Day; NZW = New Zealand white; PND = postnatal day; SC = subcutaneous

[a] For single-dose toxicity and repeat-dose toxicity studies, the highest NOAEL (no observed adverse-effect level) is underlined.

[b] The NOAEL was 3 mg/kg/day in the pups given plecanatide on PNDs 14 through 111 and 300 mg/kg/day in the pups given plecanatide on PNDs 21 through 111.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Pharmacology/Toxicology Reviews of IND 74,883 by David B. Joseph, PhD dated 7/2/2008, and by Yuk-Chow Ng, PhD dated 2/2/2010, 5/27/2010, 7/25/2013, 8/2/2013, and 6/11/2014 are referenced.

4 Pharmacology

4.1 Primary Pharmacology

SP-304 (Plecanatide): Stimulation of Intracellular cGMP Synthesis in T84 Cells (Study No. SP-PH-001)	
Materials and Methods	**Results and Conclusion**
The ability of plecanatide to stimulate cGMP production was studied in T84 human colon carcinoma cells in vitro. Plecanatide (N-terminal amino acid sequence NDE), uroguanylin (UG, NDD), and 2 related peptides, SP-302 (NEE) and SP-303 (NED), were tested at concentrations ranging from 10^{-9} M to 10^{-5} M. Cell lysates derived from cells exposed for 30 minutes to the peptides were assayed for cGMP levels using a commercial enzyme-linked immunosorbent assay (ELISA).	All 4 peptides increased cGMP production in a concentration-dependent manner. At a concentration of 10^{-6} M, cGMP levels were 54% higher in plecanatide-treated cells compared with UG-treated cells. Plecanatide stimulated cGMP production in T84 colon carcinoma cells with a half-maximal effective concentration (EC_{50}) of 1.1 x 10^{-7} M. Plecanatide was 2 to 3 fold more potent than UG and the other related test peptides in this bioassay, and induced

	higher cGMP levels (>50% greater) compared with the other peptides.

Studies on SP-304 (Plecanatide) Thermostability, pH Dependency, and Topoisomeric Stability (Study No. SP-PH-004)	
Materials and Methods	**Results and Conclusion**
Thermostability of plecanatide was examined after it was incubated at 95°C for up to 90 minutes, and the ability of plecanatide to stimulate cGMP synthesis was measured after 30-minute incubations with T84 cells at different pH. The conformational makeup (topoisomeric composition) of plecanatide and UG was analyzed by high-performance liquid chromatography (HPLC) following a 16-hour incubation at pH 3.0 and 37°C.	Plecanatide retained 100% activity after 90 minutes at 95°C, as assessed by the cGMP T84 cell assay. In contrast, UG lost about 15% of its activity after 90 minutes. The ability of plecanatide to stimulate cGMP synthesis in cells incubated at pH ranging from 6.0 to 6.5 was greater than that of UG and 2 related peptides, SP-302 and SP-303, used in the study. Maximum plecanatide activity was observed at pH 6.0. After a 16-hour incubation at 37°C and pH 3.0, UG showed 2 major peaks and 2 minor peaks. This result is consistent with the interconversion of UG between 2 solution conformers, one biologically active and the other biologically inactive. In contrast, plecanatide showed only a single peak. The preparation of plecanatide used for the study was found to be fully active using the cGMP T84 cell assay, indicating that the single peak shown in the HPLC represents the fully active conformer of plecanatide.

Stimulation of Intracellular cGMP Synthesis in T84 Cells: SP-304 (Plecanatide) & SP-338, A Comparative Study (Study No. SP-PH-008)	
Materials and Methods	**Results and Conclusion**
The efficacy of plecanatide and its metabolite SP-338 was compared in a cGMP production assay in T84 human colon carcinoma cells. Plecanatide and SP-338 were tested in vitro at concentrations ranging from 10^{-9} to 10^{-5} M (1 nM to 10 µM). Following a 30-minute incubation with the test compound, cell lysates were prepared from cultured T84 cells, and cGMP levels were measured using a commercial ELISA.	Both peptides increased cGMP levels in a concentration-dependent manner. The EC_{50} for plecanatide in the stimulation of cGMP production was 1.2×10^{-7} M (120 nM), whereas SP-338 was slightly less potent, with an EC_{50} value of 1.7×10^{-7} M (170 nM). Plecanatide induced slightly higher levels of cGMP compared with SP-338 in this study. These data suggest that the plecanatide metabolite SP-338 is biologically active and slightly less potent than plecanatide.

Biological Activity of Potential Plecanatide Metabolites (Study No. SP-PH-011)	
Materials and Methods	**Results and Conclusion**
The biological activity of plecanatide and potential plecanatide metabolites in stimulating cGMP production was studied in T84 human colon carcinoma cells in vitro. The compounds evaluated were plecanatide; fully reduced	Plecanatide potently stimulated cGMP production in T84 cells with an EC_{50} of under 1.0×10^{-7} M (100 nM). In contrast, linear plecanatide exhibited no activity in this

plecanatide (linear plecanatide); SP-338; linear SP-338; and clipped-SP-338, a putative metabolite formed as a result of cleavage of the bond between Leu6-Cys7 in SP-338. These compounds were incubated with T84 human colon carcinoma cells for 30 minutes at concentrations ranging from 10^{-9} M to 10^{-5} M. Cyclic GMP levels in culture extracts were determined by ELISA.	assay, suggesting that the 2 disulfide bonds present in the plecanatide molecule are required for pharmacological activity. Linearization of SP-338 eliminated the in vitro biological activity of SP-338, whereas intact SP-338 exhibited potent in vitro activity with an EC_{50} of 1.18×10^{-7} M for stimulation of cGMP production. Clipped-SP-338 exhibited no biological activity, suggesting that the clipped form of the peptide is also inactive. Hence, both plecanatide and SP-338 were biologically active, but linear plecanatide, linear SP-338, and clipped-SP-338 were inactive. These results suggest that the disulfide bonds present in both plecanatide and SP-338 are critical for biological activity, and that cleavage of SP-338 between amino acids 6 and 7 eliminates its biological activity.

SP-304 (Plecanatide): Binding Affinity to the Guanylate Cyclase C Receptor (Study No. SP-PH-003)

Materials and Methods	Results and Conclusion
The binding affinity of plecanatide to GC-C was examined in a T84 human colon carcinoma cell binding assay. The binding affinity of plecanatide, UG, and 2 other structurally related peptides, SP-302 and SP-303, were assessed in competitive binding assays with [^{125}I]-bacterial enterotoxin (ST) peptide in cultured human colon carcinoma T84 cells. EC_{50} values for various peptides were defined as the concentration at which 50% of ^{125}I-labeled ST peptides were displaced from GC-C in the binding assay.	Plecanatide showed high binding affinity to GC-C, as assessed by its ability to displace ^{125}I-ST peptides from GC-C. The plecanatide concentration that displaced 50% of radiolabeled ST peptide was 10^{-10} M, approximately 10-fold lower than that observed with endogenous peptide UG (10^{-9} M) and SP-302 and SP-303 (10^{-9} M).

SP-304 (Plecanatide): Synergistic Effects with Phosphodiesterase Inhibitors on cGMP Stimulation and Caspase-3 Activity in T84 Human Colon Carcinoma Cells (Study No. SP-PH-002)

Materials and Methods	Results and Conclusion
To compare the ability of plecanatide and phosphodiesterase (PDE) inhibitors, both individually and when given in combination, to stimulate cGMP production and caspase-3 activity, the study was conducted in T84 human colon carcinoma cells using two common PDE inhibitors, exisulind (sulindac sulfone) and zaprinast, as positive controls. PDE inhibitors are known to inhibit intracellular breakdown of cGMP and would be expected to increase endogenous steady-state cGMP levels. Caspase-3 activity is a measure of programmed cellular death (apoptosis). T84 colon carcinoma cells were incubated with plecanatide (0.1 µM), exisulind (100 µM), or zaprinast (100 µM) alone, and with the combination of plecanatide (0.1 µM) plus exisulind (100 µM), or plecanatide (0.1 µM) plus zaprinast (100 µM). Production of intracellular cGMP was measured in cell lysates using ELISA. Caspase-3 activity was examined by a colorimetric assay using the release of the chromophore p-nitroanilide.	Treatment with plecanatide alone resulted in a modest increase in cGMP levels (54 pmol/well), while exisulind or zaprinast alone had virtually no effect. The combination of plecanatide with zaprinast elicited the greatest stimulation of cGMP (224 pmol/well), a 4-fold enhancement over plecanatide alone. Similarly, the combination of plecanatide with exisulind also showed considerable enhancement (181 pmol/well) relative to control. Plecanatide produced about a 4-fold enhancement of caspase-3 activity over control cells, whereas exisulind alone had little or no effects. The combination of plecanatide plus exisulind increased caspase-3 activity about 2.5-fold over that of plecanatide alone, and about 9-fold over that of exisulind alone. Thus, stimulation of

	intracellular cGMP in T84 cells was synergistically enhanced by the combination of plecanatide with cGMP-specific PDE inhibitors. Similarly, the activity of caspase-3 was also considerably enhanced with the combination of plecanatide and exisulind in T84 colon carcinoma cells.

Effect of pH on Plecanatide and Uroguanylin Mediated Activation of Guanylate Cyclase-C (Study No. SP-PH-020)

Objectives: To examine the effect of pH on the ability of plecanatide and UG to activate GC-C signaling and identify key amino acids in the primary structure of plecanatide that play a role in this process.

Methods and Materials: The effect of pH on intracellular cGMP production was studied in T84 cells. Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) was adjusted to pH 5 and 8 and the cells were treated with multiple concentrations of plecanatide, UG, *Escherichia coli* enterotoxin heat-stable peptide (STp), or linaclotide. Intracellular cGMP levels were determined from cell lysates using a commercial ELISA.

The effect of pH on binding affinities of plecanatide, UG, STp, and linaclotide for GC-C expressed on T84 cells was assessed using competitive binding assays with ^{125}I-STp. The pH of the cell culture medium was adjusted to 5 or 8. T84 cell monolayers were incubated with 170 pM (~120,000 cpm) ^{125}I-STp in the presence or absence of the competitor test peptides for 1 hour at 37°C. Following the incubation, cells were solubilized and bound radioactivity was determined. Binding of ^{125}I-STp in the absence of competitor was considered as 100%.

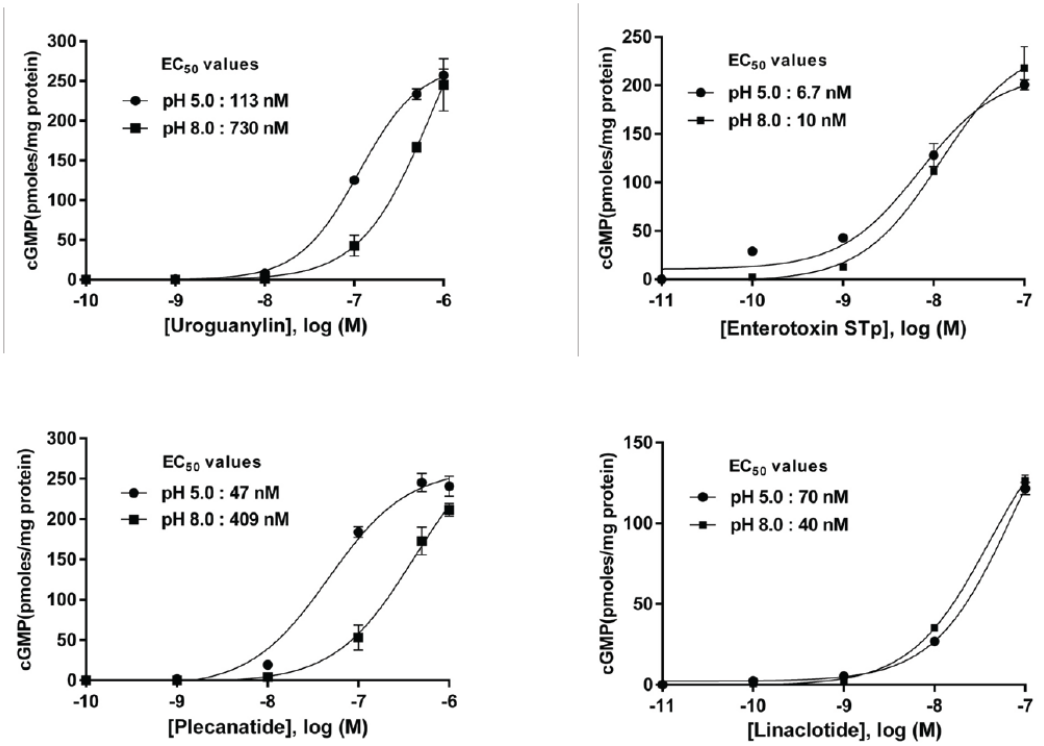
Plecanatide and UG contain charged amino acids at the N-terminal that STp and linaclotide lack. Contribution of N-terminal amino acids in plecanatide to pH-dependent activation of GC-C was studied in T84 cells. Truncated peptides SP-326 (plecanatide 2-16), SP-328 (plecanatide 3-16), and SP-330 (plecanatide 4-16) with sequential deletions of N-terminal amino acids from plecanatide were synthesized and evaluated for their ability to stimulate cGMP production and bind GC-C on T84 cells at pH 5 and 8.

The effect of pH on GC-C agonist-mediated stimulation in *Isc* (a measure of movement of chloride ions) across T84 cell monolayers and mouse intestinal tissues was measured using an Ussing chamber. T84 cells cultured on permeable membranes or freshly harvested mouse tissues were mounted on specific sliders and fitted onto the Ussing chamber such that mucosal and serosal surfaces were in contact with Krebs Ringer buffer solution in apical and basolateral chambers, respectively. In addition to imposing a chloride gradient by using sodium gluconate in place of NaCl and a higher concentration of $CaCl_2$ (4 mM), Krebs solution, pH 5 (80 mM 2-[N-morpholino] ethanesulfonic acid [MES] instead of sodium bicarbonate) or pH 8 (80 mM sodium bicarbonate) was used to bathe the apical surface of cells and tissues. The

temperature was maintained at 37°C and buffer was gassed with carbogen (mixture of 95% O2 and 5% CO2). Each agonist was tested at 0.1 µM concentration.

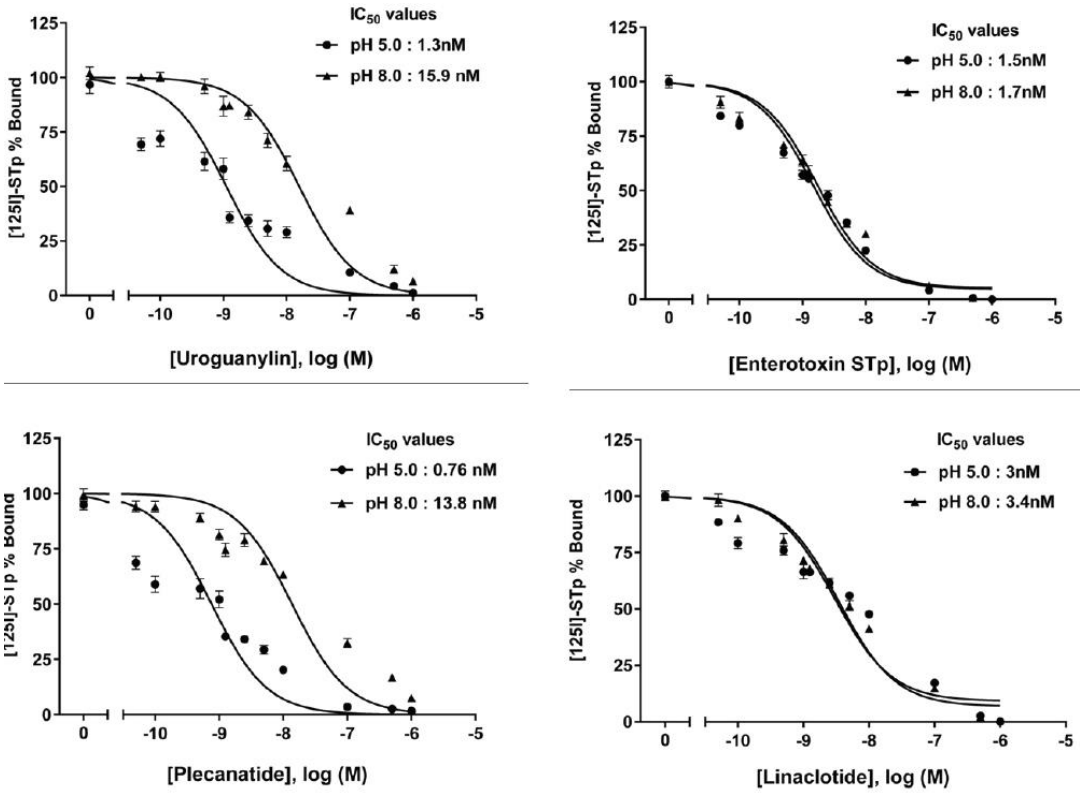
Results and Conclusions: The data demonstrated that cGMP-stimulatory activities of UG and plecanatide were modulated by a change in acidity of the medium. At pH 5, the EC_{50} values for UG and plecanatide were 113 nM and 47 nM, respectively. Considerable increases in EC_{50} values for both UG (730 nM) and plecanatide (409 nM) were observed at pH 8. Thus, a distinct shift towards the left was observed in dose response of these peptides at pH 5 as compared to those at pH 8. By contrast, cGMP-stimulatory activities of STp and linaclotide were not appreciably different at pH 5 and pH 8, indicating that STp- and linaclotide-mediated stimulation of cGMP in T84 cells were pH-independent. The data are summarized in the Sponsor's figures below.

Effects of pH on cGMP-Stimulatory Activities of GC-C Agonists in T84 Cell Bioassay



The binding affinities of UG and plecanatide were at least an order of magnitude higher (lower half-maximal inhibitory concentration [IC_{50}] values) at pH 5 as compared with pH 8. The IC_{50} values of UG and plecanatide at pH 5 were 1.3 nM and 0.76 nM, respectively. By comparison, IC_{50} values for UG and plecanatide at pH 8 were 15.9 nM and 13.8 nM, respectively. Binding affinities of STp and linaclotide were not appreciably different at pH 5 and 8. These results demonstrate that the binding affinities of UG and plecanatide are distinctively enhanced under acidic conditions, while those for STp and linaclotide are unaffected by changes in pH. The data are shown in the Sponsor's figures below.

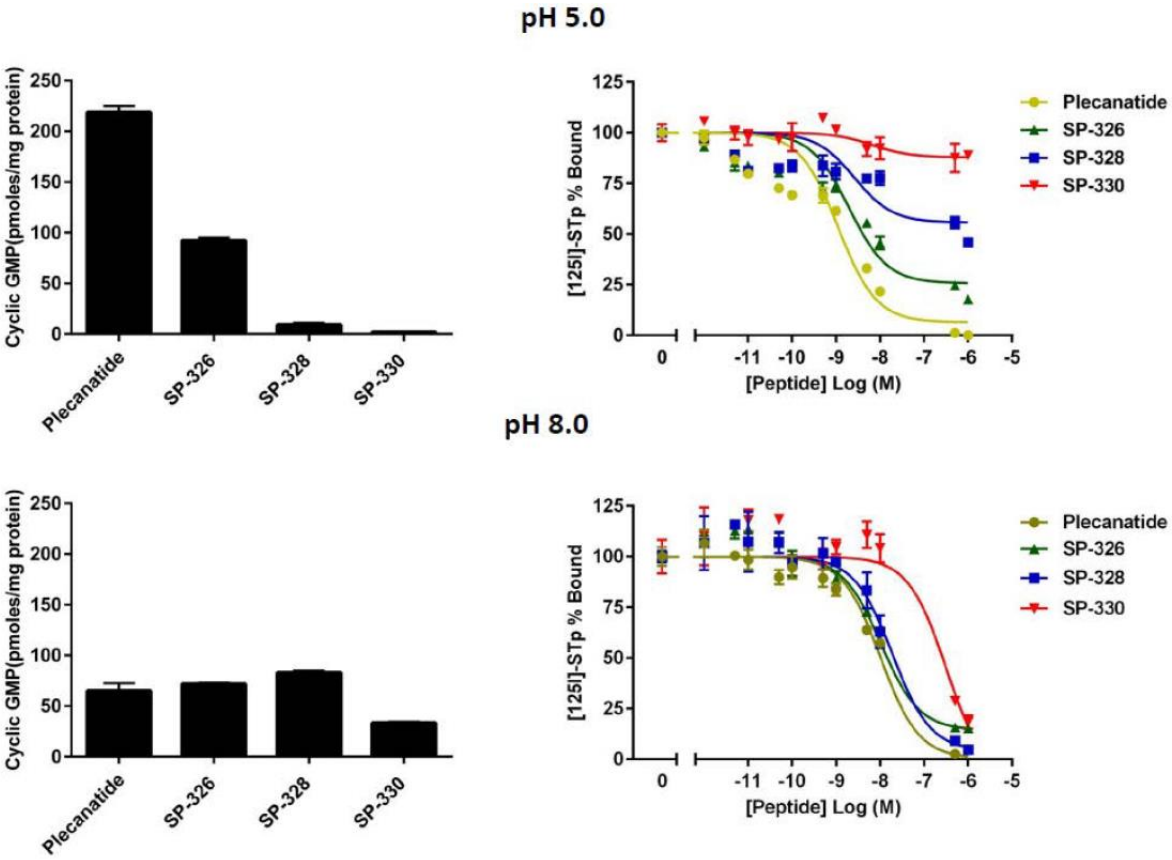
Effects of pH on Binding Affinities of GC-C Agonists



In addition, epithelial cells derived from naïve mice (n = 6 to 10) were used to evaluate binding affinities of plecanatide and linaclotide at pH 5 and 8. Approximately $2x10^6$ cells from proximal intestine and colon were incubated with approximately 500 pM (350,000 cpm) ^{125}I-STp in the presence or absence of each competitor peptide. GC-C binding was assessed as in the case of T84 cells. Consistent with the results obtained with T84 cells, the GC-C binding affinity of plecanatide was much higher at pH 5 as compared with that at pH 8 for both the proximal intestinal and colon cells. By contrast, the GC-C binding affinity of linaclotide was not appreciably different at pH 5 and 8 for either of these cells.

The cGMP-stimulatory activity at pH 5 diminished with sequential deletion of Asn1 (SP-326), Asp2 (SP-328), and Glu3 (SP-330). The cGMP-stimulatory activity of plecanatide was significantly reduced at pH 8 as compared with that observed at pH 5. SP-326 and SP-328 treatment did not alter cGMP levels at pH 8; however, the cGMP-stimulatory activity of SP-330 was reduced at pH 8, possibly due to peptide instability. The data are summarized in the Sponsor's figures.

Role of Charged Amino Acids at the N-Terminus of Plecanatide in pH-Mediated Activation of GC-C

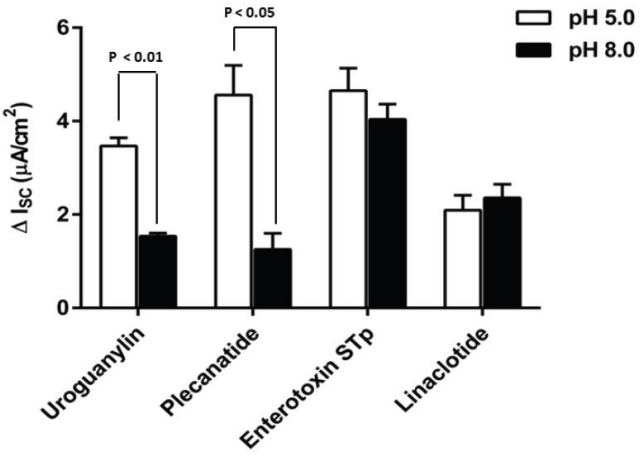


GC-C = guanylate cyclase C; GMP = guanosine monophosphate; M = molar; STp = heat-stable peptide
Source: Study SP-PH-020

Similarly, the binding affinities of SP-326, SP-328, and SP-330 at pH 5 diminished with the sequential deletion of N-terminal amino acids of plecanatide. No appreciable difference in the binding affinities of these truncated peptides was observed at pH 8, although overall binding of radiolabeled STp was lower when compared with that at pH 5. These findings suggest that the charged N-terminal amino acids influence the cGMP-stimulatory activity and GC-C binding affinity at pH 5 but not at pH 8.

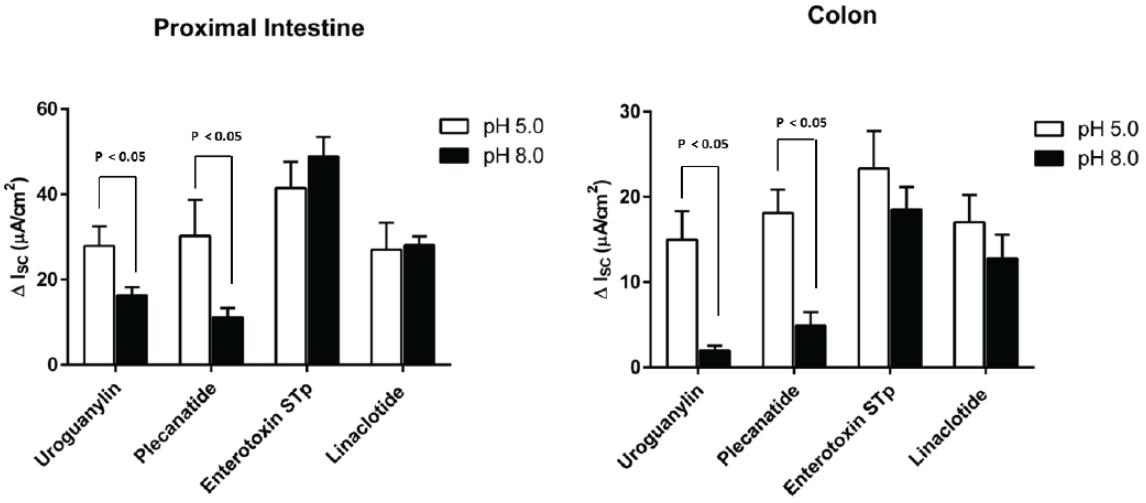
In T84 cells, the enhancement of *Isc* by plecanatide and UG was statistically higher at pH 5 as compared with that at pH 8, while *Isc* stimulation by STp and linaclotide were similar at pH 5 and 8. The data are shown in the Sponsor's figures below.

Effects of pH on GC-C Agonist-Stimulated *Isc* Across T84 Cell Monolayers



The effect of pH on *Isc* enhancement by GC-C agonists was further confirmed by examining freshly harvested proximal intestine and colon tissues of mice. Consistent with the results obtained using the T84 cells, the functional activities of UG and plecanatide to enhance Isc were statistically higher at pH 5 as compared to that at pH 8 in proximal intestine as well as colon tissues. By contrast, the stimulation of Isc by STp and linaclotide were comparable at pH 5 and 8 in either of the tissue segments.

Effects of pH on GC-C Agonist-Stimulated *Isc* Across Mouse Intestinal Tissues



These results suggest that *Isc* stimulation by plecanatide and UG is pH dependent while that for STp and linaclotide is pH independent in this functional assay.

Evaluation of the Bioactivity of Peptides that Target Receptor Guanylyl Cyclase C (Study No. SP-PH-010)

Objectives: To examine the ability of plecanatide, UG, and SP-338 to stimulate cGMP production in T84 human colon carcinoma cells in vitro, to measure the affinity of the

peptides for human GC-C, and to evaluate their potency in a ligated intestinal loop assay in rats.

Methods and Materials: In the cGMP assay, human colon carcinoma T84 cells were incubated with peptides for 30 minutes at 37^{o}C in medium containing 500 µM 3-isobutyl-1-methylxanthine (IBMX). Peptides were tested at 10^{-9} to 10^{-4} M. Cell lysates were prepared and cGMP was measured using a radioimmunoassay. Values were calculated from 3 assays, with duplicate wells in each assay.

In the GC-C binding assay, membranes were prepared from human embryonic kidney (HEK) cells stably expressing human GC-C. ^{125}I-labeled STY72F was used as the radioligand, and 40 to 50 µg of membrane protein was incubated with approximately 100,000 cpm of radiolabeled ST in the presence of various concentrations of UG, plecanatide, or SP-338 peptides for 1 hour at 37°C. Radioactivity associated with the membrane was measured. IC_{50} values were calculated from the available data.

In the ligated rat ileal loop assay, two young adult female Wistar rats (approximately 30 to 35 days of age) were provided only water for 12 hours and then anesthetized. Intestines were surgically exposed, and 4 to 6 loops (1 cm in length with an approximate 0.5 to 1 cm gap between each loop) were ligated only in the ileal region of the small intestine in each rat. Plecanatide, UG, or SP-338 at 1 nM concentrations, or vehicle (phosphate-buffered saline (PBS)), were injected into separate loops (10 µL per loop) in each rat, and the incision was closed with sutures. Rats were maintained under anesthesia for 1 hour, after which time rats were sacrificed. The loops were dissected and weighed. The fluid from each loop was collected and quantified.

Results and Conclusions: Plecanatide, UG, and SP-338 potently stimulated cGMP production in T84 cells. Plecanatide was the most potent with an EC_{50} value of 480 nM, and UG was nearly equipotent with an EC_{50} of 560 nM. SP-338 was less potent with an EC_{50} value of 2000 nM.

In the receptor binding assay, all 3 peptides displaced binding of the ^{125}I-labelled STY72F peptide. The IC_{50} for plecanatide was 1.9 nM compared with 2.8 nM for UG. SP-338 was only slightly less potent, with an IC_{50} of 5 nM.

In the ligated rat ileal loop assay, plecanatide elicited slightly greater fluid accumulation compared to SP-338 and UG. The data are summarized in the Sponsor's table below.

Plecanatide, Uroguanylin, and SP-338 Mediated Increase in Fluid Accumulation Ratio in Ligated Ileal Loops in Rats

Treatment	Fluid Accumulation Ratio*		
	Rat 1	Rat 2	Mean ± SD
PBS	0.04	0.04	0.04 ± 0.00
Uroguanylin	0.30	0.38	0.34 ± 0.06
Plecanatide	0.30	0.55	0.43 ± 0.18
SP-338	0.25	0.43	0.34 ± 0.13

PBS = phosphate-buffered saline; SD = standard deviation; SP-338 = des-leucine[16] plecanatide
*Fluid accumulation ratio determined by calculating [(x-y)/y] where x = weight of fluid filled dissected loop, and y = weight of the loop without the fluid. The weight difference (x-y) corresponds to the amount of fluid present in the loop.

These data demonstrate that plecanatide, along with the related compound UG and metabolite SP-338, can bind GC-C expressed in HEK cells membranes with high affinity and potently stimulate cGMP production in T84 cells. Plecanatide was slightly more potent than SP-338 and UG in these assays. These peptides also induced fluid secretion in ligated segments of rat ileum.

Influence of Plecanatide (SP-304) and Its Biological Active Metabolite SP-338 on Intestinal Transit and Fluid Secretion in Ligated Duodenal Loops of Rats (Study No. SP-PH-016)

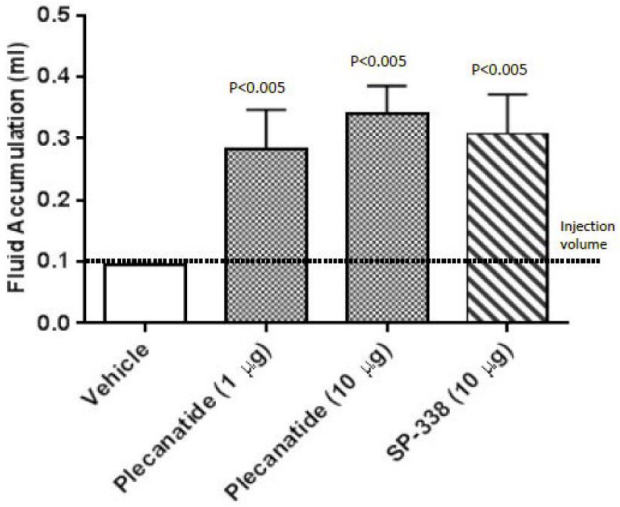
Objectives: To evaluate the effect of plecanatide and its biologically active metabolite, SP-338, on intestinal transit in rats and fluid secretion in rat ligated duodenal loops.

Materials and Methods: Intestinal transit was examined in female CD rats (9 to 10 per group) by oral administration of vehicle, 0.05, 0.5, or 5 mg/kg plecanatide or SP-338 followed immediately with an oral gavage of charcoal meal. The distance traveled by the leading edge of the charcoal between pyloric sphincter and ileocecal junction as a percentage of the total length of the intestine was used to calculate intestinal transit. Stimulation of fluid secretion was assessed by injecting 1 or 10 µg of plecanatide or 10 µg of SP-338 directly into ligated duodenal loops in rats (n = 8 to 13 for plecanatide and n = 4 for SP-338).

Results and Conclusions: A single oral dose of plecanatide given immediately prior to charcoal meal resulted in statistically significant, dose-dependent increases in small intestinal transit (11.9%, 23.1%, and 24.7% increase at 0.05, 0.5, and 5 mg/kg, respectively, compared to controls). Oral administration of SP-338 enhanced GI transit by 7.4%, 13.9%, and 13.5% at 0.05, 0.5, and 5 mg/kg, respectively, compared to controls. Statistical significance was achieved at 0.5 and 5 mg/kg SP-338. Plecanatide and SP-338 stimulated fluid secretion by 3 to 3.6-fold in ligated rat duodenal loops after a 30-minute incubation. The data on fluid secretion are shown in the Sponsor's figure below.

Plecanatide and SP-338 Mediated Fluid Accumulation in

Rat Ligated Duodenal Loops



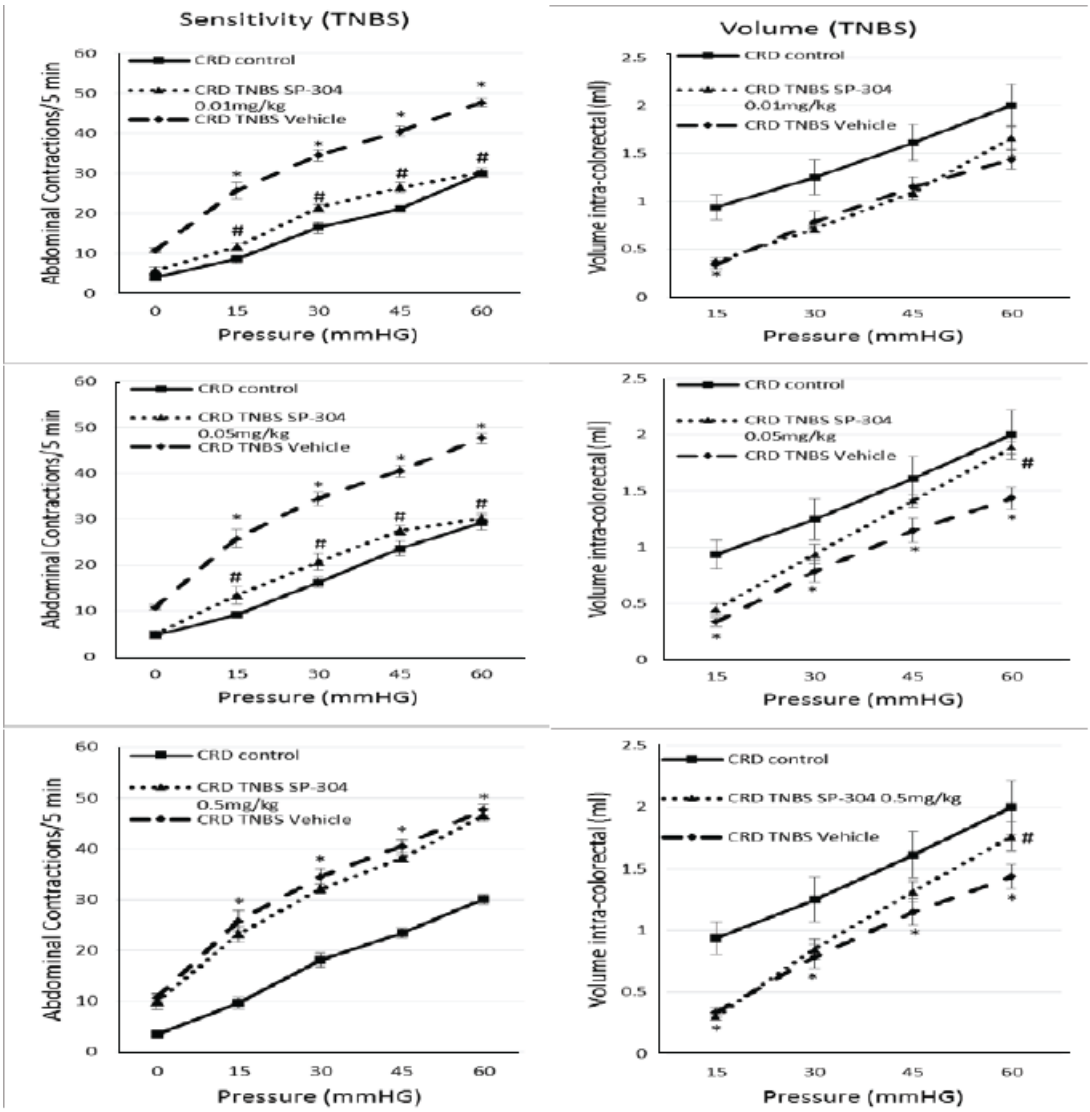
The results demonstrate that plecanatide and its biologically active metabolite, SP-338, accelerate intestinal transit in a dose-dependent manner. Furthermore, both plecanatide and SP-338 stimulate fluid secretion in duodenum of rats.

Influence of Plecanatide (SP-304) on Inflammation (TNBS) and Stress-induced Colorectal Hypersensitivity to Distension in Rats (Study No. SP-PH-019)

Objectives: To evaluate the effects of plecanatide on visceral allodynia produced by TNBS-induced colonic inflammation and visceral hyperalgesia produced by a restraint condition in rats.

Materials and Methods: Male rats were implanted with electromyography electrodes in striated abdominal muscles to measure contractions (i.e., cramping), and colonic volume changes in response to colorectal distension (CRD). Colorectal distension was induced by inserting a balloon connected to a barostat into the anus and inflating it progressively from 0 to 60 mmHg in 15-mmHg steps, each step lasting 5 minutes. The assessments included baseline responses, responses after administration of TNBS, and responses after exposing the animals to partial restraint, in the presence or absence of plecanatide treatment.

Results and Conclusions: Following intracolonic administration of TNBS, abdominal contractions increased at all pressure levels in the control group. Oral doses of 0.01 and 0.05 mg/kg plecanatide significantly reduced the number of abdominal contractions to near the basal condition. However, the responses at 0.5 mg/kg were not different from controls. This apparent inconsistency was not explained. Volume changes in plecanatide-treated groups at the higher pressure levels were similar to controls. The data are summarized in the Sponsor's figures below.



CRD = colorectal distension; SEM = standard error of the mean; SP-304 = plecanatide; TNBS = trinitrobenzenesulfonic acid

Left panel showing effect on abdominal contractions (mean ± SEM) with increasing pressure; right panel showing the effect on colonic volume (mean ± SEM) with increasing pressure. *= $p \leq 0.05$ vs. control; # = $p \leq 0.05$ vs TNBS vehicle.

In the CRD test performed 30 minutes after a 2-hour session with partial restraint, the abdominal response to CRD was significantly enhanced for the 3 higher pressures. As observed in the inflammatory model, oral doses of 0.01 and 0.05 mg/kg plecanatide exerted a significant reduction in hypersensitivity at pressures of 30, 45, and 60 mmHg. At 1 and 5 mg/kg plecanatide, no clear antinociceptive effect was observed, despite a tendency to reduce the stress-induced hypersensitivity at 30 mmHg. Plecanatide did not affect the decrease in colonic muscular tone triggered by stress.

In summary, lower doses of plecanatide reduced hypersensitivity in both inflammatory (TNBS-induced colitis) and non-inflammatory (stress due to partial restraint) conditions.

In addition, 0.05 and 0.5 mg/kg plecanatide partially restored the colonic smooth muscle elasticity altered by inflammation but not that triggered by stress.

Assessment of SP-304 (Plecanatide) in DSS and TNBS Murine IBD Models (Study No. 06-119)

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

TNBS-Induced Colitis:

Methods: Male BDF1 mice were treated orally with 0 (vehicle), 0.5, 2.5, 5, or 50 mg/kg/day SP-304 for six days (8 mice/group). The vehicle was PBS, and the dose volume was 10 ml/kg. Mice in the 5 mg/kg/day group were given two daily doses (2.5 mg/kg bid). As a positive control, one group of mice was treated orally with 80 mg/kg/day sulfasalazine for six days. To induce colitis, the mice were treated with a single intra-rectal administration of 100 μl TNBS in 50% ethanol, at four hours after the second daily administration of study drug. An additional control group was treated orally with PBS for six days, in the absence of TNBS treatment. Study parameters included clinical signs, stool consistency, rectal bleeding, bodyweight, and occult blood. The mice were sacrificed at 4-6 hr after the final dose. The large intestine was weighed and examined for histopathologic changes.

Results: Two mice in the 2.5 mg/kg/day group and one mouse in the sulfasalazine group died before study termination. SP-304 produced a dose-dependent decrease in bodyweight. All TNBS-treated groups exhibited diarrhea. The severity of diarrhea was greater in the 2.5 mg/kg bid group. Fecal blood was greater at 2.5 mg/kg bid, and lowest at 0.5 and 50 mg/kg/day. Rectal bleeding occurred in the 2.5 mg/kg/day group, the 2.5 mg/kg bid group, and the sulfasalazine group (1/8 mice in each group). As expected, colon weight in the TNBS-treated groups was increased. The highest mean colon weight occurred in the 2.5 mg/kg bid and 50 mg/kg/day groups. The lowest severity of colonic injury was observed in the 0.5 mg/kg/day group.

DSS-Induced Colitis:

Methods: Male BDF1 mice were treated orally with 0 (vehicle, one group dosed once daily, one group dosed bid), 0.005, 0.05, 0.5, 2.5, 5, or 50 mg/kg/day SP-304 for seven days (10 mice/group). The vehicle was PBS, and the dose volume was 10 ml/kg. Mice in the 5 mg/kg/day group were given two daily doses (2.5 mg/kg bid). As a positive control, one group of mice was treated orally with 80 mg/kg/day sulfasalazine for seven days. To induce colitis, the mice were provided with drinking water containing 5% DSS. An additional control group was treated orally with PBS for seven days, in the absence of DSS treatment. Study parameters included clinical signs, stool consistency, rectal bleeding, bodyweight, and occult blood. The mice were sacrificed at 4-6 hr after the final dose. The large intestine was weighed and examined for histopathologic changes.

Results: One mouse in the vehicle control group died on day 7, and one mouse in the 50 mg/kg/day group was sacrificed on day 6 due to poor health. Bodyweight was decreased in all DSS-treated groups. However, the effect on bodyweight was lowest in the 0.005 and 0.05 mg/kg/day groups. The 2.5 mg/kg bid group exhibited partial suppression of diarrhea and rectal bleeding. Fecal blood was present in all DSS-treated groups, with little difference among the groups. Colon weight in the 0.005 and 0.05 mg/kg/day group was lower compared with the other groups. The appearance of intestinal crypts was healthiest in the 0.005 mg/kg/day group.

Conclusions: SP-304 exhibited some ability to attenuate the TNBS- and DSS-induced colitis in mice. However, this effect was not dose dependent. The active doses in the TNBS and DSS models were 0.5 mg/kg/day and 0.005-0.05 mg/kg/day, respectively.

Anti-Inflammatory Activity of SP-304 (Plecanatide) in an Animal Model of Experimental Colitis in BALB/c Mice (Study SP-PH-005)

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

Methods: Female BALC/c mice were treated orally with 0 (vehicle), 0.5, 1, or 2.5 mg/kg/day SP-304 for seven days (10 mice/group). The vehicle was PBS, and the dose volume was 10 ml/kg. On day 2, the mice were treated with a single intra-rectal administration of 0.5 mg TNBS in 50% ethanol, to induce colitis. The mice were sacrificed at 24 hr after the final dose. Histopathologic examination was performed on the colon. Sections of transverse colon tissue from some of the mice were cultured for measurement of cytokine expression. After 24 hr of incubation, the supernatants from the tissue cultures were collected for cytokine measurement using an ELISA method.

Results: SP-304 produced a reduction in inflammation at all dose levels, as indicated by a dose-dependent decrease in the inflammation scores. The inflammation score was reduced by approximately 75% at the highest dose. Reductions in the levels of the pro-inflammatory cytokines IL-23, TNF, IL-4, and IL-5 were observed.

Conclusions: SP-304 produced a dose-dependent reduction in TNBS-induced colonic inflammation.

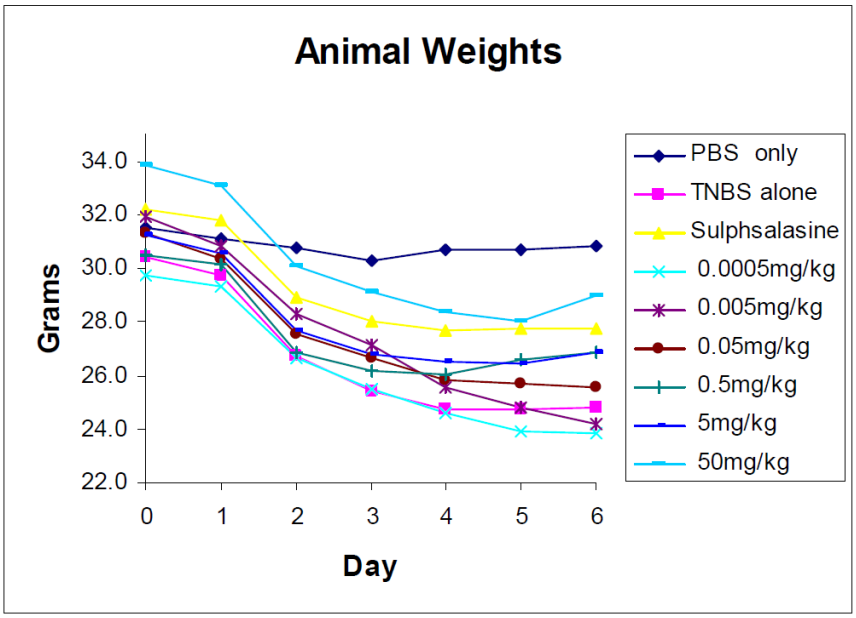
Efficacy Analysis of SP-304 (Plecanatide) in a TNBS IBD Mouse Model (Study No. 06-169)

Objective: To study the potential efficacy of plecanatide in the TNBS-induced mouse model of colitis over a wide range of doses.

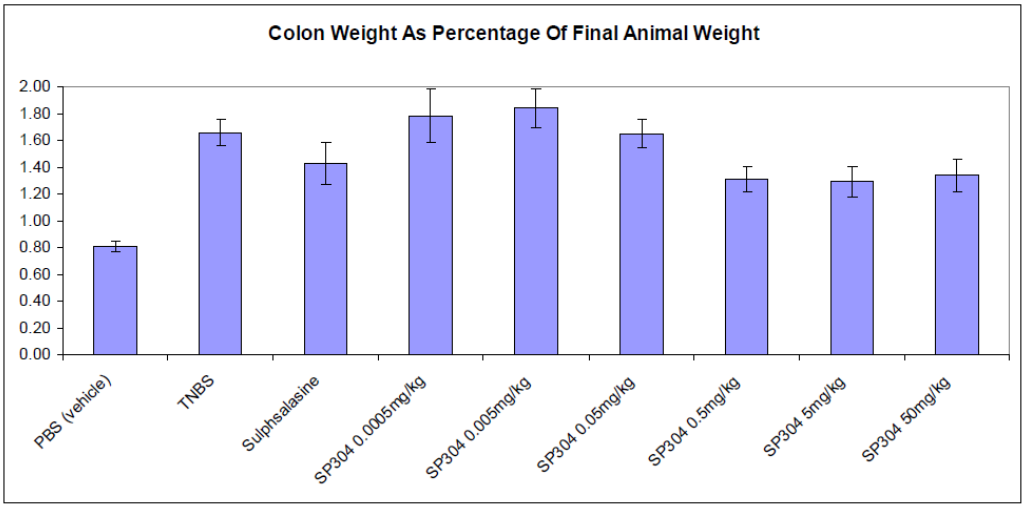
Materials and Methods: Groups of 10 *Helicobacter pylori*-free BDF1 male mice were treated by oral gavage once daily for 7 days with PBS (control), 0.0005, 0.005, 0.05,

0.5, 5, or 50 mg/kg plecanatide in a dose volume of 10 mL/kg. A positive control group was dosed once daily for 7 days with 80 mg/kg sulfasalazine. Four hours following the second dose (day 1), mice were treated rectally with 100 µL TNBS. Assessments included daily bodyweights and general clinical observations, as well as daily observations of stool consistency, fecal blood, rectal bleeding, and occult blood. The animals were also observed at 1 and 4 hours after dosing each day, and were sacrificed on day 7. The distal large intestine was removed and weighed. A gross necropsy was conducted, and the distal colon was examined histologically. A histological score was determined based on the total score from 5 separate sections of distal colon.

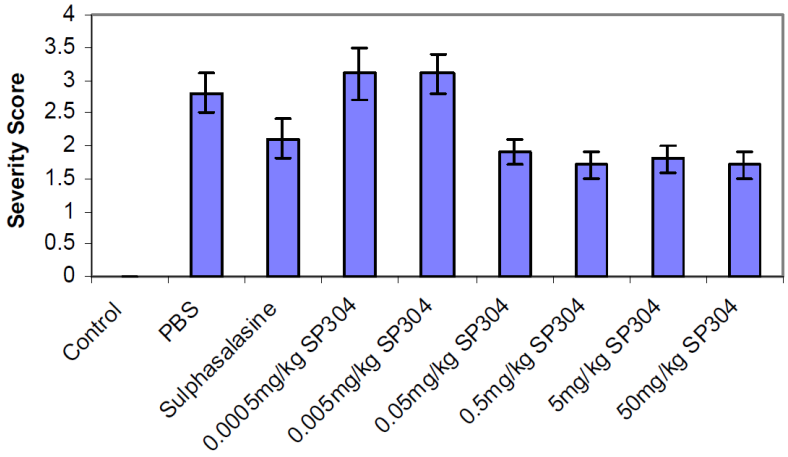
Results and Conclusions: Seven of the 90 animals died before study completion. The Sponsor suggested that the deaths were due to the effects of TNBS, and that no adverse effects were associated with oral treatment with plecanatide. There was no transient onset of diarrhea following dosing at early time points. However, there was a dramatic drop in bodyweight in all groups following TNBS administration. Animals treated with the three highest doses of plecanatide (0.5, 5, and 50 mg/kg) lost slightly less weight than did those at the lower doses of plecanatide or those treated only with TNBS. The data are shown in the Sponsor's figure below.



TNBS treatment increased colon size, and the higher doses of plecanatide produced a slight attenuation of the increase in colon size; however, the results were not statistically significant. The data are shown in the Sponsor's figure below.



Control TNBS-treated mice exhibited softer stools and more blood in the feces. Sulfasalazine treatment delayed the onset of diarrhea and reduced fecal blood. Increases in soft stools and diarrhea were observed at the higher doses of plecanatide. Treatment with 0.05 to 50 mg/kg plecanatide decreased the histological severity score relative to controls, as shown in the Sponsor's figure below.



Taken together, the results suggested that plecanatide at 0.5 to 50 mg/kg produced a slight reduction in the severity of TNBS-induced colonic disease, relative to controls treated only with PBS and TNBS. While the histological scores improved with the higher doses of plecanatide (0.5 to 50 mg/kg), the severity of diarrhea was somewhat worse in these animals compared with the lower dose groups. This effect was likely related to the pharmacological activity of plecanatide.

4.2 Secondary Pharmacology

Effect of Plecanatide on Off-target Receptor and Cytochrome P450 Binding In Vitro (Study No. AB20754)

Materials and Methods	Results and Conclusion
To examine the potential for plecanatide to inhibit or affect radioligand receptor binding or metabolic enzyme activity in vitro, plecanatide was evaluated at a single concentration (10 µM) in a broad panel of 73 targets that included neurotransmitter, hormone, cytokine, corticosteroid, and ion channel receptors, as well as a series of cytochrome P450 metabolic enzymes.	No positive binding or interactions were observed with plecanatide in this study. These results suggest that plecanatide does not interact with any of these receptors, ion channels, or metabolic enzymes.

Effect of Plecanatide on Serotonin (5-Hydroxytryptamine) Receptor Binding and Activity (Study No. AB23825)	
Materials and Methods	**Results and Conclusion**
The objective of this study was to evaluate the potential for plecanatide to bind to serotonin receptors (5-HT_4) in vitro or to activate or inhibit 5-HT_4 receptors in isolated rat esophagus. Plecanatide binding to 5-HT_4 receptors was assessed in homogenates of the striatum of the guinea pig brain using [^{3}H]GR-113808 as the competitive radioligand in the presence of 0.1, 1, or 10 µM plecanatide. The criterion for a positive interaction was ≥50% inhibition of radioligand binding. The potential for plecanatide to activate or inhibit 5-HT_4 receptors was assessed in isolated rat esophagus. Agonist activity was defined as ≥50% relaxation of carbachol (3 µM)-induced contraction relative to 0.1 µM serotonin. Antagonist activity was defined as ≥50% inhibition of 0.1 µM serotonin-induced relaxation.	No significant binding to 5-HT_4 receptors was observed in the radioligand binding assay, and no significant agonist or antagonist activity was observed in the functional assay. Therefore, plecanatide did not significantly interact with 5-HT_4 receptors in these assays.

4.3 Safety Pharmacology

Effect of Plecanatide on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study No. 120924 (b) (4))

Materials and Methods: The human embryonic kidney cell line HEK293 was stably transfected with hERG cDNA. Whole cell recordings were performed using a patch clamp amplifier. Phase 1 of the study was a concentration range-finding experiment. The effects of plecanatide (30 µM) on the activation of hERG current and steady state hERG current were measured. The voltage was clamped at –80 mV. hERG channel current was activated by increasing the voltage to +20 mV for 1 sec, followed by a gradual repolarization to –80 mV. Each recording ended with an application of a supramaximal concentration of the reference substance (500 nM E-4031) to assess the contribution of endogenous currents. The remaining unblocked current was subtracted from the data. Four cells per condition were tested. In phase 2 of the study, plecanatide was tested at 1000 µM (n = 3). The vehicle for plecanatide was HEPES-buffered physiological saline.

Results and Conclusions: Plecanatide inhibited hERG current by 0.9 ± 0.3% at 30 µM and 7.5 ± 0.8% at 1000 µM versus 1.9 ± 1.0% with vehicle. The positive control

compound terfenadine (60 nM) inhibited hERG channel current by 80%. Thus, plecanatide has no significant effect on the hERG potassium channels.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

A Pharmacological Assessment of the Effect of SP-304 (Plecanatide) on the Cardiovascular System of the Cynomolgus Monkey Using Telemetry (Study 692345)

Methods: Four male Cynomolgus monkeys (age 3.5-5 years, 3.2-4.7 kg) were implanted with telemetry devices to record hemodynamic and ECG data. The monkeys were treated orally with escalating doses of SP-304. The intended dose levels were 0 (vehicle), 0.1, 25, and 2000 mg/kg. However, the reported drug concentration for the high-dose group was about 25% lower than the intended concentration. Therefore, the high-dose is estimated to be approximately 1500 mg/kg. The interval between the first and second doses (vehicle and 0.1 mg/kg) was at least two days, followed by seven-day dosing intervals thereafter. The drug was administered as a suspension in 0.5% w/v carboxymethylcellulose using a dose volume of 15 ml/kg. Baseline data was obtained through a 24-hr recording of blood pressure and ECG. Blood pressure and ECG was recorded for about 2 hr prior to each dose through 24 hr post-dose. Data was reported as the mean values from 1-hr intervals, which were derived from the mean values from 5-minute recording intervals. ECG tracings were obtained at 7 min, 30 min, and at 1, 1.5, 2, 3, 4, 5, 6, 7, 8, and 24 hr post-dose. The tracings were evaluated by a veterinary cardiologist for waveform abnormalities. QT_c was calculated for each monkey using the following formula:

$QT_c = QT - M(RR-500)$
M = slope of regression line from RR vs. QT plot from individual monkeys

Results: Soft feces was observed at 0.1 and 25 mg/kg, 4 to 6 hr after dosing (1/4 and 2/4 monkeys, respectively). At 2000 mg/kg, all animals exhibited green liquid feces (slight to severe) at 4-8 hr after dosing.

The cardiovascular data was expressed as the change from pre-dose (baseline) values and as the absolute values. The following cardiovascular parameters were either unchanged or minimally affected: mean arterial pressure, systolic pressure, and diastolic pressure (slight reduction from baseline and vehicle control values at 2000 mg/kg, 3-7 hr); pulse pressure and QRS. No qualitative abnormalities of ECG tracings were observed.

Heart rate in the 0.1, 25, and 2000 mg/kg groups was increased by 10.1 $\pm$ 5.4, 15.9 $\pm$ 8.9, and 25.3 $\pm$ 2.9 bpm (mean $\pm$ SEM), respectively, at 0-1 hr post-dose relative to baseline values. The vehicle control group value was -0.1 $\pm$ 5.2 bpm. The absolute heart rate values in the 0 (vehicle), 0.1, 25, and 2000 mg/kg groups were 134.1 $\pm$ 7.6,

141.4 ± 3.5, 155.9 ± 8.6, 162.1 ± 11.2 bpm, respectively, at 0-1 hr post-dose. Heart rate was increased from the baseline (+19.9 ± 5.9 bpm) and vehicle control values (+58.5 bpm) in the 2000 mg/kg at 18-20 hr post-dose.

PR relative to baseline was decreased in the 2000 mg/kg group during most of the recording period, starting at 30 min post-dose. This effect does not appear to be drug-related. The decreased PR relative to baseline values can be attributed to the higher baseline (pre-dose) values in the 2000 mg/kg group, relative to the other groups. Furthermore, the absolute post-dose values in the 2000 mg/kg group were similar to those in the 0, 0.1, and 25 mg/kg groups.

QT_c was prolonged in the 25 mg/kg group at 19-22 hr post-dose, whereas no change in QT_c occurred in the 0.1 or 2000 mg/kg groups at any time-point. The magnitude of prolongation at 25 mg/kg was up to 46.4 ± 28.9 msec, relative to the baseline value. The maximum prolongation was associated with an absolute value of 290.1 ± 25.2 msec in the 25 mg/kg group, as compared to the control value of 244.3 ± 1.6 msec. Given the latency of this effect relative to the observed t_{max} in monkeys from the single-dose toxicity study, and the absence of dose dependence, the QT_c prolongation at 25 mg/kg is not considered to be drug-related.

Conclusions: SP-304 produced a small but dose-dependent increase in heart rate at oral dose levels of 0.1, 25, and 2000 mg/kg. A transient slight decrease in systolic and diastolic pressure occurred in the 2000 mg/kg group. No other parameters, including QT_c, were affected by SP-304.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

Compound SP-304: A safety Pharmacology Study of the Central Nervous System Employing a Functional Observational Battery (FOB) Following Single Oral Administration in CD-1 Mice (Study 15056)

Methods: Crl:CD1 (ICR) mice (age 8-9 weeks; males: 30.9-35.7 g; females: 23.4-28.7 g) were treated with a single oral administration of 0 (vehicle), 20, 200, or 2000 mg/kg SP-304 (5 mice/sex/group). The drug was administered as a solution in distilled water, using a dose volume of 30 ml/kg. A functional observational battery to assess behavioral, neurological, and autonomic function was performed pre-treatment, 0.5, 1.5, 4, and 24 hr post-dose.

Results: No drug-related signs were observed. Body temperature was slightly reduced (1.5-2°C) in the 2000 mg/kg group at 30 min post-dose, and at 90 min in males only. No other autonomic effects were observed. Behavioral and neurological parameters were unaffected.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

Evaluation of Respiratory Function following Oral Gavage Administration of SP-304 (Plecanatide) in Mice (Study 1275MS58.001)

Methods: Male Crl:CD®(ICR) mice (age 4 weeks; 20-27 g) were treated with a single oral administration of 0 (vehicle), 20, 200, or 2000 mg/kg SP-304 (5 mice/group). The drug was administered as a solution in distilled water, using a dose volume of 30 ml/kg. Tidal volume, minute volume, and respiratory rate were measured by placing mice in a head-out plethysmographic chamber. Respiratory parameters were recorded prior to dosing and at 0.25, 1, 3, and 6 hr after dosing.

Results: No drug-related effects were observed. The administration of vehicle produced reductions in respiratory rate (22% relative to pre-dose value), tidal volume (6%), and minute volume (28%). The maximum effects were observed at 1 hr. Groups treated with SP-304 showed similar effects, which are assumed to be related to the vehicle.

Conclusions: This study was flawed due to the use of an extremely high dose volume (30 ml/kg) (Nelson H. Wilson et al. "Short-term, subchronic, and chronic toxicology studies." Principles and methods of toxicology, 4th edition. Ed. A. Wallace Hayes, 2001. Ch. 19, pg 926.). It is likely that respiratory function in all groups was disrupted by the high dose volume, as suggested by the similarity of effects in vehicle and treatment groups. Therefore, the results are considered to be inconclusive with respect to the evaluation of drug effects on respiratory function. Under the study conditions, SP-304 did not affect respiratory function in mice.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

Pharmacological Effects of SP-304 (Plecanatide) on the Gastrointestinal System of the Albino Mouse (Charcoal Propulsion Study) (Study No. 692342)

Methods: Male Crl:CD1 (ICR) mice (age 7 weeks; 25.8-33.6 g) were treated with a single oral administration of SP-304 or vehicle, followed by oral administration of 0.3 ml of 10% activated charcoal suspension in 5% acacia at 7, 30, or 90 min post-dose. The mice were fasted overnight prior to the experiment. For each time-point, the dose levels tested were 0 (vehicle), 20, 200, and 2000 mg/kg SP-304 (8 mice/group/time-point). The drug was administered as a solution in deionized water, using a dose volume of 30 ml/kg. The positive control group was treated orally with 10 mg/kg metoclopramide HCl at 30 min before administration of the charcoal suspension. After a 20-min period following charcoal administration, the mice were sacrificed and the stomach and intestines were removed. Stomachs were weighed with and without contents to evaluate drug effects on emptying. The distance of charcoal migration from the pyloric

sphincter to cecum was measured. The distance traveled by charcoal as percentage of the total length of small intestine was calculated.

Results: The results are shown in the table below.

Parameter	Vehicle	20 mg/kg SP-304	200 mg/kg SP-304	2000 mg/kg SP-304	10 mg/kg Metoclopramide
	Charcoal Given at 7 Minutes Post-Dose				
Net Weight of Stomach Contents (g)	0.176 ± 0.097	0.199 ± 0.127	0.251 ± 0.071	0.635 ± 0.303	-
Distance of Charcoal Migration (% intestinal length)	62.0 ± 12.7	71.6 ± 15.1	73.3 ± 16.6	48.0 ± 16.2	-
	Charcoal Given at 30 Minutes Post-Dose				
Net Weight of Stomach Contents (g)	0.174 ± 0.063	0.253 ± 0.064	0.313 ± 0.16	0.920 ± 0.081	0.111 ± 0.03
Distance of Charcoal Migration (% intestinal length)	44.6 ± 12.5	81.2 ± 15.3	74.7 ± 17.2	5.0 ± 8.5	57.1 ± 6.1
	Charcoal Given at 90 Minutes Post-Dose				
Net Weight of Stomach Contents (g)	0.125 ± 0.059	0.134 ± 0.075	0.213 ± 0.07	0.576 ± 0.203	-
Distance of Charcoal Migration (% intestinal length)	51.6 ± 8.1	67.6 ± 7.0	76.0 ± 22.7	51.6 ± 26.8	-

Conclusions: This study was severely flawed due to the use of an extremely high dose volume (30 ml/kg) (Nelson H. Wilson et al. "Short-term, subchronic, and chronic toxicology studies." Principles and methods of toxicology, 4th edition. Ed. A. Wallace Hayes, 2001. Ch. 19, pg 926). The high dose volume could have altered the effect of SP-304 on gastric emptying and intestinal motility. Therefore, no conclusions about the effects of SP-304 can be derived from the study. Under the study conditions, SP-304 produced a dose-dependent increase in the net weight of stomach contents, indicative of reduced gastric emptying. A marked reduction in charcoal migration through the small intestine occurred at 2000 mg/kg, but only when the drug was given at 30 min before administration of charcoal. Metoclopramide, the positive control drug, produced the expected effects.

Renal effects: No studies were submitted.

Abuse liability: No studies were submitted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Brief Summary

Plecanatide is rapidly absorbed and digested/metabolized following oral administration. Oral bioavailability is extremely low (<0.1% in mice and monkeys). SP-338, a 15-mer C-terminal des-leucine metabolite, was found to be the only active metabolite. *In situ* metabolic studies in ligated intestinal loops were conducted, and based on the peptides identified in these studies a putative metabolic degradation pathway for plecanatide was proposed. The metabolism of plecanatide and SP-338 is accelerated under reducing conditions. Plecanatide did not bind to human serum albumin (HSA) or human α1-acid glycoprotein (AGP). Neither plecanatide nor SP-338 is an inhibitor of CYP2C9 and CYP3A4, or an inducer of CYP3A4 cytochrome P450 enzymes. Furthermore, they are not substrates or inhibitors of the P-gp or BCRP transporters. The major route of elimination of ^{14}C-derived radioactivity following a single oral administration of ^{14}C-plecanatide to rats is from expired air, with lesser amounts in feces and urine.

5.1.2 Methods of Analysis

[see under individual study reviews]

5.1.3 Absorption

Plecanatide: Bioavailability and Vehicle Comparison Study in CD-1 Mice (Study No. 1896-020)

Experimental Design and Methods: Male and female CD-1 mice, 28.3 to 32.3 g and 23.4 to 26.5 g, respectively, were treated once with 1 mg/kg plecanatide by intravenous (36/sex) or oral (gavage) administration (32/sex). The intravenous formulation was prepared in sterile water for injection. Oral plecanatide formulations were prepared in distilled water or 0.1 M sodium phosphate buffer, pH 6.91. The dose volume was 5 ml/kg for the intravenous route and 10 ml/kg for the oral route. The design of study is shown in the Sponsor's table below.

Study Design

Group Assignments			
Group Number	Treatment	Number of Animals	
		Male	Female
1	1 mg/kg [a]	36	36
2	600 mg/kg [b]	32	32
3	600 mg/kg [c]	32	32

[a] Plecanatide in sterile water for injection, USP (intravenous)
[b] Plecanatide in distilled water (oral gavage)
[c] Plecanatide in 0.1M sodium phosphate buffer, pH 6.91 (oral gavage)

Blood samples were collected from 4 animals/sex/group/time point at 2.5, 5, 15, 30 minutes, 1, 1.5, 2, 2.5, and 3 hours post-dose for group 1, and at 5, 15, 30 minutes, 1,

1.5, 2, 2.5, and 3 hours post-dose for groups 2 and 3. Plasma plecanatide and SP-338 concentrations were determined using LC-MS/MS with an LLOQ of 1 ng/mL for plecanatide and 0.775 ng/mL for SP-338.

Results and Conclusions: The Sponsor determined that there were no sex differences in the systemic exposure (AUC and C_{max}) of plecanatide or SP-338 after IV or oral administration. Therefore, data for males and females were combined in subsequent analysis. The values for the pharmacokinetic parameters for plecanatide and SP-338 are shown in the Sponsor's table below.

Pharmacokinetic Parameters

Group Number*	Analyte	C_0 (ng/mL)	AUC_{3h} (ng•h/mL)	AUC_{3h}/Dose (ng•h/mL/mg/kg)	C_{max} (ng/mL)	T_{max} (h)	F (%)
1	Plecanatide	8670	882	882	4510	0.042	NA
	SP-338	NA	4.08	4.08	6.02	0.500	NA
2	Plecanatide	NA	285	0.476	657	0.083	0.05
	SP-338	NA	22.4	0.0373	21.1	0.083	NA
3	Plecanatide	NA	318	0.530	224	0.083	0.06
	SP-338	NA	39.7	0.0662	45.6	0.250	NA

* Groups 1, 2, and 3 were dosed with plecanatide at 1 mg/kg (IV), 600 mg/kg (oral in water) and 600 mg/kg (oral in 0.1M sodium phosphate buffer, pH 6.9), respectively.

AUC_{3h} = area under the time-concentration curve from time 0 to 3 hours; C_0 = plasma concentration at time 0; C_{max} = maximum plasma concentration; F = bioavailability; IV = intravenous; NA = not applicable because C_0 could not be evaluated in plasma immediately after oral dosing or SP-338 after IV dosing of plecanatide; T_{max} = time to maximum concentration

After IV administration, plecanatide plasma concentrations were quantifiable up to 2 hours post-dose, and peak plecanatide plasma concentrations were estimated at time zero (C_0 = 8670 ng/mL). Plecanatide systemic exposure (AUC_{3h}) was 882 ng•h/mL in mice following IV administration of 1 mg/kg, with a C_{max} of 4510 ng/mL at 2.5 minutes. Following oral administration, plecanatide concentrations were similar between mice receiving the 600 mg/kg plecanatide in distilled water (group 2) or 0.1 M sodium phosphate buffer (group 3). Plecanatide plasma concentrations were quantifiable up to 3 hours post-dose, and peak plecanatide plasma concentrations (T_{max}) were observed at 5 minutes post-dose in both oral formulations. Plecanatide systemic exposure (AUC_{3h}) and dose-normalized systemic exposures (AUC_{3h}/dose) were similar between group 2 (plecanatide in distilled water) and group 3 (plecanatide in 0.1 M sodium phosphate buffer) animals where AUC_{3h} values were 285 ng•h/mL and 318 ng•h/mL, respectively. When compared with a single IV (bolus) injection of 1 mg/kg plecanatide (groups 2 and 3 versus group 1), dose-normalized systemic exposure was lower following a single oral administration of 600 mg/kg plecanatide. Oral bioavailability was approximately 0.05% and 0.06% in groups 2 and 3, respectively.

Following a single IV (bolus) injection of 1 mg/kg plecanatide, SP-338 plasma concentrations were quantifiable up to 1.5 hours post-dose, and peak SP-338 (C_{max}) plasma concentration (6.02 ng/mL) was observed at 30 minutes post-dose (T_{max}). SP-338 systemic exposure (AUC_{3h}) was 4.08 ng•hr/mL for the mice in group 1, which is less than the systemic exposure (AUC_{3h}) to plecanatide (882 ng•h/mL) following an IV

dose of 1 mg/kg in mice. Following an oral administration of 600 mg/kg plecanatide in distilled water (group 2) or 0.1 M sodium phosphate buffer (group 3), SP-338 plasma concentrations were quantifiable up to 3 hours post-dose, and peak SP-338 plasma concentrations were observed at 5 or 15 minutes after oral administration. SP-338 systemic exposures (AUC_{3h}) were similar between group 2 (plecanatide in distilled water) and group 3 (plecanatide in 0.1 M sodium phosphate buffer) where AUC_{3h} values were 22.4 ng•h/mL and 39.7 ng•h/mL, respectively. SP-338 systemic exposure (AUC_{3h}) was less than plecanatide systemic exposure (AUC_{3h}) in mice following oral dosing.

In summary, systemic exposure (AUC and C_{max}) to plecanatide and its metabolite, SP-338, were independent of sex. Systemic exposures to plecanatide and SP-338 were similar between the 2 oral formulations tested (water and 0.1 M sodium phosphate buffer, pH 6.91). Oral bioavailability of plecanatide following administration of either the water or 0.1 M phosphate buffer formulation was 0.05% and 0.06%, respectively. Systemic exposure to SP-338 in mice was lower following an IV bolus injection (1 mg/kg) or oral administration (600 mg/kg) of plecanatide when compared to systemic exposure to plecanatide.

Plecanatide: Bioavailability and Vehicle Comparison Study in Cynomolgus Monkeys (Study No. 1896-019)

Experimental Design and Methods: The animals (4/sex/group) were fasted for at least 8 hours prior to dosing and through 1 hour post-dose. Plecanatide was administered once by either IV injection (1 mg/kg, group 1) or oral gavage (100 mg/kg, groups 2 and 3) at a dose volume of 5 mL/kg. Oral plecanatide formulations were prepared in distilled water (group 2) or 0.1 M sodium phosphate buffer, pH 6.91 (group 3). Blood samples were collected via the femoral vein/artery. Samples were collected at 2, 5, 15, 30 minutes, 1, 2, and 3 hours post-dose for group 1, and at 0.5, 15, 30 minutes, 1, 1.5, 2, 2.5, and 3 hours post-dose for groups 2 and 3. Plasma plecanatide and SP-338 concentrations were measured using LC-MS/MS with an LLOQ of 1 ng/mL for plecanatide and 0.775 ng/mL for SP-338.

Results and Conclusions: The mean plecanatide and SP-338 PK parameters following a single IV bolus injection of 1 mg/kg plecanatide or oral gavage administration of 100 mg/kg plecanatide in distilled water or 0.1 M sodium phosphate buffer in Cynomolgus monkeys (males and females combined) are provided in the Sponsor's table below.

Group Number*	Analyte	C_0 (ng/mL)	AUC_{3h} (ng•h/mL)	AUC_{3h}/Dose (ng•h/mL)/ (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	F (%)
1	Plecanatide	11500	3550	3550	8100	0.042	0.513	NA
	SP-338	55.9	80.5	80.5	51.9	0.50	0.815	NA
2	Plecanatide	NA	217	2.17	172	0.50	0.674	0.06
	SP-338	NA	262	2.62	162	1.0	0.651	NA
3	Plecanatide	NA	293	2.93	188	1.0	0.599	0.08
	SP-338	NA	224	2.24	153	1.0	0.646	NA

*Groups 1, 2, and 3 were dosed with plecanatide at 1 mg/kg (intravenous), 100 mg/kg (oral in water), and 100 mg/kg in 0.1M sodium phosphate buffer, 6.9), respectively.

AUC_{3h} = area under the time-concentration curve from time 0 to 3 hours; C_0 = plasma concentration at time 0; C_{max} = maximum plasma concentration; F = bioavailability; IV = intravenous; NA = not applicable because C_0 could not be evaluated in plasma immediately after oral dosing or SP-338 after IV dosing of plecanatide; T_{max} = time to maximum concentration

The Sponsor determined that there were no sex-related differences in the systemic exposure (AUC and C_{max}) to plecanatide or SP-338 after IV or oral administration. Therefore, data for males and females were combined in subsequent analysis. Plecanatide plasma concentrations following IV or oral administrations were quantifiable up to 3 hours post-dose. Individual peak plecanatide plasma concentrations (C_{max}) were observed by 0.042 and 0.5 hour post-dose in the IV and oral administration groups, respectively. In the IV group, individual peak plecanatide plasma concentrations were estimated at C_0, and C_{max} values, approximately 30% lower than the predicted C_0 values, were also reported for comparison with the groups that received plecanatide by oral administration. Mean systemic exposure (AUC_∞) was lower following a single oral administration of 100 mg/kg in either water or 0.1 M phosphate buffer when compared with a single IV injection of 1 mg/kg plecanatide, as were the dose-normalized systemic exposure (AUC_{3h}/dose). Mean oral bioavailability (F) for groups 2 and 3 relative to group 1 was calculated to be 0.06% and 0.08%, respectively. Plecanatide was eliminated rapidly with a mean $t_{1/2}$ of 31 minutes following IV dosing, and 40 minutes and 36 minutes in group 2 and 3 animals, respectively. No differences in plecanatide exposure were observed when given orally in water or 0.1M phosphate buffer, pH 6.9.

SP-338 plasma concentrations were quantifiable up to 2 or 3 hours post-dose following either IV or oral administration of plecanatide. Individual peak SP-338 plasma concentrations (C_{max}) were observed by 0.5 or 1 hour post-dose in the IV or oral administration group, respectively. In contrast to mice, the systemic exposures to SP-338 and plecanatide following oral administration were similar in monkeys. However, similar to mice, the mean systemic exposure of SP-338 (AUC_{3h}) in monkeys following IV dosing was much lower compared with mean systemic exposure of plecanatide. Individual metabolite to parent drug ratios ranged from 0.00495 to 0.0478 following IV dosing. The mean dose-normalized systemic exposure (AUC_{3h}/dose) of SP-338 was lower following a single oral administration of 100 mg/kg plecanatide when compared with a single IV (bolus) injection of 1 mg/kg plecanatide (group 2 and group 3 versus group 1). SP-338 was eliminated rapidly with a mean $t_{1/2}$ of 49 minutes following IV

dosing and a mean $t_{1/2}$ of 38.8 minutes and 39.1 minutes following oral administration in water or 0.1 M phosphate buffer, respectively.

In summary, systemic exposure to plecanatide and its active metabolite SP-338 following oral administration of plecanatide (100 mg/kg) was similar between the sexes. No differences in systemic exposure to plecanatide and SP-338 were observed when plecanatide was dosed in either of the two oral formulations tested (water and 0.1 M sodium phosphate buffer, pH 6.9), a result in contrast to that observed in CD-1 mice. Systemic exposure to SP-338 in monkeys following an IV bolus injection of plecanatide (1 mg/kg) was lower than systemic exposure to plecanatide. Oral bioavailability of plecanatide in monkeys following oral administration in water or 0.1 M phosphate buffer formulations was 0.06% and 0.08%, respectively.

5.1.4 Distribution

Binding of Test Compound Plecanatide to Human Serum Albumin (HSA) and Human α1-Acid Glycoprotein (AGP) (Study RSN00008)

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

Methods: Binding of SP-304 to HSA (human serum albumin) and AGP (human α_1-acid glycoprotein) was performed using fluorescent probes. For HSA, dansyl amide and dansyl sarcosine probes were used to detect binding at sites IIA and IIIA, respectively. Quenching of intrinsic HSA tryptophan fluorescence was used to measure binding to HSA in general. Binding to AGP site I was measured using quinaldine red probe, and general binding to AGP was measured through quenching of intrinsic AGP tryptophan fluorescence. The positive control drugs for HSA and AGP binding were iophenoxate and chlorpromazine, respectively.

Results: The results are shown in the table below.

	HSA/Site IIA	HSA/Site IIIA	HSA/QIF	AGP/Site I	AGP/QIF
Drug	K_i (μM)	K_i (μM)	K_d (μM)	K_i (μM)	K_d (μM)
SP-304	NB	195	NB	NB	NB
Iophenoxate	6.8	13.2	2.7	223	NB
Chlorpromazine	NB	NB	180	3.1	19.6

QIF: quenching of intrinsic HSA tryptophan fluorescence
NB: no binding detected

SP-304 exhibited weak binding to HSA site IIIA, and no binding to AGP. Iophenoxate, which binds to HSA, demonstrated a 6.8 μM K_d for HSA site IIA, and a 13.2 μM K_d for HSA site IIIA. No significant binding by iophenoxate was observed for AGP. Chlorpromazine, which binds to AGP, demonstrated a strong interaction (3.1 μM K_d) for

AGP site I. It did not show any binding to HSA by probe displacement, and showed weak binding (180 µM K_d) to HSA by QIF.

Determining the Substrate and Inhibition Potential of Plecanatide for P-gp and BCRP (Study 13SYNRP1A)

Methods: Evaluation of plecanatide as a substrate of P-gp and/or BCRP (breast cancer resistance protein) was carried out at a concentration of 20 µM in Caco-2 cells. The test concentration for the substrate assessment was selected based on the expected plecanatide concentration on the luminal side of the GI wall with the highest anticipated dose level (21.4 µM). Permeability was evaluated in both the apical-to-basolateral (AP-to-BL) and basolateral-to-apical (BL-to-AP) directions by placing plecanatide in Hanks' Balanced Salt Solution containing 10 mM HEPES and 25 mM D-glucose (HBSSg) buffer, on the donor side (AP for AP-to-BL; BL for BL-to-AP) and HBSSg buffer on the receiver side.

Caco-2 cells were used to determine the inhibition potential of plecanatide toward either P-gp or BCRP. Inhibition of P-gp and BCRP transporters was assessed at 2 µM, a concentration expected to represent approximately 10% of the maximum plecanatide concentration within the lumen of the GI tract. Transport of the P-gp probe substrate digoxin (10 µM) and transport of the BCRP probe substrate cladribine (10 µM) across Caco-2 cell monolayers were used as markers of P-gp and BCRP activity, respectively.

Results and Conclusions: The efflux ratio of plecanatide in Caco-2 cell was less than 2.0, indicating that plecanatide is not a substrate of P-gp or BCRP under the test conditions. Plecanatide (2 µM) did not inhibit transport of P-gp probe substrate digoxin or BCRP probe substrate cladribine across Caco-2 cell monolayers, suggesting that plecanatide is not a significant P-gp or BCRP inhibitor under these test conditions. Therefore, plecanatide is not a substrate of either P-gp or BCRP in vitro, nor is plecanatide an inhibitor of the activity of either transport proteins under the test conditions.

Determining the Substrate and Inhibition Potential of SP-338 for P-gp and BCRP (Study 13SYNRP6A)

Methods: Evaluation of SP-338 as a substrate of P-gp and/or BCRP was conducted at concentrations of 0.5, 2, and 10 µM in Caco-2 cells. Permeability was evaluated in both the AP-to-BL and BL-to-AP directions by placing SP-338 in HBSSg buffer on the donor side (AP for AP-to-BL; BL for BL-to-AP) and HBSSg buffer on the receiver side.

Caco-2 cells (clone C2BBe1) were used to determine the inhibition potential of SP-338 toward either P-gp or BCRP. Transport of the P-gp probe substrate digoxin (10 µM) and transport of the BCRP probe substrate cladribine (10 µM) across Caco-2 cell monolayers was used as markers of P-gp and BCRP activity, respectively. Inhibition of P-gp and BCRP transporters was assessed at 2 µM SP-338.

Results and Conclusions: No data were obtained for SP-338 at 0.5 and 2 µM as the analyte was below the limit of quantification of the bioanalytical method. When tested at 10 µM, the efflux ratio of SP-338 was less than 2.0, indicating that SP-338 is not a substrate of P-gp or BCRP under the test conditions.

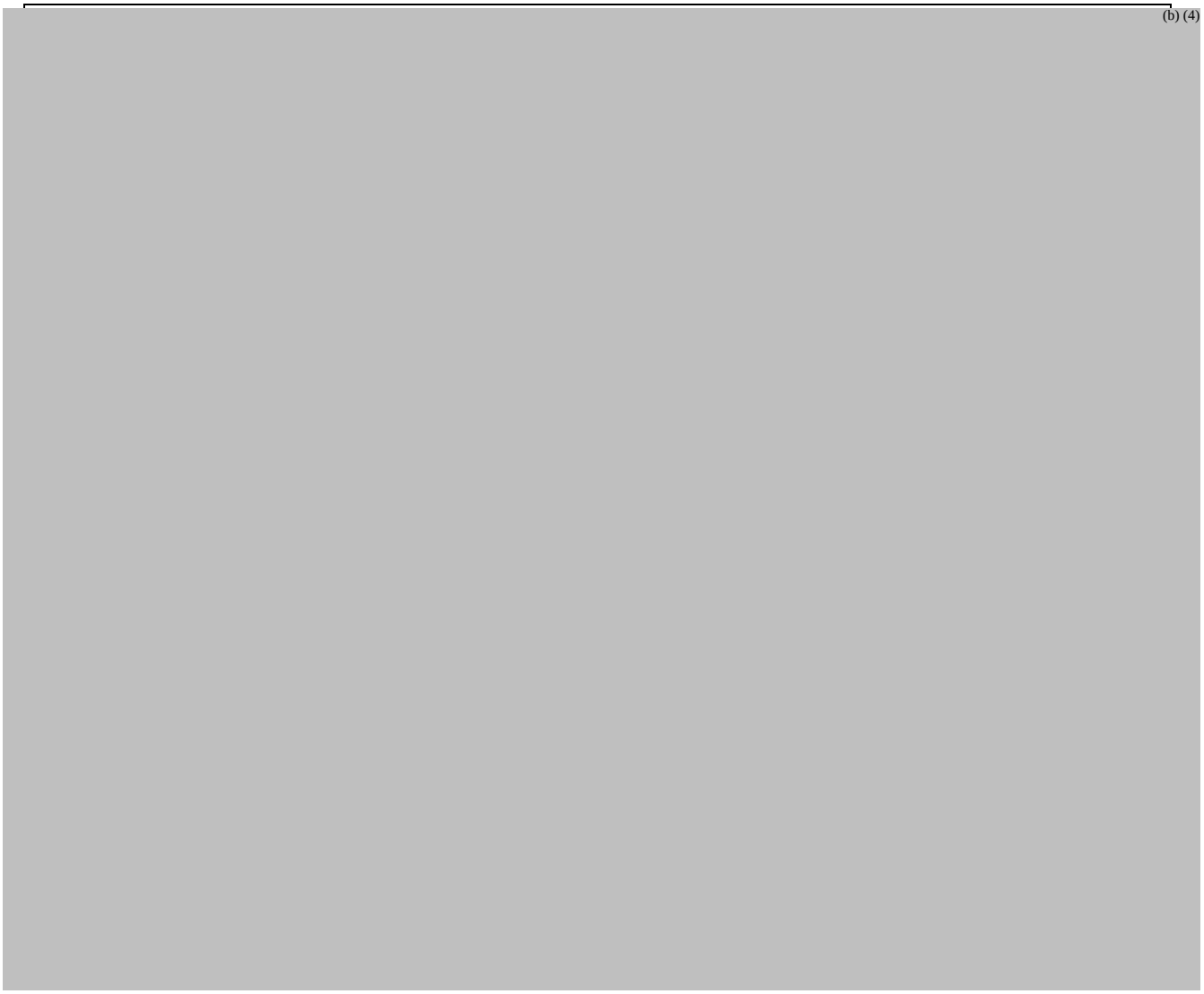
In the presence of 2 µM SP-338, the percent inhibition towards P-gp and BCRP was 15.9% and 6.62%, respectively. Since the percent inhibition values were less than 50%, the results indicated that SP-338 was not a significant P-gp or BCRP inhibitor under the test conditions.

Therefore, under the test conditions of the study, SP-338 is neither a substrate nor an inhibitor of P-gp or BCRP.

5.1.5 Metabolism

***In Vitro* Metabolism**

(b) (4)



(b) (4)

6. Assessment of Plecanatide Stability and Metabolite Identification After Treatment with Simulated Intestinal Fluid in the Presence and Absence of Reducing Agents or with Rat Intestinal Fluid (Study No. SP-PH-015)	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
The studies were conducted to evaluate the stability of plecanatide in: 1) SIF in the presence or absence of the reducing agent DTT; 2) a reducing power regenerating system, glutaredoxin; and 3) rat duodenal fluid. Peptide stability was assessed by monitoring residual biological activity subsequent to incubation in these systems and	The metabolism of plecanatide and SP-338 is accelerated in the presence of reducing agents. The $t_{1/2}$ of plecanatide in SIF was approximately 82 to 91 minutes. A greater than 2.5-fold decrease in $t_{1/2}$ was observed when plecanatide was incubated with SIF in the presence of 1 mM DTT ($t_{1/2}$ approximately 32 minutes). A faster reduction in

expressed as the percentage of cGMP produced in T84 human colon carcinoma cells as compared to vehicle-treated peptide. Plecanatide was incubated in SIF, SIF plus DTT or glutaredoxin, and rat duodenal fluid, and samples were collected over a period of 1 to 6 hours. Iodoacetamide was added to the samples to alkylate reduced cysteine residues, and the samples were analyzed by LC-MS to detect and identify metabolites of plecanatide degradation.	biological activity of the peptide was observed in the presence of glutaredoxin, a reducing power regenerating system ($t_{1/2}$ approximately 14 minutes). The $t_{1/2}$ of plecanatide in rat intestinal fluid obtained from ligated duodenal loops was approximately 9 minutes, suggestive of more rapid reduction and degradation of the peptide by the peptidases and reducing environment of the intestinal fluid. The $t_{1/2}$ of SP-338 (~ 7 minutes) was similar to that of plecanatide in duodenal fluid.

7. Assessment of the Stability of Plecanatide in Surgically Ligated Rat Intestinal Loops (Study No. 13SYNRP2)	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
Stability of plecanatide was assessed by injecting 0.3 mL of plecanatide at a concentration of 3.33 mg/mL directly into ligated intestinal loops (approximately 2 cm in length) in anesthetized male Sprague-Dawley rats. The fluid from each loop was recovered from the duodenum, jejunum, or ileum at 5, 15, and 45 minutes after administration (2 rats/time point). Blank phosphate buffer was also administered in each region of the intestine and collected 15 minutes post-injection from separate animals. Only the samples from the duodenal loops were evaluated. Detection and quantification of plecanatide metabolites at each time point was performed using HRAMS quantification. Plecanatide concentration of 3.33 mg/mL was used as 100% to determine the percentage remaining at each time point.	Plecanatide underwent rapid metabolism when incubated in the duodenum. The degradation rate was high in the first 5 minutes of incubation with 16%, 1.9%, and 0.023% of the parent peptide remaining after 5, 15, and 45 minutes, respectively. Metabolite SP-338 appeared in the intestinal fluid as early as 5 minutes, and also underwent rapid degradation over the time of incubation. The estimated $t_{1/2}$ of plecanatide in the duodenum was 1.83 minutes, less than the first sampling time point (5 minutes). Based on relative abundance, 14 major putative metabolites (M1 through M14) were detected using LC-HRAMS analysis. Detection of metabolites M1 through M3 indicates that plecanatide is reduced (opening of the disulfide bonds) in duodenal fluid.

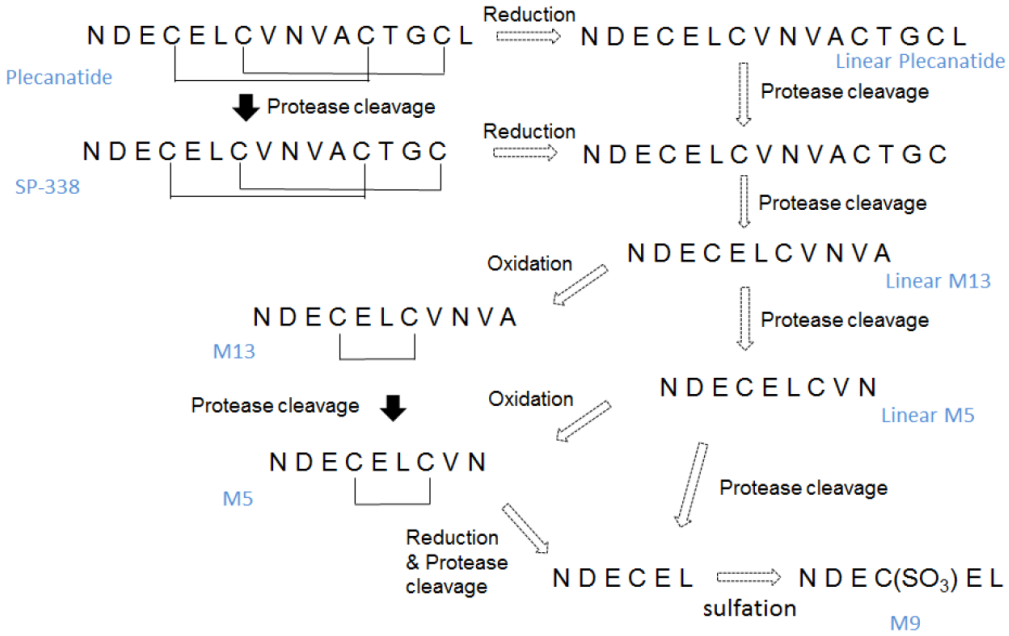
Assignment of the Sequences of Metabolites M5, M9, and M13 of Plecanatide Identified in Study 13SYNRP2 (Study No. 13SYNRP2R1)

Experimental Design and Methods: Fourteen major putative metabolites (M1 through M14) were detected using LC-HRAMS analysis in a previous study (13SYNRP2). The objective of the current study was to assign amino acid sequences to plecanatide metabolites M5, M9, and M13. Samples of fluid collected from rat ligated duodenal loops in the previous study were kept at -80°C prior to analysis.

Results and Conclusions: The amino acid sequences of M5, M9, and M13 were reported to be the following:

M5: Asn-Asp-Glu-Cys-Glu-Leu-Cys-Val-Asn (disulfide bond between Cys residues)
M9: Asn-Asp-Glu-Cys(-SO3)-Glu-Leu
M13: Asn-Asp-Glu-Cys-Glu-Leu-Cys-Val-Asn-Val-Ala (disulfide bond between Cys residues).

Taken together, results from this study and Study 13SYNRP2 demonstrate that, in addition to the biologically active metabolite SP-338, 14 other metabolites were detected in rat ligated intestinal loops. The Sponsor proposed the following degradation pattern of plecanatide (diagram taken from Sponsor's study report).



Assessment of the Stability of Plecanatide in Surgically Ligated Rat Intestinal Loops (Study No. 14SYNRP2R3-A)

Experimental Design and Methods: The objective of this study was to assess the stability of plecanatide *in situ* in the rat ligated intestinal loop. Female Sprague-Dawley rats were anesthetized, and the duodenum, jejunum, and ileum ligated using suture threads to form a 2-cm loop. Plecanatide, 0.2 mL in phosphate buffer (3.33 mg/mL), or blank phosphate buffer (control) was administered into the lumen of each of the ligated loops. Stability was assessed following incubation in the loops for 2, 5, 10, 20, 30, and 60 minutes (3 rats/time-point for plecanatide treatment, 1 rat/time-point for control). The concentration of plecanatide and its metabolite, SP-338, were quantified using LC-MS/MS. The nominal plecanatide concentration of 3.33 mg/mL was used as 100% to determine the percentage remaining at each time point.

Results and Conclusions: Plecanatide exhibited rapid metabolism in the duodenum, followed by jejunum and ileum. The concentrations of plecanatide in the samples recovered from duodenum and jejunum after 60 minutes incubation were below 1% of the initial dose. The remaining concentration of plecanatide in the ligated ileum segment reached a plateau at 10 minutes, with an average concentration of approximately 40%. The replicates from time points 20, 30, and 60 minutes were variable. The $t_{1/2}$ of plecanatide in the duodenum and jejunum was estimated to be 0.89 and 1.05 minutes, respectively.

The appearance of metabolite SP-338 followed the degradation profile of plecanatide, with a rapid appearance of high concentrations in the first 20 minutes following injection into duodenal and jejunal loops, and a slower appearance in the ileum samples. SP-338 concentration in the duodenum increased from 8% to 18% of the dosed concentration of plecanatide in the first 20 minutes, and declined to less than 1% after 1 hour incubation. A similar profile was observed in the jejunum, where the concentration of SP-338 was approximately 5% to 8% of the initial plecanatide concentration for the first 20 minutes. In the ileum, the concentrations of SP-338 increased gradually, reaching up to 32% of the dose after 60 minutes incubation.

The metabolite profiling was performed using pooled samples across all rats at the same time point in each segment. The data revealed the presence of multiple metabolites at similar intensities. Based on the matching of the retention times and mass measurements, the putative metabolites M5 and M13 were confirmed as identical to those identified in Study 13SYNP2R1.

In summary, plecanatide exhibited fast metabolism in the ligated upper small intestine segments. Faster degradation was observed in the duodenum, with a $t_{1/2}$ of less than 1 minute. The $t_{1/2}$ of plecanatide in the jejunum was 1.05 minutes. The $t_{1/2}$ of plecanatide in rat ileum could not be determined, as the remaining concentrations of plecanatide in ileum samples were variable and trended between 34% and 39% at all incubation times, suggesting that plecanatide metabolism pathways may have been rapidly inhibited and/or saturated in this segment. The intestinal loop samples revealed the presence of multiple metabolites at similar intensities. Based on the matching of the retention times and mass measurements, the putative metabolites M5 and M13 were confirmed as identical to those identified in Study 13SYNP2R1.

Reanalysis of Plecanatide Metabolites in Rat Duodenum Samples Pooled Across Multiple Time Points to Establish Their Relative Levels and Perform Tentative Structure Assignments (Study No. 14SYNRP2R3-B)

Experimental Design and Methods: The objective of this study was to perform re-analysis of pooled rat duodenal samples from Study 14SYNRP2R3-A in order to group metabolites detected during previous work according to their relative abundance. Duodenal samples for each animal (n = 3) were pooled across all time points to generate representative samples, mimicking the AUC of plecanatide and its metabolites. Samples were then re-analyzed by LC-MS/MS.

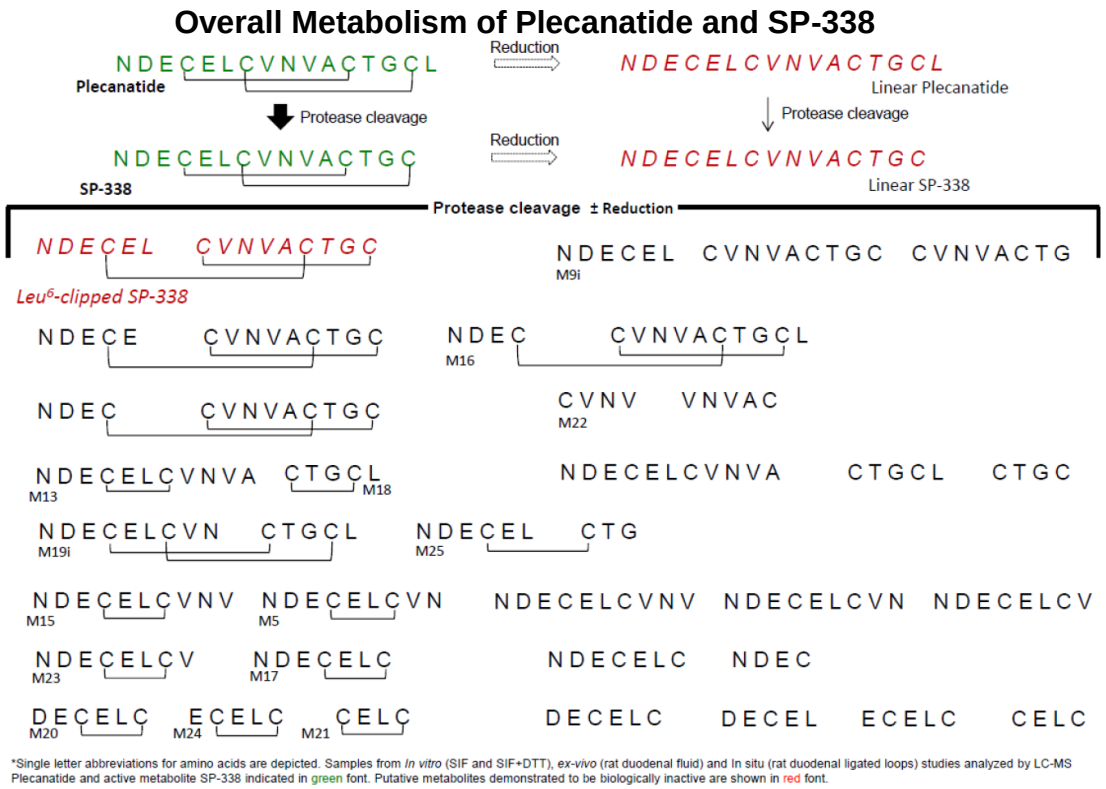
The overall profiles of putative metabolites in pooled duodenal samples were very similar between the 3 rats. The amino acid sequences of plecanatide and SP-338 and suggested sequences of 14 putative metabolites are shown the Sponsor's figure below. Mass spectrometry data supported the assignment of these sequences.

Sequence of Plecanatide and Suggested Sequences of Putative Metabolites

Analyte	Sequence
Plecanatide	Asn-Asp-Glu-Cys-Glu-Leu-Cys-Val-Asn-Val-Ala-Cys-Thr-Gly-Cys-Leu
SP-338	Asn-Asp-Glu-Cys-Glu-Leu-Cys-Val-Asn-Val-Ala-Cys-Thr-Gly-Cys-~~Leu~~
M15	Asn-Asp-Glu-Cys-Glu-Leu-Cys-Val-Asn-Val-~~Ala-Cys-Thr-Gly-Cys-Leu~~
M5	Asn-Asp-Glu-Cys-Glu-Leu-Cys-Val-Asn-~~Val-Ala-Cys-Thr-Gly-Cys-Leu~~
M17	Asn-Asp-Glu-Cys-Glu-Leu-Cys-~~Val-Asn-Val-Ala-Cys-Thr-Gly-Cys-Leu~~
M13	Asn-Asp-Glu-Cys-Glu-Leu-Cys-Val-Asn-Val-Ala-~~Cys-Thr-Gly-Cys-Leu~~
M16	Asn-Asp-Glu-Cys-~~Glu-Leu~~-Cys-Val-Asn-Val-Ala-Cys-Thr-Gly-Cys-Leu
M18	~~Asp-Asp-Glu-Cys-Glu-Leu-Cys-Val-Asn-Val-Ala~~-Cys-Thr-Gly-Cys-Leu
M9i	Asn-Asp-Glu-Cys-Glu-Leu-~~Cys-Val-Asn-Val-Ala-Cys-Thr-Gly-Cys-Leu~~
M19i	Asn-Asp-Glu-Cys-Glu-Leu-Cys-Val-Asn-Val-Ala-Cys-Thr-Gly-Cys-Leu
M20	~~Asn~~ Asp-Glu-Cys-Glu-Leu-Cys-~~Val-Asn-Val-Ala-Cys-Thr-Gly-Cys-Leu~~
M21	~~Asn-Asp-Glu~~-Cys-Glu-Leu-Cys-~~Val-Asn-Val-Ala-Cys-Thr-Gly-Cys-Leu~~
M22	~~Asn-Asp-Glu-Cys-Glu-Leu~~ Cys-Val-Asn-Val ~~Ala-Cys-Thr-Gly-Cys-Leu~~
M23	Asn-Asp-Glu-Cys-Glu-Leu-Cys-Val-~~Asn-Val-Ala-Cys-Thr-Gly-Cys-Leu~~
M24	~~Asn-Asp~~-Glu-Cys-Glu-Leu-Cys-~~Val-Asn-Val-Ala-Cys-Thr-Gly-Cys-Leu~~
M25	Asn-Asp-Glu-Cys-Glu-Leu-~~Cys-Val-Asn-Val-Ala~~-(N-formyl)Cys-Thr-Gly-~~Cys-Leu~~

Note: Underlined and colored cysteine moieties indicate matched linkages of disulfide bonds. Crossed-out amino acids indicate amino acid lost due to degradation.

As demonstrated in previous studies (13SYNRP2 and 14SYNRP2R3-A), plecanatide is rapidly metabolized in rat duodenum with a $t_{1/2}$ ranging between 0.9 to 1.8 minutes. In addition to confirming the generation of the biologically active metabolite SP-338 and metabolites identified in Study 13SYNRP2R1, this study has assigned putative structures to an additional 12 metabolites. Based on the structure of the metabolites, the Sponsor provided the following overall schematic representation of the metabolism of plecanatide (figure taken from the Sponsor's study report).



In conclusion, SP-338 is produced upon removal of C-terminal leucine from plecanatide. The putative metabolite Leu6 clipped SP-338 can result from an internal cleavage at Leu6-Cys7 within SP-338. In a reducing environment, several peptide fragments of 4 to 9 amino acids were identified, suggesting complete reduction and proteolytic degradation of plecanatide or SP-338. LC-MS analysis also revealed a series of low molecular weight peaks, expected to be fragments consisting of 2 to 4 amino acids. SP-338 is the only metabolite of plecanatide that has been demonstrated to bind GC-C with relatively high affinity and induce cGMP synthesis in a dose-dependent manner. Leu6 clipped SP-338, linear plecanatide, and linear SP-338 were biologically inactive. Additional internally cleaved metabolites and small fragments identified in these studies are not expected to be pharmacologically active. In rat duodenal fluid, plecanatide appears to be reduced (metabolites M1 through M3 detected in Study 13SYNRP2). A series of metabolites were detected subsequent to ex vivo or in situ incubation with rat duodenal fluid. The structures for several metabolites were elucidated using MS/MS analysis. Results suggest that plecanatide is cleaved from the carboxyl terminal, amino terminal, and internally within the molecule by intestinal peptidases. Oxidation of free cysteines during recovery of the fluid from ligated duodenal loops could have resulted in the formation of disulfide bonds not present in the original plecanatide.

5.1.6 Excretion

Pharmacokinetics and Excretion of Radioactivity in Rats Following a Single Oral Dose of ^{14}C-Plecanatide (Study No. 1896-023)

Study Design and Methods: To determine the plasma-time course and routes and rates of excretion of plecanatide, radioactivity was measured in urine, feces, plasma, and tissues following a single oral dose of ^{14}C-plecanatide in rats. The design of the study is summarized in the Sponsor's table below.

Excretion Study of Plecanatide in Rats

Group Number	Target Dose Level (mg/kg)	Dose Volume (mL/kg)	Target Dose Radioactivity (µCi/kg)	Samples Collected	Number of Male Animals
1	30	5	50	Plasma, Urine, Feces, Cage Residues, Carcass	2[a]
2	30	5	50	Plasma	12[a]
3	30	5	50	Urine, Feces, Cage Residues, Expired Air, Tissues, Carcass	4

[a]Animals in groups 1 and 2 were dual cannulated (portal and jugular vein).

Plecanatide was administered in a single dose by oral gavage at an average dose of 28.0 mg/kg with 14.08 µCi/animal (50.3 µCi/kg). Morbidity, mortality, injury, and the availability of food and water were observed twice daily. Bodyweights were measured and recorded prior to dosing (day -1). Blood samples were collected at designated time points up to 4 hours post-dose in group 1 and up to 72 hours post-dose in group 2 for plasma radioactivity analysis. All plasma samples from groups 1 and 2 were also analyzed for unlabeled plecanatide.

Results and Conclusions: The total elimination of ^{14}C-derived radioactivity was 21.7%. (see table from study report below). The major route of elimination of radioactivity was expired air (16.52%, measured as CO_2), with lesser amounts excreted in feces (4.01%) and urine (1. 11%). Methane and other gases formed as a result of fermentation of amino acids were not measured in this study. At 72 hours post-dose, the radioactivity recovered in visceral tissues and carcass was determined to achieve mass balance. The liver and kidney contained 5.85% and 0.89% of the dose, respectively. The GI tract and its contents/rinses accounted for 10.67% of the dose and the carcass accounted for 42.6%, the highest amount of ^{14}C-derived radioactivity recovered in group 3 animals; there was a total mass balance yield of 74.9% of the dose (see Sponsor's table below).

Summary of the ^{14}C-Dervied Radioactivity Recovery 72 Hours Post-Dose

Sample	Time Point (Hours Post-Dose)	Mean Percentage ^{14}C-Derived Radioactivity Recovery	SD
Expired Air (as CO_2)	72	16.52	1.99
Urine		1.10	0.24
Feces		4.01	0.71
Cage Rinse		0.04	0.03
Cage Wipe		0.05	0.03
Carcass		42.63	2.04
Kidney		0.89	0.04
Liver		5.85	0.55
Large Intestine		0.74	0.10
Large Intestine Contents/Rinse		0.20	0.05
Small Intestine		2.03	0.24
Small Intestine Contents/Rinse		0.34	0.15
Upper GI Tract (Esophagus/Stomach)		0.46	0.02
Upper GI Tract Contents/Rinse		0.05	0.02
TOTAL		**74.92**	**2.69**

In group 1 and 2 animals, the plasma radioactivity concentration over time is consistent with rapid absorption. However, radioactivity in plasma was measurable with a T_{max} of 4 hours (last time point measured) in group 1 animals and 8 hours in group 3 animals. Plasma radioactivity was still measurable at 72 hours post-dose (group 3 animals).

Mean plasma concentrations of unlabeled plecanatide samples from the portal and jugular veins measured in group 1 and 2 animals were measured by LC-MS/MS. The analysis of unlabeled plecanatide showed that the absorption of plecanatide is low (<10 ng/mL) and rapid (T_{max} approximately 5 minutes), and all plasma samples were below the limit of quantitation by 1 hour (portal vein) or 2 hours (jugular vein) post dose. These results suggest that the ^{14}C-derived radioactivity observed in plasma samples up to 72 hours post-dose is not associated with intact plecanatide (LC-MS/MS analysis). It was suggested that plecanatide is rapidly catabolized and the ^{14}C-derived radioactivity is incorporated into other proteins or molecules, explaining the measurable radioactivity observed in plasma, tissues, and carcass tested at 72 hours post-dose.

In conclusion, the results from the excretion study with ^{14}C-plecanatide indicate that the major route of elimination of radioactivity is expired air, with lesser amounts in feces and urine. Catabolism of ^{14}C-plecanatide followed by incorporation of ^{14}C into other proteins/molecules likely accounts for the presence of radioactivity in plasma, tissues, and carcass at 72 hours post-dose.

5.1.7 Pharmacokinetic Drug Interactions

No studies were submitted.

5.1.8 Other Pharmacokinetic Studies

Induction and Inhibition of Cytochrome P450

In Vitro Metabolism Studies of Plecanatide (Study No. 13SYNRP1B)	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
The potential inhibition and induction of relevant gut CYP450 enzymes by plecanatide were studied in vitro by incubation with human liver microsomes (HLM) and known substrates for specific CYP450 isozymes. In the inhibition study, CYP2C9 and CYP3A4 were evaluated. Diclofenac was used as a substrate for CYP2C9, and testosterone and midazolam were used as substrates for CYP3A4. Sulfaphenazole and ketoconazole were used as positive control inhibitors for CYP2C9 and CYP3A4, respectively. Plecanatide (5 µM) was incubated with pooled HLM in phosphate buffer, and CYP probe substrates. Formation of CYP probe metabolite was determined by LC-MS/MS. The potential for induction of CYP3A4 in human hepatocytes by plecanatide was evaluated. For cytotoxicity assays, freshly plated hepatocytes were treated with induction medium spiked with plecanatide at final concentrations of 0.03, 0.1, 0.3, 1, or 3 µM. Vehicle (negative) controls were treated in parallel with induction medium containing the same content of solvent. Cytotoxicity was assessed using a standard MTS viability assay. CYP3A4 induction was determined by incubation of hepatocytes for 72 hours in induction medium spiked with plecanatide at final concentrations of 0.03, 0.3, or 3 µM. Positive controls were treated in parallel with induction medium spiked with 50 µM rifampicin. Midazolam was used as the CYP3A4 probe substrate. CYP3A4 enzyme induction was evaluated by assessing both CYP3A4 enzymatic activities (by measuring the formation of the CYP3A4 probe substrate metabolite, 1'-hydroxymidazolam) as well as by quantitative polymerase chain reaction (qPCR) on total cellular RNA from treated hepatocytes. Metabolite detection was performed using LC-MS/MS.	Plecanatide did not inhibit CYP2C9-mediated metabolism of diclofenac or CYP3A4-mediated metabolism of testosterone in vitro. Plecanatide produced a negligible 6.4% inhibition of CYP3A4-mediated metabolism of midazolam. Positive control inhibitors sulfaphenazole and ketoconazole produced significant inhibition of CYP2C9 and CYP3A4, respectively. Therefore, plecanatide did not inhibit the CYP450 isozymes CYP2C9 or CYP3A4, which are predominant in the GI tract. Viability of human hepatocytes at all plecanatide concentrations (0.03 µM to 3 µM) was greater than 70% of the vehicle control. Cytotoxicity of plecanatide was minimal and not concentration dependent. The results showed that plecanatide did not increase either CYP3A4 activity or mRNA (<2-fold induction) at concentrations up to 3 µM, suggesting that plecanatide is unlikely to be an inducer of CYP3A4 in vivo.

In Vitro Metabolism Studies of SP-338 (Study No. 13SYNRP6B)	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
The potential for inhibition of CYP2C9 and CYP3A4 by SP-338 was evaluated using HLM as described in the previous study with plecanatide. The potential for induction of CYP3A4 by SP-338 was	The enzyme inhibition by SP-338 at 5 µM was 16% for CYP2C9, 1.1% for CYP3A4 with testosterone as the probe substrate, and 6.3% for CYP3A4 with midazolam as the probe substrate.

evaluated using human hepatocytes as described in the previous study with plecanatide. Fresh human hepatocytes were treated with SP-338 (0.03, 0.3, and 3 µM). CYP3A4 enzyme activity was measured by determining the formation of CYP3A4 probe metabolite by LC-MS/MS. CYP3A4 mRNA was measured by qPCR.	Cytotoxicity test with one human hepatocyte donor showed that the viability of human hepatocytes after treatment with SP-338 at 0.03 µM to 3 µM was >90% of the vehicle control, suggesting that SP-338 has no cytotoxicity under the test conditions. SP-338 did not increase either CYP3A4 enzyme activity or CYP3A4 mRNA expression (<2-fold versus vehicle control and <20% of positive control) at concentrations up to 3 µM in any of the three tested human hepatocyte donors. The results suggest that SP-338 is unlikely to be an inhibitor of CYP2C9 or CYP3A4, or an inducer of CYP3A4 in vivo.

5.2 Toxicokinetics

See toxicity studies in General Toxicology.

6 General Toxicology

6.1 Single-Dose Toxicity

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

SP-304: A Single Dose Oral Toxicity Study in CD-1 Mice

Key study findings: a single oral dose of 20, 200, and 2000 mg/kg SP-304 to mice was well tolerated; deaths occurred at 200 and 2000 mg/kg/day but do not appear to be drug-related; no target organs of toxicity were clearly identified; glandular cell hyperplasia in stomach occurred in the 2000 mg/kg/day group (2/20 mice); the NOAEL is considered to be 2000 mg/kg/day.

Study no.: 70474

Volume #, and page #: Vol. 4, Pg. 57

Conducting laboratory and location: (b) (4)

Date of study initiation: 11-9-2007
GLP compliance: yes

QA report: yes (x) no ( )

Drug, lot #, and % purity: Lot # B05137, >90% and B05369, >95%

Methods: Crl:CD1(ICR) mice (age 8-10 weeks; males: 24.2-40.1 g; females: 20.2-33.0 g) were treated with a single oral dose of 0 (vehicle), 20, 200, or 2000 mg/kg SP-304 (10 mice/sex/group in main study, 20 mice/sex/group for recovery phase). The toxicokinetic groups were treated with the same dose levels (12 mice/sex/group in treatment groups, 4 mice/sex in control group). The dose volume was 30 ml/kg, and the vehicle was distilled water. The main study groups were sacrificed on day 2, and the recovery groups were sacrificed on day 15.

RESULTS:

Mortality: Mortality checks were performed once daily during all phases of the study. No treatment-related deaths occurred. One female in the 200 mg/kg recovery group died on day 15. No clinical signs were observed in this animal prior to death. The most likely cause of death was considered to be bilateral acute hemorrhage of the adrenal inner cortex, an effect that was not considered as drug-related. One female in the 2000 mg/kg recovery group was sacrificed on day 2 due to poor health condition. The clinical signs in this animal included piloerection, ptosis, hypoactivity, hypothermia, slight pallor, thin appearance, and severe dehydration. The most likely cause of death was dehydration, an effect that was not considered as drug-related given the absence of diarrhea.

Clinical Signs: Animals were observed twice daily during the treatment and recovery periods. No drug-related effects were observed.

Bodyweight: Body weights were measured on days -7, -1, 7, and 14. Weight gain was not affected in any treatment groups.

Food Consumption: After dosing of the animals, food consumption was measured in the recovery groups on days 1-8 and 8-14. Food intake was not affected.

Ophthalmoscopy: The procedure was performed once prior to dosing and on days 2 and 15. No drug-related effects were observed.

ECG: Not performed.

Hematology: Blood samples were collected on the day of sacrifice (days 2 and 15). No effects were observed.

Clinical Chemistry: Blood samples were collected on the day of sacrifice (days 2 and 15). No effects were observed on day 2. One female (# 4530F) in the
2000 mg/kg group exhibited a marked increase in ALT (19.8-fold greater than the mean control value). The same animal also had an AST value 12.7-fold greater than the mean control value.

Urinalysis: Not performed.

Organ Weights: Organs were collected on the day of sacrifice (table below was taken from Sponsor's application). Absolute and relative weights were recorded for the main study and recovery groups. There were no drug related effects.

ORGANS/TISSUES	Retain (•)	Weigh (√)	Examine (€)
Adrenals	•	√	€
Animal identification	•		
Aorta (thoracic)	•		€
Blood	•		€
Bone marrow smears (3)	•		
Brain	•	√	€
Cecum	•		€
Colon	•		€
Epididymides	•d		€
Esophagus	•		€
Eyes	•a		€
Femur + marrow	•		€
Gallbladder	•		€
Heart	•	√	€
Kidneys	•	√	€
Liver (2 lobes)	•	√	€
Lungs + bronchi (2 Lobes)	•bc	√	€
Lymph node, mandibular	•		€
Lymph node, mesenteric	•		€
Mammary gland (inguinal)	•		€
Optic nerves	•a		€
Ovaries	•	√	€
Pancreas	•		€
Pituitary	•	√	€
Prostate	•	√	€
Rectum	•		€
Salivary gland (mandibular)	•		€
Sciatic nerve	•		€
Seminal vesicles	•		€
Skeletal muscle	•		€

ORGANS/TISSUES	Retain (•)	Weigh (√)	Examine (€)
Skin + subcutis (inguinal)	•		€
Small intestine, duodenum	•		€
Small intestine, jejunum	•		€
Small intestine, ilcum	•		€
Spinal cord (cervical)	•		€
Spleen	•	√	€
Sternum + marrow	•		€
Stomach	•		€
Testes	•d	√	€
Thymus	•	√	€
Thyroid lobes + parathyroids	•	√	€
Tongue	•		€
Trachea	•c		€
Urinary bladder	•		€
Uterus	•	√	€
Vagina	•		€
Abnormal findings	•		€
Additional Tissues presented below			

a	Davidson's fluid (euthanized animal only)
b	Lungs were infused with 10% neutral buffered formalin (euthanized animal only)
c	Lungs weighed with trachea
d	Bouin's fluid (euthanized animal only)

Notes
Paired organs, weighed together
Parathyroids, mammary gland and optic nerves were examined histologically, only if present in routine sections

Three femoral bone marrow smears were prepared from each euthanized animal and stained with Modified Wright's stain but were not examined.

Samples of gross lesions were retained at the macroscopic evaluation.

Gross Pathology: Observations were made at sacrifice and immediately after premature death.

Premature Deaths

No abnormalities were observed.

Main Study

No drug-related effects were observed.

Recovery Groups

No drug-related effects were observed.

Histopathology: Tissues/organs from animals treated with 0, 20, 200, or 2000 mg/kg SP-304 were collected, prepared, and examined. The tissues examined are listed in the table above taken from Sponsor's submission.

Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Premature Deaths

The following lesions were observed in the 200 mg/kg female: hemorrhage of inner adrenal cortex, congestion and hemorrhage in kidney, atrophy/hypoplasia of pancreas, lymphoid atrophy in spleen, hemorrhage and lymphoid hypocellularity in thymus.

No abnormalities were observed in the 2000 mg/kg female.

Main Study

No drug-related effects were observed.

Recovery Groups

Stomach: Glandular cell hyperplasia was observed in the 2000 mg/kg group (2/20 mice). Stomach was not examined in the 20 and 200 mg/kg groups.

Toxicokinetics: Plasma concentrations of SP-304 and its metabolite SP-338 (15 amino acid peptide) were measured. SP-338 is formed through removal of the C-terminal leucine. The results are shown in the table below taken from the Sponsor's submission. The lower limit of detection was 25 ng/ml for SP-304 and 50 ng/ml for SP-338. After oral dosing at 200 and 2000 mg/kg, plasma concentrations of SP-338 and SP-304 were observed to increase in a dose related manner. Mean plasma concentrations of SP-304 were usually greater than SP-338. Some gender differences were noted at 2000 mg/kg which suggested that exposure to both SP-304 and SP-338 was greater in female animals compared to males.

Analyte	Gender	Group	Dose	C_{max} (ng/mL)	t_{max} (hrs)	$t_{1/2}$ (hrs)	AUC_{0-t}* (ng.hr/mL)	t** (hrs)	AUC_{0-inf} (ng.hr/mL)
SP-304	Male	3	200 mg/kg	268	0.25	***	101	1	***
		4	2000 mg/kg	5626	0.25	1.6	9254	8	9360
	Female	3	200 mg/kg	148	1.00	***	62	1	***
		4	2000 mg/kg	15,767	1.00	0.2	15,709	2	15,969
SP-338	Male	3	200 mg/kg	197	0.25	***	25	0.25	***
		4	2000 mg/kg	2029	0.25	0.8	2305	4	2410
	Female	3	200 mg/kg	93	1.00	***	46	1	***
		4	2000 mg/kg	11,720	1.00	0.2	10,187	2	10,262

* AUC_{0-t} = area-under-the-curve from the time of dosing (0 hrs) until the last measurable timepoint
** t = time of last measurable concentration
*** Unable to be calculated from the available data

Value are the mean of 2-3 mice/sex/group.

Conclusions: A single oral dose of 20, 200, and 2000 mg/kg SP-304 to mice was well tolerated. The deaths which occurred at 200 and 2000 mg/kg/day do not appear to be drug-related. No target organs of toxicity were clearly identified, although glandular cell hyperplasia in stomach occurred in the 2000 mg/kg/day group (2/20 mice). Some gender differences were noted at 2000 mg/kg which suggested that exposure to both SP-304 and SP-338 was greater in female animals compared to males. The NOAEL is considered to be 2000 mg/kg/day.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

SP-304: A Single Dose Oral Toxicity Study in Cynomolgus Monkeys

Key study findings: the study was deficient in the number of animals used; diarrhea was observed at 25, 250, and 2000 mg/kg, which was likely due to the pharmacological activity of SP-304; the tolerated dose in cynomolgus monkeys is considered to be 250 mg/kg

Study no.: 30145

Volume #, and page #: Vol. 6, Pg. 1

Conducting laboratory and location: (b) (4)

Date of study initiation: 11-16-2007

GLP compliance: yes

QA report: yes (x) no ( )

Drug, lot #, and % purity: Lot # B05116, >94%; B05150, >92%; B05158, >91%; B05196, >90%; B05239, >90%; and B05369, >95%

Methods: Cynomolgus monkeys (males: 2.4-3.4 kg; females: 2.1-2.9 kg) were treated with a single oral dose of 0 (vehicle), 0.1, 25, 250, or 2000 mg/kg SP-304 (2 monkeys/sex/group in main study, 2 monkeys/sex/group for recovery phase). The monkeys were described as "young adults", but the age range was not stated. The dose volume was 15 ml/kg, and the vehicle was 0.5% carboxymethylcellulose. The main study groups were sacrificed on day 2, and the recovery groups were sacrificed on day 15.

Results:

Mortality: Mortality checks were performed once daily. No deaths occurred.

Clinical Signs: Clinical signs were recorded twice daily. In addition, special attention was paid to stool production.
Main animals:
-Day 1: 2, 4, 6, and 8 hours post-dosing
-Day 2: During regular detailed clinical examination (prior to necropsy)

Recovery animals:
-Day 1: 2, 4, 6, and 8 hours post-dosing
-Days 2 and 3: Two times in the morning, with a minimum of 2 hours between the two occasions and two times in the afternoon with a minimum of 2 hours between the two occasion.

Groups treated with 25, 250, and 2000 mg/kg exhibited diarrhea and changes in stool color. Loose or liquid feces were observed in these groups. The severity of diarrhea was dose dependent. The clinical signs occurred at 2-8 hr post-dose in the 25 mg/kg group, 2 hr to 2 days post-dose in the 250 mg/kg group, and 2 hr to 3 days post-dose in the 2000 mg/kg group.

Bodyweight: After dosing of the animals, bodyweight was measured in the recovery groups on days 7 and 14. Weight gain was not affected.

Food Consumption: Not measured.

Ophthalmoscopy: No drug-related effects were observed.

ECG: Not performed.

Hematology: Blood was collected from main study animals on day 2 and from recovery animals on the day of sacrifice. No effects were observed on days 2 or 15. PT and APTT were not affected.

Clinical Chemistry: Blood was collected from main study animals on day 2 and from recovery animals on the day of sacrifice. No effects were observed on days 2 or 15.

Urinalysis: Urine was collected from main study animals on day 2 and from recovery animals on the day of sacrifice. No effects were observed on days 2 or 15.

Organ Weights: adrenals, brain, heart, kidneys, liver, lungs & trachea, pituitary, prostate, spleen, testes, thymus, thyroid lobes & parathyroids, uterus; absolute and relative weights were recorded for the main study and recovery groups.

Main Study

Pituitary: Absolute weight in the 2000 mg/kg males was increased by 120%.

Prostate: Absolute weight was increased by 44-184% in all treatment groups. This effect was not dose dependent. Relative weight in all treatment groups was increased by 36-104% (not dose dependent).

Thyroid lobes and parathyroids: Absolute weight in the 250 and 2000 mg/kg females was decreased by 70.4% and 48.3%, respectively, and relative weight was decreased by 69.8% and 50.8%, respectively.

Recovery Phase

Lungs: Absolute weight in the 2000 mg/kg males was increased by 35%.

Ovaries: Absolute weight in the 250 and 2000 mg/kg females was increased by 127% and 179%, respectively. Relative weight in the 250 and 2000 mg/kg females was increased by 133% and 175%, respectively.

Prostate: Absolute weight was increased by 79-253% in all treatment groups. This effect was not dose dependent. Relative weight in all treatment groups was increased by 81-206% (not dose dependent).

Thymus: Absolute weight in the 250 and 2000 mg/kg males was increased by 121% and 194%, respectively. Relative weight in the 250 and 2000 mg/kg males was increased by 131% and 74%, respectively. Absolute and relative weight in the 2000 mg/kg females was reduced by 40%.

Gross Pathology:

Main Study
Spleen: Pale area was observed in the 0.1 and 2000 mg/kg groups (1/4 monkeys in each group).

Testes: Dark discoloration of testes occurred in the 2000 mg/kg group (1/2 males).

Recovery Phase

Pituitary: Dark discoloration occurred in the 2000 mg/kg group (1/4 monkeys).

Spleen: Pale area was observed in the 2000 mg/kg group (1/4 monkeys).

Histopathology:

The following tissues were examined in all groups:

ORGANS/TISSUES	Retain (•)	Weigh (√)	Examine (€)
Adrenals	•	√	€
Animal identification	•		
Aorta (thoracic)	•		€
Blood			
Bone marrow smears (3)	•		
Brain	•	√	€
Cecum	•		€
Colon	•		€
Epididymides	•d		€
Esophagus	•		€
Eyes	•a		€
Femur + marrow	•		€
Gallbladder	•		€
Heart	•	√	€
Kidneys	•	√	€
Liver (2 lobes)	•	√	€
Lungs + bronchi (2 Lobes)	•bc	√	€
Lymph node, mandibular	•		€
Lymph node, mesenteric	•		€
Mammary gland (thoracic)	•		€
Optic nerves	•a		€
Ovaries	•	√	€
Pancreas	•		€
Pituitary	•	√	€
Prostate	•	√	€
Rectum	•		€
Salivary gland (mandibular)	•		€
Sciatic nerve	•		€
Seminal vesicles	•		€
Skeletal muscle	•		€

ORGANS/TISSUES	Retain (•)	Weigh (√)	Examine (€)
Skin + subcutis (thoracic)	•		€
Small intestine, duodenum	•		€
Small intestine, jejunum	•		€
Small intestine, ileum	•		€
Spinal cord (cervical)	•		€
Spleen	•	√	€
Sternum + marrow	•		€
Stomach	•		€
Testes	•d	√	€
Thymus	•	√	€
Thyroid lobes + parathyroids	•	√	€
Tongue	•		€
Trachea	•c		€
Urinary bladder	•		€
Uterus	•	√	€
Vagina	•		€
Abnormal findings	•		€
Additional Tissues presented below			

a	Davidson's fluid
b	Lungs were infused with 10% neutral buffered formalin
c	Lungs weighed with trachea
d	Bouin's fluid

Notes

Paired organs, weighed together

Parathyroids and mammary gland were examined histologically, only if present in routine sections

Three femoral bone marrow smears were prepared from each euthanized animal and stained with Modified Wright's stain but were not examined.

Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Main Study

Kidneys: Mononuclear cell infiltrate (minimal) occurred in the 0.1, 25, and 2000 mg/kg groups (1/4, 3/4, and 2/4 monkeys, respectively). Interstitial mineralization occurred in the 2000 mg/kg group (1/4 monkeys).

Rectum: Congestion and hemorrhage was observed in the 2000 mg/kg group (1/4 monkeys).

Skeletal Muscle: Necrosis was observed in the 2000 mg/kg group (1/4 monkeys).

Thymus: Involution occurred in the 2000 mg/kg group (1/4 monkeys).

Recovery Phase

Kidneys: Mononuclear cell infiltrate (minimal) occurred in the 0.1, 25, and 250 mg/kg groups (2/4, 1/4, and 3/4 monkeys, respectively).

Lungs: Fibroblast proliferation was observed in the 25 and 2000 mg/kg groups (1/4 monkeys in each group). Mononuclear cell infiltrate occurred in the 2000 mg/kg group.

Pituitary: Congestion and hemorrhage occurred in the 2000 mg/kg group (1/4 monkeys).

Thymus: Involution was observed in the 25 and 2000 mg/kg groups (1/4 monkeys in both groups).

Toxicokinetics: Plasma concentrations of SP-304 and its metabolite SP-338 (15 amino acid peptide) were measured. SP-338 is formed through removal of the C-terminal leucine. The results are shown in the table below. The lower limit of detection was 6.25 ng/ml for SP-304 and 12.5 ng/ml for SP-338. The data are shown in the tables below taken from the Sponsor's submission.

Table 3: Pharmacokinetic Parameters for SP-304 in Monkey Plasma

Gender	Group	Dose (mg/kg)	Animal #	C_{max} (ng/mL)	t_{max} (hrs)	t½ (hrs)	AUC_{0-t} * (ng.hr/mL)	t ** (hrs)	AUC_{0-inf} (ng.hr/mL)
Male	3	25 mg/kg	3001A	6	1.00	***	2	1.0	***
			3002B	6	0.25	***	2	0.5	***
			3003C	6	0.25	***	5	1.0	***
			3004D	0	***	***	0	***	***
			Average:	**5**	**0.50**	--	**2**	--	--
			SD:	**3**	**0.43**	--	**2**	--	--
	4	250 mg/kg	4002B	102	0.25	***	52	2.0	***
			4003C	945	1.00	0.5	1250	4.0	1254
			4004D	349	0.50	0.6	330	2.0	371
			4101A	33	0.25	***	18	2.0	***
			Average:	**357**	**0.50**	**0.5**	**412**	--	**812**
			SD:	**415**	**0.35**	**0.1**	**576**	--	**625**
	5	2000 mg/kg	5001A	382	0.25	1.5	602	8.0	615
			5002B	1665	0.50	2.1	1883	8.0	1976
			5003C	3510	0.50	5.0	4362	8.0	5278
			5104D	253	0.50	0.5	425	4.0	429
			Average:	**1452**	**0.44**	**2.3**	**1818**	--	**2075**
			SD:	**1513**	**0.13**	**1.9**	**1816**	--	**2244**
Female	3	25 mg/kg	3501A	6	0.25	***	2	0.5	***
			3502B	6	0.50	***	1	0.5	***
			3503C	6	0.25	***	5	1.0	***
			3504D	0	***	***	0	***	***
			Average:	**5**	**0.33**	--	**2**	--	--
			SD:	**3**	**0.14**	--	**2**	--	--
	4	250 mg/kg	4501A	190	1.00	0.6	289	2.0	346
			4502B	88	0.50	0.4	80	2.0	84
			4503C	165	0.50	0.5	227	2.0	253
			4504D	73	0.25	0.4	76	2.0	79
			Average:	**129**	**0.56**	**0.5**	**168**		**190**
			SD:	**57**	**0.31**	**0.1**	**107**		**132**
	5	2000 mg/kg	5501A	423	0.50	2.1	1110	8.0	1194
			5502B	9845	1.00	1.6	15438	8.0	15597
			5503C	1585	1.00	3.0	2360	8.0	2527
			5604D	781	0.25	***	756	8.0	***
			Average:	**3158**	**0.69**	**2.2**	**4916**	--	**6439**
			SD:	**4484**	**0.38**	**0.7**	**7048**	--	**7959**

* AUC_{0-t} = area-under-the-curve from the time of dosing (0 hrs) until the last measurable timepoint
** t = time of last measurable concentration
***These parameters were unable to be calculated from the available data.

Table 4: Pharmacokinetic Parameters for SP-338 in Monkey Plasma

Gender	Group	Dose (mg/kg)	Animal #	C_{max} (ng/mL)	t_{max} (hrs)	t½ (hrs)	AUC_{0-t} * (ng.hr/mL)	t ** (hrs)	AUC_{0-inf} (ng.hr/mL)
Males	3	25 mg/kg	3001A	0	***	***	0	***	***
			3002B	13	0.50	***	2	0.5	***
			3003C	31	1.00	0.8	39	2.0	53
			3004D	0	***	***	0	***	***
			Average:	**11**	**0.75**	**0.8**	**10**	--	**53**
			SD:	**15**	**0.35**	--	**19**	--	--
	4	250 mg/kg	4002B	61	0.50	1.9	87	2.0	185
			4003C	1096	1.00	0.7	1377	4.0	1407
			4004D	159	1.00	0.7	320	4.0	333
			4101A	26	2.00	***	35	2.0	***
			Average:	**335**	**1.13**	**1.1**	**455**	--	**641**
			SD:	**510**	**0.63**	**0.7**	**627**	--	**667**
	5	2000 mg/kg	5001A	309	1.00	1.3	959	8.0	982
			5002B	353	2.00	4.1	1349	8.0	1774
			5003C	349	1.00	***	1099	8.0	***
			5104D	268	1.00	***	487	6.0	***
			Average:	**319**	**1.25**	**2.7**	**973**	--	**1378**
			SD:	**40**	**0.50**	**2.0**	**362**	--	**560**
Female	3	25 mg/kg	3501A	0	***	***	0	***	***
			3502B	13	0.50	***	8	1.0	***
			3503C	0	***	***	0	***	***
			3504D	0	***	***	0	***	***
			Average:	**3**	**0.50**	--	**2**	--	--
			SD:	**6**	--	--	**4**	--	--
	4	250 mg/kg	4501A	256	2.00	0.5	589	4.0	598
			4502B	61	0.50	1.6	77	2.0	147
			4503C	162	1.00	0.7	288	4.0	301
			4504D	154	1.00	0.4	155	2.0	173
			Average:	**158**	**1.13**	**0.8**	**277**	--	**305**
			SD:	**80**	**0.63**	**0.5**	**226**	--	**207**
	5	2000 mg/kg	5501A	527	0.50	3.6	2063	8.0	2629
			5502B	1080	1.00	4.1	2731	8.0	3184
			5503C	819	0.50	5.0	2615	8.0	3335
			5604D	783	0.50	3.7	1258	8.0	1596
			Average:	**802**	**0.63**	**4.1**	**2167**	--	**2686**
			SD:	**226**	**0.25**	**0.6**	**672**	--	**788**

* AUC_{0-t} = area-under-the-curve from the time of dosing (0 hrs) until the last measurable timepoint
** t = time of last measurable concentration
***These parameters were unable to be calculated from the available data.

Dose-related, although not always dose-proportional, exposure to SP-304 and SP-338 in plasma was observed at 250 and 2000 mg/kg. SP-304 concentrations were usually greater than SP-338 in the first 1 to 2 hours post-dosing, but at later time points, SP-338 concentrations were usually comparable to or greater than SP-304. Mean values for the terminal $t_{1/2}$ were greater at 2000 mg/kg (2.2 to 4.1 hours) compared to 250 mg/kg (0.5 to 1.1 hour). Consistent with results from the mice study, some gender differences were noted at 2000 mg/kg which suggested that exposure to SP-304 was greater in female animals compared to males.

Conclusions: The study was deficient in the number of animals used (2/sex/group in the main study, 2/sex/group in the recovery phase). The Sponsor should increase the animal numbers to

4/sex/group in future studies. Diarrhea was observed at 25, 250, and 2000 mg/kg. This effect was likely related to the pharmacological activity of SP-304. Kidney may have been a target organ of toxicity, based on the incidence of mononuclear cell infiltrate at 0.1, 25, 2000 mg/kg in the main study and at 0.1, 25, 250 mg/kg in the recovery groups. The severity was described as minimal, and the incidence was not dose related. Thymus involution was observed in the 2000 mg/kg group in the main study and in the 25 and 2000 mg/kg groups in the recovery group. The lesions observed in microscopic examination are not considered to be dose-limiting toxicity. Based on the severity of diarrhea in the 2000 mg/kg group, the tolerated dose is considered to be 250 mg/kg. Some gender differences were noted at 2000 mg/kg, suggesting that exposure to SP-304 was greater in female animals compared to males.

Pilot Oral Dose Escalation Toxicity Study in Monkeys (Study No. 30169)

This non-GLP study was performed in 4 separate single-dose phases, with a recovery or clearance period between each single dose. Two female monkeys in the control group received vehicle (distilled water) only on each dosing day, and 2 females were treated with a single dose of plecanatide at 1, 10, 25, or 50 mg/kg by oral gavage. Upon completion of Phase 1 dosing, all 4 animals were observed for 33 days. After completion of Phase 2 dosing at 10 mg/kg, all animals were observed for an additional 7 days. Animals were then treated with vehicle or 25 mg/kg plecanatide and observed for 12 days (Phase 3), then were treated with either vehicle or 50 mg/kg plecanatide and observed for an additional 7 days (Phase 4). The parameters included mortality, clinical observations, body weights, and food consumption.

In study Phase 1, there were no mortalities, no clinical signs, and no changes in body weights or food consumption that could be attributed to plecanatide at 1 mg/kg. Similarly, in Phase 2, no mortalities or changes in body weights or food consumption were attributed to plecanatide at 10 mg/kg. At this dose, plecanatide produced mild diarrhea as indicated by the presence of small amounts of loose feces in the cage tray of both animals. Both animals recovered by 24 hours post-dosing. At 25 mg/kg (Phase 3), there were no mortalities, no changes in body weights or food consumption, and no adverse clinical signs that could be attributed to the administration of plecanatide. Plecanatide at 50 mg/kg (Phase 4) produced mild diarrhea as indicated by the presence of small amounts of liquid/loose and pale feces in the cage tray of 1 animal. Therefore, a single dose of plecanatide by oral gavage at 1 mg/kg was well tolerated. Plecanatide at 10 and 50 mg/kg produced mild diarrhea. The Sponsor considered the NOEL to be 1 mg/kg.

6.2 Repeat-Dose Toxicity

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

Pilot Oral Dose Range-Finding Study of SP-304 in Cynomolgus Monkeys

Key study findings: the maximum tolerated dose appears to be 100 mg/kg; lesions were observed throughout the GI tract.

Study no.: 00722-07246

Volume #, and page #: Vol. 9, pg. 1

Conducting laboratory and location: (b) (4)

Date of study initiation: 6-29-2007

GLP compliance: No

QA report: yes ( ) no (x)

Drug, lot #, and % purity: B04642, B05239, B05093, B05137, B05172 (purity not stated)

Methods: Adult female Cynomolgus monkeys (1.8-3.1 kg, age not stated) were used. The objective of the study was to evaluate the toxicity of SP-304 when administered once daily for three consecutive days or as a single dose. The dose levels used for 3-day administration were 1, 10, 50, 100, 200, and 1000 mg/kg/day. The single-administration dose levels were 0 (vehicle), 10, 100, 250, 500, and 1000 mg/kg. Five animals were used in the study. The study design is shown in the table below (taken from the study report).

Group	Test Session	SP-304 Dosage mg/kg	Volume mL/kg	Conc. mg/mL	Animal No.	Dose Day(s) (Day(s) of Study)
CTA1	1	50	10	5	1	1, 2, 3
CTA1	2	10	10	1	1	15, 16, 17
CTA1	3	Not Dosed	Not Applicable	Not Applicable	1	Not Applicable
CTA2	1	1000	20	50	2	1, 2, 3
CTA2	2	1	10	0.1	2	15, 16, 17
CTA2	3	10	10	1	2	30, 31, 32
CTA2	4	50	10	5	2	37, 38, 39
CTA2	5	1000*	20	50	2	44
CTA2	6	500*	20	25	2	51
CTA2	7	250*	20	12.5	2	58
CTA2	8	100*	20	5	2	65
CTA2	9	10*	20	0.5	2	72
CTA3	4	10	10	1	3	37, 38, 39
CTA3	5	1000*	20	50	3	44
CTA3	6	500*	20	25	3	51
CTA3	7	250*	20	12.5	3	58
CTA3	8	100*	20	5	3	65
CTA3	9	10*	20	0.5	3	72
CTA4	4	100	10	10	4	37, 38, 39
CTA4	5	1000*	20	50	4	44
CTA4	6	500*	20	25	4	51
CTA4	7	250*	20	12.5	4	58
CTA4	8	100*	20	5	4	65
CTA4	9	10*	20	0.5	4	72
CTA5	4	200	10	20	5	37, 38, 39
CTA5	6	0 (vehicle)*	20	0	5	51
CTA5	7	250*	20	12.5	5	58
CTA5	8	0 (vehicle)*	20	0	5	65
CTA5	9	10*	20	0.5	5	72

*Single dose only

The test article was administered as a solution in phosphate buffered saline (pH 7.4). The animals were sacrificed at 14 days after the final dose.

Results:

Mortality: Animals were observed at least twice daily. Animal # 1 was sacrificed on day 46 due to recurring diarrhea. It is unlikely that the recurring diarrhea was drug-related, given that this sign was not generally correlated with the animal's dosing schedule. This animal exhibited fecal staining of the perianal region during the pretest examination, but otherwise appeared healthy.

Clinical Signs: Beginning after the first dose, and continuing through the day of scheduled sacrifice, examinations for general signs of toxicity, including fecal and urine quality, were conducted for all animals twice daily. Bowel movements were documented twice daily. Particular attention was paid to gastrointestinal signs (including diarrhea, soft or watery stool). Diarrhea or loose feces was usually observed at 3.5-24 hr after dosing, as described below. Animal # 1: Watery diarrhea occurred on most days through day 46 (day of sacrifice), although dosing occurred only on days 1-3 (50 mg/kg/day) and 15-17 (10 mg/kg/day). Loose feces was

also observed.

Animal # 2: Watery diarrhea was observed after administration of 250 mg/kg and higher. Loose feces occurred after administration of 100 mg/kg and higher.

Animal # 3: Watery diarrhea occurred after administration of 500 and 1000 mg/kg. Loose feces was observed after treatment with 250 and 1000 mg/kg.

Animal # 4: Watery diarrhea occurred after administration of 1000 mg/kg. Loose feces was observed at dose levels of 250, 500, and 1000 mg/kg.

Animal # 5: Loose feces occurred after treatment with 10 and 250 mg/kg.

Bodyweight: Body weights were recorded on a day prior to, or on the day of each dose, or at least weekly throughout the study. A fasted body weight was recorded prior to scheduled euthanasia. Animal # 1 lost about 10% of its initial bodyweight (3.1 kg) prior to being sacrificed. All other animals gained weight during the study. However, the data is difficult to interpret, given the absence of any control animals and the varying dose levels used for each animal.

Food Consumption: Food consumption was performed daily throughout the study for all surviving animals. Incomplete consumption of daily diet occurred frequently (18 days) in animal #1.

Hematology: Whole blood was collected from all animals once prior to the initial administration, and once from all surviving animals on the day of scheduled sacrifice. Lymphocyte count and eosinophil count in all of the surviving animals was reduced by 43-52% and 44.4-66.7%, respectively, relative to the baseline values.

Clinical Chemistry: No substantial changes relative to baseline values were observed.

Urinalysis: The urine parameters were within the normal reference range.

Gross Pathology: The animal that was sacrificed prematurely had liquid feces in the cecum and colon. These observations were correlated with acute inflammation in the microscopic examination. No abnormalities were found in the surviving animals.

Histopathology: The following organs/tissues were examined: cecum, colon, duodenum, ileum, jejunum, and stomach (cardia, fundus, and pylorus).

The animal that was sacrificed prematurely exhibited the following lesions: acute inflammation in cecum and colon; diffuse lymphocytic inflammation in the cecum and colon (mild to moderate); dilatation of villous lacteals in jejunum; mucosal/submucosal lymphoid nodule in cardial region of stomach; karyorrhectic debris around villus tip in duodenum.

Lesions in the surviving animals are described below.

Cecum: Karyorrhectic debris in superficial lamina propria (4/4 monkeys); balantidium infection and diffuse lymphocytic inflammation in one monkey.

Colon: Balantidium infection (4/4 monkeys, moderately severe in one); diffuse lymphocytic inflammation (2/4 monkeys). Karyorrhectic debris in superficial lamina propria (4/4 monkeys).

Duodenum: Karyorrhectic debris around villus tip (4/4 monkeys); dilatation of villous lacteals (1/4 monkeys).

Ileum: Karyorrhectic debris around villus tip (4/4 monkeys); dilatation of villous lacteals (1/4 monkeys); increased eosinophilic infiltrate (2/4 monkeys).

Jejunum: Karyorrhectic debris around villus tip (4/4 monkeys); dilatation of villous lacteals (3/4 monkeys); increased eosinophilic infiltrate (1/4 monkeys).

Stomach (cardia): Diffuse lymphocytic inflammation (1/4 monkeys); mucosal/submucosal lymphoid nodule (3/4 monkeys, mild to moderate).

Stomach (fundus): Acute inflammation (1/4 monkeys); diffuse lymphocytic inflammation (1/4 monkeys, moderate); karyorrhectic debris in superficial lamina propria; mucosal/submucosal lymphoid nodule (2/4 monkeys, minimal to moderate); vacuolization of macrophages in superficial lamina propria (1/4 monkeys).

Stomach (pylorus): Diffuse lymphocytic inflammation (2/4 monkeys, mild); mucosal/ submucosal lymphoid nodule (2/4 monkeys).

Conclusions: The maximum tolerated dose appears to be 100 mg/kg, given that watery diarrhea occurred at doses of 250 mg/kg and higher. The histopathology data is difficult to evaluate, given the absence of any control animals and the varying dose levels used for each animal. However, lesions were observed throughout the GI tract.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 2/2/2010). The review is included verbatim below.

SP-304: 5-Day Oral Toxicity Pilot Study in CD-1 Mice

In a 5-day oral (gavage) toxicity pilot study in CD-1 mice, animals (6/sex) were treated with 1200 mg/kg/day SP-304 for 5 days to investigate the tolerability of SP-304. This study was not conducted under Good Laboratory Practice regulations. Toxicity was evaluated by monitoring clinical signs, body weight, food consumption, and clinical pathology parameters (hematology and serum chemistry). On day 5, the animals were sacrificed at 30 min after dosing and subjected to complete gross necropsy. The Sponsor only reported the raw data of the measured parameters from individual animals, and no statistical analysis was performed. Gross pathological findings included bloated/ distended and fluid filled stomach, duodenum, jejunum,

and/or cecum, with the highest occurrence being observed in the jejunum of all 12 animals. The oral administration of SP-304 to CD-1 mice for 5 days at 1200 mg/kg/day appears to be well tolerated.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

Seven-Day Oral Dose Range-Finding Study of SP-304 in Mice

Key study findings: weight gain in the recovery groups for days 7-14 was decreased by 26-53%; because of the increased levels of ALT and TBIL, the NOAEL or tolerated dose could not be established from this study.

Study no.: 0722-07281

Volume #, and page #: Vol. 8. Pg. 70

Conducting laboratory and location: (b) (4)

Date of study initiation: 9-8-2007
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: B05172, 91.9%; B05239, 92.9%

Methods: Male Hsd:ICR (CD-1) mice (age 6-8 weeks, 25-30 g) were treated orally with 0 (vehicle), 20, 200, or 2000 mg/kg/day SP-304 (6 mice/group in the main study). The recovery and toxicokinetic groups were treated with the same dose levels (6 mice/group). The dose volume in the 0, 20, 200, and 2000 mg/kg/day groups was 30, 20, 20, and 30 ml/kg, respectively. The test article was administered as a solution in phosphate buffed saline. The main study groups were sacrificed on day 8, and the recovery groups were sacrificed after a 14-day recovery period (day 22). Microscopic examination was performed on cecum, colon, duodenum, ileum, jejunum, and stomach (control and high-dose groups only).

Results:

Mortality: Animals were observed twice daily. No deaths occurred.

Clinical Signs: Animals were observed twice daily after dosing, except on Day 4, when animals were observed once. No signs were observed.

Bodyweight: Body weights were recorded on days -1, 7, 14, and 21. Weight gain during the main study (days 1-7) increased by 31%, 6%, and 25% in the 20, 200, and 2000 mg/kg/day groups (not significant). Weight gain in the recovery groups for days 7-14 was decreased by 26-53%.

Food Consumption: Food consumption was recorded weekly. No effects were observed.

Ophthalmoscopy: Not performed.

ECG: Not performed.

Hematology: Whole blood was collected at each scheduled euthanasia. In the main study groups, absolute neutrophil count increased by 35.9% and 23.4% at 20 and 200 mg/kg/day, respectively; platelet count increased by 10.7%, 31.5%, and 21.6% at 20, 200, and 2000 mg/kg/day, respectively; reticulocyte count increased by 20.9% and 20.2% at 200, and 2000 mg/kg/day, respectively. In the recovery group, absolute neutrophil count decreased by 41.3%, 25.9%, and 26.9% at 20, 200, and 2000 mg/kg/day, respectively.

Clinical Chemistry: Whole blood was collected from the second three toxicology animals/group at each scheduled euthanasia. In the main studies groups, ALT was increased by 107-113% in the 20, 200, and 2000 mg/kg/day groups, TBIL (total bilirubin) increased by 230% and 140% in the 20 and 2000 mg/kg/day, respectively.

Urinalysis: Not performed.

Organ Weights: Pre-necropsy fasted body weight, and necropsy weights of the organs (adrenal, brain, epididymides, heart, kidneys, liver, spleen, testes, thymus, and thyroid/parathyroids) were recorded. Absolute weight, relative weight (organ/bodyweight), and organ/brain weight ratio was reported. No effects were observed in the main study or recovery groups.

Gross Pathology: The abdominal, thoracic, and cranial cavities and their contents were examined. No abnormalities were observed in the main study or recovery groups.

Histopathology: Examination was performed on cecum, colon, duodenum, ileum, jejunum, and stomach (control and high-dose groups only). No drug-related effects were observed. Minimal individual cell necrosis of epithelium was observed in the pylorus of a few of the stomach sections from both groups. Vacuolation of the glandular epithelium near the crypts was observed in the ileum of one control and one high-dose male from the recovery sacrifice.

Toxicokinetics: Results will be included in the final report.

Conclusions: Weight gain during the main study (days 1-7) increased by 31%, 6%, and 25% in the 20, 200, and 2000 mg/kg/day groups (not significant). Weight gain in the recovery groups for days 7-14 was decreased by 26-53%. In the main study groups, absolute neutrophil count increased by 35.9% and 23.4% at 20 and 200 mg/kg/day, respectively; platelet count increased by 10.7-31.5% in the 20, 200, and 2000 mg/kg/day groups; reticulocyte count increased by 20.9% and 20.2% at 200, and 2000 mg/kg/day, respectively. In the recovery group, absolute neutrophil count decreased by 25.9-41.3% in the 20, 200, and 2000 mg/kg/day groups. In the main studies groups, ALT was increased by 107-113% in the 20, 200, and 2000 mg/kg/day groups, bilirubin increased by 230% and 140% at 20 and 2000 mg/kg/day, respectively. These changes are suggestive of liver injury, and, therefore, the NOAEL or tolerated dose cannot be

established from this study.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 5/27/2010). The review is included verbatim below.

A Pilot Single-Dose and 14-Day Repeated Dose Range-Finding Oral Toxicity Study with SP-304 in Rats

Key study findings: oral administration of up to 50 mg/kg/day for 14 days and 500 mg/kg/day for seven days appeared to be well tolerated in rats; a single oral administration of 2000 mg/kg was also well tolerated

Study no.: 018683
Volume #, and page #: Vol. 8, pg. 179
Conducting laboratory and location: (b) (4)

Date of study initiation: 10-4-2005
GLP compliance: No
QA report: yes ( ) no (x)
Drug, lot #, and % purity: B04119, >90%; B04180, >90%

Methods: Male Sprague Dawley (Crl:CD®(SD)) rats were used in two studies. The rats were seven weeks old at the time of study initiation. In the initial study, rats were treated orally with 0 (vehicle), 5, or 50 mg/kg/day SP-304 for 14 days (3 rats/group). Additional groups (150 and 500 mg/kg/day) were added to the study at nine days after the start of dosing in the original groups. The duration of treatment for the 150 and 500 mg/kg/day groups was limited to seven days, due to a shortage of test article. The 150 and 500 mg/kg/day dose levels were selected based on the absence of observed effects in the 5 and 50 mg/kg/day groups. The bodyweight in the 0, 5, and 50 mg/kg/day groups was 133-175 g, and the bodyweight in the 150 and 500 mg/kg/day groups was 194-228 g at the time of treatment initiation. All rats were sacrificed the day after the final dose.

In the second study, male rats (161-176 g) were treated with a single oral administration of 0 (vehicle) or 2000 mg/kg SP-304 (3 rats/group). The rats were sacrificed following a 14-day observation period. For both studies, the dose volume was 10 ml/kg. The drug was administered as a solution in phosphate buffered saline (pH 7.5).

Results (14/7-Day Study):

Mortality: Animals were observed twice daily. No deaths occurred.

Bodyweight: Body weights were measured on day -6, -1, 0, and 7 in the 14-day treatment group; however, body weight data for day 14 at the time of sacrifice were missing. In the 7-day treatment group, body weights were measured on day -6, -1, and 7. Weight gain was unaffected.

Food Consumption: Food and water consumption was measured weekly and daily, respectively, starting the week prior to dosing. Food and water intake were unaffected.

Hematology: Blood samples were collected on day 14 and day 7 prior to termination. No effects were observed. PT and APTT were unaffected.

Clinical Chemistry: Blood samples were collected on day 14 and day 7 prior to termination. No effects on clinical chemistry were observed.

Organ Weights: Absolute weight, relative weight (organ/bodyweight), and organ/brain weight ratio was reported.

Adrenals: Absolute weight and organ/brain weight ratio were reduced by 22% in the 500 mg/kg/day group.

Thymus: Absolute weight in the 50, 150, and 500 mg/kg/day groups was decreased by 19%, 18%, and 34%, respectively. Relative weight was reduced by 30% in the 500 mg/kg/day group. Organ/brain weight ratio was decreased by 35% in the 500 mg/kg/day group.

Gross Pathology: Small thymus was observed in the 150 mg/kg/day group (1/3 rats).

Histopathology: The following organs/tissues were examined in the control and 500 mg/kg/day groups: cecum, colon, duodenum, esophagus, gross lesions, ileum, jejunum, kidneys, liver, rectum, spleen, and stomach.

Liver: Hepatocellular vacuolization (minimal) was observed in the 500 mg/kg/day group (1/3 rats).

Kidney: Focal bilateral basophillic tubules (1/3 rats) and unilateral basophilic tubules (1/3 rats) were observed in the 500 mg/kg/day group.

Results (Single-Dose Study):

Mortality: Animals were observed daily. No deaths occurred.

Clinical Signs: Observations were recorded at 1, 2.5 and 4 hours post-dose and daily thereafter. No abnormal signs were observed.

Bodyweight: Body weights were measured on Day -6, -1, 0, 7, and 13. Weight gain was not affected by treatment.

Food Consumption: Food consumption was recorded on Day -1, 7, and 13. Water consumption was recorded daily starting on Day -3. Food and water consumption were unaffected.

Hematology: Blood samples were collected on Day 14 prior to termination. No effects were

observed. PT and APTT were unaffected.

Clinical Chemistry: Blood samples were collected on Day 14 prior to termination. No effects were observed.

Organ Weights: Absolute weight, relative weight (organ/bodyweight), and organ/brain weight ratio was reported. No effects were observed.

Gross Pathology: Multiple areas (1 mm) with red discoloration in lungs was observed in the 2000 mg/kg group (1/3 rats).

Histopathology: The following organs/tissues were examined in both groups: cecum, colon, duodenum, esophagus, gross lesions, ileum, jejunum, kidneys, liver, rectum, spleen, and stomach.

Liver: Focal necrosis (minimal) occurred in the 2000 mg/kg group (1/3 rats).

Conclusions: Oral administration of up to 50 mg/kg/day for 14 days and 500 mg/kg/day for seven days appeared to be well tolerated in rats. A single oral administration of 2000 mg/kg was also well tolerated. Effects in liver were observed at 500 mg/kg/day in the repeat-dose study, and 2000 mg/kg in the single-dose study. The severity of these effects was minimal, and the relationship to drug administration is uncertain.

SP-304: A Repeat-Dose Oral Range-Finding Toxicity Study in Cynomolgus Monkeys (Study No. 30155)

In a pilot non-GLP study, two groups of 2 female cynomolgus monkeys were dosed orally with 100 or 250 mg/kg/day plecanatide for 14 days. There were no control animals in the study. Parameters evaluated in this study included mortality, clinical observations, body weights, food consumption, clinical pathology, and TK. After 14 days of dosing, the animals were observed for an additional 14 days. The animals were then subjected to a full gross necropsy that included organ weights, and select tissues from the 250 mg/kg/day animals were examined histopathologically, including cecum, colon, esophagus, rectum, small intestine (duodenum, jejunum, ileum), and stomach. There were no deaths or drug-related changes in body weights, food consumption, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), or organ weights. There were no drug-related macroscopic or microscopic findings. Administration of plecanatide at 100 and 250 mg/kg/day resulted in diarrhea and changes in the color and/or odor of the stools. In summary, oral administration of plecanatide for 14 days to female cynomolgus monkeys at 100 and 250 mg/kg/day produced predictable effects based on the pharmacology, which included mild to moderate diarrhea and changes in the color and/or odor of the stools. No other adverse changes were observed.
The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 2/2/2010). The review is included verbatim below.

SP-304: 28-Day Oral Toxicity Study in CD-1 Mice With a 2-Week Recovery

Key study findings: deaths occurred in the 2000/1200 mg/kg/day mice; plasma exposure of SP-304 was higher in females than in males; no target organs of toxicity were clearly identified; the NOAEL is considered to be 200 mg/kg/day.

Study no.: VMF00007

Volume #, and page #: Vol.1, page 1

Conducting laboratory and location: (b) (4)

Date of study initiation: 7-21-2009 (report dated 12-29-2009)

GLP compliance: Yes

QA report: yes (x) no ( )

Drug, lot #, and % purity: 034E09, 96.4%; FUROG0801A, 96.5%

METHODS: CD-1 mice (b) (4) 8-10 weeks of age (20.7 g to 37.8 g) were used in the study. The experimental design is detailed in the table below (taken from the Sponsor):

Text Table 2
Experimental Design

Group Number	Number of Animals[a]						Test Article	Dosage Level (mg/kg/day)	Dose Conc (mg/mL)	Dosage Volume (mL/kg)	Dosing Regimen	Necropsy Day
	Main Study		Recovery[b]		Satellite[c]							
	M	F	M	F	M	F						
1	20	20	[redacted]	[redacted]	18	18	Control Article	0	0	20/10[d]	Oral gavage on Days 1–28	Day 29 (all animals)
2	5	5	[redacted]	[redacted]	24	24	SP-304 Lot A	20	2	10		
	5	5			24	24	SP-304 Lot B					
3	5	5			24	24	SP-304 Lot A	200	20			
	5	5			24	24	SP-304 Lot B					
4	5	5	5	5	24	24	SP-304 Lot A	2000/ 1200[e]	100/ 60[e]	20	Oral gavage on Days 1–14	Day 15 (all animals)
	5	5	5	5	24	24	SP-304 Lot B					
5	5	5	5	5	18	18	Control Article	0	0	20	Oral gavage on Days 1–28	Day 29 (main study) Day 43 (recovery)
6	5	5	5	5	24	24	SP-304 Lot A	1000	50			
	5	5	5	5	24	24	SP-304 Lot B					

Lot A = SP-304, Lot No. 034E09; Lot B = SP-304, Lot No. FUROG0801A.

[a] In each of Groups 2–4 and 6, half of the animals received SP-304, Lot A (Lot No. 034E09) throughout the dosing period, and half of the animals received SP-304, Lot B (Lot No. FUROG0801A) throughout the dosing period (see *Test Article Lot Number Assignment for Dose Administration*, Appendix 5).

[b] Because of mortality noted in Group 4, the necropsy day for both the Group 4 main study and recovery animals was changed to Day 15 by Amendment No. 5. Consequently, the necropsy day for the Group 1 recovery animals was changed to Day 29, and these animals were considered to be part of the main study group.

[c] In Groups 2–4, satellite animals scheduled for TK blood collection before dosing, 15 minutes after dosing, and 1 and 4 hours after dosing received SP-304 Lot A (Lot No. 034E09), and animals scheduled for TK blood collection at 5 and 30 minutes after dosing and 2 and 8 hours after dosing received Lot B (Lot No. FUROG0801A).

[d] The dose volume was 20 mL/kg on Days 1–14. After termination of the Group 4 animals, the dose volume was changed to 10 mL/kg for Days 15–28.

[e] SP-304 was administered at a dose level of 2000 mg/kg/day and a concentration of 100 mg/mL to Group 4 males on Days 1–5 and Group 4 females on Days 1–6. Due to mortality noted in the group, the dose level was amended to 1200 mg/kg/day at a concentration of 60 mg/mL, which was administered to Group 4 males on Days 6–14 and Group 4 females on Days 7–14.

In the main study groups, the animals were treated with 0 (vehicle), 20, 200, and 1000 mg/kg/day SP-304 by oral route (gavage) for 28 days and they were sacrificed on day 29.

In the recovery groups, the animals were treated with 0 (vehicle) and 1000 mg/kg/day SP-304 for 28 days and sacrificed on day 43 after a 2-week recovery period.

In the satellite groups for toxicokinetic measurements, animals were treated with 0, 20, 200, and 1000 mg/kg/day SP-304 for 28 days and sacrificed on day 29. Some of the 1000 mg/kg/day animals were allowed to recover for 2 weeks and sacrificed on day 43.

In the main study, recovery phase, and satellite groups, some of the animals were initially dosed

at 2000 mg/kg/day and subsequently lowered to 1200 mg/kg/day between days 6 and 7 due to intolerability at 2000 mg/kg/day. Because of morbidity and early deaths, all 2000/1200 mg/kg/day animals were sacrificed on day 15.

The dose volume was 10 or 20 ml/kg, and the vehicle was 0.5% carboxymethylcellulose.

RESULTS:

Mortality: Animals were observed twice daily. There were 22 unscheduled deaths during the course of the study. The following table summarizes the mortality data (taken from the Sponsor):

Summary of Mortality

Group(Dose)	Sex	Animal Number	Disposition	Day
Main Study Animals				
2 (20 mg/kg/day)	Female	89	Moribund	18
4 (2000/1200 mg/kg/day)	Male	43	Moribund	1
		47	Moribund	8
		49	Moribund	9
		50	Moribund	5
		53	Moribund	7
		60	Moribund	6
	Female	101	Moribund	2
		103	Moribund	4
		104	Moribund	6
		113	Moribund	6
		118	Found Dead	7
6 (1000 mg/kg/day)	Male	465	Moribund	7
Satellite Animals				
4 (2000/1200 mg/kg/day)	Male	263	Moribund	6
		278	Moribund	5
	Female	427	Moribund	6
		428	Moribund	7
		432	Moribund	6
		433	Moribund	5
		435	Moribund	8
		441	Moribund	6
		447	Moribund	8

All except two deaths occurred in the animals administrated 2000/1200 mg/kg/day SP-304 (8/68 males and 12/68 females). Clinical signs noted at the time of sacrifice in the majority of the animals included lethargy, cold to touch, squinted eyes, labored breathing, and/or abdominal bloating. The deaths are considered to be drug-related. A gross finding at necropsy was the presence of fluid in the intestinal tract. Microscopically, there was minimal to mild edema in the mucosa of the small intestine. However, cause of the deaths could not be determined.

There was one death in the 1000 mg/kg/day males (1/68 males). Clinical signs were similar to that observed in the 2000/1200 mg/kg/day animals. Whether this death was drug-related is uncertain.

There was also one death in the 20 mg/kg/day females (1/58 females). Because of the lack of dose dependency and the very low incidence, it is unlikely that the death was related to SP-304.

Cageside Observations: Cageside observations for main study and recovery animals were performed at least once daily, starting on day 1. On dosing days, cageside observations were performed 1 to 4 hours after dosing. In the recovery groups, cageside observations were not performed on days when clinical observations were scheduled.

No drug-related effects were observed during the course of the study in animals that survived to the scheduled sacrifice. In the 2000/1200 mg/kg/day animals (early termination on day 15), the following observations were noted: lethargy, partial disuse, hunched posture, and shivering.

Clinical Signs: Clinical observations were performed for main study and recovery animals on day -1 and at least once weekly thereafter. On dosing days, clinical observations were performed before dosing. During the recovery period, clinical observations were performed at least once weekly. A final clinical observation was performed on the day of necropsy. Some of the clinical signs included: oily fur, rough hair coat, urine stain, abrasion, alopecia, apparent blood, cold to touch, dry red material, soft feces, and lethargy. Because of the lack of dose dependency and/or incidences that were no higher than that observed in controls, these clinical signs are not considered drug-related.

Bodyweight: Body weights were measured before dosing, approximately weekly thereafter, and on the day before necropsy. No drug-related effects on bodyweight gain were noted in the main study groups, the recovery groups, or the satellite groups.

Food Consumption: Food consumption was measured weekly, beginning on day -1 for main study and recovery animals, except on days after overnight fast. No drug-related changes were observed. A statistically significant increase (up to 14.5%) in food consumption in the 1000 mg/kg/day males was noted. However, the increases occurred both before and after study initiation.

Ophthalmic Examinations: No drug-related effects were observed.

Hematology: Blood was collected on the day of sacrifice (15, 29, and 43), as described in the table below (taken from the Sponsor):

Text Table 3
Blood Sample Collection Schedule

Time Point	Hematology	Serum Chemistry	Toxicokinetics[a,b]
Day 1: Predose, 15 minutes postdose, and 8 hours postdose (Group 1 and 5 satellite animals)			X
Day 1: Predose; 5, 15, and 30 minutes postdose; and 1, 2, 4, and 8 hours postdose (Group 2–4 and 6 satellite animals)			X
Day 28: Predose, 15 minutes postdose, and 8 hours postdose (Group 1 and 5 satellite animals)			X
Day 14[c] / 28: Predose; 5, 15, and 30 minutes postdose; and 1, 2, 4, and 8 hours postdose (Groups 2–4 and 6 satellite animals)			X
Day 15[d] /29 (First 2 main study animals from each group and/or lot for Groups 2, 3, 5, and 6 and the first 10 animals from Group 1)	X		
Day 15[d] /29 (Last 3 main study animals from each group and/or lot for Groups 2, 3, 5, and 6 and the last 10 animals from Group 1)		X	
Day 43 (First 2 recovery animals from each group and/or lot for Groups 5 and 6)	X		
Day 43 (Last 3 recovery animals from each group and/or lot for Groups 5 and 6)		X	
Volume of Whole Blood	Maximum obtainable	Maximum obtainable	Maximum obtainable
Anticoagulant	EDTA	None[e]	K_3EDTA

There were sporadic changes in some of the measured parameters. However, the changes were small in magnitude and lack dose-dependency. None of the changes are considered drug-related.

Mean values for samples collected on day 15 from animals dosed at 2000/1200 mg/kg/day (day of sacrifice) were comparable to values from the control group on days 29 and 43.

Prothrombin time and activated partial thromboplastin time were not measured.

Clinical Chemistry: Blood was collected on the day of sacrifice (days 15, 29 and 43), as described in the table above.

A decrease in alanine aminotransferase (ALT) (-42%) was observed in the 200 mg/kg/day males on day 29; however, the change was not dose-dependent. The clinical significance of a decreased ALT is uncertain.

Mean values for samples collected on day 15 from animals dosed at 2000/1200 mg/kg/day (day of sacrifice) were comparable to values from the control group on days 29 and 43.

Urinalysis: Urine samples were collected on days 29 (main study animals) and 43 (recovery animals), and on day 15 (2000/1200 mg/kg/day animals only). The parameters examined included: volume, color, clarity, specific gravity, microscopic evaluation of urine sediment, and

urine test strip analysis (pH, protein, glucose, bilirubin, ketones, blood, urobilinogen, nitrites, and leukocytes). The urine parameters were within the normal reference range.

Organ Weights: Organs were collected on the day of sacrifice (days 15, 29, and 43). Absolute weight, relative weight (organ/bodyweight), and organ/brain weight ratio was reported. A final, fasted body weight was measured on the day of necropsy for main study and recovery animals for the calculation of organ to bodyweight ratios.

Organs Weighed at Necropsy

Adrenal gland (paired) [a]	Prostate gland
Brain	Salivary gland, mandibular (paired)
Epididymis (paired)	Seminal vesicles (paired)
Heart	Spleen
Kidney (paired)	Testis (paired)
Liver	Thymus
Lung	Thyroid gland with parathyroid gland (paired)[a]
Ovary (paired) [a]	Uterus
Pituitary gland[a]	

[a] Weighed after fixation.

Main Study (day 29):

Adrenal glands: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio in the 20 mg/kg/day males was decreased by 23%, 25%, and 23%, respectively. Absolute weight in the 20 and 200 mg/kg/day females was increased by 24% and 14%, respectively. Organ/brain weight ratio in the 20 mg/kg/day females was increased by 26%.

Epididymides: Absolute weight in the 200 and 1000 mg/kg/day males was increased by 18% and 12%, respectively. Organ/bodyweight ratio was increased by 17% in the 200 mg/kg/day males.

Kidney: Absolute weight and organ/brain weight ratio in the 1000 mg/kg/day males was increased by 18%.

Liver: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio in the 1000 mg/kg/day males was increased by 17%, 9%, and 17%, respectively. Absolute weight, organ/bodyweight, and organ/brain weight ratio in the 1000 mg/kg/day females was increased by 11%, 6%, and 15%, respectively.

Pituitary: Absolute weight in the 1000 mg/kg/day males was increased by 33%. Organ/bodyweight ratio in the 200 mg/kg/day males was increased by 18%. Organ/brain weight ratio in the 1000 mg/kg/day males was increased by 33%.

Seminal Vesicles: Absolute weight and organ/brain weight ratio in the 1000 mg/kg/day males was increased by 11%.

Thyroid gland: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio in the 200 mg/kg/day females was decreased by 23%.

Salivary gland: Organ/bodyweight ratio was decreased by 17% in the 1000 mg/kg/day males.

Recovery Phase (day 43):

Adrenal Glands: Absolute weight, organ/body weight ratio, and organ/brain weight ratio in the 1000 mg/kg/day males was increased by 23%, 32%, and 25%, respectively.

Liver: Absolute weight and organ/body weight ratio in the 1000 mg/kg/day females was increased by 10% and 5%, respectively.

Spleen: Absolute weight and organ/brain weight ratio in the 1000 mg/kg/day females was increased by 19% and 16%, respectively.

2000/1200 mg/kg/day group (day 15):

Organ weights in the 2000/1200 group were comparable to that of the controls in the main study or recovery phase.

Gross Pathology: Observations were made at sacrifice (days 15, 29, and 43) and immediately after premature death.

Premature Deaths

No abnormalities were observed.

Main Study

No drug-related effects were observed.

Recovery Phase

No drug-related effects were observed.

Histopathology: Tissues and organs were collected on the day of sacrifice: day 29 for the main study groups, day 43 for the recovery groups, and day 15 for the 2000/1200 mg/kg/day animals. The following organs/tissues from all the above groups were examined (table taken from the Sponsor):

Tissues Collected and Examined at Necropsy

Adrenal gland (paired)	Lymph node, mesenteric
Animal identification	Mammary gland
Aorta	Nerve, optic [b]
Bone, femur	Nerve, sciatic
Bone, sternum	Ovary (paired)
Bone marrow, femur	Pancreas
Bone marrow smear[a]	Parathyroid gland[c]
Brain (cerebrum, cerebellum, brain stem)	Pituitary gland
Cervix	Prostate gland
Epididymis (paired)	Salivary gland (paired)
Esophagus	Seminal vesicle (paired)
Eye (paired)[b]	Skeletal muscle
Gallbladder	Skin
Harderian gland (paired)	Spinal cord (cervical, thoracic, lumbar)
Heart	Spleen
Intestine, cecum	Stomach (nonglandular and glandular)
Intestine, colon	Testis (paired)[d]
Intestine, duodenum	Thymus
Intestine, ileum (with Peyer's patch [c])	Thyroid gland (paired)
Intestine, jejunum	Tongue
Intestine, rectum	Trachea
Kidney (paired)	Urinary bladder
Liver	Uterus
Lung	Vagina
Lymph node, mandibular	Gross lesions/masses

Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Premature deaths

Epididymis: minimal spermatic alteration (1/6 M)

Intestine, duodenum: mucosal edema (6/6 M and 3/4 F); epithelial apoptosis (1/4 F)

Intestine, ileum: mucosal edema (3/6 M)

Intestine, jejunum: mucosal edema (6/6 M and 4/4 F)

Liver: bile duct ectasia (1/6 M); focal mineralization (1/6 M); focal macrophage pigmentation (1/6 M)

Lung: acute hemorrhage (1/6 M and 1/5 F)

Pituitary gland: congestion (1/5 F)

Thymus: apoptosis (4/6 M and 1/5 F); atrophy (2/5 F)

Tongue: epithelial edema (1/5 F)

Main Study

Epididymis: unilateral hypospermia was observed in the 1000 mg/kg/day group (1/10 M).

Intestine, duodenum: mucosal edema was observed in the control group (2/25 M and 2/25 F) and 1000 mg/kg/day group (2/10 M).

Intestine, ileum: mucosal edema was observed in the control group (3/25 M and 3/25 F), 200 mg/kg/day group (3/10 F), and 1000 mg/kg/day group (1/10 M and 1/10 F).

Intestine, jejunum: mucosal edema was observed in the control group (4/25 M and 5/25 F), 200 mg/kg/day group (2/10 M and 3/10 F), and 1000 mg/kg/day group (2/10 M).

Ovary: cyst was observed in the 1000 mg/kg/day group (1/10 F).

Testis: unilateral atrophy was observed in the 1000 mg/kg/day group (1/10 M).

Other sporadic lesions were observed in various tissues; however, those changes lack dose-dependency and/or the incidences were no higher than that observed in the controls.

Recovery Phase

Bone marrow (femur): mild granulopoiesis was observed in the 1000 mg/kg/day group (1/9 M) and minimal granulopoiesis was observed in 1000 mg/kg/day group (2/10 F).

Intestine, ileum: minimal mucosal edema was observed in the 1000 mg/kg/day group (1/9 M).

Intestine, jejunum: minimal mucosal edema was observed in the 1000 mg/kg/day group (1/10 F).

Pancreas: focal necrosis was observed in the 1000 mg/kg/day group (1/10 F).

Toxicokinetics: Blood samples were collected according to the schedule described in the table above. Blood samples were collected from the first 3 remaining satellite animals/sex/group at each time point. For the 2000/1200 mg/kg/day animals, blood samples were collected on day 14. Data for the toxicokinetic parameters are summarized in the table below (table from the Sponsor).

Group	Dosage (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (h)	T_{last} (h)	$AUC_{(0-t)}$ (ng·h/mL)	$AUC_{(0-inf)}$ (ng·h/mL)	$T_{1/2}$ (h)
Day 1								
3	200	M	43.6	0.0833	0.50	14.1	a	a
		F	34.3	0.25	0.50	13.9	b	b
6	1000	M	1400	0.0833	1.00	278	a	a
		F	2068	0.50	2.00	1044	a	a
4	2000/1200	M	3490	0.0833	4.00	978	a	a
		F	2093	0.25	2.00	1215	1252	0.381
Day 28[c]								
3	200	M	135	0.0833	2.00	57.8	a	a
		F	522	0.0833	0.25	d	d	0.0764
6	1000	M	1306	0.0833	0.50	199	202	d
		F	978	0.0833	2.00	317	a	a
4	2000/1200	M	1587	0.0833	1.00	286	292	0.162
		F	2825	0.0833	2.00	494	526	0.838

C_{max} = maximum observed concentration after dosing; T_{max} = time of maximum observed concentration after dosing; T_{last} = time of last quantifiable concentration after dosing; $AUC_{(0-t)}$ = area under the concentration versus time curve from time zero to T_{last}; $AUC_{(0-inf)}$ = area under the concentration versus time curve from time zero to infinity; $T_{1/2}$ = apparent terminal elimination half-life; Rsq = coefficient of determination for the terminal elimination phase regression model.

[a] Value not reported because the $AUC_{(0-inf)}$ was extrapolated by more than 20% or Rsq was < 0.8.

[b] There was no reportable result as the terminal phase could not be identified.

[c] Samples collected from animals dosed at 2000/1200 mg/kg/day were collected on Day 14 rather than Day 28.

[d] Value was not considered reportable because the number of quantifiable plasma concentrations was less than 3.

In the 20 mg/kg/day animals (not reported in the table), with the exception of one animal, there were no quantifiable drug levels on day 1. By day 28, SP-304 levels were detected at 15 minutes post-dose (11.3 ng/ml) in the males, and at 5 minutes (39.4 ng/ml) and 15 minutes post-dose (32.9 ng/ml) in the females.

SP-304 exposure was higher in females than in males. The increase in AUC_{0-t} was disproportionately high relative to dose increment between 200 and 1000 mg/kg/day (e.g., 75-fold increase in AUC_{0-t} was observed for 1000 mg/kg/day females on day 1). For the 1000 to 2000 mg/kg/day dose increment, a more moderate increase in exposure was observed. SP-304 tended to accumulate after repeated administration for 28 days at 200 mg/kg/day. At higher dose levels, SP-304 exposure generally tended to decrease after repeated administration. $T_{1/2}$ was estimated from the 200 and 2000/1200 mg/kg/day animals; the values ranged from 0.076 to 0.838 hour.

SP-338, a metabolite that was identified in the studies submitted in the original IND, was not measured in the current study.

Conclusions: Oral administration of SP-304 to CD-1 mice for 28 days was well tolerated at doses of 1000 mg/kg/day or less. Daily oral doses at 2000/1200 mg/kg/day were associated with mortality. There was 1 death at 1000 mg/kg/day, but it is uncertain whether the death was treatment-related. There were no microscopic findings that could explain the early deaths. Although mucosal edema of the small intestine was a common lesion observed in the SP-304 treated animals, the lesion was also prevalent among the controls. No target organs of toxicity

were clearly identified at any dose level. Plasma exposure of SP-304 was generally low and was higher in females than in males. The half-life appears to be less than 1 hour. Because of the intolerability of SP-304 at 2000/1200 mg/kg/day, and the uncertainty about the single death at 1000 mg/kg/day, the NOAEL (no observed adverse effect level) is considered to be 200 mg/kg/day.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 2/2/2010). The review is included verbatim below.

SP-304: 28-Day Oral Toxicity Study in Cynomolgus Monkeys With a 2-Week Recovery

Key study findings: changes in fecal consistency were observed at 1, 10, and 75 mg/kg/day and the severity and frequency were higher in females than males; plasma exposure levels were generally higher in females than in males (up to 5-fold higher on day 1); urinary bladder is considered a target organ of toxicity; the tolerated dose in cynomolgus monkeys is considered to be 10 mg/kg/day.

Study no.: VMF00009

Volume #, and page #: Vol. 3, pg. 1

Conducting laboratory and location: (b) (4)

Date of study initiation: 8-14-2009 (report dated)

GLP compliance: Yes

QA report: yes (x) no ( )

Drug, lot #, and % purity: 034E09, 96.4%; FUROG0801A, 96.5%

METHODS: Cynomolgus monkeys (males and females: 2.2-3.1 kg) were treated with oral (gavage) dose of 0 (vehicle), 1, 10, or 75 mg/kg/day SP-304 for 28 days (4 monkeys/sex/group in the main study and 1 monkey/sex/group at 0 and 75 mg/kg/day in the recovery phase). The monkeys were described as "young adults", but the age range was not stated. The dose volume was 15 ml/kg, and the vehicle was water. The main study animals were sacrificed on day 29 and the recovery animals were sacrificed on day 43.

RESULTS:

Mortality: Animals were observed twice daily. No deaths occurred.

Clinical Signs: Clinical observations were performed on day -1 and weekly thereafter. On

dosing days, clinical observations were performed before dosing. During the recovery period, clinical observations were performed at least once weekly. Cageside observations were performed at least once daily, starting on day -7. On dosing days, cageside observations were performed 1 to 4 hours after dosing. In the recovery groups, cageside observations were performed on days when clinical observations were not scheduled.

SP-304 treatment was associated with a slight increase in the number of emesis events in males; however, the increase was not dose related. Summary of the emesis events is shown in the table below (taken from the Sponsor):

Number of Emesis Events During the Dosing Phase

Group No. (Dose Level)	Males			Females		
	Total Number of Animals	Number of Animals Affected	Number of Observations	Total Number of Animals	Number of Animals Affected	Number of Observations
1 (0 mg/kg/day)	5	2	8	5	2	6
2 (1 mg/kg/day)	4	3	13	4	2	2
3 (10 mg/kg/day)	4	1	2	4	1	1
4 (75 mg/kg/day)	5	3	17	5	1	2

Fecal Observations: Observations included, but were not limited to, color and the consistency of stool. Fecal consistency was scored as 1, 2, or 3 for well formed, mushy/loose, or watery feces, respectively.

Main animals:
-Daily: 3 hours pre-dosing, 3 hours post-dosing, and at the end of the day.

Recovery animals:
3 times daily with a minimum of 3 hours between each observation

Summary of the fecal consistence observations is shown in the following table (taken from the Sponsor):

Summary of Fecal Consistency Scores and Incidence of Fecal Consistency Scoring of 2 and/or 3 in Groups 1–4 During the Dosing Phase

Group No. (Dose Level)	Fecal Consistency Scores[a,b] (Incidence [%])[c]					
	Males			Females		
	Score of 2	Score of 3	Score of 2 and 3	Score of 2	Score of 3	Score of 2 and 3
1 (0 mg/kg/day)	58 (6.9)	3 (0.2)	61 (7.1)	38 (4.5)	0 (0.0)	38 (4.5)
2 (1 mg/kg/day)	44 (6.5)	9 (0.9)	53 (7.4)	100 (14.9)	30 (3.0)	130 (17.9)
3 (10 mg/kg/day)	106 (15.8)	48 (4.8)	154 (20.5)	120 (17.9)	213 (21.1)	333 (39.0)
4 (75 mg/kg/day)	146 (17.4)	255 (20.2)	401 (37.6)	188 (22.4)	198 (15.7)	386 (38.1)

[a] Fecal consistency score = number of observations × severity score.
[b] The total number of fecal observations was 420 for the males and females of Groups 1 and 4. The total number of fecal observations was 336 for the males and females of Groups 2 and 3.
[c] Incidence = (number of observations for Score 2 or 3/total number of fecal observations) × 100%.

SP-304 administration was associated with dose-dependent changes in fecal consistency scores, indicating production of mushy/loose and/or watery feces. The severity and frequency of fecal consistency changes were greater in females at 1 and 10 mg/kg/day, as evidenced by the greater fecal consistency scores, higher incidence of scoring of 2 and/or 3, and earlier onset of fecal consistency changes in the females. At 75 mg/kg/day, the total fecal consistency scores and the incidence of fecal consistency changes were similar between the males and females, but the onset of fecal consistency changes was earlier in the females.

Bodyweight: Body weights were measured on day -7, -1, and weekly thereafter. Additional measurements on days 35 and 42 were made in the recovery animals. Weight gain was unaffected by drug treatment. Mean body weights on day -1 for control males and females were 2.66±0.28 kg and 2.54±0.23 kg, respectively, and on day 28 for control males and females were 2.84±0.28 kg and 2.88±0.47 kg, respectively.

Food Consumption: Food consumption was measured daily beginning on day -7. Food consumption was unaffected by drug treatment.

Physical Examinations: Physical examinations were performed on days 27 and 42 for males and on days 26 and 41 for females. There were no abnormal findings attributable to SP-304 administration.

Ophthalmic Examinations: No drug-related effects were observed.

Electrocardiography Report: ECG measurements were recorded from all animals on days -11 to -2, on days 26 and 27 for main study animals, and on days 39 and 40 for recovery animals. All animals were in sinus rhythm and ECGs were qualitatively within normal limits.

Hematology: Blood was collected pre-dose on day -6 or -10 for males and day -11 for females, and on day 29 and 43 from main study animals and recovery animals, respectively. No drug-related effects were observed in any of the measured parameters. Coagulation parameters,

including prothrombin time, activated partial thromboplastin time, and fibrinogen concentrations were unaffected by drug treatment.

Blood Sample Collection Schedule

Time Point	Hematology	Coagulation	Serum Chemistry	Toxicokinetics[a]
Before initial treatment (all animals)	X	X	X	
Day 1[b]: predose and 5, 15, and 30 minutes and 1, 2, 4, and 8 hours after dose (main study and recovery animals)				X
Day 28[b]: predose and 5, 15, and 30 minutes and 1, 2, 4, and 8 hours after dose (main study and recovery animals)				X
Day 29 (all animals)	X	X	X	
Day 43 (recovery animals)	X	X	X	
Volume of Whole Blood	1.3 mL	1.3 mL	1.8 mL	1 mL
Anticoagulant	EDTA	Sodium Citrate	None[c]	K_3EDTA

Clinical Chemistry: Blood was collected pre-dose on day -6 or -10 for males and day -11 for females, and on day 29 and 43 from main study animals and recovery animals, respectively. No drug-related effects were observed in any of the measured parameters.

Urinalysis: Urine samples were collected at necropsy on days 29 (main study animals) and 43 (recovery animals). The parameters examined included: volume, color, clarity, specific gravity, microscopic evaluation of urine sediment, and urine test strip analysis (pH, protein, glucose, bilirubin, ketones, blood, urobilinogen, nitrites, and leukocytes). No drug-related effects in any of the measured parameters were observed.

Organ Weights: The following organs were collected on the day of sacrifice (days 29 and 43) (table taken from the Sponsor):

Organs Weighed at Necropsy

Adrenal gland (paired)	Prostate gland
Brain	Salivary gland, mandibular (paired)
Epididymis (paired)	Seminal vesicles (paired)
Heart	Spleen
Kidney (paired)	Testis (paired)
Liver	Thymus
Lung	Thyroid gland with parathyroid gland (paired)
Ovary (paired)	Uterus
Pituitary gland	

Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reported for main study groups and recovery groups. The only statistically significant organ weight changes were observed in the main study animals:

Heart: Absolute weight in the 75 mg/kg/day males was increased by 22.4%; however, there were no differences in the organ/bodyweight ratio or the organ/brain weight ratio.

Thymus: Absolute weight and organ/brain weight ratio in the 75 mg/kg/day females was decreased by 49% and 50%, respectively.

Gross Pathology: Observations were made at sacrifice (days 29 and 43).

Main Study:

Lymph node, mesenteric: tan accumulation was observed in the 75 mg/kg group (1/4 M).

Seminal vesicle: dark discoloration was observed in the 75 mg/kg/day group (1/4 M).

Stomach: thick and firm mucosa of the fundus was observed in the 75 mg/kg/day group (2/4 F).

Thymus: small thymus size was observed in the 75 mg/kg/day group (1/4 F).

Thyroid gland: multiple bilateral cysts were observed in the 75 mg/kg/day group (1/4 M).

There were other sporadic lesions in various tissues, however, they either lacked dose-dependency or the incidences were no higher than that observed in the controls.

Recovery Phase:

No drug-related effects were observed.

Histopathology: The following tissues and organs were collected and examined from animals treated at 0, 1, 10, or 75 mg/kg/day SP-304 in the main study groups and at 0 or 75 mg/kg/day in recovery groups (table taken from the Sponsor):

Tissues Collected and Examined at Necropsy

Adrenal gland (paired)	Lymph node, mesenteric
Animal identification	Mammary gland
Aorta	Nerve, optic (paired)[a]
Bone, femur	Nerve, sciatic
Bone, sternum	Ovary (paired)
Bone marrow, sternum	Pancreas
Bone marrow smear	Parathyroid gland[b]
Brain (cerebrum, cerebellum, brain stem)	Pituitary gland
Cervix	Prostate gland
Epididymis (paired)	Salivary gland, mandibular (paired)
Esophagus	Seminal vesicle (paired)
Eye (paired)[a]	Skeletal muscle
Gallbladder	Skin
Heart	Spinal cord (cervical, thoracic, lumbar)
Intestine, cecum	Spleen
Intestine, colon	Stomach (cardiac, fundic, pyloric)
Intestine, duodenum	Testis (paired)[c]
Intestine, ileum (with Peyer's patch[b])	Thymus
Intestine, jejunum	Thyroid gland (paired)
Intestine, rectum	Tongue
Kidney (paired)	Trachea
Lacrimal gland (paired)	Urinary bladder
Liver	Uterus
Lung	Vagina
Lymph node, mandibular	Gross lesions/masses

[a] Fixed in Davidson's Solution.
[b] Examined only when present in the routine section.
[c] Fixed in Modified Davidson's Solution.

Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

Main Study

Adrenal gland: Mononuclear cell infiltration of the cortex was observed in the 10 mg/kg/day group (1/4 M) and the 75 mg/kg/day group (1/4 M).

Brain: Mononuclear cell infiltration was observed in the 75 mg/kg/day group (1/4 F).

Gall bladder: Mononuclear cell infiltration was observed in the 75 mg/kg/day group (1/4 M).

Intestine, cecum: Balantidiasis was observed in the 75 mg/kg/day group (1/4 M); multifocal brown pigmentation was observed in the 75 mg/kg/day group (1/4 M).

Lacrimal gland: Lymphocytic infiltration was observed in the 10 mg/kg/day group (1/4 F) and in the 75 mg/kg/day group (1/4 F).

Lymph node, mesenteric: Unilateral mineralization was observed in the 75 mg/kg/day group (1/4 M); marked unilateral necrosis was observed in the 75 mg/kg/day group (1/4 M).

Pancreas: Focal acinar cell atrophy was observed in the 75 mg/kg/day group (1/4 M).

Salivary gland, mandibular: Lymphocytic infiltration was observed in the 10 mg/kg/day group (2/4 M) and the 75 mg/kg/day group (1/4 M).

Thymus: Atrophy was observed in the 75 mg/kg/day group (1/4 F).

Thyroid gland: Multiple cysts were observed in the 10 mg/kg/day group (1/4 M) and the 75 mg/kg/day group (1/4 F); single cyst was observed in the 75 mg/kg/day group (1/4 F).

Urinary bladder: Focal hemorrhage of the serosa was observed in the 75 mg/kg/day group (1/4 M); mononuclear cell infiltration was observed in the 1 mg/kg/day group (1/4 M and 1/4 F), the 10 mg/kg/day group (2/4 M and 1/4 F), and the 75 mg/kg/day group (2/4 F).

There were other sporadic lesions in various tissues; however, they either lacked dose-dependency or the incidences were no higher than that observed in the controls.

Recovery Phase

Intestine, duodenum: Mixed cell infiltration of the muscularis was observed in the 75 mg/kg/day group (1/1 F).

Kidney: Mononuclear cell infiltration was observed in the 75 mg/kg/day group (1/1 M).

Stomach: Mononuclear cell infiltration was observed in the 75 mg/kg/day group (1/1 M and 1/1 F).

Urinary bladder: Mononuclear cell infiltration was observed in the 75 mg/kg/day group (1/1 F).

Toxicokinetics: Blood samples were collected on days 1 and 28 prior to dosing, at 5, 15, 30 minutes, and 1, 2, 4, and 8 hours after dose. There was no systemic exposure at 1 mg/kg/day SP-304, and exposure at 10 mg/kg/day was negligible. At 75 mg/kg/day, maximum SP-304 plasma concentrations were observed between 5 and 30 minutes after dosing, and the levels declined rapidly thereafter. SP-304 was no longer quantifiable at 2 hours after dosing. $T_{1/2}$ was 0.428 ± 0.228 h (n = 3) and 0.636 h (n = 2) on days 1 and 28, respectively, in the 75 mg/kg/day females. The exposure in females ($AUC_{(0-t)}$ = 92.8±18.3 ng·h/ml) was approximately 5-fold higher than in males ($AUC_{(0-t)}$ = 18.8±8.83 ng·h/ml) on day 1. On day 28, exposure was decreased in females (-22.8%) and increased in males (110%), but was still approximately 2-fold higher in females than in males. The toxicokinetics data at 75 mg/kg/day are summarized in the table below:

Table: Toxicokinetics data at 75 mg/kg/day

Sex	C_{max} (ng/ml)	T_{max} (min)	$AUC_{(0-t)}$ (ng·h/ml)	$AUC_{(0-inf)}$ (ng·h/ml)	$T_{1/2}$ (h)
Day 1					
M	29.1±14.2	5	18.8±8.83		
F	136±49.8	30	92.8±18.3	115±12.6	0.428±0.228
Day 28					
M	42.4±22.4	5	39.5±35.8		
F	86.2±27.6	5	71.6±27.9	84.6	0.636

SP-338, a metabolite that was identified in the studies submitted in the original IND, was not measured in the current study.

Conclusions: Oral administration of SP-304 for 28 days was well tolerated in cynomolgus monkeys at dose levels up to 75 mg/kg/day. Findings attributable to SP-304 administration included dose-dependent, reversible increases in fecal consistency scores at ≥ 10 mg/kg/day in males and at ≥ 1 mg/kg/day in females. The increases in fecal scores indicate formation of loose or watery feces. The changes in stool form is considered to be a pharmacological response. Microscopic findings were observed in the following tissues: adrenal gland, brain, gall bladder, cecum, lacrimal gland, mesenteric lymph node, pancreas, mandibular salivary gland, thymus, thyroid gland, and urinary bladder. Most of the microscopic findings were reversible; however, mononuclear cell infiltration of the urinary bladder was evident in a 75 mg/kg/day recovery female (1/1 animal). Urinary bladder is considered a target organ of toxicity, based on the presence of mononuclear cell infiltrate at all 3 doses administered, and hemorrhage in the 75 mg/kg/day group. It appears that the mononuclear cell infiltration may not be completely reversible. Plasma exposure levels of SP-304 at 1 and 10 mg/kg/day were negligible. At 75 mg/kg/day, exposure levels were 2- to 5-fold higher in females than in males. The tolerated dose is considered to be 10 mg/kg/day.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 8/2/2013). The review is included verbatim below.

Study title: Plecanatide: 13-week oral toxicity study in mice with a 2-week recovery period

Study no.:	1896-001
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/20/2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SP-304, FUROG1001, and 96.2%

Key Study Findings

Animals (10/sex/group) were treated with 0 (vehicle), 20, 200, or 800 mg/kg/day plecanatide for 13 weeks. Recovery animals (6/sex/group) were treated with 0 or 800 mg/kg/day plecanatide for 13 weeks, followed by a 2-week recovery period. There were four deaths; however, these were not considered as drug-related because there were no consistent clinical observations which indicated specific toxicity and no dose relationship. Furthermore, the deaths generally occurred early in the study with the remaining mice surviving to termination. There were no clear drug-related clinical signs, although some of the 800 mg/kg/day animals showed unkempt appearance. Microscopic findings were observed in the following tissues: tibiofemoral joint synovial hyperplasia/hypertrophy (1/10 F in the 200 mg/kg/day group and 4/20 mice in the 800 mg/kg/day group), tibiofemoral joint subacute/chronic inflammation (1/10 F in the 800 mg/kg/day group), liver mononuclear cell infiltration (3/19 in the control group, 9/20 in the 200 mg/kg/day group, and 9/20 in the 800 mg/kg/day group), ovary follicular cyst (2/10 F in the 800 mg/kg/day group), and pituitary gland focal necrosis (1/10 M in the 200 mg/kg/day group and 3/20 in the 800 mg/kg/day group). At the end of the 2-week recovery period, liver mononuclear cell infiltration (2/6 M), synovial hyperplasia/ hypertrophy (1/6 F), and ovary follicular cyst (1/6 F) were still evident and appear not to be completely reversible. Plasma concentrations of plecanatide in the control or the 20 mg/kg/day mice were not measurable. Systemic exposure levels increased in approximate proportion to dose between 200 and 800 mg/kg/day, and there was no significant drug accumulation after repeated dosing. NOAEL is considered to be 20 mg/kg/day based on microscopic findings at 200 and 800 mg/kg/day.

Methods

Doses:	0, 20, 200, and 800 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg/dose
Formulation/Vehicle:	solution/0.01 M sodium phosphate buffer, pH 7.0
Species/Strain:	Mouse/Crl:CD1 (ICR)
Number/Sex/Group:	Main study: 10/sex/group Recovery: 6/sex/group at 0 and 800 mg/kg/day
Age:	6 weeks of age
Weight:	M: 20 to 35 g; F: 18-25 g
Satellite groups:	Toxicokinetic groups: 15/sex/group at 0 mg/kg/day and 45/sex/group at 20, 200, and 800 mg/kg/day
Unique study design:	N/A
Deviation from study protocol:	Minor deviations occurred that did not affect the quality or integrity of the study.

The following table shows the design of the study (taken from Sponsor's study report).

Study Design

GROUP	DOSE LEVEL	NUMBER OF ANIMALS-MAIN STUDY COHORT													
		TOTAL		CLINICAL PATHOLOGY[a]				NECROPSY				MICROSCOPIC PATHOLOGY			
				TERM		REC		TERM		REC		TERM		REC	
	MG/KG	M	F	M	F	M	F	M	F	M	F	M	F	M	F
1	0	16	16	10	10	6	6	10	10	6	6	10	10	A.R.	A.R.
2	20	10	10	10	10	-	-	10	10	-	-	A.R.[b]	A.R.	-	-
3	200	10	10	10	10	-	-	10	10	-	-	A.R.	A.R.	-	-
4	800	16	16	10	10	6	6	10	10	6	6	10	10	A.R.	A.R.

a: Hematology and clinical chemistry at termination and recovery necropsies.
b: A.R. = As Required: 1) Target tissues identified by Group 4 evaluations; 2) Tissues in all animals found dead or euthanized in a moribund condition,
TERM = Terminal necropsy; REC = Recovery necropsy.

Observations and Results

Mortality

Animals were observed twice daily. Four animals were found dead or euthanized *in extremis*, as follows: a control female on day 52, a 200 mg/kg/day TK male on day 2, two 800 mg/kg/day TK males on days 6 and 27. The deaths were not considered drug-related because there were no clinical observations which indicated specific toxicity and no dose relationship. Furthermore, the deaths generally occurred early in the study with the remaining mice surviving to termination.

Clinical Signs

A detailed clinical examination was performed during the acclimation period (days -6 to -1) and daily during the treatment and recovery period. Examinations during the dosing period were conducted at 30 to 90 minutes post-dose. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior.

Main study:

Pelage/skin: Unkempt appearance was observed in the control group (1/10 F), 200 mg/kg/day group (1/10 M), and 800 mg/kg/day group (3/10 M and 1/10 F). Skin cold to touch was observed in the 800 mg/kg/day group (1/10 M).

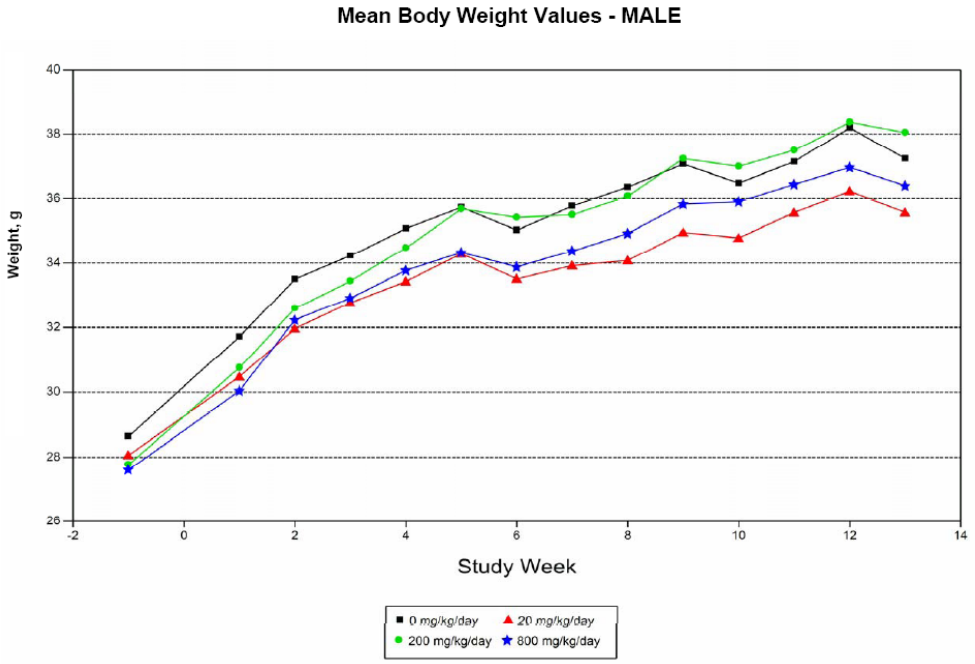
There were other sporadic clinical observations; however, these changes either lacked dose-dependency and/or the incidences were no higher than that observed in the controls.

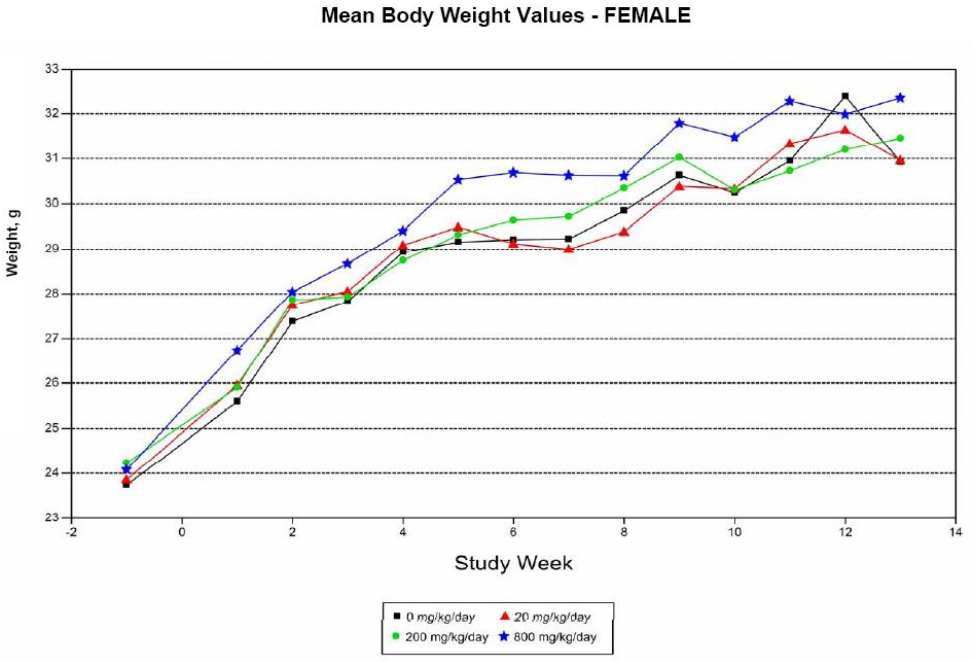
Recovery Phase:

There were no drug-related clinical signs.

Body Weights

Body weights were measured and recorded two days following arrival, prior to randomization, and weekly beginning in week -1 during the study. The body weights during the treatment period are presented in the two figures below (taken from the Sponsor's study report).





There were no significant drug-related changes in the body weights during the treatment and recovery period.

Feed Consumption

Food consumption was measured and recorded for the main study animals weekly during the study.

There were no significant drug-related effects on food consumption.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted on all animals pre-test and on main study animals prior to terminal and recovery necropsies.

Retinal atrophy was the most frequently observed change; however, the incidence was comparable among the groups.

ECG

N/A

Hematology

Hematology evaluations were conducted in four or five animals/sex/group in the main study animals at the terminal necropsy on day 91, and in three animals/sex/group at the recovery necropsy on day 105.

The hematologic parameters evaluated included hematocrit (Hct), hemoglobin (Hb), erythrocyte count (RBC), reticulocyte count (Retic), leukocyte count (total and absolute differential), mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet count, and blood cell morphology.

There were no meaningful drug-related effects on the hematology parameters. One of the five 800 mg/kg/day males showed slightly decreased erythrocytes, hemoglobin, and hematocrit at termination, and one recovery 800 mg/kg/day male showed slightly decreased erythrocytes, hemoglobin, and hematocrit, as well as mildly increased neutrophils and lymphocytes. However, there were no significant dose-dependent changes in the average means of these parameters. Thus, the changes are not considered adverse.

Clinical Chemistry

Clinical chemistry evaluations were conducted in the main study at the terminal necropsy on day 91 and in three animals/sex/group at the recovery necropsy on day 105.

The following parameters were evaluated: alkaline phosphatase (AP), total bilirubin (with direct bilirubin if total bilirubin exceeds 1 mg/dL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, total protein, albumin, globulin and A/G (albumin/globulin) ratio, glucose, calcium, phosphorus, amylase, and lipase.

There were no meaningful drug-related changes in clinical chemistry parameters at the terminal or recovery phase. There were sporadic significant changes in ALT (M: -30% in the 800 mg/kg/day recovery group), total protein (F: -8.4% in the 800 mg/kg/day group), and globulin (M: -10% in the 800 mg/kg/day recovery group); however, these changes are not considered adverse because they lack dose-dependency, occurred in only in a single sex, and/or there were no significant changes at the terminal necropsy.

Urinalysis

N/A

Gross Pathology

Observations were made at sacrifice (days 91 and 105) and immediately after premature death.

Premature Deaths

There were no drug-related macroscopic findings.

Main Study

There were no meaningful drug-related macroscopic findings.

Recovery Phase

There were no meaningful drug-related macroscopic findings.

Organ Weights

Organs were collected on the day of sacrifice (days 91 and 105). The following organs were collected and their weights were measured (table taken from the Sponsor's study report).

Organs or Tissues to be Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 4	2, 3	Rec[a]
Adrenal gland	X	X	X		
Aorta		X	X		
Bone with bone marrow, femur		X	X		
Bone with bone marrow, sternum		X	X		
Bone marrow smear[b]		X			
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X		
Epididymis	X	X	X		
Esophagus		X	X		
Eye (with optic nerve)		X	X		
Gallbladder		X	X		
GALT[c]		X	X		
Heart	X	X	X		
Joint, tibiofemoral		X	X		
Kidney	X	X	X		
Lacrimal gland, exorbital		X	X		
Large intestine, cecum		X	X		
Large intestine, colon		X	X		
Large intestine, rectum		X	X		
Larynx		X	X		
Liver	X	X	X		

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 4	2, 3	Rec[a]
Lung with bronchi	X	X	X		
Lymph node, mandibular		X	X		
Lymph node, mesenteric		X	X		
Mammary gland (process females only)		X	X		
Nerve, sciatic		X	X		
Ovary	X	X	X		
Oviducts		X	X		
Pancreas		X	X		
Pituitary	X	X	X		
Prostate		X	X		
Salivary gland, mandibular[d]	X	X	X		
Salivary gland, parotid		X	X		
Salivary gland, sublingual		X	X		
Seminal vesicles		X	X		
Skeletal muscle, biceps femoris		X	X		
Skin		X	X		
Small intestine, duodenum		X	X		
Small intestine, ileum		X	X		
Small intestine, jejunum		X	X		
Spinal cord, cervical		X	X		
Spinal cord, lumbar		X	X		
Spinal cord, thoracic		X	X		
Spleen	X	X	X		
Stomach, glandular		X	X		
Stomach, nonglandular		X	X		
Target Organs[e]		X	X	X	X
Testis	X	X	X		
Thymus	X	X	X		
Thyroid gland (with parathyroid)[f]	X	X	X		

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination 1, 4	2, 3	Rec[a]
Tongue		X	X		
Trachea		X	X		
Ureters		X	X		
Urinary bladder		X	X		
Uterus with cervix		X	X		
Vagina		X	X		
Gross lesions		X	X	X	X
Tissue masses with regional lymph node[g]		X	X	X	X

[a] *Recovery*
[b] *Bone marrow smears will be prepared only for animals necropsied at scheduled intervals. Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost).* [b]
[c] *Gut Associated Lymphoid Tissue*
[d] *The combined weight of the right mandibular/sublingual salivary gland will be obtained.*
[e] *Target organs (and target organ gross lesions) will be designated by the Study Director, Pathologist and/or Sponsor based on experimental findings (additional cost).*
[f] *Parathyroids cannot always be identified macroscopically. They will be examined if in the plane of section and in all cases where they are noted as grossly enlarged.*
[g] *A regional lymph node drains the region where a tissue mass is located. A regional lymph node may not always be identified when a mass is present.*

Absolute weight, relative weight (organ/bodyweight), and organ/brain weight ratio were reported. There were no meaningful drug-related organ weight changes at the terminal necropsy. A statistically significant increase in pituitary gland weight (+24%) was present at the recovery phase necropsy in the 800 mg/kg/day males. However, the increase was considered to be incidental since there were no microscopic correlates, no similar pituitary weight increases in the females, and there were no pituitary weight changes at the terminal necropsy. There were other sporadic organ weight changes; however, these changes lacked dose-dependency and microscopic correlates.

Histopathology

Tissues and organs were collected on the day of sacrifice (day 91 for the main study groups, and day 105 for the recovery groups). The organs/tissues that were examined are listed in the table above.

Adequate Battery - Yes

Peer Review - No

Histological Findings

Main Study

Joint, tibiofemoral: Synovial hyperplasia/hypertrophy was observed in the 200 mg/kg/day (1/10 F) and 800 mg/kg/day groups (2/10 M and 2/10 F). Subacute/chronic inflammation was observed in the 800 mg/kg/day group (1/10 F).

Liver: Mononuclear cell infiltration was observed in the control group (1/10 M and 2/9 F),

200 mg/kg/day group (3/10 M and 6/10 F), 800 mg/kg/day group (4/10 M and 5/10 F).

Ovaries: Follicular cyst was observed in the 800 mg/kg/day group (2/10 F).

Pituitary gland: Focal necrosis was observed in the 200 mg/kg/day group (1/10 M) and 800 mg/kg/day group (1/10 M and 2/10 F).

Recovery Phase

Joint, tibiofemoral: Synovial hyperplasia/hypertrophy was observed in the 800 mg/kg/day group (1/6 F).

Liver: Mononuclear cell infiltration was observed in the 800 mg/kg/day group (2/6 M).

Ovaries: Follicular cyst was observed in the 800 mg/kg/day group (1/6 F).

Special Evaluation

N/A

Toxicokinetics

Blood samples were collected from three animals/sex/group (TK groups). The table below shows the blood collection schedule (taken from Sponsor's study report).

Blood Collection for Plasma Concentration			
Groups	Number of Animals per Interval	Study Interval	Interval (Hour Postdose)
Control group	3/sex	Days 1 and 90	0.25 and 1 hour after dosing on Day 1; Predose, 0.25, and 1, hour after dosing on Day 90
Treated groups	3/sex	Days 1 and 90	0.0833, 0.25, 0.5, 1, 1.5, 2, and 3 hours after dosing on Day 1; Prior to dosing and at 0.0833, 0.25, 0.5, 1, 1.5, 2, and 3 hours after dosing on Day 90
Special Instructions			
The test animals will not be fasted before blood collection. After completion of blood collection, these animals will be euthanized and discarded without further evaluation.			

The lower limit of quantitation for plecanatide was 10 ng/ml. The results from days 1 and 90 are shown in the following table (taken from the Sponsor's study report).

Day	Dose (mg/kg/day)	Sex	$AUC_{0\text{-tlast}}$ (hr•ng/mL)	C_{max} (ng/mL)	T_{max} (hr)
1	20	M	0	0	NC
		F	0	0	NC
		Combined	0	0	NC
	200	M	NC	24.4	0.0833
		F	NC	45.0	0.0833
		Combined	NC	34.7	0.0833
	800	M	26.2	126	0.0833
		F	34.6	85.8	0.0833
		Combined	30.4	106	0.0833
90	20	M	0	0	NC
		F	0	0	NC
		Combined	0	0	NC
	200	M	9.02	25.5	0.0833
		F	NC	22.5	0.0833
		Combined	9.02	24.0	0.0833
	800	M	37.9	132	0.0833
		F	53.2	154	0.0833
		Combined	45.5	143	0.0833

NC = not calculated; insufficient data.

Plecanatide was not detected in plasma at 20 mg/kg/day. Maximum plasma levels of plecanatide were achieved by 5 minutes after oral administration. The increases in $AUC_{0\text{-tlast}}$ values on day 90 in the 200 and 800 mg/kg/day mice were generally proportional to dose. There was no significant drug accumulation between day 1 and day 90 after repeated dosing. There were no significant sex differences in plecanatide exposure.

Dosing Solution Analysis

The concentration determination of SP-304 in the dosing formulation samples was performed in accordance with the validated method. Analysis of preliminary mixes showed that the preparation procedure used for the study produced homogeneous mixtures. Analyses conducted during the treatment period confirmed that dosing solutions contained drug concentrations approximately equal to the nominal (intended) concentrations.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 7/25/2013). The review is included verbatim below.

Study title: Plecanatide: 13-week oral toxicity study in rats

Study no.:	1896-013
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/14/2012

GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide;110425 / 120709; 97.6-98.6% / 97.0%

Key Study Findings

Animals (10/sex/group) were treated with 0 (vehicle), 30, 100, or 300 mg/kg/day plecanatide for 13 weeks. There were no deaths during the study. A dose-dependent decrease in bodyweight gain in the females, although not statistically significant, was noted; a 14.8% decrease, compared to controls, was observed in the 300 mg/kg/day females. This decrease in bodyweight gain appears to correlate with significant decreases in food consumption in the 300 mg/kg/day females. There were no meaningful drug-related clinical signs, and no changes in hematology or clinical chemistry were observed. There were no drug-related macroscopic or microscopic findings. The NOAEL in males is considered to be 300 mg/kg/day. The NOAEL in females is considered to be 100 mg/kg/day based on the decrease in bodyweight gain. The maximum tolerated dose (MTD) is considered to be 100 mg/kg/day in females. MTD in the males was not established due to the absence of adverse effects (i.e. MTD > 300 mg/kg/day). Plecanatide was detected in plasma at all dose levels. There was some drug accumulation between day 1 and day 84 after repeated dosing. There were no consistent sex-related differences in plecanatide exposures overall

Methods

Doses:	0, 30, 100, and 300 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg/dose
Formulation/Vehicle:	solution/Distilled water
Species/Strain:	Rat/CD® [Crl:CD®(SD)]
Number/Sex/Group:	Main study: 10/sex/group
Age:	6 weeks of age
Weight:	M: 233-269 g; F: 171-205 g
Satellite groups:	Toxicokinetic groups: 6/sex/group at 0 mg/kg/day and 21/sex/group at 30, 100, and 300 mg/kg/day
Unique study design:	N/A
Deviation from study protocol:	Minor deviations occurred that did not affect the quality or integrity of the study.

The following table shows the design of the study (taken from Sponsor's study report).

Study Design

Group	Dose Level (mg/kg/day)	Dose Volume (mL/kg/dose)	Dose Concentration (mg/mL)	Number of Animals	
				Males	Females
Main Study					
1	0	10	0	10	10
2	30	10	3	10	10
3	100	10	10	10	10
4	300	10	30	10	10
Toxicokinetic (TK) Study					
5	0	10	0	6+1*	6+1*
6	30	10	3	21+2*	21+2*
7	100	10	10	21+2*	21+2*
8	300	10	30	21+2*	21+2*

**Additional animals/sex/treatment group included as replacement animals.*

Observations and Results

Mortality

Animals were observed twice daily. There were no deaths.

Clinical Signs

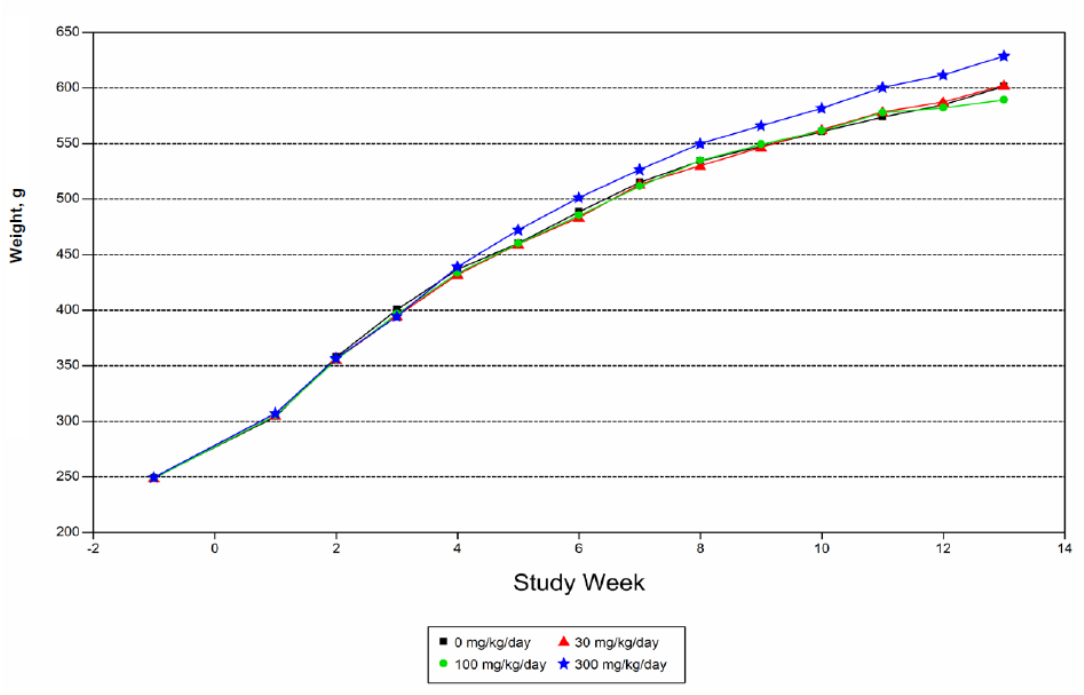
A detailed clinical examination was performed on the main study animals prior to dosing and weekly during the treatment period. On occasion, clinical observations were recorded at unscheduled intervals for all animals. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior. Cageside clinical observation was performed once daily (1 to 2 hours postdose) during the treatment period.

There were no drug-related clinical signs during the study.

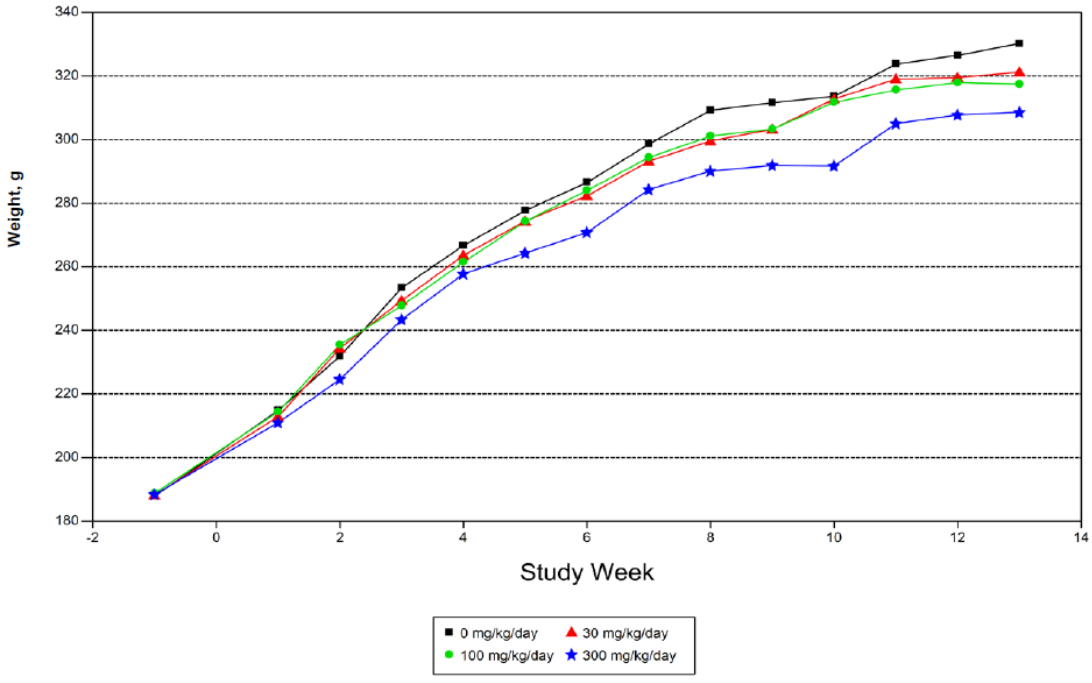
Body Weights

Body weights were measured and recorded two days following arrival, prior to randomization (week -1), and weekly during the study. The body weights during the treatment period are presented in the following figures (taken from the Sponsor's study report). Absolute body weight and bodyweight gains are shown in the following table.

Mean Body Weight Values - MALE



Mean Body Weight Values - FEMALE



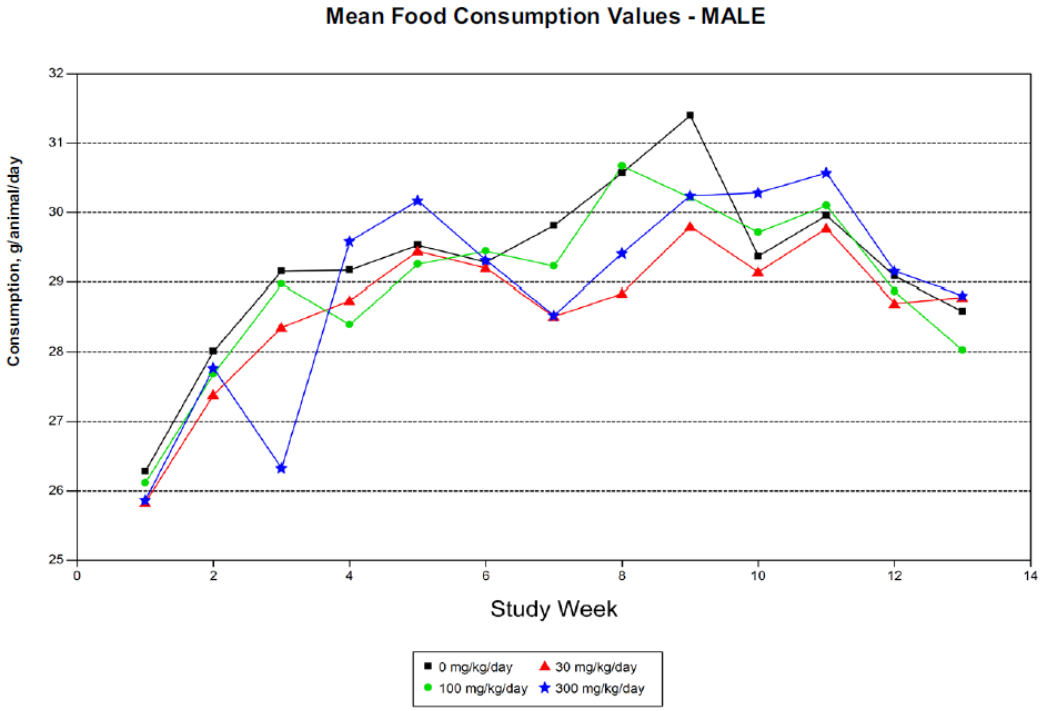
Absolute body weight and bodyweight gain

Parameter	Males				Females			
Dose (mg/kg/day)	**0**	**30**	**100**	**300**	**0**	**30**	**100**	**300**
BW (g), wk -1	249	249	249	250	188	188	189	188
BW (g), wk 13	602	603	589	629	330	321	317	309
BW, % of control	100	100.2	97.8	104.5	100	97.3	96.1	93.6
ΔBW (g)	353	354	340	379	142	133	128	121
BW gain, % of control	100	100.3	96.3	107.4	100	93.7	90.1	85.2

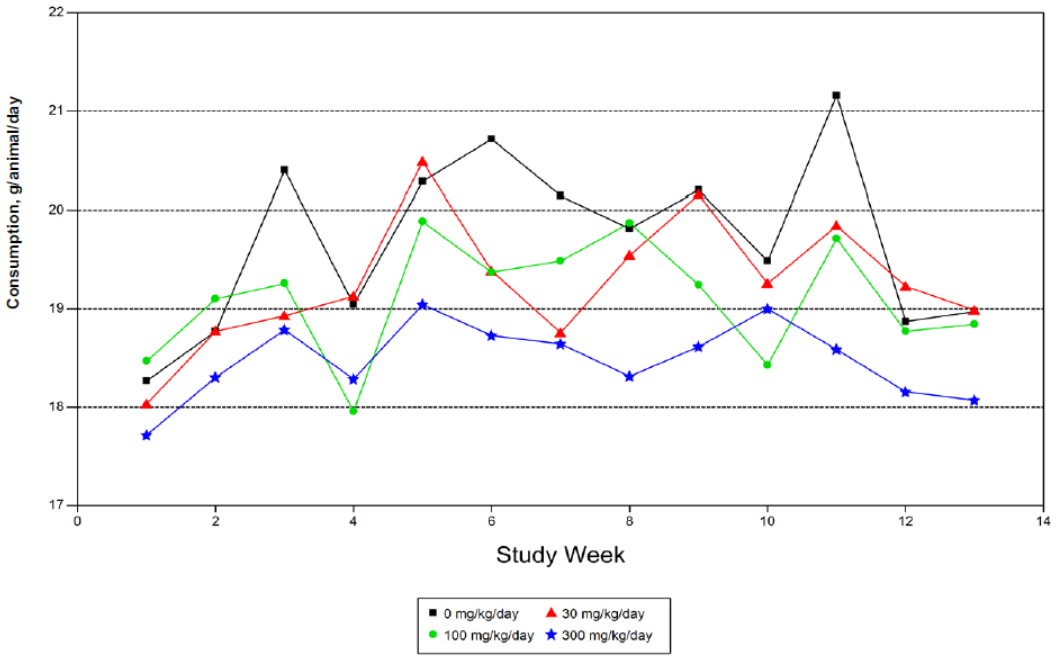
There were no statistically significant changes in body weight or bodyweight gain during the treatment period. However, there was a dose-dependent trend in a decrease in bodyweight gain in females, reaching a 14.8% decrease at the high dose, compared to controls.

Feed Consumption

Food consumption was measured and recorded weekly during the study. The figures and tables below show food consumption for the animals during the study (taken from the Sponsor's study report).



Mean Food Consumption Values - FEMALE



Summary of Food Consumption Values - MALE

Study Interval (Week)	0 mg/kg/day			30 mg/kg/day			100 mg/kg/day			300 mg/kg/day		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
1	26.28	1.034	10	25.83	0.846	10	26.12	1.208	10	25.87	0.608	10
2	28.01	1.222	10	27.39	1.121	10	27.69	0.778	10	27.77	1.819	10
3	29.16	1.456	10	28.36	0.834	10	28.97	0.280	10	26.33	7.299	10
4	29.17	1.562	10	28.74	1.104	10	28.40	0.748	10	29.59	1.248	10
5	29.53	1.306	10	29.44	1.196	10	29.26	0.580	10	30.17	1.381	10
6	29.29	2.373	10	29.20	1.447	10	29.44	0.809	10	29.31	1.812	10
7	29.81	1.676	10	28.51	1.732	10	29.23	0.661	10	28.53	1.775	10
8	30.57	2.297	10	28.84	0.620	10	30.67	0.947	10	29.41	1.655	10
9	31.40	2.488	10	29.80	1.011	10	30.21	1.560	10	30.24	2.417	10
10	29.37	1.963	10	29.14	1.146	10	29.71	1.014	10	30.29	1.881	10
11	29.96	2.090	10	29.77	1.173	10	30.10	0.540	10	30.57	2.283	10
12	29.09	2.425	10	28.70	1.393	10	28.87	0.518	10	29.16	1.930	10
13	28.59	2.076	10	28.79	1.065	10	28.03	1.374	10	28.81	1.952	10

Summary of Food Consumption Values - FEMALE

Study Interval (Week)	0 mg/kg/day			30 mg/kg/day			100 mg/kg/day			300 mg/kg/day		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
1	18.27	1.668	10	18.03	0.982	10	18.47	1.664	10	17.72	1.007	10
2	18.77	0.924	10	18.77	0.810	10	19.10	1.006	10	18.30	0.572	8
3	20.40	0.817	10	18.93[a]	1.234	10	19.26	1.034	10	18.79[b]	1.163	10
4	19.04	0.751	10	19.13	1.341	10	17.96	0.875	10	18.29	1.091	10
5	20.29	1.207	10	20.49	1.012	10	19.89	0.605	10	19.04[b]	0.505	10
6	20.71	1.767	10	19.39[a]	0.828	10	19.37[a]	0.908	10	18.73[b]	0.973	10
7	20.14	2.007	10	18.76[a]	1.032	10	19.49	0.677	10	18.64[a]	0.798	10
8	19.81	1.677	10	19.54	0.922	10	19.87	1.631	10	18.31[a]	0.845	10
9	20.20	1.607	10	20.16	0.572	10	19.24	0.885	10	18.61[a]	1.459	10
10	19.49	1.606	10	19.26	0.684	10	18.43	0.968	10	19.00	1.410	10
11	21.16	1.018	10	19.84[a]	0.896	10	19.71[b]	1.087	10	18.59[b]	1.120	10
12	18.87	1.646	10	19.23	0.790	10	18.77	0.777	10	18.16	0.576	10
13	18.97	1.346	10	18.99	1.721	10	18.84	0.751	10	18.07	1.115	10

[a] Significantly different from control; ($p<0.05$)
[b] Significantly different from control; ($p<0.01$)

Significant decreases in food consumption (3-12%) were observed in the 300 mg/kg/day females at most intervals, starting from week 3. A few other statistically significant differences were noted at the lower doses; however, these decreases were sporadic and did not occur in a dose-related manner.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted on all animals pretest and prior to terminal necropsies.

Chorioretinal hyperplasia and retinal atrophy were noted in a few animals at terminal interval; however, these findings did not occur in a dose-dependent manner and are not considered drug-related.

ECG

N/A

Hematology

Blood samples were collected at sacrifice (week 13) on 10 animals/sex/group in the main study groups. Animals were fasted overnight prior to blood collection. The following parameters were examined:

Hemoglobin concentration
Hematocrit
Erythrocyte count
Platelet count
Mean corpuscular volume

Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Total leukocyte count
Differential leukocyte count
Reticulocyte count

There were no meaningful changes in the parameters examined. However, it is noted that statistically significant decreases in erythrocytes (-6.4%), and hematocrit (-4.8%) were observed in the 300 mg/kg/day males.

Coagulation values, including activated partial thromboplastin time and prothrombin time, were evaluated at the terminal necropsy at week 13. There were no drug-related meaningful changes.

Clinical Chemistry

Clinical chemistry evaluations were conducted at terminal necropsy on week 13.

The following parameters were evaluated: alkaline phosphatase (AP), total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, total protein, albumin, globulin and A/G (albumin/globulin) ratio, glucose, calcium, phosphorus, amylase, and lipase.

There were no drug-related meaningful changes in the parameters examined. However, it is noted that a statistically significant decrease in calcium, -4.4% and -4.8%, occurred in the 100 and 300 mg/kg/day females, respectively. Significant decreases in total protein (-6.3%) and albumin (-7.7%) were also noted in the 300 mg/kg/day females, compared to controls.

Urinalysis

Urine samples were obtained in a 16-hour overnight collection period at termination. Animals were fasted but had access to water during the collection period.

Urinalysis parameters, including urine volume and specific gravity, were evaluated at terminal necropsy on week 13. There were no drug-related meaningful changes in the parameters examined.

Gross Pathology

Necropsy examinations were performed on animals at the scheduled terminal necropsies on week 13.

There were no meaningful drug-related macroscopic findings at terminal necropsy.

Organ Weights

Organs were collected on the day of sacrifice (week 13). The following organs were collected and their weights were measured (table taken from the Sponsor's study report).

Organs or Tissues to be Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			1, 4	2, 3
Adrenal glands	X	X	X	
Aorta		X	X	
Bone with bone marrow, femur		X	X	
Bone with bone marrow, sternum		X	X	
Bone marrow smear[a]		X		
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X	
Epididymides		X	X	
Esophagus		X	X	
Eyes (with optic nerve)		X	X	
Gallbladder		X	X	
GALT[f]		X	X	
Heart	X	X	X	
Joint, tibiofemoral		X	X	
Kidneys	X	X	X	
Lacrimal glands, exorbital		X	X	
Large intestine, cecum		X	X	
Large intestine, colon		X	X	
Large intestine, rectum		X	X	
Larynx		X	X	
Liver	X	X	X	
Lung with bronchi	X	X	X	
Lymph node, mandibular		X	X	
Lymph node, mesenteric		X	X	
Mammary gland (process females only)		X	X	
Nerve, sciatic		X	X	
Ovaries	X	X	X	

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination 1, 4	Microscopic Examination 2, 3
Oviducts		X	X	
Pancreas		X	X	
Pituitary gland	X	X	X	
Prostate gland	X	X	X	
Salivary gland, mandibular[b]	X	X	X	
Salivary gland, parotid		X	X	
Salivary gland, sublingual		X	X	
Seminal vesicles	X	X	X	
Skeletal muscle, biceps femoris		X	X	
Skin		X	X	
Small intestine, duodenum		X	X	
Small intestine, ileum		X	X	
Small intestine, jejunum		X	X	
Spinal cord, cervical		X	X	
Spinal cord, lumbar		X	X	
Spinal cord, thoracic		X	X	
Spleen	X	X	X	
Stomach, glandular		X	X	
Stomach, nonglandular		X	X	
Target Organs[c]		X	X	X
Testes	X	X	X	
Thymus	X	X	X	
Thyroid gland (with parathyroid)[d]	X	X	X	
Tongue		X	X	
Trachea		X	X	
Ureters		X	X	
Urinary bladder		X	X	
Uterus with cervix	X	X	X	
Vagina		X	X	
Gross lesions		X	X	X

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination 1, 4	Microscopic Examination 2, 3
Tissue masses with regional lymph node[e]		X	X	X

[a]Bone marrow smears will be collected, stained, and coverslipped only for main study animals necropsied at the scheduled interval. Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost).
[b]The combined weight of the right mandibular/sublingual salivary gland will be obtained.
[c]Based on experimental findings, the Study Pathologist will identify potential target organs to the Study Director. The designation of potential target organs to be evaluated for the study (additional cost) will then be communicated by the Study Director after discussion with the Sponsor.
[d]Parathyroid glands cannot always be identified macroscopically. They will be examined if in the plane of section and in all cases where they are noted as grossly enlarged.
[e]A regional lymph node drains the region where a tissue mass is located. A regional lymph node may not always be identified when a mass is present.
[f]Gut-Associated Lymphoid Tissue

Absolute weight, relative weight (organ/bodyweight), and organ/brain weight ratio were reported. There were no drug-related meaningful organ weight changes. In the heart, absolute weight, organ/body weight, and organ/brain weight ratio decreased significantly by 13.6, 11.9, and 9.9%, respectively, in the 30 mg/kg/day females, and absolute weight and organ/brain weight ratio decreased significantly by 13.1 and 9.6%, respectively, in the 300 mg/kg/day females. These changes were not dose-dependent and no similar heart weight decreases in males were observed.

Histopathology

Tissues and organs were collected on the day of sacrifice (week 13). The organs/tissues that were examined are listed in the table above.

Adequate Battery - Yes
Peer Review – No

Histological Findings

There were no meaningful drug-related microscopic findings. Sporadic findings, including minimal cardiomyopathy in the heart, chronic progressive nephropathy in the kidneys, and mononuclear cell infiltration in the liver were observed. However, the incidence of these findings in the 300 mg/kg/day animals was no higher than that of the controls. Therefore, these changes are not considered to be drug-related.

Special Evaluation

N/A

Toxicokinetics

Blood (plasma) were collected by sublingual vein from two cohorts of three control animals/sex (one cohort/time-point) and seven cohorts of three treated animals/sex/group (one cohort/time-point) on Days 1 and 84. Blood collection occurred at approximately 0 (predose, day 84 only), 5, 15, and 30 minutes and at 1, 2, 4, and 8 hours postdose from plecanatide-dosed animals, and at approximately 5 and 30 minutes postdose from control animals. The animals were not fasted prior to blood collection. Plasma levels of plecanatide were measured (lower limit of quantification was 1 ng/ml). The results in male and female rats on day 1 and day 84 are shown in the following table (taken from the Sponsor's study report).

Dose (mg/kg)	Day	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{Tlast} (ng*hr/mL)	$AUC_{(0\text{-}8hr)}$ (ng*hr/mL)
30	1	Female	26.7	0.083	8.61	9.74
		Male	68.6	0.083	12.8	14.2
	84	Female	45.2	0.083	22.7	24.9
		Male	17.5	0.083	12.8	16.4
100	1	Female	68.6	0.083	30.9	31.9
		Male	1230	0.083	187	193
	84	Female	172	0.083	63.7	64.5
		Male	103	0.083	54.5	57.4
300	1	Female	222	0.083	127	127
		Male	597	0.083	152	164
	84	Female	2120	0.5	974	974
		Male	213	0.083	385	385

Plecanatide was detected in plasma at all dose levels. Maximum plasma levels of plecanatide were achieved by 5 minutes after oral administration. There appears to be drug accumulation between day 1 and day 84 after repeated dosing, with the exception of the 100 mg/kg males which displayed a decrease. The accumulation, in terms of increases in C_{max} and AUC, in the 300 mg/kg/day females on day 84 was particularly large compared to that in the 100 mg/kg/day females or the 300 mg/kg/day males. However, the marked increase in the 300 mg/kg/day females was due to a clear outlier in the 30 minute time-point cohort (95.4, 303, and 5950 ng/mL). There were no consistent sex-related differences in plecanatide exposure overall.

Dosing Solution Analysis

The concentration determination of SP-304 in the dosing formulation samples was performed in accordance with the validated method. Analysis of preliminary mixes showed that the preparation procedure used for the study produced homogeneous mixtures. Analyses conducted during the treatment period confirmed that dosing solutions contained drug concentrations approximately equal to the nominal (intended) concentrations.

Study title: Plecanatide: 13-week Oral Toxicity Study in Cynomolgus Monkeys with a 2-week Recovery Period

Study no.:	1896-002
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/20/2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide, lot#: P381009, P381012, and RD071310; purity: 94.6%, 94.8%, 95.9%

Key Study Findings

- Animals (4/sex/group) were treated with 0 (vehicle), 1, 10, or 100 mg/kg/day plecanatide for 13 weeks. Recovery animals (2/sex/group) were treated with 0 or 100 mg/kg/day plecanatide for 13 weeks, followed by a 2-week recovery period.

- There were no deaths during the study.

- Red discolored skin and dry skin were noted in the 100 mg/kg/day males and females.

- A dose-related increase in the incidence of loose/liquid stools occurred in males and females. This is considered a pharmacologic effect of plecanatide, and is not considered as adverse.

- The incidence of sinus tachycardia was greater in the drug-treated groups compared to controls, although there was no clear dose relationship. The effect was not considered as adverse because sinus tachycardia is considered to be a normal variant in monkeys. There were no significant changes in RR, PR, or QT_c intervals, or QRS duration.

- There were no drug-related effects on hematology, clinical chemistry, or urinalysis parameters.

- There were no meaningful organ weight changes, or macroscopic or microscopic findings.

- The NOAEL was considered to be 100 mg/kg/day, the highest dose studied.

Methods

Doses:	0, 1, 10, and 100 mg/kg/day
Frequency of dosing:	once daily
Route of administration:	oral gavage
Dose volume:	5 mL/kg/dose
Formulation/Vehicle:	solution/distilled deionized water
Species/Strain:	Cynomolgus Monkey (Macaca fascicularis)
Number/Sex/Group:	Main study: 4/sex/group Recovery: 2/sex each at 0 and 100 mg/kg/day
Age:	27 to 49 months of age
Weight:	M: 2.29 to 3.16 kg F: 2.30 to 3.02 kg
Satellite groups:	N/A
Unique study design:	N/A
Deviation from study protocol:	During week 13, animals in the 10 mg/kg/day group inadvertently received formulations that were incorrectly prepared to deliver 1 mg/kg/day (due to a dilution error). The deviation did not affect the quality or integrity of the study.

Study Design

GROUP	DOSE LEVEL MG/KG	NUMBER OF ANIMALS													
		TOTAL		CLINICAL PATHOLOGY				NECROPSY				MICROSCOPIC PATHOLOGY			
				TERM		REC		TERM		REC		TERM		REC	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F
1	0	6	6	4	4	2	2	4	4	2	2	4	4	A.R.	A.R.
2	1	4	4	4	4	-	-	4	4	-	-	4	4	-	-
3	10	4	4	4	4	-	-	4	4	-	-	4	4	-	-
4	100	6	6	4	4	2	2	4	4	2	2	4	4	A.R.	A.R.

A.R. = As Required: 1) Target tissues identified by Group 4 evaluations; 2) Tissues in all animals found dead or euthanized in a moribund condition; and 3) gross lesions
TERM = Terminal necropsy; REC = Recovery necropsy.

Observations and Results

Mortality

Animals were observed for morbidity, mortality, injury, and the availability of food and water at least twice daily throughout the duration of the study.

No deaths occurred during the study.

Clinical Signs

A detailed clinical examination of each animal was performed on days -7 and -1 during

the acclimation period and then daily (30 to 90 minutes post-dose) during the study.

Main study:

Pelage/Skin: Red discolored skin was observed in the control group (1/4 M and 1/4 F) 10 mg/kg/day group (1/4 F), and 100 mg/kg/day group (2/4 M and 3/4 F). Dry skin was observed in the 100 mg/kg/day group (2/4 M and 3/4 F).

Recovery group:

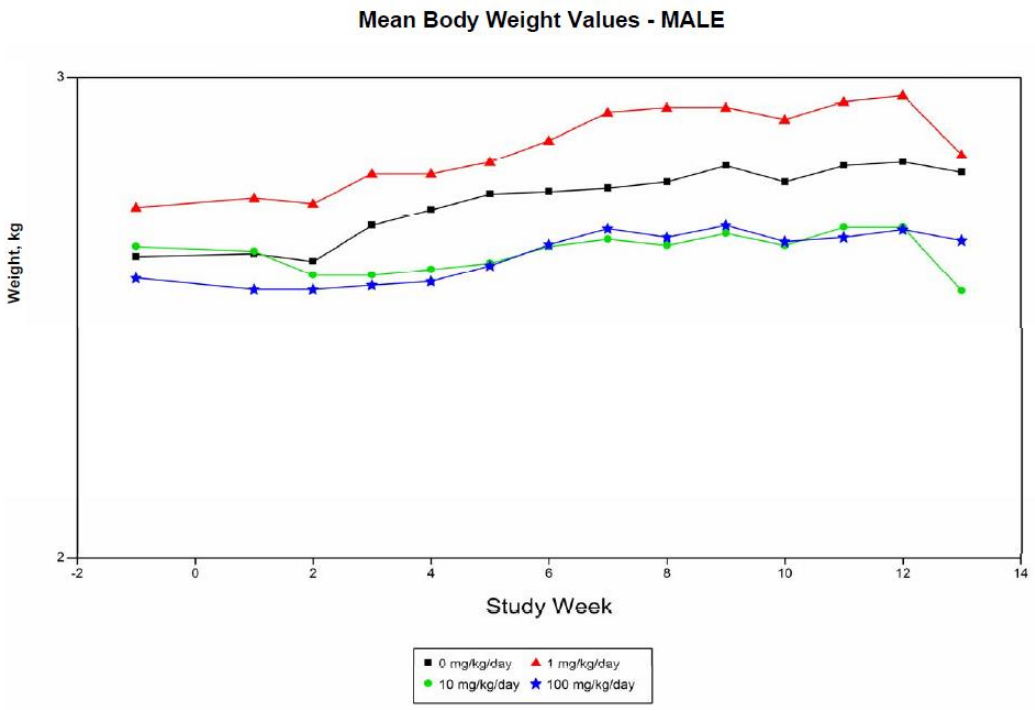
Pelage/skin: Red discolored skin was observed in the 100 mg/kg/day group (1/2 M). Dry skin was observed in the 100 mg/kg/day group (1/2 M).

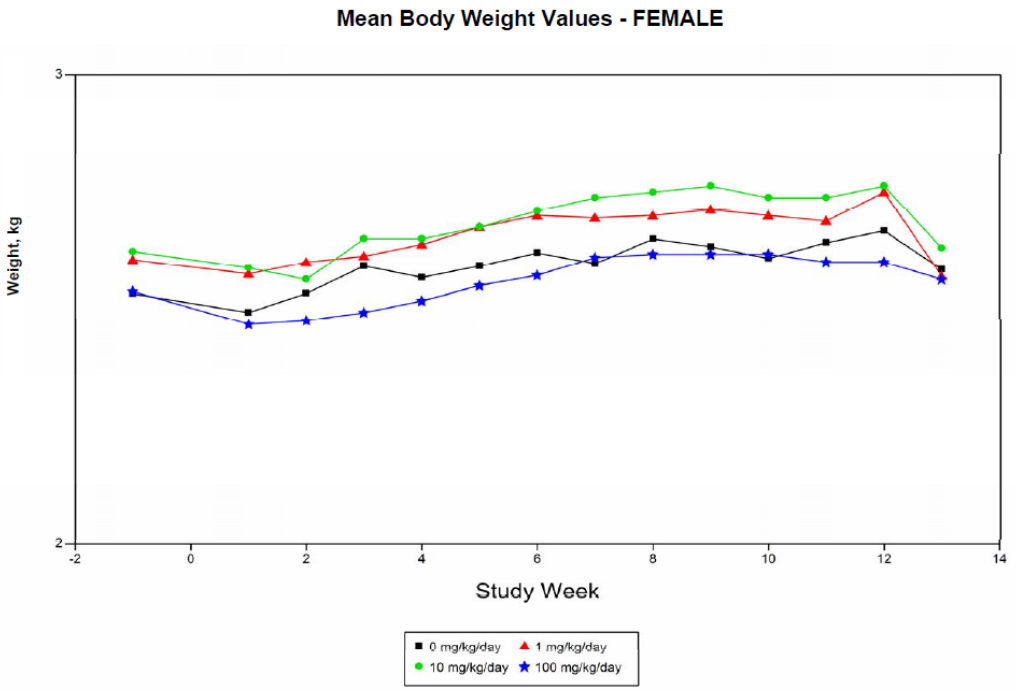
Thus, skin lesions were observed in the 100 mg/kg/day males and females. The skin lesions were not completely reversed in the 100 mg/kg/day males after the 2-week recovery period.

Body Weights

Bodyweights for all animals were measured and recorded at transfer, prior to randomization (day -1), and weekly beginning in week -1.

There were no significant drug-related changes in bodyweight or bodyweight gain.





Feed Consumption

Not measured.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted on all animals during the pre-dose period, and prior to the terminal and recovery phase necropsies.

There were no drug-related ophthalmoscopic findings.

ECG

Animals received an electrocardiographic examination during the pre-dose period, the last week of dosing (day 85), and during the recovery period (day 100). The examinations during the last week of dosing were conducted approximately 2.5 to 3.5 hours after daily dosing. Standard 10-lead ECGs were recorded at 50 mm/sec. The RR, PR, and QT intervals and QRS duration were measured, and heart rate was determined. Corrected QT (QT_c) interval was calculated using Bazett's correction.

Sinus tachycardia, defined as an average heart rate greater than 270 beats per minute for a single interval, occurred in 14 animals at 15 intervals (see Sponsor's table below).

Sinus Tachycardia in Monkeys

	Study Interval			
	Pretest		Terminal	Recovery
Group	Bradycardia	Tachycardia	Tachycardia	Tachycardia
1 (0 mg/kg/day)		103 (M)	126 (F)	
2 (1 mg/kg/day)			107 (M) 109 (F) 110 (F) 127 (M) 129 (F)	
3 (10 mg/kg/day)			111 (M)[a] 133 (F)[a]	
4 (100 mg/kg/day)	137 (M)	136 (M)	116 (M) 117 (M) 118 (F) 135 (M)	116 (M)

a. Due to an error in the preparation of the formulation for the 10 mg/kg/day group in Week 13 of the study, this group was given an actual dose of approximately 1 mg/kg/day during this examination.

The incidence of sinus tachycardia was greater in the drug-treated groups compared to controls, although there was no clear dose relationship. However, the incidence of sinus tachycardia was greatest following drug exposure, and the group mean heart rate was statistically greater than interval matched vehicle control values in the males following the 100 mg/kg/day dose (+16.9% in males). Thus, there was a drug-related effect on the incidence of sinus tachycardia at the high dose. There were no significant changes in RR, PR, or QT_c intervals, or in QRS duration.

The effect was not considered adverse because sinus tachycardia was considered by the Sponsor to be a normal variant in monkeys, and it did not result in QT_c prolongation or a qualitative change in the ECG.

Hematology

Blood samples (approximately 4.8 to 5.8 mL) were collected from the femoral vein on all animals pretest and prior to the terminal and recovery necropsies. There were no significant drug-related changes in hematology parameters, including coagulation values, at terminal sacrifice or after the recovery period.

Clinical Chemistry

There were no significant drug-related changes in clinical chemistry parameters at terminal sacrifice or after the recovery period.

Urinalysis

There were no significant drug-related changes in urinalysis parameters (volume, color and appearance, specific gravity, pH, protein, glucose, bilirubin, ketones, blood,

urobilinogen and microscopy of centrifuged sediment) at terminal sacrifice or after the recovery period.

Gross Pathology

Observations were made at terminal sacrifice on day 91 in the main study groups or day 105 in the recovery groups.

There were no drug-related macroscopic findings at terminal sacrifice or after the recovery period.

Organ Weights

Organs were collected at sacrifice on day 91 for the main study groups and day 105 for the recovery groups. The following organs or tissues were weighed (table taken from the study report):

Organs or Tissues to be Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			Terminal	Recovery
Adrenal gland	X	X	X	
Aorta		X	X	
Bone with bone marrow, femur		X	X	
Bone with bone marrow, rib		X	X	
Bone with bone marrow, sternum		X	X	
Bone marrow smear[a]		X		
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X	
Epididymis	X	X	X	
Esophagus		X	X	
Eye (with optic nerve)		X	X	
Gallbladder		X	X	
GALT[b]		X	X	
Heart	X	X	X	
Joint, tibiofemoral		X	X	
Kidney	X	X	X	
Large intestine, cecum		X	X	
Large intestine, colon		X	X	
Large intestine, rectum		X	X	
Larynx		X	X	
Liver	X	X	X	
Lung with bronchi	X	X	X	

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			Terminal	Recovery
Trachea		X	X	
Ureters		X	X	
Urinary bladder		X	X	
Uterus with cervix		X	X	
Vagina		X	X	
Gross lesions		X	X	X
Tissue masses with regional lymph node[f]		X	X	X

[a] *Bone marrow smears will be prepared only for animals necropsied at scheduled intervals. Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost).*
[b] *Gut Associated Lymphoid Tissue*
[c] *Only the right mandibular salivary gland will be weighed.*
[d] *Target Organs (and target organ gross lesions) will be designated by the Study Director, Pathologist and/or Sponsor based on experimental findings (additional cost).*
[e] *Parathyroids cannot always be identified macroscopically. They will be examined if in the plane of section and in all cases where they are noted as grossly enlarged.*
[f] *A regional lymph node drains the region where a tissue mass is located. A regional lymph node may not always be identified when a mass is present.*

Main Study:

Pituitary gland weight (absolute and relative to the body and brain weights) were higher in the 100 mg/kg/day males (+41% organ/bodyweight) and in the females at all dose levels (+17% to +44% organ/bodyweight) compared to controls. Mandibular salivary gland weights (absolute and relative to body and brain) were lower in 100 mg/kg/day females (-33% organ/bodyweight) compared to controls. The differences in organ weights were not statistically significant.

Recovery group:

Testes and epididymides weights (absolute and relative to body and brain) were lower in the 100 mg/kg/day males.. In the 100 mg/kg/day females, heart, ovaries, pituitary gland, thymus, and thyroid/parathyroid gland weights were different from the controls. However, the differences in organ weights were not statistically significant. In addition, with the exception of pituitary gland weights, the observed changes in organ weights in the recovery groups were not observed in the main study groups.

Histopathology

Tissues and organs were collected at sacrifice on day 91 in the main study groups, and day 105 in the recovery groups. The organs/tissues examined are listed in the above table.

Adequate Battery - Yes

Peer Review - No

Histological Findings

Main Study**:**

Adrenal glands: Mineralization was observed in 1/4 females in the 100 mg/kg/day group.

Esophagus: Lymphocytic infiltration was observed in 1/4 males in the 100 mg/kg/day group.

Femur bone: Hemorrhage was observed in 1/4 females in the 100 mg/kg/day group.

Kidneys: Lymphocytic infiltration was observed in 1/4, 1/4, and 4/4 females in the control, 1, and 100 mg/kg/day groups, respectively.

Large intestine (cecum): Pigmented macrophages was observed in 1/4 females in the 100 mg/kg/day group.

Lung: Eosinophilic perivascular/peribronchiolar infiltration was observed in 1/4 males in the 100 mg/kg/day group.

Salivary gland (mandibular): Lymphocytic infiltration was observed in 2/4, 2/4, and 4/4 females in the 1, 10, and 100 mg/kg/day groups, respectively. Mineralization was observed in 1/4 males in the 100 mg/kg/day group.

Salivary gland (sublingual): Lymphocytic infiltration was observed in 2/4, 1/4, and 3/4 females in the control, 10, and 100 mg/kg/day groups, respectively.

Urinary bladder: Lymphocytic infiltration was observed in 2/4, 1/4 , 2/4, and 1/4 males in the control, 1, 10, and 100 mg/kg/day groups, respectively, and in 1/4, 1/4, 2/4, and 3/4 females in the control, 1, 10, and 100 mg/kg/day groups, respectively.

The microscopic changes listed above are not considered as drug-related because they lacked dose-dependency and/or occurred in a single sex.

Recovery group**:**

No drug-related effects were observed.

Special Evaluation

Fecal Observations and Scoring

A fecal examination for characterization (color and quantity) and scoring of fecal consistency was conducted three times per day during the study. In addition, the presence of a sulfur odor was recorded when observed. Baseline fecal examinations and scoring were conducted during the acclimation period (days -7 to -1). During the dosing period, examinations and scoring were conducted pre-dose and at 2 hr (±15 minutes) and 5 hr (±30 minutes) post-dose. Fecal examinations and scoring during the recovery period were conducted at least three hours apart. Fecal consistency (stool form) was scored as 1 (well formed), 2 (mushy/loose), or 3 (watery). If no feces were present during an examination, a score of 0 was recorded. For each week and for the cumulative score, the number of scores in category was tabulated for each animal. These counts were then converted to percentages using the following methods: 1) the denominator for the count of examinations with no feces present was the total number of examinations (usually 21) during the week; and 2) the denominator for the counts of the examinations with each of the other categories was the total number of examinations with feces present; each week the percentages of examinations with normal (well formed), mushy/loose, or watery feces summed to 100 for each animal. These individual percentages in each category were then averaged by dose level and sex. The table below summarizes the results.

Stool Form Evaluation at Week 13

Sex	Male				Female			
Dose (mg/kg/day)	**0**	**1**	**10**	**100**	**0**	**1**	**10**	**100**
No Feces[1]	7.4	4.2	6.9	4.6	4.6	16.7	4.2	6.5
Well formed[2]	93.1	78.8	69.6*	55.4*	88.3	73.4	76.9	55.4*
Loose[2]	5.9	7.4	18.2	15.4	10.7	9.6	12.8	15.7
Liquid[2]	1.0	13.9	12.2	29.2	1.0	17.0	10.3	28.9

Cumulative Stool Form Evaluation

Sex	Male				Female			
Dose (mg/kg/day)	**0**	**1**	**10**	**100**	**0**	**1**	**10**	**100**
No Feces[1]	7.5	5.4	5.8	4.0	10.2	9.8	5.1	4.6
Well formed[2]	90.4	87.7	69.3*	58.5*	94.4	75.3	65.4	58.6
Loose[2]	6.6	4.9	13.8	14.5	5.2	13.1	15.9	11.4
Liquid[2]	3.0	7.5	16.8	27.0	0.3	11.6	18.6	30.0

* Significantly different from controls

1 Percent based on incidence of absence of feces ÷ total # of examinations

2 Percent based on incidence of stool form ÷ # of examinations with feces present

A dose-related decrease in the incidence of normal feces, and a corresponding increase in the incidence of loose/liquid stools occurred in males and females, although at 1 mg/kg/day the females had more significant changes than the males. The changes were present for the duration of the treatment period. During the recovery period, fecal consistency for the treated animals was comparable to that of the controls. The increase in the incidence of loose/liquid stools is a pharmacologically-expected effect of

plecanatide, and is not considered adverse. It was reported that there was no indication of dehydration in the monkeys, and no microscopic alterations in the GI tract associated with the treatment. A strong sulfur odor was noted in the animal rooms on days 1, 2, 13, and 23 following dosing and prior to dosing on day 7. A few males and females at ≥10 mg/kg/day had green discolored feces on a few occasions. However, this did not occur in a dose-related pattern, and the number of instances over the 13-week treatment period was minimal.

Toxicokinetics

Blood samples were collected on day 1 prior to dosing, and on days 1 and 90 at 5, 15, 30 minutes, and 1, 1.5, 2, and 3 hours after dosing.

There was only negligible systemic exposure at 1 mg/kg/day. The exposure increased with dose between 10 to 100 mg/kg/day. At 100 mg/kg/day, the maximum plecanatide plasma concentrations were observed between 15 and 60 minutes after dosing (T_{max}= 0.52±0.39 hr), and the levels declined rapidly thereafter. $T_{1/2}$ was 0.61 ± 0.27 hr (n = 7) and 0.65 ± 0.20 hr (n = 5) on days 1 and 90, respectively, in the 100 mg/kg/day animals. There were no significant sex-related differences in systemic exposure. The toxicokinetics data are summarized in the table below taken from the study report.

Mean (±SD) Toxicokinetic Parameters in Male and Female Monkeys

Day	Dose (mg/kg/day)	Sex	$AUC_{0\text{-tlast}}$ (hr•ng/mL)	C_{max} (ng/mL)	$t_{1/2}$ (hr)	T_{max} (hr)
1	1	M	0±0 (4)	0±0 (4)	NC	NC
		F	0±0 (3)	3.10±6.20 (4)	NC	0.0833 (1)
		Combined	0±0	1.55±4.38	NC	0.0833
	10	M	12.0±16.9 (2)	14.4±14.4 (4)	NC	0.583±0.382 (3)
		F	2.48±4.96 (4)	8.10±16.2 (4)	NC	0.0833 (1)
		Combined	5.64±9.80	11.2±14.6	NC	0.458±0.400
	100	M	70.8±30.0 (6)	75.4±47.2 (6)	0.424±0.00819 (3)	0.639±0.417 (6)
		F	73.7±47.2 (6)	61.3±46.2 (6)	0.751±0.281 (4)	0.403±0.347 (6)
		Combined	72.3±37.8	68.3±45.1	0.611±0.265	0.521±0.386
90	1	M	9.00±15.6 (3)	8.83±11.9 (4)	NC	0.750 (2)
		F	0±0 (3)	3.13±6.25 (4)	NC	0.500 (1)
		Combined	4.50±11.0	5.98±9.29	NC	0.667±0.288
	10*	M	0±0 (4)	0±0 (4)	NC	NC
		F	0±0 (4)	0±0 (4)	NC	NC
		Combined	0±0	0±0	NC	NC
	100	M	83.3±39.8 (5)	62.3±47.1 (6)	0.700±0.262 (3)	0.750±0.500 (6)
		F	76.6±25.0 (5)	59.7±27.2 (6)	0.585 (2)	0.681±0.382 (6)
		Combined	80.0±31.5	61.0±36.7	0.654±0.202	0.715±0.426

NC = not calculated; insufficient data.
Where no SD is given, the mean is comprised of fewer than 3 observations. N is given in parentheses following the mean and SD.
*Animals actually got 1 mg/kg/day for the last week of treatment. Therefore, these parameters are not correct for a 10 mg/kg/day dose.

Dosing Solution Analysis

The concentration determination of plecanatide in the dosing formulation samples was performed in accordance with the validated method. Analyses conducted during the treatment period showed that the concentrations of dose formulations at 0.2, 2.0, and 20.0 mg/ml, with the exception of the 2.0 mg/ml samples from week 13, were approximately within 10% of the target value. Back-up sample analysis confirmed the lower concentration (0.2 mg/ml) measured in the samples from the nominal 2.0 mg/mL formulations from week 13, and thus a dilution error was suspected in the formulation preparation.

The following study was reviewed under IND 74,883 (Pharmacology/Toxicology review dated 8/2/2013). The review is included verbatim below.

Study title: Plecanatide: 26-week oral toxicity study in mice with a 4-week recovery period

Study no.:	1896-008
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/12/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide (b) (4) 110111 and 110425, and 96.5% and 97.8%

Key Study Findings

Animals (20/sex/group) were treated with 0 (vehicle), 20, 60, 150, or 400 mg/kg/day plecanatide for 26 weeks. Recovery animals (10/sex/group) were treated with 0, 150, or 400 mg/kg/day plecanatide for 26 weeks, followed by a 4-week recovery period. Eighteen animals were found dead or euthanized *in extremis* as follows: 3, 3, 6, 3, and 3 in the control, 20, 60, 150, and 400 mg/kg/day groups, respectively (20 mice/sex/main study group). The deaths are not considered drug-related because there were no clinical observations which indicated toxicity, no consistent macroscopic or microscopic findings that support a particular cause of death in these animals, and there was no dose relationship. Significant decreases in absolute reticulocyte count were observed in the 400 mg/kg/day males (-26%) and females (-25%) during the treatment period, and the effect was reversible after the recovery period. Sciatic nerve axonal/myelin degeneration was observed in the 400 mg/kg/day males and females (1/39 in the controls and 5/39 in the 400 mg/kg/day animals). AUC could not be measured in the 20 or 60 mg/kg/day mice on day 1, or in the 20 mg/kg/day mice at week 26. The increase in AUC_{tlast} was generally dose proportional between 150 and 400 mg/kg/day. There was no significant drug accumulation between day 1 and week 26 after repeated dosing. During week 26, the average C_{max} value for both sexes in the 20 mg/kg/day mice was 25.9 ng/mL, and the average C_{max} and $AUC_{0\text{-}tlast}$ values for both sexes in the

400 mg/kg/day mice were 150 ng/mL and 30.7 ng·h/mL, respectively. There were no significant sex differences in plecanatide exposures on day 1 or week 26. The NOAEL is considered to be 150 mg/kg/day based on a significant decrease in absolute reticulocyte count and sciatic nerve axonal/myelin degeneration at 400 mg/kg/day plecanatide.

Methods

Doses:	0, 20, 60, 150, and 400 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg/dose
Formulation/Vehicle:	Distilled deionized water
Species/Strain:	Mouse/Crl:CD1 (ICR)
Number/Sex/Group:	Main study: 20/sex/group Recovery: 10/sex/group at 0, 150, and 400 mg/kg/day
Age:	6 weeks
Weight:	M: 27 to 34 g; F: 21-26 g
Satellite groups:	Toxicokinetic groups: 17/sex/group at 0 mg/kg/day and 47/sex/group at 20, 60, 150, and 400 mg/kg/day
Unique study design:	N/A
Deviation from study protocol:	Minor deviations occurred that did not affect the quality or integrity of the study.

The following table shows the design of the study (taken from Sponsor's study report).

Study Design

Group	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dose Volume (mL/kg/dose)	Number of Animals	
				Males	Females
Main Study					
1	0	0	10	30[a]	30[a]
2	20	2	10	20	20
3	60	6	10	20	20
4	150	15	10	30[a]	30[a]
5	400	30	10	30[a]	30[a]
Toxicokinetic (TK) Study					
6	0	0	10	15+2*	15+2*
7	20	2	10	45+2*	45+2*
8	60	6	10	45+2*	45+2*
9	150	15	10	45+2*	45+2*
10	400	30	10	45+2*	45+2*

[a]10 animals/sex will be designated for a 4-week recovery period.

**Additional animals/sex/treatment group included as replacement animals.*

Observations and Results

Mortality

Animals were observed twice daily. The following table lists the animals that were either found dead or euthanized *in extremis* (taken from Sponsor's study report). The table also lists the causes of death.

Unscheduled Deaths			
Animal Number	**Dose Level**	**Sex**	**Cause of Death**
88[*]	60 mg/kg/day	M	Undetermined (DOS; Day 5)
426[*]	0 mg/kg/day	F	Dosing injury (DOS; Day 6)
440[*]	0 mg/kg/day	F	Dosing injury (DOS; Day 6)
3027	0 mg/kg/day	M	Skin inflammation/necrosis (E; Day 155)
3549	20 mg/kg/day	F	Lymphoid tumor (DOS; Day 80)
3674[b]	20 mg/kg/day	F	Undetermined (DOS; Day 23)
3694[b]	20 mg/kg/day	F	Undetermined (E; Day 156)
3052	60 mg/kg/day	M	Skin inflammation/necrosis (E; Day 155)
3054	60 mg/kg/day	M	Skin inflammation/necrosis (E; Day 155)
3057	60 mg/kg/day	M	Urogenital inflammation/obstruction/calculi (E; Day 165)
3219[b]	60 mg/kg/day	M	Urogenital inflammation/obstruction/calculi (E; Day 180)
3236[b]	60 mg/kg/day	M	Skin inflammation/necrosis (E; Day 119)
3078	150 mg/kg/day	M	Skin inflammation/necrosis (E; Day 165)
3079	150 mg/kg/day	M	Skin inflammation/necrosis (E; Day 165)
3099	150 mg/kg/day	M	Skin inflammation/necrosis (E; Day 165)
3616	400 mg/kg/day	F	Undetermined (DOS; Day 169)
3628[a]	400 mg/kg/day	F	Skin inflammation/necrosis (E; Day 206)
3629[a]	400 mg/kg/day	F	Lymphoid tumor (DOS; Day 203)

DOS - Died on Study (Found Dead)
E - Euthanized *in extremis*
M - Male F - Female
[a]Recovery animal [b]Toxicokinetic (TK) animal [*]Animal replaced shortly after study start

Animals were observed twice daily. Eighteen animals were found dead or euthanized *in extremis*, as follows: 3, 3, 6, 3, and 3 in the control, 20, 60, 150, and 400 mg/kg/day groups, respectively (20 mice/sex/main study group). Of the 18 unscheduled deaths, 4 occurred during the first month of treatment and are not considered drug-related. Eight of the dead mice had skin inflammation/necrosis and had to be euthanized *in extremis*. There was no apparent dose-dependency in the frequency of the skin inflammation/ necrosis (1 at 0 mg/kg/day, 3 at 60 mg/kg/day, 3 at 150 mg/kg/day, and 1 at 400 mg/kg/day). Lymphoid tumors were observed in two of the dead mice (1 at 20 mg/kg/day and 1 at 400 mg/kg/day). None of the deaths are considered as drug-related, because there were no clinical observations that indicated toxicity, no consistent macroscopic or microscopic findings that support a particular cause of death in these animals, and there was no dose relationship.

Clinical Signs

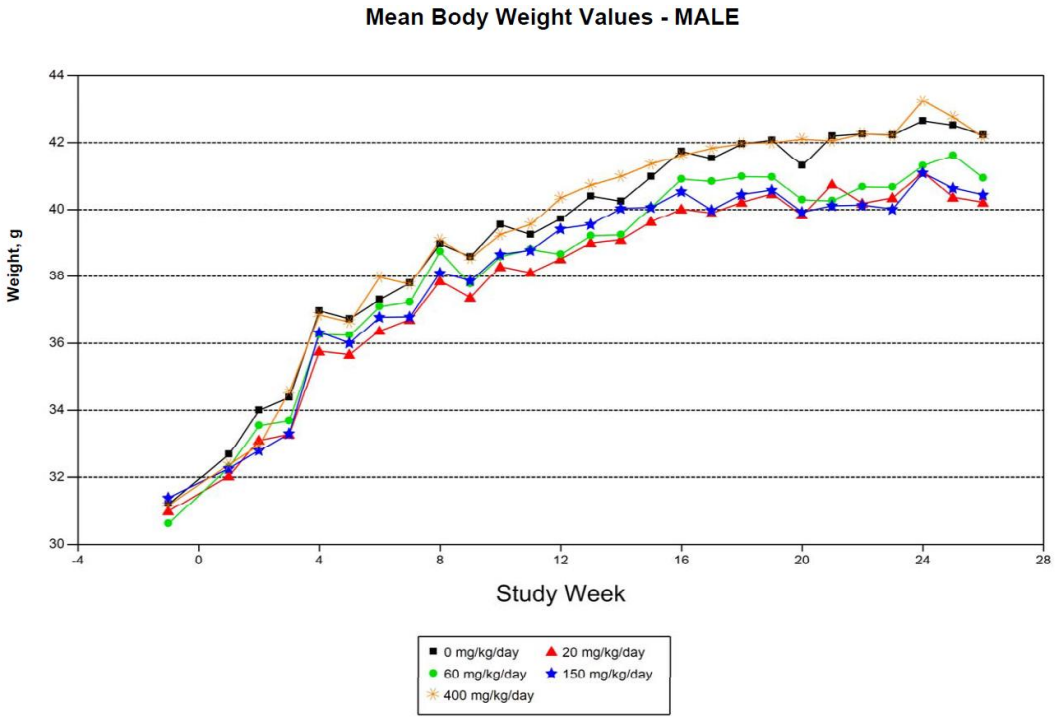
A detailed clinical examination was performed on the main study animals prior to dosing and weekly during the treatment and recovery periods. On occasion, clinical observations were recorded at unscheduled intervals for all animals. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects,

autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior. Cageside clinical observation was performed once daily (30 to 90 minutes postdose) on main study animals during the treatment period.

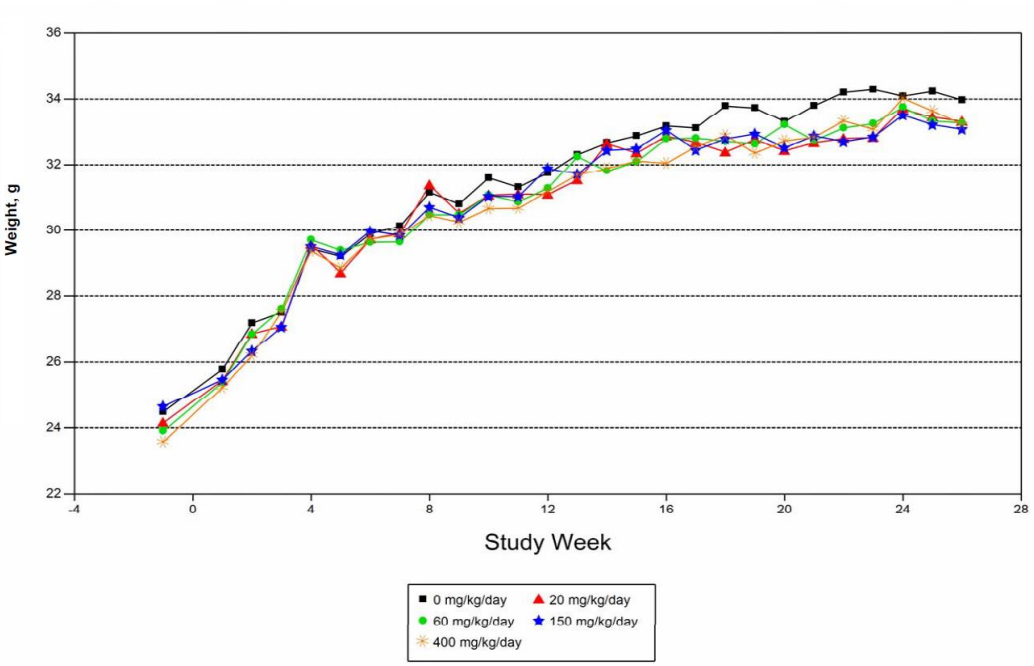
There were no drug-related clinical signs during either the treatment or recovery period.

Body Weights

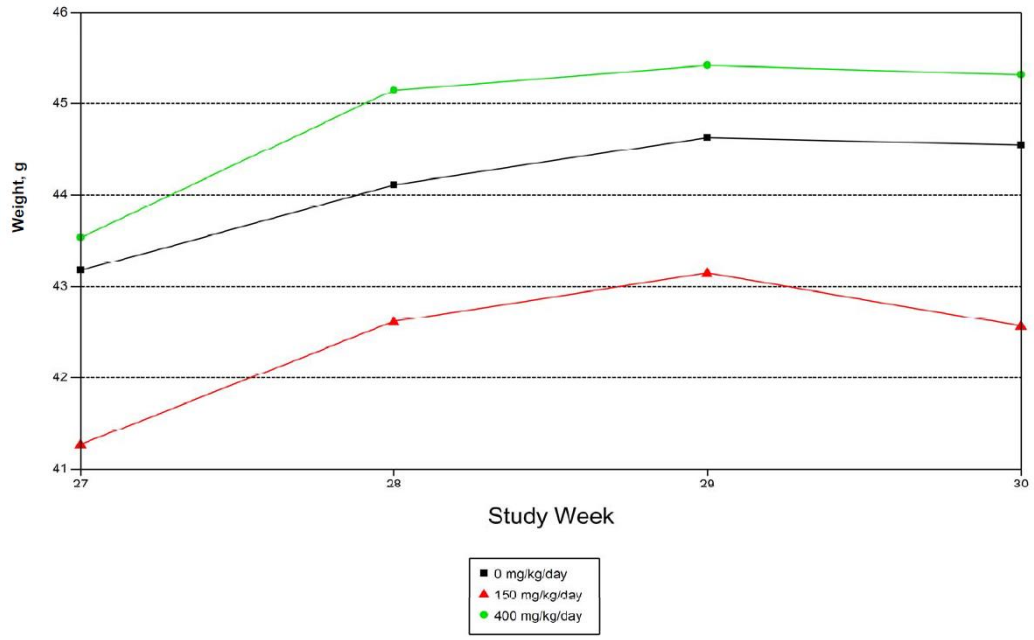
Body weights were measured and recorded three days following arrival, prior to randomization (day -1), and weekly during the study. The body weights during the treatment and recovery periods are presented in the following charts (taken from the Sponsor's study report).

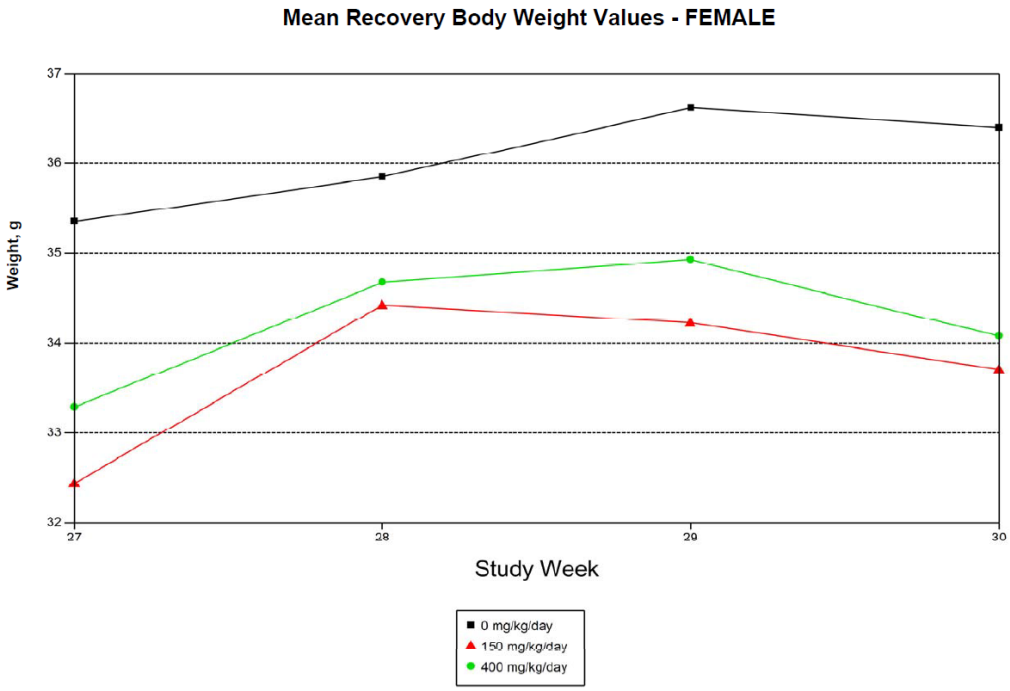


Mean Body Weight Values - FEMALE



Mean Recovery Body Weight Values - MALE

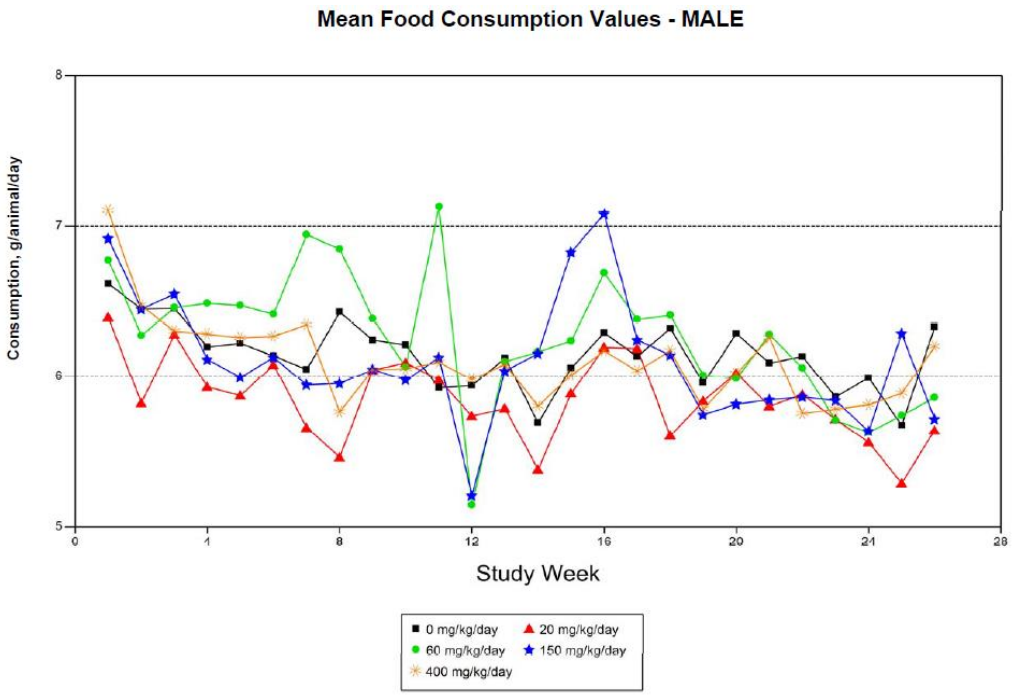


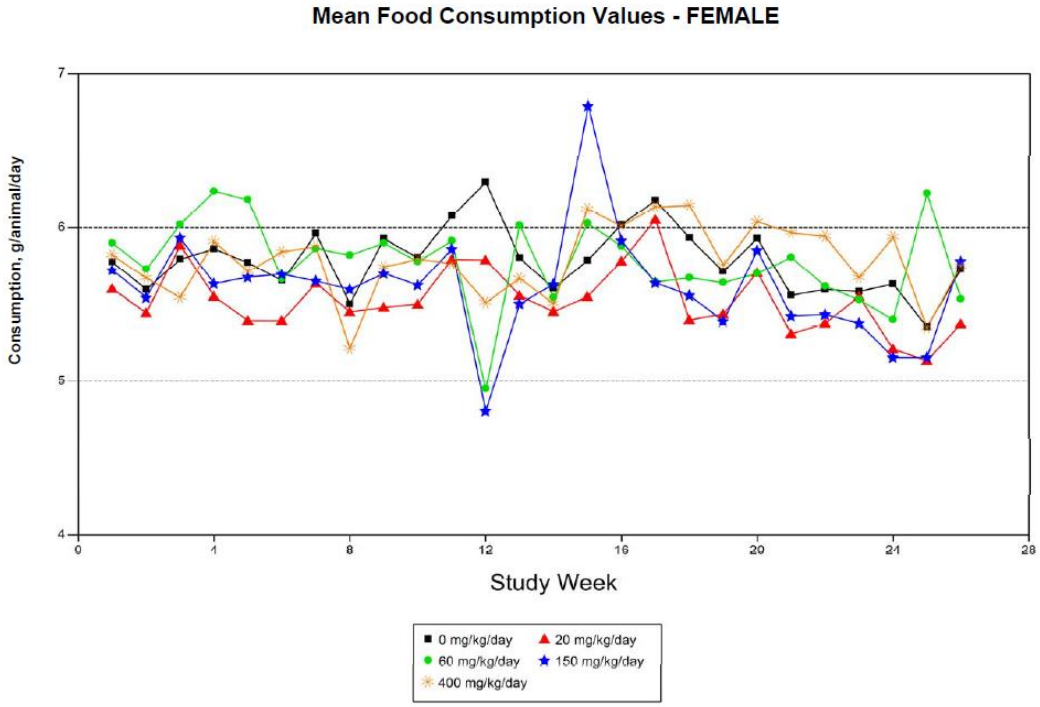


There were no significant drug-related changes in body weights during the treatment or recovery period.

Feed Consumption

Food consumption was measured and recorded weekly for the main study animals during the study. The tables below show the food consumption for the main study animals during the treatment period (taken from the Sponsor's study report).





There were no significant drug-related changes in food consumption during either the treatment or recovery period.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted on all animals pretest and on main

study animals prior to terminal necropsies.

Corneal opacity, blepharitis, and retinal atrophy were the most frequently observed changes; however, the incidences did not occur in a dose-dependent manner and are not considered drug-related.

ECG

N/A

Hematology

Hematology evaluations were conducted on approximately 10 animals/sex/group in the main study groups at necropsy on week 26, and approximately 5 animals/sex/group at the recovery necropsy at week 30. Blood samples also were collected from all animals euthanized *in extremis*.

The hematologic parameters evaluated included hematocrit (Hct), hemoglobin (Hb), erythrocyte count (RBC), reticulocyte count (Retic), leukocyte count (total and absolute differential), mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelet count, and blood cell morphology.

There were dose-related decreases in absolute reticulocyte count during the treatment period, and the decrease was statistically significant at 400 mg/kg/day in both males and females. The changes are shown in the table below.

Changes in reticulocyte count at weeks 26 and 30

Parameter	Week	Sex	% change compared to controls			
			20 mg/kg/day	60 mg/kg/day	150 mg/kg/day	400 mg/kg/day
Reticulocytes	26	M	-13%	-23%	-24%	-26%*
		F	-1%	-17%	-8%	-25%*
	30(R)	M			+5%	-1%
		F			+3%	+2%

* $p < 0.05$
R: recovery

There were other sporadic changes; however, the changes were small in magnitude, not dose-dependent, and/or occurred only in one gender. There were no meaningful differences between treated and control animals during the recovery period.

Clinical Chemistry

Clinical chemistry evaluations were conducted on approximately 10 animals/sex/group in the main study groups at necropsy at week 26, and approximately 5 animals/sex/ group at the recovery necropsy at week 30. Blood samples also were collected from all animals euthanized *in extremis*.

The following parameters were evaluated: alkaline phosphatase (AP), total bilirubin (with direct bilirubin if total bilirubin exceeds 1 mg/dL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, total protein, albumin, globulin and A/G (albumin/globulin) ratio, glucose, calcium, phosphorus, amylase, and lipase.

There were no meaningful drug-related changes in clinical chemistry parameters at the terminal or recovery phase. However, the following changes were observed. These changes are not considered adverse because they lack dose-dependency and/or occurred only in the recovery phase.

Parameter	Week	Sex	% change compared to controls			
			20 mg/kg/day	60 mg/kg/day	150 mg/kg/day	400 mg/kg/day
AP (U/L)	30(R)	M				+59%
Total Bilirubin	30(R)	M				+43%
AST (U/L)	26	F			+25%*	+5%
	30(R)	M F			+19% -25%	+22% -21%
ALT (U/L)	26	M F			+38%* +41%*	+28% +8%
	30 (R)	M F			+25% -33%	+46% -25%
Lipase (U/L)	30 (R)	M				+17%

* $p < 0.05$

Urinalysis

N/A

Gross Pathology

Observations were made at sacrifice (weeks 26 and 30) or immediately after premature death. Necropsy examinations were performed on animals euthanized *in extremis*, animals found dead, all surviving animals at the scheduled terminal and recovery necropsies, and various toxicokinetic (TK) animals found dead or euthanized *in extremis.*

Premature Deaths

There were no meaningful drug-related macroscopic findings at necropsy in animals euthanized *in extremis*. There were sporadic observations that lacked dose-dependency or occurred only in a single sex.

Main Study

There were no meaningful drug-related macroscopic findings at terminal necropsy. However, the following changes were noted:

Lymph node: Enlarged mandibular node was observed in the 20 mg/kg/day (2/20) and 400 mg/kg/day (2/20) males.

Recovery Phase

There were no meaningful drug-related macroscopic findings.

Organ Weights

Organs were collected on the day of sacrifice (weeks 26 and 30). The following organs were collected and their weights were measured (table taken from the Sponsor's study report).

Organs or Tissues to be Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 5	2, 3, 4	Rec[a]
Adrenal gland	X	X	X		
Aorta		X	X		
Bone with bone marrow, femur		X	X		
Bone with bone marrow, sternum		X	X		
Bone marrow smear[b]		X			
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X		
Epididymis	X	X	X		
Esophagus		X	X		
Eye (with optic nerve)		X	X		
Gallbladder		X	X		
GALT[c]		X	X		
Heart	X	X	X		
Joint, tibiofemoral		X	X	X	X
Kidney	X	X	X		
Lacrimal gland, exorbital		X	X		
Large intestine, cecum		X	X		
Large intestine, colon		X	X		
Large intestine, rectum		X	X		
Larynx		X	X		

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 5	2, 3, 4	Rec[a]
Liver	X	X	X		
Lung with bronchi	X	X	X		
Lymph node, mandibular		X	X		
Lymph node, mesenteric		X	X		
Mammary gland (process females only)		X	X		
Nerve, sciatic		X	X		
Ovary	X	X	X		
Oviducts		X	X		
Pancreas		X	X		
Pituitary	X	X	X	X	X
Prostate		X	X		
Salivary gland, mandibular[d]	X	X	X		
Salivary gland, parotid		X	X		
Salivary gland, sublingual		X	X		
Seminal vesicles		X	X		
Skeletal muscle, biceps femoris		X	X		
Skin		X	X		
Small intestine, duodenum		X	X		
Small intestine, ileum		X	X		
Small intestine, jejunum		X	X		
Spinal cord, cervical		X	X		
Spinal cord, lumbar		X	X		
Spinal cord, thoracic		X	X		
Spleen	X	X	X		
Stomach, glandular		X	X		
Stomach, nonglandular		X	X		
Target Organs[e]		X	X	X	X
Testis	X	X	X		
Thymus	X	X	X		

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 5	2, 3, 4	Rec[a]
Thyroid gland (with parathyroid)[f]	X	X	X		
Tongue		X	X		
Trachea		X	X		
Ureters		X	X		
Urinary bladder		X	X		
Uterus with cervix		X	X		
Vagina		X	X		
Gross lesions		X	X	X	X
Tissue masses with regional lymph node[g]		X	X	X	X

[a] *Recovery*
[b] *Bone marrow smears will be prepared only for animals necropsied at scheduled intervals. Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost). Bone marrow smears will not be collected from animals that do not survive until terminal necropsy.*
[c] *Gut Associated Lymphoid Tissue*
[d] *The combined weight of the right mandibular/sublingual salivary gland will be obtained.*
[e] *Target organs (and target organ gross lesions) will be designated by the Study Director, Pathologist and/or Sponsor based on experimental findings (additional cost).*
[f] *Parathyroids cannot always be identified macroscopically. They will be examined if in the plane of section and in all cases where they are noted as grossly enlarged.*
[g] *A regional lymph node drains the region where a tissue mass is located. A regional lymph node may not always be identified when a mass is present.*

Absolute weight, relative weight (organ/bodyweight), and organ/brain weight ratio were reported. There were no drug-related meaningful organ weight changes at the terminal or recovery phase. However, the following changes were observed:

Main Study

Adrenal glands: Absolute weight, organ/body weight, and organ/brain weight ratio increased by 44%, 45%, and 41%, respectively, in 150 mg/kg/day males compared to controls, and absolute weight, organ/body weight, and organ/brain weight ratio increased by 22%, 24%, and 22%, respectively, in 400 mg/kg/day males compared to controls.

Recovery Phase

Adrenal gland: Absolute weight, organ/body weight, and organ/brain weight ratio increased by 50%, 33%, and 45%, respectively, in 400 mg/kg/day males compared to controls. Absolute weight, organ/body weight, and organ/brain weight ratio increased

by 31%, 41%, and 34%, respectively, in the 150 mg/kg/day females, and decreased by 25%, 20%, and 23%, respectively, in the 400 mg/kg/day females, compared to controls.

Ovary: Absolute weight, organ/body weight, and organ/brain weight ratio decreased by 32%, 32%, and 34%, respectively, in the 400 mg/kg/day females compared to controls.

Pituitary gland: Absolute weight, organ/body weight, and organ/brain weight ratio decreased by 27%, 23%, and 26%, respectively, in the 150 mg/kg/day females, and decreased by 27%, 24%, and 25%, respectively, in the 400 mg/kg/day females, compared to controls.

Salivary gland (mandibular/submandibular): Absolute weight, organ/body weight, and organ/brain weight ratio decreased by 18%*, 13%, and 18%*, respectively, in the 150 mg/kg/day females, and 14%*, 10%, and 13%, respectively, in the 400 mg/kg/day females, compared to controls.

Spleen: Absolute weight, organ/body weight, and organ/brain weight ratio decreased by 34%, 40%, 36%, respectively, in 400 mg/kg/day males compared to controls.

Thymus: Absolute weight, organ/body weight, and organ/brain weight ratio decreased by 43%, 39%, and 44%, respectively, in 400 mg/kg/day males, and decreased by 37%, 35%, and 37%, respectively, in the 400 mg/kg/day females, compared to controls.

Thyroid/parathyroid gland: Absolute weight, organ/body weight, and organ/brain weight ratio decreased 25%, 16%, and 18%, respectively, in the 400 mg/kg/day females compared to controls.

Statistically significant increases in mandibular/submandibular salivary gland weights were observed at the recovery phase necropsy in the 150 and 400 mg/kg/day females. These increases are considered to be incidental since there were no microscopic correlates and no similar weight increases in males.

Histopathology

Tissues and organs were collected on the day of sacrifice: week 26 for the main study groups, week 30 for the recovery groups. The organs/tissues that were examined are listed in the table above.

Adequate Battery - Yes
Peer Review – Yes

Histological Findings

<u>Premature Deaths</u>

There were no meaningful drug-related microscopic findings in the animals that died prematurely.

Main Study

The following microscopic findings were observed.

Organ/Tissue	Sex	End of treatment period					Recovery		
		Dose (mg/kg/day)					Dose (mg/kg/day)		
		0	20	60	150	400	0	150	400
Joint (tibiofemoral)									
Degenerative joint disease	M	0/19	2/19	0/17	0/17	1/19	0/10	0/10	0/10
Kidneys									
Cyst	M	0/19		1/3		1/20			0/1
Lymphocytic infiltration	F	4/20		1/1		7/20			0/0
Lung									
Lymphocytic infiltration	M	0/19		0/1		1/20			0/1
Subacute/chronic inflammation	M	0/19		0/1		1/20			0/1
Pigmented alveolar macrophages	M	0/19		0/1		1/20			0/1
Alveolar histiocytosis	F	1/20				3/20			0/0
Lymph node (mandibular)									
Medulla lymphocyte/plasmacyte hyperplasia	M	0/18	2/2	0/1		2/20			0/1
Lymph node (mesenteric)									
Amyloid	M	0/19				1/20			0/1
Mammary gland									
Glandular dilatation	F	0/20				1/19			0/0
Nerve (sciatic)									
Axonal/myelin	M	0/19	0/0	0/0	0/0	2/20	0/0	0/0	0/1
Degeneration	F	1/20	0/0	0/0	0/0	3/19	0/0	0/0	0/0
Ovary									
Cyst	F	1/20	2/2	1/1	1/1	2/19	1/1		0/0
Pancreas									
Subacute/chronic Inflammation	M	0/19				1/20			0/1
Pituitary									
Cyst	M	0/19	1/20	2/17	1/17	0/20	0/10	0/10	0/10
	F	0/20	0/19	0/20	0/20	1/19	0/10	0/10	0/8
Prostate gland									
Lymphocytic infiltration	M	0/19				1/20			0/1
Salivary gland (mandib.)									
Lymphocytic infiltration	F	0/20				5/20			0/0
Small intestine (ileum)									

Amyloid	M	0/19				2/20			0/1
Lymphocytic infiltration	M	0/19				1/20			0/1
Stomach (glandular)									
Keratin cyst	M	0/19				1/20			0/1
Lymphocytic infiltration	M	0/19				1/20			0/1
Thymus									
Lymphoid generalized Depletion	F	1/20		0/1		3/20			0/0
Urinary bladder									
Lymphocytic infiltration	F	3/20				5/19			0/0
Uterus with cervix									
Cystic endometrial Hyperplasia	F	11/20	1/1		2/2	14/19	1/1		1/1
Vagina									
Acute inflammation	F	0/20				1/19			0/0

Sciatic nerve axonal/myelin degeneration was observed in the 400 mg/kg/day males and females (1/39 in controls and 5/39 in 400 mg/kg/day animals). Dose-dependency of these changes cannot be verified because tissues in the 20, 60, and 150 mg/kg/day animals were not evaluated. Sciatic nerve was examined in one recovery male in the 400 mg/kg/day group. The other changes are not considered as drug-related because these occurred at very low frequency, lack dose-dependency, and/or occurred in a single sex.

Special Evaluation

N/A

Toxicokinetics

Blood samples were collected from three toxicokinetic (TK) animals/sex/group via cardiac puncture. For TK animals at 0 mg/kg/day, samples were collected at 15 minutes and 3 hours post-dose on day 1, and pre-dose, 15 minutes, and 3 hours post-dose at week 26. For TK animals at 20, 60, 150, and 400 mg/kg/day, samples were collected at 2, 5, 15, and 30 minutes, 1, 1.5, and 3 hours post-dose on day 1, and pre-dose, 2, 5, 15, and 30 minutes, 1, 1.5, and 3 hours post-dose at week 26. The animals were not fasted prior to blood collection. Plasma levels of plecanatide were measured (lower limit of quantification was 10 ng/ml). The results in male and female mice on day 1 and week 26 are shown in the following table (taken from the Sponsor's study report).

Toxicokinetic Parameters for Plecanatide in Male and Female Mice

Day/Week	Dose (mg/kg/day)	Sex	AUC_{0-3} (hr•ng/mL)	$AUC_{0-tlast}$ (hr•ng/mL)	C_{max} (ng/mL)	T_{max} (hr)
Day 1	20	M	NC	NC	4.50	0.0830
		F	NC	NC	13.2	0.0830
		Combined	NC	NC	8.83	0.0830
	60	M	0	0	0	NC
		F	NC	NC	17.9	0.0830
		Combined	0	0	8.95	0.0830
	150	M	NC	NC	77.2	0.0830
		F	15.5	13.6	93.3	0.0830
		Combined	15.5	13.6	85.2	0.0830
	400	M	28.0	25.4	180	0.0830
		F	36.6	35.6	197	0.0830
		Combined	32.3	30.5	189	0.0830
Week 26	20	M	NC	NC	3.80	0.0330
		F	NC	NC	48.0	0.0830
		Combined	NC	NC	25.9	0.0580
	60	M	NC	NC	23.2	0.0830
		F	19.5	9.76	77.7	0.250
		Combined	19.5	9.76	50.4	0.167
	150	M	20.2	15.0	72.3	0.0830
		F	9.04	7.10	47.7	0.0830
		Combined	14.6	11.0	60.0	0.0830
	400	M	44.5	35.4	182	0.0830
		F	31.7	26.0	117	0.0330
		Combined	38.1	30.7	150	0.0580

NC = not calculated; insufficient data.

Plecanatide was detected in plasma at all dose levels. However, due to the limited number of time-points with detectable drug levels, AUC could not be measured in the 20 or 60 mg/kg/day mice on day 1, or in the 20 mg/kg/day mice at week 26. Maximum plasma levels of plecanatide were achieved by 5 minutes after oral administration. The increases in $AUC_{0-tlast}$ were generally dose proportional between 150 and 400 mg/kg/day. There was no significant drug accumulation between day 1 and week 26 after repeated dosing. There were no significant sex-related differences in plecanatide exposures on day 1 or week 26.

Dosing Solution Analysis

The concentration determination of SP-304 in the dosing formulation samples was performed in accordance with the validated method. Analysis of preliminary mixes showed that the preparation procedure used for the study produced homogeneous mixtures. Analyses conducted during the treatment period confirmed that dosing solutions contained drug concentrations approximately equal to the nominal (intended) concentrations.

Study title: Plecanatide: A 39-week Oral (Gavage) Toxicity Study in Cynomolgus Monkeys

Study no.:	1896-009
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/12/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide, lot # 110111(97.5%), 110425 (97.8%)

Key Study Findings

- Animals (4/sex/group) were treated with 0 (vehicle), 2, 10, or 100 mg/kg/day plecanatide for 39 weeks.
- There were no deaths during the study.
- A drug-related increase in the incidence of loose/watery stools occurred in males and females at doses ≥ 2 mg/kg/day. This is considered a pharmacologic effect of plecanatide, and is not considered as adverse.
- A slightly higher incidence of sinus tachycardia was observed in the 100 mg/kg/day group. The effect was not considered adverse because sinus tachycardia is considered to be a normal variant in monkeys. There were no significant drug-related changes in RR, PR, or QT_c intervals, or in QRS duration.
- There were no drug-related effects on hematology, clinical chemistry, or urinalysis parameters.
- There were no meaningful organ weight changes, or macroscopic or microscopic findings.
- The NOAEL was considered to be 100 mg/kg/day, the highest dose studied.

Methods

Doses:	0 (vehicle), 2, 10, and 100 mg/kg/day
Frequency of dosing:	once daily
Route of administration:	oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	distilled deionized water
Species/Strain:	Cynomolgus Monkey (Macaca Fascicularis)

Number/Sex/Group:	4/sex/group
Age:	27 to 43 months of age
Weight:	M: 2.43 to 2.96 kg F: 2.51 to 2.86 kg
Satellite groups:	N/A
Unique study design:	N/A
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

Observations and Results

Mortality

Animals were observed for morbidity, mortality, injury, and the availability of food and water at least twice daily throughout the duration of the study.

No deaths occurred during the study.

Clinical Signs

A detailed clinical examination of each animal was performed weekly during the study.

Notable clinical signs:

Behavior/Activity: Inappetence was observed in 1/4 males in the 100 mg/kg/day group, and in 2/4, 2/4, and 1/4 females in the control, 10, and 100 mg/kg/day groups, respectively.

Stool form: Soft feces was observed in 3/4, 2/4, 4/4, and 4/4 males in the control, 2, 10, and 100 mg/kg/day groups, respectively, and in 4/4, 3/4, 4/4, and 4/4 females in the control, 2, 10, and 100 mg/kg/day groups, respectively. Watery feces was observed in 2/4, 4/4, 4/4, and 4/4 males in the control, 2, 10, and 100 mg/kg/day groups, respectively, and in 4/4, 4/4, and 4/4 females in the 2, 10, and 100 mg/kg/day groups, respectively. Gray discolored feces was observed in 2/4 females in the 100 mg/kg/day group.

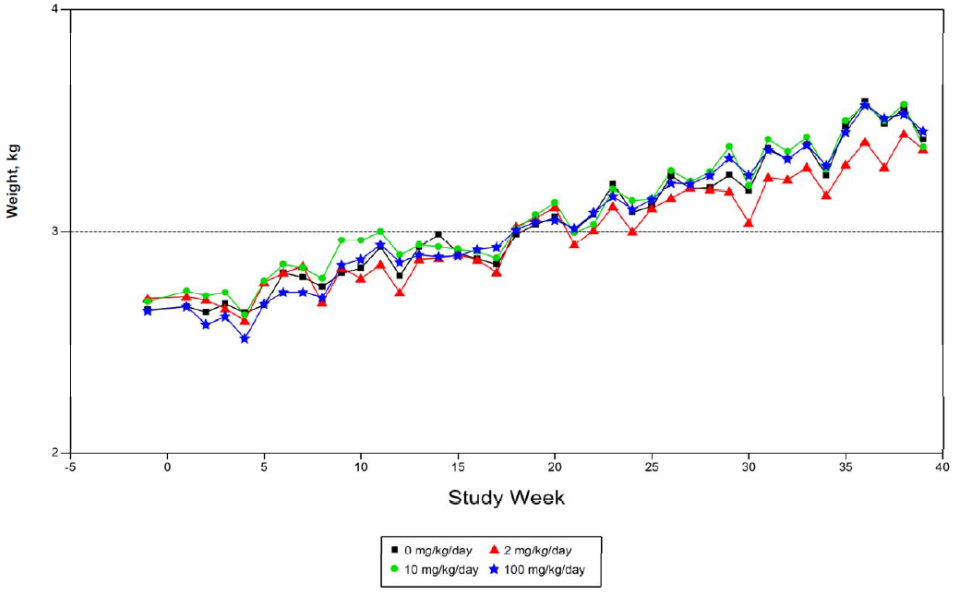
Pelage/Skin: Abrasion was observed in 1/4, 1/4, and 4/4 males in the 2, 10, and 100 mg/kg/day groups, respectively. Red discolored skin was observed in 1/4, 1/4, and 1/4 females in the 2, 10, and 100 mg/kg/day groups, respectively. Red/black discolored skin was observed in 2/4 males in the 100 mg/kg/day group. Dry skin was observed in 1/4 females in the 100 mg/kg/day group.

Body Weights

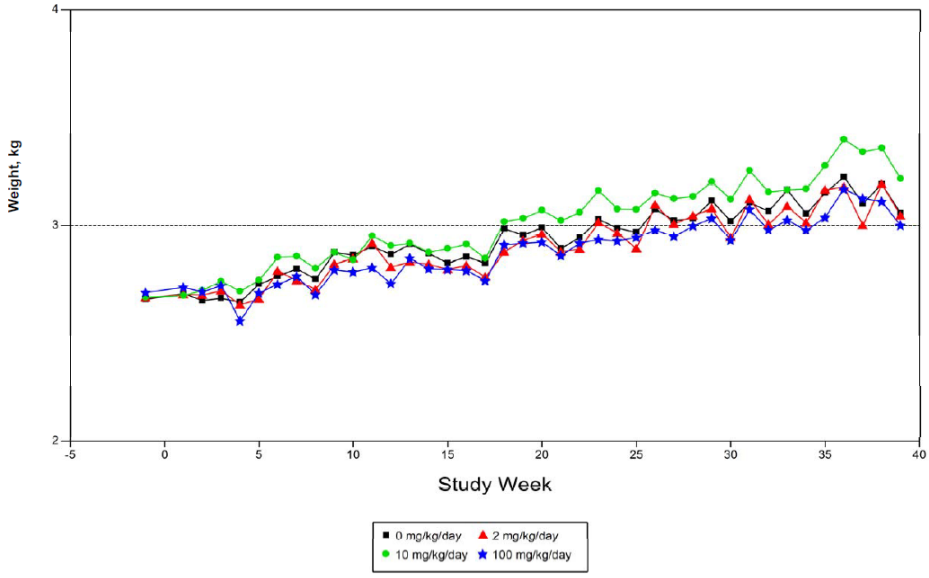
Bodyweights for all animals were measured and recorded at transfer, prior to randomization, and weekly beginning in Week -1. The data are shown in the Sponsor's

figures below.

Bodyweights in Males



Bodyweights in Females



There were no significant drug-related changes in bodyweight or bodyweight gain.

Feed Consumption

Not measured.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted on all animals during the pre-dose

period and prior to terminal necropsies. There were no drug-related ophthalmoscopic findings.

ECG

Animals received an electrocardiographic examination during the pre-dose period and during week 39. The examinations during the last week of dosing were conducted approximately 4.5 to 5.5 hours after daily dosing. Standard 10-lead ECGs were recorded at 50 mm/sec. The RR, PR, and QT intervals, and QRS duration were measured, and heart rate was determined. Corrected QT (QT_c) interval was calculated using Bazett's correction

All animals were in sinus rhythm or sinus arrhythmia; the Sponsor indicated that both are normal rhythms in monkeys. Sinus tachycardia, defined as an average heart rate greater than 270 beats per minute for a single interval, occurred in 8 animals at 9 intervals. The incidence of sinus tachycardia was greatest in the high dose group (Group 1: 1 animal, 1 instance, which was pretest; Group 2: 2 animals, 2 instances, both of which were pre-test; Group 3: 1 animal, 1 instance, which was pretest; Group 4: 4 animals, 5 instances, 3 of which were pre-test). The incidence of sinus tachycardia was greatest during the last week of dosing in the high dose group; however, as 6 of the 8 instances occurred pre-test, the rhythm is not considered as drug-related. All of the electrocardiograms were qualitatively and quantitatively within normal limits.

Hematology

Blood samples (approximately 4.8 to 5.8 mL) were collected during the pre-dose period and weeks 26 and 39 from the femoral vein from all animals. There were no significant drug-related changes in hematology parameters, including coagulation values.

Clinical Chemistry

There were no significant drug-related changes in clinical chemistry parameters at week 26 or terminal sacrifice.

Urinalysis

Urine samples were collected from all animals during the pre-dose period and weeks 26 and 39. There were no significant drug-related changes in urinalysis parameters at termination sacrifice.

Gross Pathology

Observations were made at terminal necropsy on week 39. There were no drug-related macroscopic findings.

Organ Weights

Organs were collected at sacrifice on week 39. The following organs or tissues were weighed (table taken from the study report):

Organs or Tissues to be Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination
Adrenal gland	X	X	X
Aorta		X	X
Bone with bone marrow, femur		X	X
Bone with bone marrow, rib		X	X
Bone with bone marrow, sternum		X	X
Bone marrow smear[a]		X	
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X
Epididymis	X	X	X
Esophagus		X	X
Eye (with optic nerve)		X	X

Gallbladder		X	X
GALT[b]		X	X
Heart	X	X	X
Joint, tibiofemoral		X	X
Kidney	X	X	X
Large intestine, cecum		X	X
Large intestine, colon		X	X
Large intestine, rectum		X	X
Larynx		X	X
Liver	X	X	X
Lung with bronchi	X	X	X
Lymph node, mandibular		X	X
Lymph node, mesenteric		X	X
Mammary gland (process females only)		X	X
Nerve, sciatic		X	X
Ovary	X	X	X
Oviducts		X	X
Pancreas		X	X
Pituitary	X	X	X
Prostate		X	X
Salivary gland, mandibular[c]	X	X	X
Salivary gland, parotid		X	X
Salivary gland, sublingual		X	X
Seminal vesicles		X	X
Skeletal muscle, rectus femoris		X	X
Skin		X	X
Small intestine, duodenum		X	X
Small intestine, ileum		X	X
Small intestine, jejunum		X	X
Spinal cord, cervical		X	X

Spinal cord, lumbar		X	X
Spinal cord, thoracic		X	X
Spleen	X	X	X
Stomach, cardia		X	X
Stomach, fundus		X	X
Stomach, pylorus		X	X
Target Organs[d]		X	X
Testis	X	X	X
Thymus	X	X	X
Thyroid gland (with parathyroid)[e]	X	X	X
Tongue		X	X
Trachea		X	X
Ureters		X	X
Urinary bladder		X	X
Uterus with cervix		X	X
Vagina		X	X
Gross lesions		X	X
Tissue masses with regional lymph node[f]		X	X

[a] *Bone marrow smears will be prepared only for animals necropsied at scheduled intervals. Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost).*
[b] *Gut Associated Lymphoid Tissue*
[c] *Only the right mandibular salivary gland will be weighed.*
[d] *Target Organs (and target organ gross lesions) will be designated by the Study Director, Pathologist and/or Sponsor based on experimental findings (additional cost).*
[e] *Parathyroids cannot always be identified macroscopically. They will be examined if in the plane of section and in all cases where they are noted as grossly enlarged.*
[f] *A regional lymph node drains the region where a tissue mass is located. A regional lymph node may not always be identified when a mass is present.*

There were no significant drug-related organ weight changes.

Histopathology

Tissues and organs were collected at necropsy on week 39. The organs/tissues examined are listed in the above table (taken from the study report):

Adequate Battery - Yes

Peer Review - No

Histological Findings

There were no drug-related microscopic findings.

Special Evaluation

Fecal Observations and Scoring

A fecal examination for characterization (color and quantity) and scoring of fecal consistency was conducted twice per day (at least 3 hours apart) starting at two weeks prior to dosing and during the first four weeks of the study. Thereafter, a fecal examination was conducted twice per day for one week in each month. During the dosing period, examinations and scoring were conducted pre-dose and at 2 hr (±15 min) and 5 hr (±30 min) post-dose. Fecal examinations and scoring during the recovery period were conducted at least three hours apart. Fecal consistency (stool form) was scored as 1 (well formed), 2 (mushy/loose), or 3 (watery). If no feces were present during an examination, a score of 0 was recorded. For each week and for the cumulative score, the number of scores in category was tabulated for each animal. These counts were then converted to percentages using the following methods: 1) the denominator for the count of examinations with no feces present was the total number of examinations during the week; and 2) the denominator for the counts of the examinations with each of the other categories was the total number of examinations with feces present; each week the percentages of examinations with normal (well formed), mushy/loose, or watery feces summed to 100 for each animal. These individual percentages in each category were then averaged by dose level and sex. The table below summarizes the results.

Stool Form Evaluation at Week 39

Sex	Male				Female			
Dose (mg/kg/day)	**0**	**2**	**10**	**100**	**0**	**2**	**10**	**100**
No Feces[1]	16.1	10.7	23.2	10.7	28.6	8.9	17.9	7.1
Well formed[2]	100.0	68.3	28.0	67.6	93.2	63.4	40.2	40.4
Mushy/Loose[2]	0.0	15.9	16.9	10.1	6.8	13.2	21.7	23.1
Watery[2]	0.0	15.9	55.1	22.3	0.0	23.4	38.1	36.5

Cumulative Stool Form Evaluation

Sex	Male				Female			
Dose (mg/kg/day)	**0**	**2**	**10**	**100**	**0**	**2**	**10**	**100**
No Feces[1]	6.4	5.7	7.3	2.2	12.4	5.1	7.1	4.0
Well formed[2]	99.5	63.4	35.9	70.6	98.8	65.4	54.1	41.6
Mushy/Loose[2]	0.5	18.0	14.6	9.7	1.2	11.7	19.1	15.1
Watery[2]	0.0	18.6	49.5	19.6	0.0	22.8	26.8	43.3

1 Percent based on incidence of absence of feces ÷ total # of examinations
2 Percent based on incidence of stool form ÷ # of examinations with feces present

A dose-related decrease in the incidence of normal feces, and a corresponding increase in the incidence of mushy/loose or watery stools occurred in both males and females.

The changes were present for the duration of the treatment period. The increase in the incidence of mushy/loose or watery stools is considered a pharmacological effect of plecanatide, and is not considered adverse. The Sponsor indicated that there was no indication of dehydration and no microscopic alterations in the GI tract associated with the treatment.

Toxicokinetics

Blood samples were collected on day 1 prior to dosing, and on days 1, week 27, and week 39 at 5, 15, 30 minutes, and 1, 1.5, 2, and 3 hours after dosing.

Toxicokinetic parameters for plecanatide were highly variable. There was only negligible systemic exposure at 2 mg/kg/day, and exposure increased with dose between 2 to 100 mg/kg/day. At 100 mg/kg/day, maximum plecanatide plasma concentration was observed at 0.29 hr (T_{max}). Systemic exposures during weeks 1 and 39 were approximately equal, with slightly lower exposure during week 27.
There were no significant sex-related differences in systemic exposure. The toxicokinetics data are summarized in the table below taken from the Sponsor's study report.

Mean (±SD) Toxicokinetic Parameters for Plecanatide in Male and Female Monkeys

Day/Week	Dose (mg/kg/day)	Sex	AUC_{0-3} (hr•ng/mL)	$AUC_{0-tlast}$ (hr•ng/mL)	C_{max} (ng/mL)	T_{max} (hr)
Day 1	2	M	0±0	0±0	0±0	NC
		F	0±0	0±0	0±0	NC
		Combined	0±0	0±0	0±0	NC
	10	M	0±0	0±0	2.68±5.35	0.500
		F	0±0	0±0	6.45±12.9	0.500
		Combined	0±0	0±0	4.56±9.36	0.500
	100	M	113±77.1	106±77.8	82.7±48.8	0.688±0.375
		F	128±83.6	116±79.1	111±106	0.563±0.315
		Combined	121±74.8	111±72.8	96.8±78.2	0.625±0.327
Week 27	2	M	0±0	0±0	0±0	NC
		F	0±0	0±0	3.18±6.35	0.250
		Combined	0±0	0±0	1.59±4.49	0.250
	10	M	0±0	0±0	0±0	NC
		F	0±0	0±0	0±0	NC
		Combined	0±0	0±0	0±0	NC
	100	M	10.6±3.66	7.71±3.58	13.1±1.43	0.167±0.0964
		F	19.7±20.7	18.7±19.1	16.8±19.4	1.11±1.64
		Combined	15.2±14.2	13.2±13.7	15.0±12.9	0.571±1.07
Week 39	2	M	0	0	0.805±0.977	0.750±0.354
		F	1.78	1.65	1.78±1.83	0.833±0.289
		Combined	0.888	0.824	1.29±1.46	0.800±0.274
	10	M	10.1±6.00	9.43±5.68	8.80±4.74	0.750±0.289
		F	8.90±7.21	8.27±7.01	7.63±6.07	0.563±0.315
		Combined	9.50±6.17	8.85±5.93	8.22±5.08	0.656±0.297
	100	M	98.1±30.7	98.1±30.7	90.6±39.6	0.438±0.125
		F	122±68.0	121±68.6	118±91.6	0.688±0.375
		Combined	110±50.4	110±50.7	104±67.0	0.291±0.563

Where no SD is given, the mean is comprised of fewer than 3 observations.

Dosing Solution Analysis

The concentration determination of plecanatide in the dosing formulation samples was performed in accordance with the validated method. Analyses conducted during the treatment period showed that the concentrations of dose formulations at 0.4, 2.0, and 20.0 mg/mL were approximately within ±10% of the nominal concentration (-2.9% to +8.5%). The 2 mg/mL samples from week 26 yielded 129.6% of nominal. An investigation was conducted but no assignable cause was determined for this result. All other samples for week 26 were within specifications. The Sponsor indicted that the single observed sample that was out of specification does not impact the study due to the direction of the variation (greater than nominal) and because this dose level (10 mg/kg/day) is below the NOAEL for this study.

7 Genetic Toxicology

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

Study title: Bacterial Reverse Mutation Assay	
Study no.:	AD27SJ.503.BTL
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/1/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide (SP-304), RD071310, 95.9%

Key Study Findings: Plecanatide was negative in the Ames test.

Methods	
Strains:	*Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537, and *Escherichia coli* WP2 *uvr*A
Concentrations in definitive study:	1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg per plate
Basis of concentration selection:	Negative findings in the initial toxicity/ mutation assay; neither precipitate nor background lawn toxicity was observed at the maximum dose tested (5000 µg/plate).
Negative control:	Sterile water
Positive control:	In the absence of metabolic activation: sodium azide (SA, 1.0 µg/plate) for TA1535 and TA100, 2-nitrofluorene (2NF, 1.0 µg/plate) for TA98, 9-aminoacridine (9AAD, 75 µg/plate) for TA1537, and methyl methanesulfonate (MMS, 1000 µg/plate) for WP2 *uvr*A. In the presence of metabolic activation: 2-aminoanthracene (2AA, 1.0 µg/plate) for TA98, TA1535, and TA1537; 2.0 µg/plate 2AA for TA100; 15 µg/plate 2AA for WP2 uvrA.
Formulation/Vehicle:	Plecanatide dissolved in sterile water
Incubation & sampling time:	The plate incorporation method was used. Aroclor 1254-induced rat liver S9 (10%) was used as the metabolic activation system. The plates (triplicate in the confirmation assay) were incubated for approximately 48 to 72 hours at 37±2^{o}C. The condition of the bacterial background lawn was evaluated for evidence of test article toxicity, and precipitate was evaluated after the incubation period by

	visual examination. Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the plate exhibited toxicity.

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

Study Criteria: For the test article to be considered as positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was greater than or equal to 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100 and WP2 *uvr*A were judged as positive if the increase in mean revertants at the peak of the dose response was greater than or equal to 2.0-times the mean vehicle control value. An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for a positive response. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response was evaluated as negative, if it was neither positive nor equivocal. These criteria are acceptable.

Results: In the initial toxicity-mutation assays, the dose levels used were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg per plate. No positive mutagenic responses were observed with any of the tester strains in the presence or absence of S9 activation. In the confirmatory mutagenicity assays, no positive mutagenic responses were observed with any of the tester strains in the presence or absence of S9 activation for plecanatide. Neither precipitate nor appreciable toxicity was observed. Results of the confirmatory mutagenicity assay are summarized in the tables below (taken from the study report).

Confirmatory Mutagenicity Assay Without S9 Activation

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**Plecanatide**	5000 µg	*17*	*5*	*1.2*	13^{A}, 23^{A}, 15^{A}
		1500 µg	*14*	*5*	*1.0*	19^{A}, 9^{A}, 13^{A}
		500 µg	*13*	*2*	*0.9*	12^{A}, 13^{A}, 15^{A}
		150 µg	*14*	*1*	*1.0*	13^{A}, 15^{A}, 15^{A}
		50 µg	*18*	*5*	*1.3*	23^{A}, 13^{A}, 17^{A}
	Water	100 µL	*14*	*2*		17^{A}, 13^{A}, 13^{A}
TA100	**Plecanatide**	5000 µg	*108*	*12*	*1.0*	121^{A}, 98^{A}, 105^{A}
		1500 µg	*116*	*12*	*1.0*	127^{A}, 119^{A}, 103^{A}
		500 µg	*97*	*9*	*0.9*	94^{A}, 89^{A}, 107^{A}
		150 µg	*110*	*14*	*1.0*	101^{A}, 127^{A}, 103^{A}
		50 µg	*101*	*5*	*0.9*	106^{A}, 101^{A}, 97^{A}
	Water	100 µL	*111*	*17*		119^{A}, 123^{A}, 92^{A}
TA1535	**Plecanatide**	5000 µg	*11*	*2*	*1.2*	12^{A}, 12^{A}, 8^{A}
		1500 µg	*7*	*2*	*0.8*	7^{A}, 5^{A}, 8^{A}
		500 µg	*13*	*4*	*1.4*	17^{A}, 12^{A}, 9^{A}
		150 µg	*10*	*2*	*1.1*	12^{A}, 9^{A}, 9^{A}
		50 µg	*14*	*2*	*1.6*	11^{A}, 15^{A}, 15^{A}
	Water	100 µL	*9*	*3*		7^{A}, 13^{A}, 8^{A}
TA1537	**Plecanatide**	5000 µg	*6*	*3*	*0.7*	5^{A}, 4^{A}, 9^{A}
		1500 µg	*6*	*3*	*0.7*	3^{A}, 8^{A}, 7^{A}
		500 µg	*7*	*2*	*0.8*	9^{A}, 5^{A}, 8^{A}
		150 µg	*4*	*1*	*0.4*	3^{A}, 5^{A}, 4^{A}
		50 µg	*4*	*2*	*0.4*	7^{A}, 3^{A}, 3^{A}
	Water	100 µL	*9*	*2*		11^{A}, 9^{A}, 7^{A}
WP2*uvr*A	**Plecanatide**	5000 µg	*47*	*2*	*1.1*	45^{A}, 46^{A}, 49^{A}
		1500 µg	*45*	*8*	*1.1*	45^{A}, 38^{A}, 53^{A}
		500 µg	*50*	*8*	*1.2*	58^{A}, 49^{A}, 42^{A}
		150 µg	*54*	*9*	*1.3*	61^{A}, 44^{A}, 56^{A}
		50 µg	*47*	*3*	*1.1*	49^{A}, 48^{A}, 44^{A}
	Water	100 µL	*42*	*9*		41^{A}, 34^{A}, 52^{A}
TA98	**2NF**	1.0 µg	*250*	*41*	*17.9*	245^{A}, 212^{A}, 294^{A}
TA100	**SA**	1.0 µg	*855*	*104*	*7.7*	942^{A}, 739^{A}, 883^{A}
TA1535	**SA**	1.0 µg	*694*	*69*	*77.1*	645^{A}, 664^{A}, 772^{A}
TA1537	**9AAD**	75 µg	*489*	*32*	*54.3*	524^{A}, 463^{A}, 479^{A}
WP2*uvr*A	**MMS**	1000 µg	*447*	*29*	*10.6*	476^{A}, 418^{A}, 448^{A}

Confirmatory Mutagenicity Assay With S9 Activation

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**Plecanatide**	5000 µg	*25*	*3*	*1.1*	23^{A}, 28^{A}, 25^{A}
		1500 µg	*25*	*4*	*1.1*	20^{A}, 28^{A}, 27^{A}
		500 µg	*20*	*2*	*0.9*	19^{A}, 23^{A}, 19^{A}
		150 µg	*29*	*9*	*1.3*	28^{A}, 20^{A}, 38^{A}
		50 µg	*19*	*3*	*0.8*	23^{A}, 17^{A}, 17^{A}
	Water	100 µL	*23*	*5*		20^{A}, 20^{A}, 28^{A}
TA100	**Plecanatide**	5000 µg	*113*	*5*	*1.0*	119^{A}, 109^{A}, 111^{A}
		1500 µg	*114*	*5*	*1.0*	109^{A}, 117^{A}, 117^{A}
		500 µg	*121*	*6*	*1.0*	115^{A}, 121^{A}, 127^{A}
		150 µg	*128*	*16*	*1.1*	114^{A}, 125^{A}, 145^{A}
		50 µg	*115*	*16*	*1.0*	117^{A}, 98^{A}, 129^{A}
	Water	100 µL	*118*	*20*		106^{A}, 141^{A}, 106^{A}
TA1535	**Plecanatide**	5000 µg	*12*	*2*	*0.9*	15^{A}, 11^{A}, 11^{A}
		1500 µg	*11*	*5*	*0.8*	9^{A}, 17^{A}, 8^{A}
		500 µg	*9*	*2*	*0.7*	11^{A}, 8^{A}, 8^{A}
		150 µg	*12*	*2*	*0.9*	11^{A}, 15^{A}, 11^{A}
		50 µg	*10*	*3*	*0.8*	9^{A}, 7^{A}, 13^{A}
	Water	100 µL	*13*	*7*		7^{A}, 11^{A}, 20^{A}
TA1537	**Plecanatide**	5000 µg	*7*	*2*	*0.6*	5^{A}, 8^{A}, 8^{A}
		1500 µg	*11*	*3*	*1.0*	13^{A}, 8^{A}, 13^{A}
		500 µg	*5*	*3*	*0.5*	3^{A}, 3^{A}, 9^{A}
		150 µg	*9*	*2*	*0.8*	7^{A}, 11^{A}, 9^{A}
		50 µg	*7*	*2*	*0.6*	5^{A}, 8^{A}, 7^{A}
	Water	100 µL	*11*	*2*		8^{A}, 12^{A}, 12^{A}
WP2*uvr*A	**Plecanatide**	5000 µg	*45*	*13*	*1.0*	45^{A}, 32^{A}, 58^{A}
		1500 µg	*45*	*7*	*1.0*	44^{A}, 52^{A}, 38^{A}
		500 µg	*41*	*4*	*0.9*	46^{A}, 38^{A}, 40^{A}
		150 µg	*49*	*8*	*1.0*	40^{A}, 54^{A}, 52^{A}
		50 µg	*44*	*12*	*0.9*	45^{A}, 56^{A}, 32^{A}
	Water	100 µL	*47*	*12*		50^{A}, 33^{A}, 57^{A}
TA98	**2AA**	1.0 µg	*294*	*8*	*12.8*	297^{A}, 300^{A}, 285^{A}
TA100	**2AA**	2.0 µg	*697*	*118*	*5.9*	637^{A}, 621^{A}, 833^{A}
TA1535	**2AA**	1.0 µg	*64*	*11*	*4.9*	57^{A}, 76^{A}, 58^{A}
TA1537	**2AA**	1.0 µg	*49*	*4*	*4.5*	49^{A}, 45^{A}, 53^{A}
WP2*uvr*A	**2AA**	15 µg	*185*	*16*	*3.9*	199^{A}, 168^{A}, 188^{A}

In summary, plecanatide was negative in the Ames test.

7.2 In Vitro Assays in Mammalian Cells

Study title: In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)	
Study no.:	AD27SJ.704.BTL
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/12/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide, RD071310, 95.9%

Key Study Findings: Plecanatide was not mutagenic in the in vitro L5178Y TK$^{+/-}$ mouse lymphoma mammalian cell gene mutation assay.

Methods	
Cell line:	L5178Y/TK$^{+/-}$ mouse lymphoma
Concentrations in definitive study:	Initial assay (4 hr treatment): 1000, 2000, 3000, 4000 and 5000 µg/ml Extended treatment assay (24 hr treatment): 1000, 2000, 3000, 4000, and 5000 µg/ml
Basis of concentration selection:	The doses of the definitive studies were selected based on results obtained in a dose-ranging study, performed in the presence (4 hr treatment) and absence (4 hr and 24 hr treatments) of metabolic activation. Plecanatide concentrations between 0.5 and 5000 µg/mL were used. Suspension growth relative to the solvent controls at 5000 µg/mL was 100% without activation with a 4-hour exposure, 102% with S9 activation with a 4-hour exposure, and 59% without activation with a 24-hour exposure.
Negative control:	Sterile distilled water
Positive control:	Without S9: Methyl methanesulfonate (MMS) 20 and 15 µg/ml in initial assay, and 7.5 and 5 µg/ml in the extended treatment assay. With S9: 7,12-dimethylbenz(a)anthracene (DMBA) 1.5 and 1.25 µg/ml in initial assay
Formulation/Vehicle:	Plecanatide dissolved in sterile distilled water
Incubation & sampling time:	Aroclor 1254-induced rat liver S9 (10%) was used as the metabolic activation

	system. Cell cultures (2/concentration) in suspension were exposed to solvent or plecanatide in the presence or absence of S-9 for 4 or 24 hours. Following treatment, cells were washed and cultured in suspension for a 24 to 72 hours expression period. Following the expression period, cells were plated (3 plates/concentration) in soft agar medium with and without the selective agent, trifluorothymidine (TFT). After the appropriate period of time the colonies were counted. The colony size distribution was determined in the controls and at all concentrations of the test article.

Study Validity:

The Sponsor's criteria for an acceptable assay are: **1)** for negative controls, the average spontaneous mutant frequency of the solvent (or vehicle) control cultures must be within 35 to 140 TFT-resistant mutants per 10^6 surviving cells. Low spontaneous mutant frequencies, i.e., 20 to 34 mutants per 10^6 surviving cells, are considered acceptable if small colony recovery is demonstrated. The average cloning efficiency of the solvent (or vehicle) controls must be between 65% and 120% and the total suspension growth between 8-32 for the 4-hour exposure and 20-180 for the 24-hour exposure; **2)** for positive controls, the mutant frequency for at least one dose of the positive controls must meet the criteria for a positive response and induce an increase in small colony mutants according to the following criteria: Induced Mutant Frequency (IMF) positive control ≥ 300 x 10^{-6} mutants with 40% small colonies or small colony IMF for positive control ≥ 150 x 10^{-6}; and **3)** Cultures treated with a minimum of four concentrations of test article must be attained and their mutant frequencies reported. The highest test article concentration must produce 80% to 90% toxicity unless limited by solubility or the maximum required concentration. In the case of a test article with a steep toxicity curve (no concentrations with 10-20% survival), the results may be considered acceptable if a concentration spacing of ≤ 2-fold is used and the highest concentration tested showed <20% survival or total kill. The criteria are acceptable and the study is deemed valid.

Study Criteria:

The Sponsor's criteria for positive results are: 1) if a concentration-related increase in induced mutant frequency was observed in the treated cultures; 2) one or more treatment conditions with 10% or greater total growth exhibited induced mutant frequencies of ≥90 mutants per 10^6 clonable cells (based on the average mutant frequency of duplicate cultures); and 3) if the average solvent control mutant

frequency was >90 mutants per 10^6 clonable cells, a doubling of mutant frequency over the background will also be required.

The Sponsor's criteria for negative results are: 1) if the treated cultures exhibited induced mutant frequencies of less than 90 mutants per 10^6 clonable cells (based on the average mutant frequency of duplicate cultures); and 2) there was no concentration-related increase in mutant frequency.

The criteria are acceptable.

Results:

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

DATA SUMMARY FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH plecanatide
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Initial Assay (4-hour exposure)

DOSE LEVEL (µg/mL)	PRECIP.	% SUSP. GROWTH	VC COLONIES PLATE COUNTS 1	2	3	MEAN	TFT COLONIES PLATE COUNTS 1	2	3	MEAN	TOTAL MUTANT FREQUENCY (PER 10^6 CELLS)	INDUCED MUTANT FREQUENCY (PER 10^6 CELLS)	% RELATIVE TOTAL GROWTH
SOLVENT A		100	140	124	155	**140**	29	20	25	**25**	35	N/A	100
SOLVENT B			136	174	168	**159**	20	27	32	**26**	33		
1000 A		91	216	197	220	**211**	51	32	23	**35**	33	**-1**	**128**
1000 B		101	179	187	181	**182**	23	20	19	**21**	23	**-12**	**124**
2000 A		96	189	190	150	**176**	59	27	24	**37**	42	**7**	**113**
2000 B		94	156	140	137	**144**	46	44	49	**46**	64	**30**	**90**
3000 A		93	144	162	139	**148**	24	33	31	**29**	40	**5**	**92**
3000 B		91	160	141	136	**146**	40	37	45	**41**	56	**22**	**89**
4000 A		102	169	175	174	**173**	29	29	39	**32**	37	**3**	**118**
4000 B		95	149	157	140	**149**	25	37	34	**32**	43	**9**	**94**
5000 A		93	175	168	168	**170**	45	33	25	**34**	40	**6**	**106**
5000 B		101	181	164	183	**176**	29	33	38	**33**	38	**4**	**119**
POSITIVE CONTROL: Methyl methanesulfonate (MMS) (µg/mL)													
20		45	36	45	37	**39**	87	68	75	**77**	390	**356**	**12**
15		50	53	51	50	**51**	103	96	138	**112**	438	**403**	**17**

MEAN SOLVENT TOTAL SUSPENSION GROWTH: 26.8
MEAN SOLVENT CLONING EFFICIENCY: 75%
MEAN SOLVENT MUTANT FREQUENCY: 34 *(PER 10^6 CELLS)*

DATA SUMMARY FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH plecanatide
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
Initial Assay (4-hour exposure)

DOSE LEVEL (µg/mL)	PRECIP.	% SUSP. GROWTH	VC COLONIES PLATE COUNTS 1	2	3	MEAN	TFT COLONIES PLATE COUNTS 1	2	3	MEAN	TOTAL MUTANT FREQUENCY (PER 10^6 CELLS)	INDUCED MUTANT FREQUENCY (PER 10^6 CELLS)	% RELATIVE TOTAL GROWTH
SOLVENT A		100	162	143	151	**152**	27	30	23	**27**	35	N/A	100
SOLVENT B			169	194	199	**187**	33	37	39	**36**	39		
1000 A		110	150	188	168	**169**	34	29	15	**26**	31	**-6**	**110**
1000 B		108	235	220	210	**222**	48	38	33	**40**	36	**-1**	**141**
2000 A		120	225	214	219	**219**	30	26	26	**27**	25	**-12**	**155**
2000 B		112	189	169	189	**182**	21	38	34	**31**	34	**-3**	**121**
3000 A		120	181	188	187	**185**	29	29	31	**30**	32	**-5**	**132**
3000 B		116	159	170	172	**167**	30	29	36	**32**	38	**1**	**114**
4000 A		113	163	165	168	**165**	32	38	27	**32**	39	**2**	**110**
4000 B		115	176	168	147	**164**	44	26	33	**34**	42	**5**	**111**
5000 A		116	201	194	203	**199**	29	27	25	**27**	27	**-10**	**137**
5000 B		124	213	160	151	**175**	24	10	32	**22**	25	**-12**	**128**
POSITIVE CONTROL: 7,12-dimethylbenz(a)anthracene (DMBA) (µg/mL)													
1.5		21	109	106	115	**110**	181	182	184	**182**	332	**295**	**14**
1.25		35	115	96	112	**108**	182	189	203	**191**	355	**318**	**22**

MEAN SOLVENT TOTAL SUSPENSION GROWTH: 22.1
MEAN SOLVENT CLONING EFFICIENCY: 85%
MEAN SOLVENT MUTANT FREQUENCY: 37 *(PER 10^6 CELLS)*

Solvent = Sterile distilled water A and B are duplicate cultures

DATA SUMMARY FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH plecanatide
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Extended Treatment Assay (24-hour exposure)

DOSE LEVEL (µg/mL)	PRECIP.	% SUSP. GROWTH	VC COLONIES				TFT COLONIES				TOTAL MUTANT FREQUENCY (PER 10^6 CELLS)	INDUCED MUTANT FREQUENCY (PER 10^6 CELLS)	% RELATIVE TOTAL GROWTH
			PLATE COUNTS				PLATE COUNTS						
			1	2	3	MEAN	1	2	3	MEAN			
SOLVENT A		100	185	192	158	**178**	25	35	27	**29**	33	N/A	100
SOLVENT B			150	151	141	**147**	27	18	24	**23**	31		
1000 A		93	149	131	132	**137**	36	11	12	**20**	29	**-3**	**79**
1000 B		91	118	124	168	**137**	23	19	21	**21**	31	**-1**	**77**
2000 A		76	152	157	157	**155**	23	18	11	**17**	22	**-10**	**73**
2000 B		95	133	141	156	**143**	10	11	15	**12**	17	**-15**	**84**
3000 A		75	205	183	164	**184**	36	29	36	**34**	37	**5**	**84**
3000 B		74	160	157	199	**172**	31	23	19	**24**	28	**-4**	**78**
4000 A		76	164	160	173	**166**	27	23	25	**25**	30	**-2**	**77**
4000 B		85	158	146	158	**154**	18	16	27	**20**	26	**-5**	**80**
5000 A		93	192	175	184	**184**	23	23	24	**23**	25	**-6**	**105**
5000 B		92	151	195	164	**170**	22	27	16	**22**	25	**-6**	**97**
POSITIVE CONTROL: Methyl methanesulfonate (MMS) (µg/mL)													
7.5		41	64	64	59	**62**	153	117	128	**133**	426	**394**	**16**
5		47	77	93	120	**97**	126	121	162	**136**	282	**250**	**28**

MEAN SOLVENT TOTAL SUSPENSION GROWTH: 38.4
MEAN SOLVENT CLONING EFFICIENCY: 81%
MEAN SOLVENT MUTANT FREQUENCY: 32 *(PER 10^6 CELLS)*

In the initial assay, no visible precipitate was present at any concentration in the treatment medium. In the non-activated system, cultures treated with concentrations of 1000, 2000, 3000, 4000, and 5000 µg/mL were cloned and produced a range in suspension growth from 91% to 102%. In the S9-activated system, cultures treated with the same concentrations were cloned and produced a range in suspension growth from 108% to 124%. No cloned cultures exhibited induced mutant frequencies that were ≥90 mutants per 10^6 clonable cells. No concentration-related increase in mutant frequency was observed in the non-activated or S9-activated systems. The total growth ranged from 89% to 128% for the non-activated cultures and 110% to 155% for the S9-activated cultures at concentrations from 1000 to 5000 µg/mL. The results of the initial assay were negative in the absence or presence of S9 activation.

Because no unique metabolic requirements were known about the test article, an extended treatment assay was performed only in the absence of S9 for a 24-hour exposure period. Colony size distributions for the positive and solvent control cultures were determined. No visible precipitate was present at any concentration in the treatment medium. Cultures treated with concentrations of 1000, 2000, 3000, 4000, and 5000 µg/mL were cloned and produced a range in suspension growth from 74% to 95%. No cloned cultures exhibited induced mutant frequencies that were ≥90 mutants per 10^6 clonable cells. No concentration-related increase in mutant

frequency was observed. The total growth ranged from 73% to 105% for non-activated cultures with a 24-hour exposure. The TFT-resistant colonies for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS and DMBA positive controls yielded the expected increase in small colonies (verifying the adequacy of the methods used to detect small colony mutants) and large colonies.

Conclusions: There were no significant or reproducible dose-dependent increases in mutant colony numbers up to the maximum plecanatide concentration, with or without metabolic activation. Plecanatide was not mutagenic in mouse lymphoma L5178Y/TK$^{+/-}$ cells in the absence or presence of metabolic activation.

7.3 In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Mouse Bone Marrow Erythrocyte Micronucleus Test Following Oral Administration of Plecanatide (SP-304)

Study no:	AD27SJ.123.BTL
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/5/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide (SP-304), RD071310, 95.9%

Key Study Findings:

Plecanatide did not increase the incidence of micronucleated PCE at any of the doses tested. Plecanatide is considered to be non-clastogenic in the in vivo mouse micronucleus assay.

Methods

Doses in definitive study:	0 (vehicle), 500, 1000, or 2000 mg/kg in the groups sacrificed at 24 hours post-dose, and 0 (vehicle) or 2000 mg/kg in the groups sacrificed at 48 hours post-dose
Frequency of dosing:	single administration
Route of administration:	oral (gavage)
Dose volume:	25 mL/kg
Formulation/Vehicle:	dissolved in 0.1M phosphate buffer, pH 7.0

Species/Strain:	mouse/ICR
Number/Sex/Group:	5/sex/group
Satellite groups:	10 toxicokinetic groups; 3/sex/group/sacrifice time-point exposed to vehicle, 500, 1000, or 2000 mg/kg plecanatide. Mice in the vehicle control group were bled at 15 minutes post-dose and test article groups were bled at 5, 15, or 45 minutes post-dose.
Basis of dose selection:	The dose selection was based on a dose range-finding study in mice (3M + 3F per group), where mice were given single oral (gavage) doses of 1000, 1300, 1750, or 2000 mg/kg plecanatide. Animals were monitored for clinical signs, mortality, and body weight change for 3 days post-dosing. Plecanatide did not produce any mortalities or significant clinical signs at 2000 mg/kg, which was chosen as the high dose in the definitive study. Two lower doses, 500 and 1000 mg/kg, were also tested.
Negative control:	0.1M phosphate buffer, pH 7.0
Positive control:	Cyclophosphamide monohydrate (50 mg/kg)

Study Design

Group/Treatment (25 mL/kg)	Number of Mice/Sex Dosed	Number of Mice/Sex Used for Bone Marrow Collection at	
		24 hrs post-dose	48 hrs post-dose
1/Vehicle Control: 0.1M phosphate buffer (pH 7.0)	10 + 3**	5	5
Test Article: plecanatide			
2/Low dose (500 mg/kg)	5 + 9**	5	-
3/Mid dose (1000 mg/kg)	5 + 9**	5	-
4/High dose (2000 mg/kg)	15* + 9**	5	5
5/Positive Control: CP (50 mg/kg)	5	5	-

*Including 5 replacement mice/sex to ensure the availability of five mice for micronucleus analysis.
**Satellite mice assigned for the toxicokinetic portion of the study.

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

Study Validity

The Sponsor considered the study as valid if 1) no mortality occurred in the main portion of the study resulting in evaluation of all 5 animals/sex/group; 2) the incidence of micronucleated polychromatic erythrocytes in the vehicle control groups did not exceed the historical vehicle control range; 3) the incidence of micronucleated polychromatic erythrocytes in the male and female positive control groups was significantly increased relative to the respective vehicle control groups ($p \leq 0.05$).

Study Criteria

A test item was classified as clastogenic by the Sponsor if the incidence of micronucleated polychromatic erythrocytes at one or more dose levels was statistically elevated relative to the vehicle control ($p \leq 0.05$). A dose-dependent increase in the incidence of micronucleated PCEs and biological relevance would also be taken into consideration during determination of the test article positivity.

Results

No mortality occurred in the dose range-finding study. Piloerection was observed in the 1000, 1300, 1750, and 2000 mg/kg groups, and lethargy was observed in the 1750 and 2000 mg/kg groups. No significant changes in bodyweight were noted.

In the definitive study, no mortality was observed in any of the treatment groups during the study period. All mice in the control article (vehicle or positive) groups and all 500 mg/kg mice appeared normal during the study period. Piloerection was observed in the 1000 and 2000 mg/kg mice, and lethargy was observed in the 2000 mg/kg mice. The clinical findings are summarized in the Sponsor's table below.

Clinical Signs in the Main Experiment

		Number of Animals With Observed Signs/Number of Surviving Animals						Number of Animals Found Dead/Total Number of Animals Dosed	
		Males			Females				
Treatment (25 mL/kg)	Observation*	Day 0	Day 1	Day 2	Day 0	Day 1	Day 2	Males	Females
0.1M phosphate buffer (pH 7.0)	Normal	10/10	10/10	5/5	10/10	10/10	5/5	0/10	0/10
Plecanatide (SP-304) 500 mg/kg	Normal	5/5	5/5	N/A	5/5	5/5	N/A	0/5	0/5
1000 mg/kg	Normal Piloerection	0/5 5/5	5/5 0/5	N/A	0/5 5/5	5/5 0/5	N/A	0/5	0/5
2000 mg/kg	Lethargy Piloerection	15/15 15/15	0/15 15/15	0/10 10/10	15/15 15/15	0/15 15/15	0/10 10/10	0/15	0/15
Cyclophosphamide 50 mg/kg	Normal	5/5	5/5	N/A	5/5	5/5	N/A	0/5	0/5

N/A = No observations are applicable since these animals were sacrificed for 24 hour bone marrow collection.
*Observations of animals assigned for toxicokinetic evaluation are not presented.

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

Summary of Micronucleus Assay Results

Treatment (PO, 25 mL/kg)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
0.1M Phosphate buffer, pH 7.0	M	24	5	0.585 ± 0.03	---	0.3 ± 0.45	3 / 10000
	F	24	5	0.536 ± 0.05	---	0.8 ± 0.45	8 / 10000
Plecanatide (SP-304) 500 mg/kg	M	24	5	0.568 ± 0.03	-3	0.3 ± 0.27	3 / 10000
	F	24	5	0.545 ± 0.06	2	0.3 ± 0.45	3 / 10000
1000 mg/kg	M	24	5	0.585 ± 0.07	0	0.3 ± 0.27	3 / 10000
	F	24	5	0.523 ± 0.07	-2	0.2 ± 0.27	2 / 10000
2000 mg/kg	M	24	5	0.552 ± 0.07	-6	0.2 ± 0.45	2 / 10000
	F	24	5	0.560 ± 0.03	4	0.6 ± 0.65	6 / 10000
Cyclophosphamide 50 mg/kg	M	24	5	0.402 ± 0.04	-31	23.6 ± 5.82	*236 / 10000
	F	24	5	0.428 ± 0.04	-20	19.3 ± 8.81	*193 / 10000
0.1M Phosphate buffer, pH 7.0	M	48	5	0.590 ± 0.03	---	0.0 ± 0.00	0 / 10000
	F	48	5	0.601 ± 0.04	---	0.5 ± 0.00	5 / 10000
Plecanatide (SP-304) 2000 mg/kg	M	48	5	0.567 ± 0.02	-4	0.3 ± 0.27	3 / 10000
	F	48	5	0.570 ± 0.04	-5	0.2 ± 0.27	2 / 10000

*Statistically significant increase compared to vehicle control, $p \leq 0.05$ (binomial distribution, Kastenbaum-Bowman Tables)
PCE: Polychromatic Erythrocytes; MPCEs (MNPCEs): Micronucleated Polychromatic Erythrocytes

Plecanatide had no effect on the incidence of micronuclei. The mean number of PCEs was not decreased after treatment with plecanatide compared to the mean value of PCEs in vehicle controls, indicating that plecanatide did not have cytotoxic effects in bone marrow. Cyclophosphamide (50 mg/kg), the positive control, induced a significant increase in micronucleated PCEs in male and female mice, compared to vehicle controls ($p \leq 0.005$).

Plasma samples from plecanatide-treated or vehicle control mice were analyzed for plecanatide concentrations. The data are shown in the Sponsor's table below.

Plasma Plecanatide Concentrations in the In Vivo Micronucleus Assay in Mice

Sex	Dose (mg/kg)	0[a]	500			1000			2000[b]		
	Interval (minutes)	15	5	15	45[c]	5	15	45	5	15	45
F	n	3	3	3	3	3	3	3	3	3	3
	Mean	0.0	**157.7**	51.7	39.2	**241.7**	138.7	57.5	**20333.3**	503.0	244.1
	Std. Dev	0.0	10.8	7.6	20.5	36.7	81.7	65.5	32451.9	560.6	200.5
M	n	3	3	3	3	3	3	3	3	3	3
	Mean	0.0	**132.7**	120.8	11.4	**329.0**	126.9	124.5	**876.7**	455.7	161.4
	Std Dev	0.0	32.7	46.5	11.0	75.3	33.0	84.3	409.4	236.6	83.0
Total	n	6	6	6	6	6	6	6	6	6	6
	Mean	0.0	**145.2**	86.3	25.3	**285.3**	132.8	91.0	**10605.0**	479.3	202.8
	Std Dev	0.0	25.7	48.2	21.2	71.4	56.1	76.8	23127.6	385.7	144.5

Mean values are presented ng/mL

a. In the initial analysis, one female (ID# 103) and two male (ID# 99 and 101) mice had values above the limit of quantification (14.8, 10.1, and 11.9 ng/mL, respectively). At the request of the Sponsor, duplicate samples from each mouse were retested to confirm the initial results, and those samples were all below the 10 ng/mL limit of quantitation (see Table 6 of Appendix V for details).

b. One female mouse (ID# 98) at the 5 minute sampling interval had an extreme value (57800 ng/mL). If that value is excluded, the mean (and SD) for the females at 2000 mg/kg is 1600.0 ng/mL (792.0) and the corresponding total mean (and Std Dev) for both sexes combined is 1166.0 (630.5).
The mean value in **bold** at each dose level in the groups given plecanatide is the maximum value among the three intervals.

c. One of the male mice (animal #125) had plecanatide values below the lower limit of quantification (BLOQ).

The data showed that measurable concentrations of plecanatide were detected in all treated animals and at all the time points tested.

In summary, plecanatide did not produce an increase in the incidence of micronucleated PCE in any of the doses tested. Plecanatide is not considered to be clastogenic in the in vivo mouse micronucleus assay.

7.4 Other Genetic Toxicity Studies

8 Carcinogenicity

Study title: Plecanatide (SP-304): A 2-Year Oral (Gavage) Carcinogenicity Study in CD-1 (ICR) Mice

Study no.:	12-2324
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	2/12/2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide (SP-304), #120810 (97.5%), #121126 (98.4%)
CAC concurrence:	Yes (meeting minutes dated 1/29/2013)

Key Study Findings

- Male and female mice (60/sex/group) received 0 (vehicle), 10, 30, or 90 mg/kg/day plecanatide (SP-304) by oral gavage.

- Due to low survival in the control males and 10 mg/kg/day females, all surviving males and females were sacrificed beginning on week 98 and 104, respectively, based on the Executive CAC recommendations.

- There were no drug-related effects on mortality rate, clinical signs, bodyweight or bodyweight gain, or food consumption in either sex.

- There were no drug-related macroscopic findings or incidence of palpable masses in either sex.

- There was no dose-dependent statistically significant increase in either rare or common tumors in either sex. A significant increase ($p = 0.039$) in the incidence of skin pleomorphic fibrosarcoma occurred in the 10 mg/kg/day males (5/60, compared to 0/60 in controls), but without a significant trend ($p=0.931$). This increase is not considered as drug-related because of the lack of dose-dependency. There were no statistically significant changes in combined benign and malignant tumor incidences.

- There were no drug-related non-neoplastic findings.

Adequacy of Carcinogenicity Study

This study used doses of 0 (vehicle), 10, 30, and 90 mg/kg/day that were recommended by the Executive CAC. The study length was acceptable since all surviving animals were sacrificed beginning on week 98 (males) or week 104 (females) based on

recommendations by the Executive CAC, due to low survival in the control males and 10 mg/kg/day females. Analysis of mortality showed no drug-related increase in mortality rate. The carcinogenicity study was conducted appropriately.

Appropriateness of Test Models

The CD-1 COBS Swiss Albino [Crl:CD-1(ICR)BR] strain is an appropriate model because this strain is known to be responsive to known carcinogens and historical control data have been established in the conducting laboratory. The test model used by the Sponsor was appropriate.

Evaluation of Tumor Findings

There were no significant drug-related neoplastic findings in male or female mice treated with plecanatide in the 2-year oral carcinogenicity study. According to the FDA statistical review by Dr. Hepei Chen dated 10/11/2016, based on the criteria of adjustment for multiple testing, the increase in the incidence of skin and subcutis pleomorphic fibrosarcoma pleomorphic fibrosarcoma was statistically significant ($p = 0.0412$, significant for rare tumor criterion) in the low dose males when compared to the vehicle control group. However, the trend test was not statistically significant ($p = 0.9091$). Therefore, the finding is not considered as biologically significant. No other statistically significant findings were noted for male or female mice. The Executive CAC noted that the study was adequate, and concluded that the study was negative for drug-related neoplasms (see meeting minutes dated 7/26/2016).

Methods

Doses:	0 (vehicle), 10, 30, 90 mg/kg/day
Frequency of dosing:	once daily
Dose volume:	5 ml/kg
Route of administration:	oral gavage
Formulation/Vehicle:	solution/distilled water
Basis of dose selection:	Executive Carcinogenicity Assessment Committee recommendations; the Committee recommended doses of 10, 30, and 90 mg/kg/day by oral gavage, based on a sufficiently high multiple of local (intestinal) exposure relative to a pharmacologically active dose in a mouse model of DSS-induced colitis, as predicted by a mg/kg comparison.
Species/Strain:	Mice/CD-1 COBS Swiss Albino [Crl:CD-1(ICR)BR]
Number/Sex/Group:	60
Age:	7 weeks old
Animal housing:	Up to 5 of the same sex and dose group in a polycarbonate body cage with a stainless steel mesh lid with bedding
Paradigm for dietary restriction:	No
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	No
Deviation from study protocol:	Due to low survival in the control males and 10 mg/kg/day females, all surviving male and female animals were sacrificed beginning on week 98 and 104, respectively, based on the Executive CAC recommendations conveyed on November 18, 2014. There were other minor deviations that did not affect the quality or integrity of the study.

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

Study Design of the 2-Year Carcinogenicity Study in Mice

Group	Treatment	Dose (mg/kg/day)	Number of animals Main study	
			Male	Female
1	Control	0	60	60
2	plecanatide	10	60	60
3	plecanatide	30	60	60
4	plecanatide	90	60	60

Observations and Results

Mortality

All animals were observed twice daily for morbidity and mortality. Due to low survival in the male control group and the 10 mg/kg/day female group, all surviving animals were sacrificed beginning on week 98 (males) or week 104 (females), based on the Executive CAC recommendations conveyed on November 18, 2014.

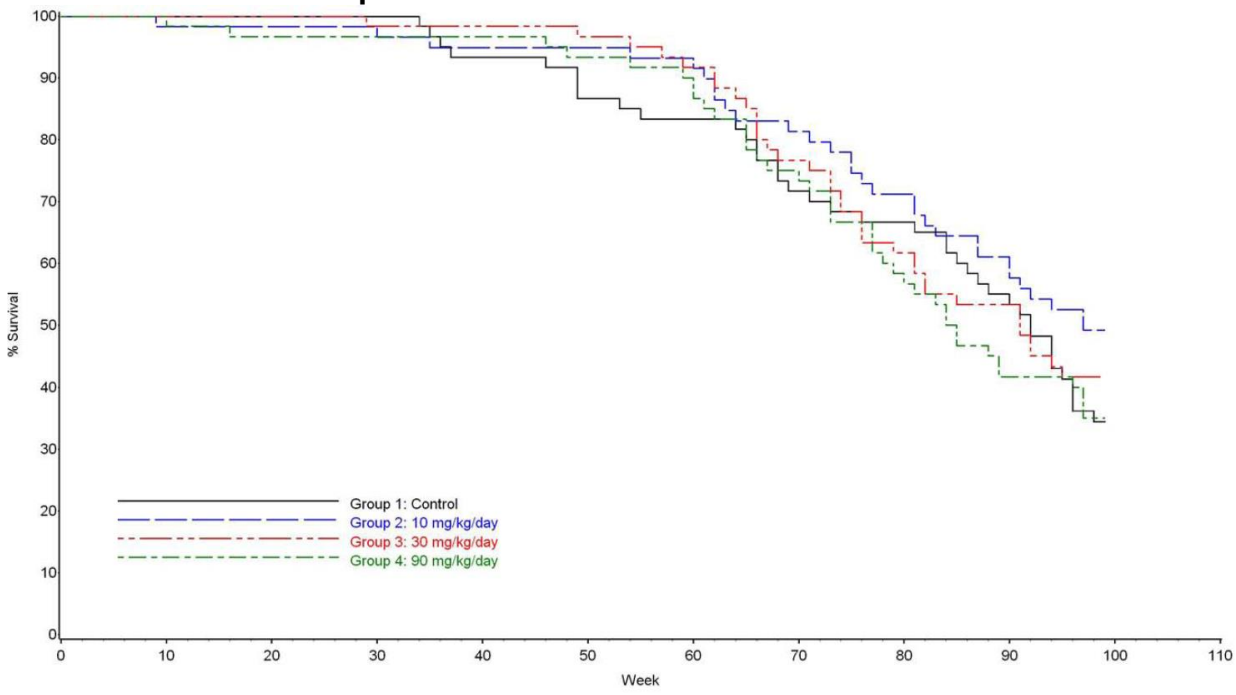
At the end of the dosing period there were no drug-related increases in the overall number of unscheduled deaths, and survival was similar among all groups for both males and females. The survival rates are summarized in the table and figures below taken from the Sponsor's study report.

Percent Survival at Termination

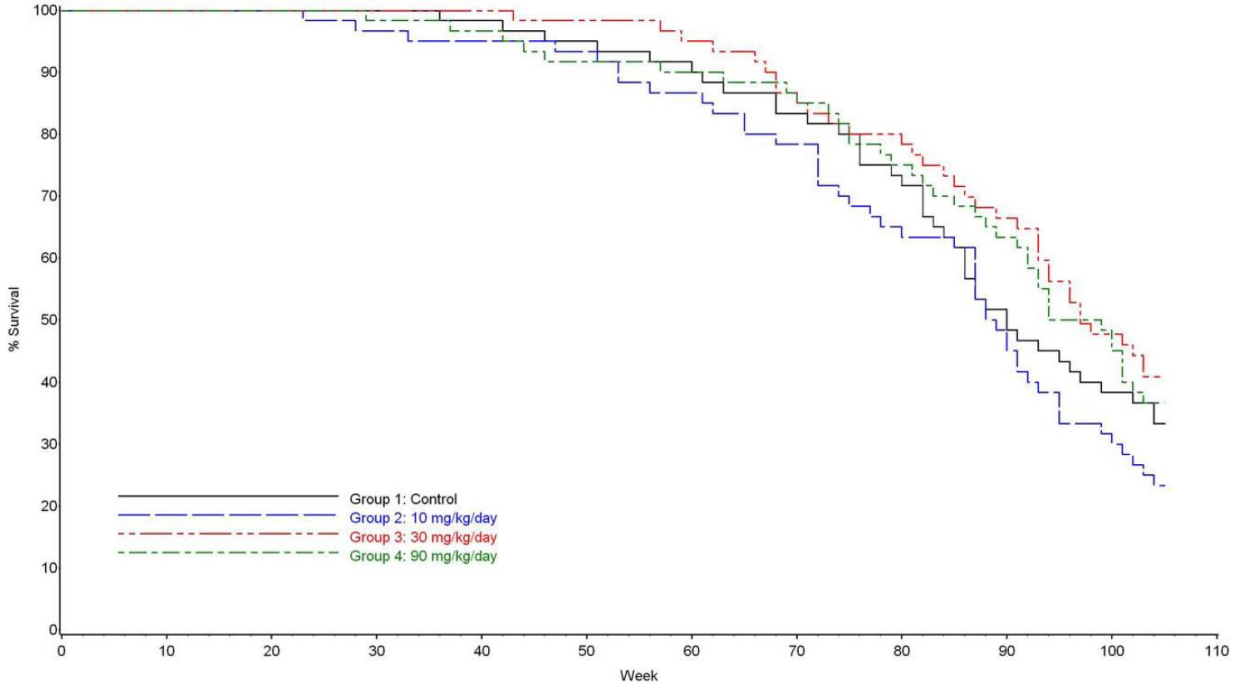
Dose (mg/kg/day)	0	10	30	90
Treatment	Vehicle	Plecanatide	Plecanatide	Plecanatide
Males	33%	48%	42%	35%
Females	33%	23%	40%	37%

[a]Initial on-test number of animals = 60/sex/group, the % survival was calculated based on the number of terminal sacrificed animals versus the initial on-test number; terminal sacrifice commenced Week 98 for males and Weeks 104 for females.

Kaplan-Meier Survival Function for Males



Kaplan-Meier Survival Function for Females



According to the FDA statistical review by Dr. Hepei Chen, dated 10/11/2016, there was no statistically significant difference in mortality rates in either male or female mice.

The table below (taken from the Sponsor's report) shows that the major cause of death was due to hematopoietic neoplasm. However, none of the causes of death showed dose-dependency. Therefore, the deaths are not considered as drug-related.

Major Causes of Death in Mice for up to 99 Weeks (Males)[1] or 105 Weeks (Females)[2]

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	10	30	90	0	10	30	90
Number of study animals	60	60	60	60	60	60	60	60
Number of Unscheduled Decedents	39	31	35	39	40	46	35	38
Hematopoietic Neoplasm	8	6	9	9	17	26	22	23
Skin Neoplasms	3	8	2	2	1	5	2	4
Chronic Progressive Nephropathy	0	1	6	2	7	5	1	1
Undetermined	3	1	1	4	2	0	1	2

1: Sacrifice initiated on Week 98

2: Sacrifice initiated on Week 104

Clinical Signs

Animals were observed twice daily for abnormalities and general condition. Detailed observations were performed twice during the pre-dose period, and once weekly during the treatment period. The observations included, but were not limited to, evaluation of the skin, fur, eyes, nose, oral cavity, abdomen, external genitalia, and respiration. Palpation of masses was performed, with recording of location and size of the masses.

There were no meaningful drug-related clinical signs. The following notable clinical signs were observed.

Clinical Signs in the 2-Year Carcinogenicity Study in Mice

		Dose (mg/kg/day)			
Clinical signs	**Sex**	0	10	30	90
Number/sex/group		60	60	60	60
Build (Deformity)					
Lower ventral surface swollen area	M	1	1	1	1
	F	1	1	0	8
Upper dorsal surface swollen area	M	0	3	2	2
	F	0	1	0	2
Skin					
Left ear encrustation	M	0	0	4	3
	F	1	0	0	2
Right ear encrustation	M	2	8	5	4
	F	0	0	1	1
Ears encrustation	M	0	0	1	2
	F	0	0	0	1
Skin color					
Dark lower ventral surface	M	0	0	0	2
	F	1	1	2	3
Right ear reddening	M	1	0	3	2
	F	0	0	1	0

These findings are not considered as drug-related because they lack dose-dependency, occurred in a single sex, and/or were in low frequency.

There were no drug-related effects on the incidence of palpable masses. The Sponsor's table below summarizes the incidence of masses.

Palpable Masses – Group Distribution

	Vehicle	Plecanatide (SP-304)		
Dose Group	1	2	3	4
Dose (mg/kg/day)		10	30	90

Group /Sex	Multiplicity ∅ 0	1	2	3	4 or more	Number of animals with palpable masses	Total number of palpable masses	Mean time of onset*
1M	30	17	4	6	2	29	51	45
2M	28	21	4	4	3	32	55	51
3M	30	19	9	2	0	30	43	54
4M	33	20	6	1	0	27	35	48
1F	50	6	4	0	0	10	14	72
2F	48	9	3	0	0	12	15	68
3F	47	11	0	0	1	12	15	87
4F	46	12	2	0	0	14	16	84

+ - Including palpable masses which regressed or were not positively identified at *post mortem* examination, ∅ - Expressed as number of animals bearing the indicated number of palpable masses, * - In weeks to onset of first recorded swelling including those found at necropsy examination.

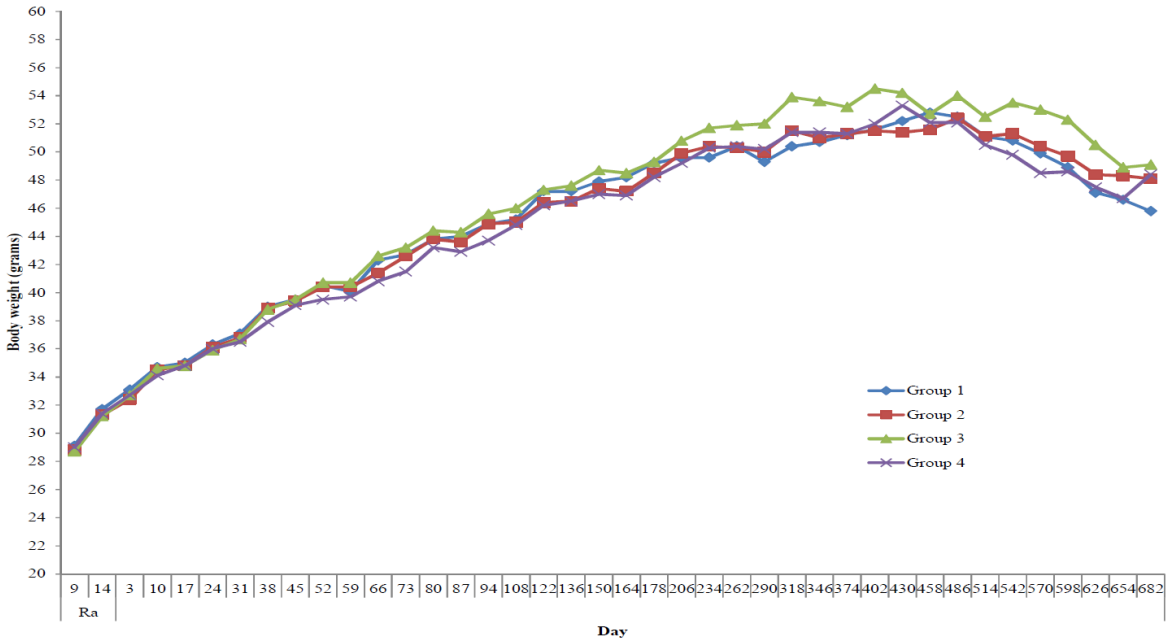
Body Weights

Animals were weighed (non-fasted) twice during the pre-dose period and weekly for the first 13 weeks, bi-weekly for weeks 14 to 26, and every 4 weeks thereafter.

There were no statistically significant changes in bodyweights throughout the treatment period. Sporadic, and sometimes statistically significant changes in bodyweight gains were noted. These changes were transient and not dose-dependent, and are not considered as drug-related. The figures below, taken from the Sponsor's study report, summarizes the effects of plecanatide on bodyweight.

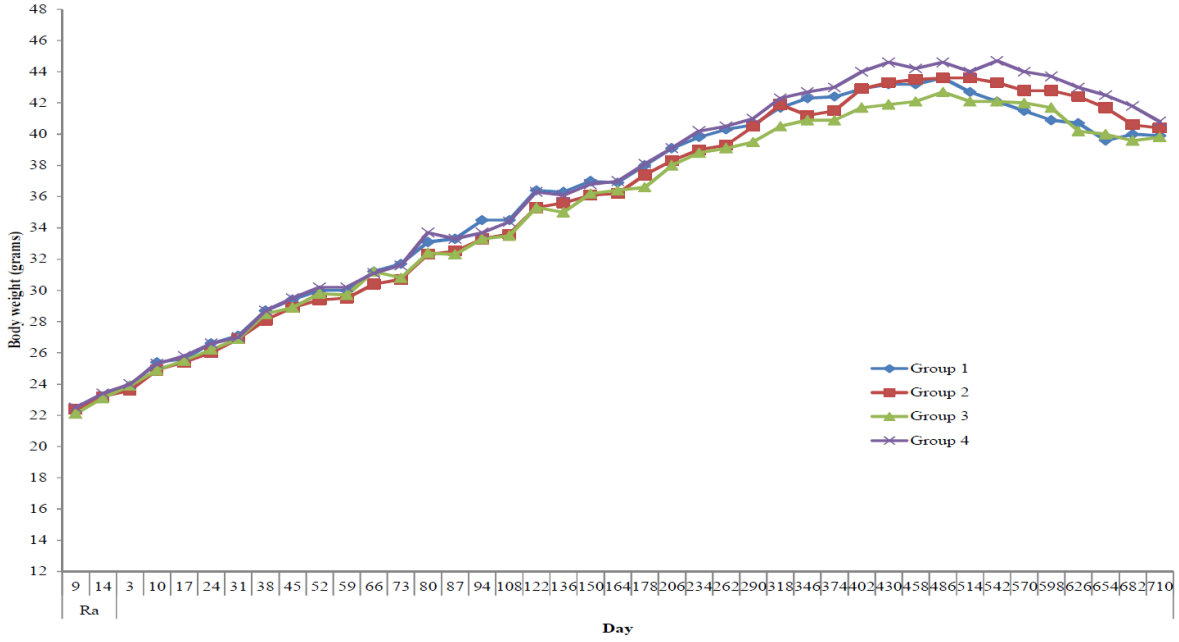
Mean Bodyweight Data – Males

	Vehicle	Plecanatide (SP-304)		
Dose Group	1	2	3	4
Dose (mg/kg/day)		10	30	90



Mean Bodyweight Data – Females

	Vehicle	Plecanatide (SP-304)		
Dose Group	1	2	3	4
Dose (mg/kg/day)		10	30	90

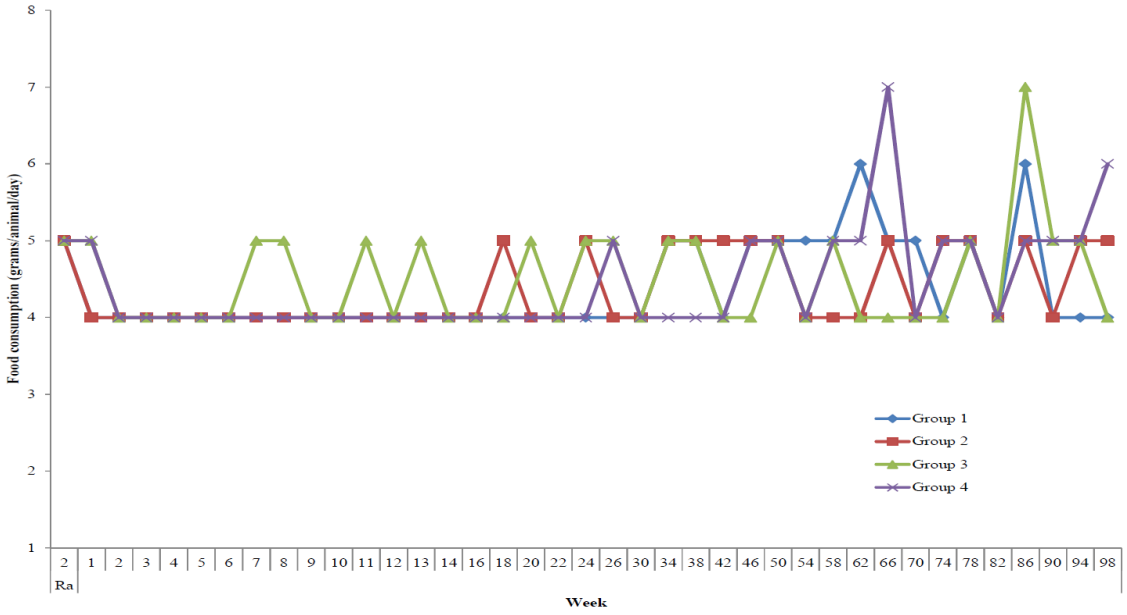


Feed Consumption

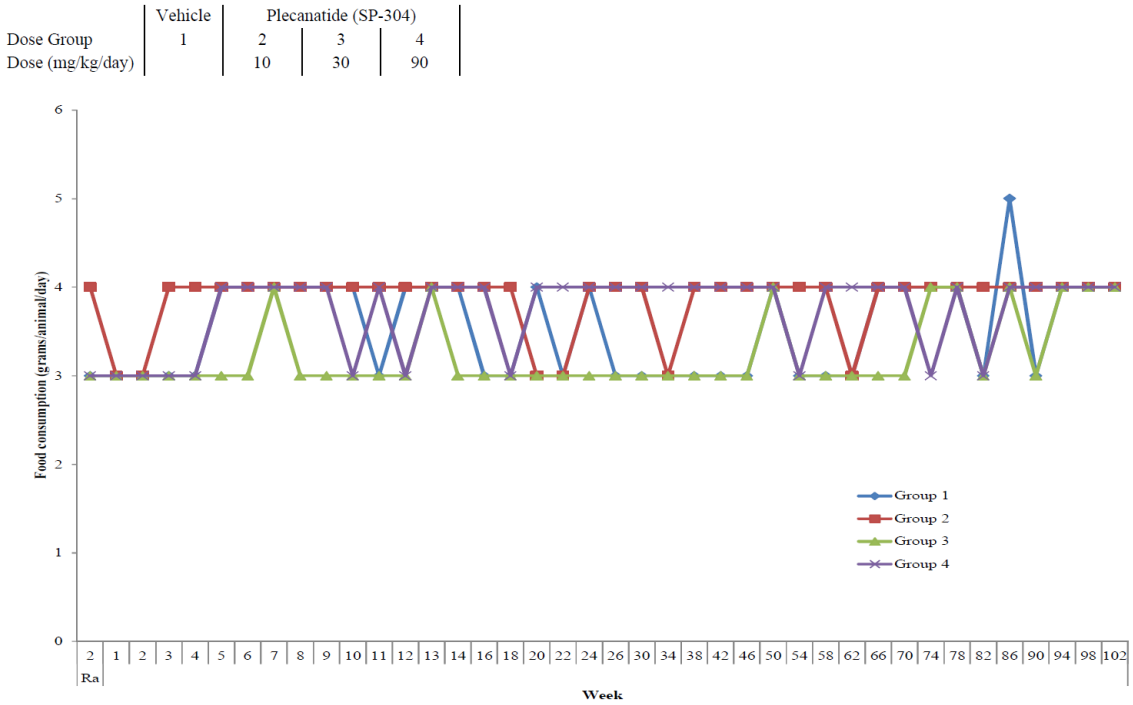
Food consumption was recorded during the pre-dose period and weekly at weeks 1 through 13, biweekly at weeks 14 to 26, and every 4 weeks thereafter. As shown in the figures below (taken from the Sponsor's report), there were no meaningful drug-related changes in food consumption, although sporadic and sometimes statistically significant changes were noted.

Food Consumption in the 2-Year Carcinogenicity Study in Male Mice

	Vehicle	Plecanatide (SP-304)		
Dose Group	1	2	3	4
Dose (mg/kg/day)		10	30	90



Food Consumption in the 2-Year Carcinogenicity Study in Female Mice



Gross Pathology

Observations were made at sacrifice or immediately after premature death. Necropsies were performed on all carcinogenicity animals during scheduled sacrifice, animals found dead, and animals sacrificed prior to study termination. The animals were examined for palpable masses, and abnormalities were identified and correlated with postmortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities.

The following noteworthy findings were observed.

Notable Macroscopic Findings in the 2-Year Carcinogenicity Study in Mice

Organ/Tissue	Sex	Doses (mg/kg/day)			
		0	10	30	90
Number/group/sex examined		60	60	60	60
Bone (Femur including joint)					
Brittle	M	0	0	2	2
	F	0	0	0	0
Kidney					
Abnormal color	M	2	1	8	2
	F	4	4	1	0
Lung and Bronchi					
Dark areas	M	3	5	3	2

	F	8	2	4	5
Lymph Node (Mesenteric) *Abnormal color*	 M F	 0 0	 1 2	 0 1	 1 2
Seminal vesicle *Enlarged*	 M	 20	 26	 28	 26
Skin and Subcutis *Encrustation*	 M F	 0 0	 1 0	 0 0	 2 2
Uterus and Cervix *Dark areas*	 F	 0	 0	 1	 2

There were no meaningful drug-related macroscopic findings. The findings lacked dose-dependency, occurred in only one sex, and/or were low in frequency.

Histopathology

All study animals, including those euthanized in moribund condition or found dead before the end of the dosing period, were submitted to full necropsy. The following tissues and organs were collected for microscopic examination during scheduled and unscheduled sacrifice, and from animals found dead during the study (table taken from Sponsor's study report).

Tissues and Organs Collected for Microscopic Examination

ORGAN NAME	PRESERVED	EXAMINED MICROSCOPICALLY
animal identification	X	
adrenals	X	X
aorta (thoracic)	X	X
bone marrow smear (femur)	X	
bone (femur including joint)	X	X
bone (sternum)	X	X
bone marrow (femoral)	X	X[a]
bone marrow (sternal)	X	X[a]
brain	X	X
cecum	X	X
colon	X	X
duodenum	X	X
epididymides	X	X
esophagus	X	X
eyes	X	X
gallbladder	X	X
Harderian gland	X	X
heart	X	X
ileum	X	X
jejunum	X	X
kidneys	X	X
lacrimal glands	X	X
liver	X	X
lungs (with mainstem bronchi)	X	X
lymph node (mesenteric, axillary)	X	X
nerve (sciatic)	X	X
optic nerve	X	
ovaries	X	X
pancreas	X	X

ORGAN NAME	PRESERVED	EXAMINED MICROSCOPICALLY
Peyer's patches\GALT	X	X[b]
pituitary	X	X
prostate	X	X
rectum	X	X
salivary glands (mandibular)	X	X
seminal vesicles	X	X
skeletal muscle, thigh	X	X
skin and mammary	X	X[b]
spinal cord (cervical, thoracic and lumbar)	X	X
spleen	X	X
stomach	X	X
testes	X	X
thymus	X	X
thyroid (with parathyroid)	X	X
tongue	X	
trachea	X	X
urinary bladder	X	X
uterus and cervix	X	X
vagina	X	X
gross lesions and masses	X	X

[a]Qualitative examination (no differential count).
[b]Mammary gland for males and GALT were evaluated only if present in routine sections.

Peer Review - Yes
A peer review was performed at (b) (4) All benign and malignant neoplasms from all animals on study and all tissues from six males and six females in the control and high-dose group animals were evaluated.

Neoplastic
In the Sponsor's analysis, non-palpable tumors were categorized as non-incidental if the tumor was a factor contributing towards the death of the animal, or as incidental otherwise. For statistical purposes, all animals that died after terminal sacrifice commenced (weeks 98 and 104 for males and females, respectively) were considered terminal, and the tumors observed in these animals were categorized as incidental. For palpable tumors, each tumor was classified as non-incidental if it was palpable before death and before the terminal sacrifice commenced, or if the tumor was a factor contributing towards the death of the animal. The tumor was classified as incidental if the tumor was first found after death and was not a factor contributing towards the death

of the animal, or if the tumor was first found in or after the first week of the terminal sacrifice.

The analyses were carried out for benign, malignant, and benign and malignant tumors combined, where appropriate. If an animal had a benign non-palpable tumor and a malignant non-palpable tumor, then only the malignant tumor was included in the analysis of both tumors together. If an animal had more than one palpable tumor of the same category, benign or malignant, then only the first observed tumor was included in the analysis. Tumor types were selected for full statistical analysis where at least 2 tumors were observed in treated groups for which all animals were examined.

One-sided positive trends in common (background incidence rate > 1%) and rare (background incidence < 1%) tumors, as identified by the study pathologist, were evaluated at the 0.005 and 0.025 significance levels, respectively. Pair-wise comparisons in common and rare tumors were evaluated at the 0.01 and 0.05 significance levels, respectively.

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

The following abbreviations were used:

B-	Benign
C-	Multicentric
D-	Multicentric benign
ECL	Enterochromaffin-like
F-	Infiltrating
GALT	Gut-associated lymphoid tissue
I-	Locally invasive
Lt	Left
M-	Malignant
M.	Muscle
MALT	Mucosa-associated lymphoid tissue
N-	Metastatic
NALT	Nasal-associated lymphoid tissue
NOS	Not Otherwise Specified
PALS	Periarteriolar lymphoid sheaths
PCR	Polymerase chain reaction
Rt	Right
S-	Systemic
V.	Vein
X-	Unknown primary

Noteworthy neoplastic tumor findings are summarized in the table below.

Summary of Noteworthy Neoplastic Changes in the 2-Year Carcinogenicity Study in Mice

Organ/Tissue	Sex	Doses (mg/kg/day)			
		Control	10	30	90
Number/group/sex examined		60	60	60	60
Adrenals					
Lymphoma	M	3	2	4	1
	F	9	17	13	16
Adrenals					
B-Subcapsular cell adenoma	M	1	1	0	0
	F	1	1	2	1
N-Sarcoma	M	0	0	0	0
	F	0	1	0	0
M-Subcapsular cell carcinoma	M	0	0	0	0
	F	0	1	0	0
Bone Marrow (Femoral)					
N-Lymphoma	M	0	3	4	1
	F	6	10	5	5
Bone Marrow (Sternal)					
N-Lymphoma	M	0	3	4	1
	F	6	9	8	7
Bone (Femur including joint)					
N-Lymphoma	M	3	2	3	1
	F	6	8	7	11
Bone (Sternum)					
N-Lymphoma	M	3	3	5	3
	F	10	10	9	18
Cecum					
Lymphoma	M	0	1	2	1
	F	2	3	0	5
Colon					
N-Lymphoma	M	0	2	1	1
	F	3	4	1	4
Duodenum					
N-Lymphoma	M	0	1	2	1
	F	2	2	1	3
Esophagus					
N-Lymphoma	M	0	0	0	3
	F	7	3	5	8
Gall Bladder					
N-Histiocytic sarcoma	M	0	1	0	0
	F	0	1	1	2
Harderian Glands					
N-Lymphoma	M	2	4	5	2
	F	7	10	11	11
Heart					
N-Lymphoma	M	4	3	5	3
	F	10	15	13	20

Hemopoietic System					
N-Lymphoma	M	7	6	12	8
	F	15	22	23	28
Jejunum					
N-Lymphoma	M	0	2	0	1
	F	0	2	0	2
Kidneys					
N-Lymphoma	M	7	4	9	4
	F	11	16	18	20
Lacrimal Glands					
N-Lymphoma	M	4	5	7	4
	F	8	17	15	14
Liver					
M-Hepatocellular carcinoma	M	8	7	7	11
	F	0	0	1	0
N-Lymphoma	M	6	5	8	3
	F	9	17	16	17
B-Hemangioma	M	0	1	2	0
	F	0	0	0	2
Lung					
N-Lymphoma	M	7	6	12	8
	F	12	18	15	18
B-Bronchiolo-alveolar adenoma	M	5	3	7	2
	F	4	5	3	4
M-Bronchiolo-alveolar carcinoma	M	4	4	5	7
	F	5	0	5	6
Lymph Node (Mesenteric)					
N-Lymphoma	M	6	4	7	7
	F	9	18	20	24
Mammary					
N-Lymphoma	F	10	16	12	12
M-Adenocarcinoma	F	3	1	3	0
M-Carcinosarcoma	F	1	0	0	0
Nerve (Sciatic)					
N-Lymphoma	M	3	1	3	1
	F	6	8	8	9
Ovary					
N-Lymphoma	F	9	16	14	16
B-Luteoma	F	1	0	1	0
B-Cystadenoma	F	0	2	1	2
B-Sertoli tumor	F	1	0	0	1
B-Mixed sex cord stromal	F	0	0	1	0
Pancreas					
N-Lymphoma	M	3	4	5	3

	F	10	16	15	12
Pituitary					
N-Lymphoma	M	0	0	0	1
	F	1	4	1	1
Prostate					
N-Histiocytic sarcoma	M	0	2	1	1
Salivary Gland (Mandibular)					
N-Lymphoma	M	4	4	4	5
	F	7	14	14	11
Seminal Vesicles					
N-Lymphoma	M	3	2	5	5
Skeletal Muscle (thigh)					
N-Lymphoma	M	3	1	3	0
	F	4	7	9	7
Skin and Subcutis					
N-Lymphoma	M	4	3	4	5
	F	4	4	13	9
M-Fibrosarcoma	M	4	2	3	2
	F	0	4	1	2
M-Fibrosarcoma (pleomorphic)	M	0	5	0	0
	F	1	0	2	2
M-sarcoma NOS	M	0	0	0	0
	F	0	0	1	0
M-schwannoma	M	0	0	0	0
	F	0	1	1	1
B-squamous cell papilloma	M	0	0	1	0
	F	0	0	0	1
M-squamous cell carcinoma	M	0	0	1	0
	F	0	0	0	1
B-hair follicle tumor	M	0	0	0	0
	F	0	1	0	0
Spleen					
N-Lymphoma	M	6	5	10	5
	F	13	16	20	19
Stomach					
N-Lymphoma	M	4	3	2	2
	F	4	12	13	13
Testis					
B-Interstitial cell adenoma	M	0	0	2	1
Thymus					
N-Lymphoma	M	4	4	6	7
	F	15	18	22	27
Thyroids					
N-Lymphoma	M	1	0	2	1
	F	0	5	4	2
Trachea					
N-Lymphoma	M	4	0	4	3
	F	3	10	9	10

Urinary Bladder *N-Lymphoma*	M F	4 10	2 13	6 15	3 18
Uterus and Cervix *N-Lymphoma*	F	9	14	9	19
B-leiomyoma	F	0	0	2	1
M-Leiomyosarcoma	F	0	0	2	1
Vagina *N-Lymphoma*	F	5	9	7	7

The Sponsor reported a significant increase (p = 0.039) in the incidence of skin fibrosarcoma in the 10 mg/kg/day male group (5/60, 8.3%), compared to controls (0/60), but without a significant trend (p=0.931). This increase is not considered as drug-related because of the lack of dose-dependency. The Sponsor reported that there were no other statistically significant changes, in either direction, in any other neoplastic lesion in males or females, even when the incidences of some of the adenoma and carcinoma were combined.

According to the FDA statistical review by Dr. Hepei Chen, dated 10/11/2016, based on the criteria of adjustment for multiple testing, the increase in the incidence of skin and subcutis pleomorphic fibrosarcoma was statistically significant (p = 0.0412, significant for rare tumor criterion) in the low dose male group when compared to the vehicle control group. However, the trend test was not statistically significant (p = 0.9091). Therefore, it is this reviewer's conclusion that the finding is not considered biologically significant because the finding had no dose relationship. No other statistically significant findings were noted for male or female mice. The summary table below is from Dr. Chen's review.

Table 4. Summary Table of Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship and/or Pairwise Comparisons of Treated Groups and Vehicle Control Group in Mice

Organ name	Tumor name	0 mg Vehicle (C) P - Trend	10 mg Low (L) P - C vs. L	30 mg Mid (M) P - C vs. M	100 mg High (H) P - C vs. H
Male-Skin and Subcutis	Fibrosarcoma, Pleomorphic	0/33 (59) 0.9091	5/39 (60) 0.0412 $	0/35 (60) NC	0/32 (59) NC

& X/YY (ZZ): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
$ = Statistically significant at 0.05 level in rare tumor for test of pairwise group comparison;
NC = Not calculable.

In addition, a request was made by this reviewer to Dr. Chen for a statistical analysis of the following tumor combinations:

1. adrenal subcapsular cell adenoma and carcinoma
2. lung bronchiolo-alveolar adenoma and carcinoma
3. mammary adenocarcinoma and carcinosarcoma

4. ovary luteoma, sertoli cell tumor, cystadenoma, and mixed sex cord stromal tumor
5. skin fibrosarcoma, pleomorphic fibrosarcoma, and not otherwise specified sarcoma
6. skin squamous cell papilloma, squamous cell carcinoma, and hair follicle tumor,

No statistically significant changes were found in any of these combinations. The following are data from Dr. Chen's statistical analysis.

Table 4A: Tumor Rates and P-Values for Trend and Pairwise Comparisons of Tumor Combinations in Male Mice

Organ name	Tumor name	0 mg/kg Vehicle (C) P - Trend	10 mg/kg Low (L) P - C vs. L	30 mg/kg Mid (M) P - C vs. M	90 mg/kg High (H) P - C vs. H
Lungs And Bronchi	Adenoma + Carcinoma, Bronchiolo-Alveolar	9/37 (59) 0.3358	6/38 (60) 0.7369	12/38 (60) 0.3296	9/36 (60) 0.5806
Skin And Subcutis	Fibrosarcoma + Fibrosarcoma, Pleomorphic + Sarcoma Nos	4/35 (59) 0.9018	8/41 (60) 0.2605	3/37 (60) 0.5317	2/33 (59) 0.6347
	Carcinoma, Squamous Cell + Papilloma, Squamous Cell	0/33 (59) 0.4139	0/37 (60) NC	2/35 (60) 0.2612	0/32 (59) NC

& X/YY (ZZ): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed
NC = Not calculable

Table 4B: Tumor Rates and P-Values for Trend and Pairwise Comparisons of Tumor Combinations in Female Mice

Organ name	Tumor name	0 mg/kg Vehicle (C) P - Trend	10 mg/kg Low (L) P - L vs. C	30 mg/kg Mid (M) P - M vs. C	90 mg/kg High (H) P - H vs. C
Adrenals	Adenoma, Subcapsular Cell + Carcinoma, Subcapsular Cell	1/40 (60) 0.5931	2/36 (60) 0.4600	2/44 (58) 0.5361	1/42 (60) 0.2593
Lungs And Bronchi	Adenoma + Carcinoma, Bronchiolo-Alveolar	9/40 (60) 0.4126	5/39 (60) 0.7967	7/44 (59) 0.6882	9/45 (60) 0.5075
Mammary	Adenocarcinoma + Carcinosarcoma	4/41 (59) 0.9586	1/36 (58) 0.7776	3/42 (56) 0.5137	0/41 (58) 0.9421
Ovaries	Luteoma + Tumor, Sertoli Cell, Benign + Cystadenoma + Tumor, Sex Cord Stromal, Mixed, Benign	2/40 (60) 0.3476	2/37 (60) 0.6623	3/43 (59) 0.5347	3/42 (58) 0.5234
Skin And Subcutis	Carcinoma, Squamous Cell + Papilloma, Squamous Cell + Tumor, Hair Follicle	0/40 (60) 0.1194	1/37 (60) 0.4805	0/43 (59) NC	2/43 (60) 0.2654

Organ name	Tumor name	0 mg/kg Vehicle (C) P - Trend	10 mg/kg Low (L) P - L vs. C	30 mg/kg Mid (M) P - M vs. C	90 mg/kg High (H) P - H vs. C
	Fibrosarcoma + Fibrosarcoma, Pleomorphic/Sarcoma Nos	1/40 (60) 0.1575	4/38 (60) 0.1636	4/45 (59) 0.2189	5/44 (60) 0.1242

& X/YY (ZZ): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed
$ = Statistically significant at 0.025 and 0.05 level for rare tumor for tests of dose response relationship and pairwise comparison, respectively
# = Statistically significant at 0.005 and 0.01 level for common tumor for tests of dose response relationship and pairwise comparison, respectively
NC = Not calculable

Non-Neoplastic

The following noteworthy microscopic findings were observed.

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

Organ/Tissue	Sex	Doses (mg/kg/day) 0	10	30	90
Number / group / sex		60	60	60	60
Adrenals					
Vascular	M	0	0	1	0
dilatation/congestion/hemorrhage	F	0	0	0	3
Focal cortical hypertrophy with	M	0	2	0	1
vacuolation	F	0	0	0	1
Bone Marrow (Femoral)					
Increased granulopoiesis	M	24	27	29	24
	F	14	18	12	21
Brain					
Mineralization	M	0	0	0	2
	F	0	0	0	0
Epididymides					
Reduced luminal sperm	M	5	9	9	7
Gall Bladder					
Lumen dilatation	M	4	3	8	7
	F	14	10	14	14
Heart					
Lumen dilatation	M	4	3	8	7
	F	14	10	14	14
Epicardial inflammatory cells infiltrate	M	0	1	0	4
	F	1	1	0	2
Amyloid	M	0	0	0	4
	F	1	0	1	0
Arteritis/Periarteritis	M	0	3	5	2
	F	0	1	1	2
Kidneys					

Inflammatory cell infiltrate	M	3	11	11	11
	F	7	6	14	7
Lymphoid/macrophage aggregates	M	3	9	7	9
	F	7	4	4	13
Lungs and Bronchi					
Lymphoid/macrophage aggregates	M	0	2	2	0
	F	1	1	3	2
Lymph Node (Mandibular)					
Lymphoid hyperplasia	M	0	0	1	2
	F	1	0	0	1
Mammary					
Secretory activity	F	17	18	22	23
Acinar hyperplasia	F	4	5	3	8
Ovaries					
Cyst	F	6	12	6	12
Preputial/Clitoral Glands					
Abscess	F	0	6	3	4
Prostate					
Inflammatory cell infiltrate	M	0	2	1	1
Salivary Gland (Mandibular)					
Lymphoid/Macrophage aggregates	M	2	6	5	9
	F	6	1	6	10
Skin and Subcutis					
Inflammatory cell infiltrate	M	0	1	2	2
	F	0	0	0	1
Preputial/Clitoral gland tissue present	M	9	12	13	11
Stomach					
Non-glandular region	M	3	6	12	10
hyperkeratosis/hyperplasia	F	14	7	6	7
Thymus					
Vascular	M	0	4	1	4
inflammation/degeneration/necrosis	F	2	2	0	1
Urinary Bladder					
Inflammation	M	0	3	3	2
	F	0	0	0	0
Urothelium hyperplasia	M	0	0	2	1
	F	0	0	0	0
Uterus and Cervix					
Cystic endometrial hyperplasia	F	31	29	39	41
Angiectasis	F	0	0	2	1

The non-neoplastic findings lacked dose-dependency, occurred in only one sex, and/or were low in frequency, and therefore are not considered to be drug-related.

Toxicokinetics

N/A

Dosing Solution Analysis

Stock assay solutions were tested appropriately for stability and drug concentration. Mean concentrations of the 2 mg/ml, 6 mg/ml, and 18 mg/ml dosing solutions ranged from 90.0% to 101.6% of nominal concentrations. Dose formulations are solutions at the concentrations used, therefore no homogeneity analysis was performed.

Study title: Plecanatide: A 104-Week Oral (Gavage) Carcinogenicity Study in Sprague-Dawley Rats

Study no.:	1896-011
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	05/08/2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide; #110826 (97.1%), #130117 (96.8%), #120709 (97.3%), #121126 (98.4%), #121026R (97.3%), #121026B (97.1%), #131021 (98.0%), #140402 (98.1%), #140109 (97.5%), #140623 (97.5%)
CAC concurrence:	Yes (meeting minutes dated 4/9/2013)

Key Study Findings

- Male and female rats (66/sex/group) received 0 (vehicle), 10, 30, or 100 mg/kg/day plecanatide (SP-304) by oral gavage.
- Due to low survival in the control groups, all surviving animals were sacrificed on week 94, based on the Executive CAC recommendations.
- There were no drug-related effects on mortality rate, clinical signs, bodyweight and bodyweight gains, or food consumption.
- There were no drug-related macroscopic findings or palpable masses.
- There was no drug-related statistically significant increase in either rare or common tumors. There were no statistically significant changes in combined benign and malignant tumor incidences.
- There were no drug-related non-neoplastic findings.

Adequacy of Carcinogenicity Study

This study used doses of 0 (vehicle), 10, 30, and 100 mg/kg/day that were recommended by the Executive CAC. The study length was acceptable since all surviving animals were sacrificed on week 94 based on recommendations by the Executive CAC, due to low survival in the control males and females. Analysis of mortality showed no drug-related increase in mortality rate. The carcinogenicity study was conducted appropriately.

Appropriateness of Test Models

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

Evaluation of Tumor Findings

There were no significant drug-related neoplastic findings in male or female rats treated with plecanatide in the 104-week oral carcinogenicity study. Based on the criteria of adjustment for multiple testing, the analysis showed a statistically significant positive trend ($p = 0.0216$, significant for rare tumor criterion) for the incidence rates of carcinoma islet cell of pancreas in male rats. However, there were no statistically significant pairwise comparisons for this tumor. Therefore, the finding is not considered biologically significant. No other statistically significant findings were noted for male or female rats. The Executive CAC noted that the study was adequate, and concluded that the study was negative for drug-related neoplasms (see meeting minutes dated 7/26/2016).

Methods

Doses:	0 (vehicle), 10, 30, and 100 mg/kg/day
Frequency of dosing:	once daily
Dose volume:	10 ml/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	solution/distilled water
Basis of dose selection:	Executive Carcinogenicity Assessment Committee recommendations; the Committee recommended doses of 0, 10, 30, and 100 mg/kg/day for female rats based on reduced bodyweight gains in females at 300 mg/kg/day, and doses of 0, 10, 30, and 100 mg/kg/day for male rats based on an anticipated large rat to human margin of local drug concentration in the intestinal tract, and the expectation that the drug concentration in rats will achieve a maximum pharmacological effect.
Species/Strain:	rat/ CD[Crl:CD(SD)]
Number/Sex/Group:	66
Age:	5 weeks old
Animal housing:	pair-housed in solid-bottom cages with bedding
Paradigm for dietary restriction:	N/A
Dual control employed:	N/A
Interim sacrifice:	N/A
Satellite groups:	N/A
Deviation from study protocol:	Due to low survival in the control groups, all surviving animals were sacrificed on week 94, based on the Executive CAC recommendations conveyed on February 2, 2015. There were other minor deviations that did not affect the quality or integrity of the study.

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

Study Design of the 104-week Carcinogenicity Study in Rats

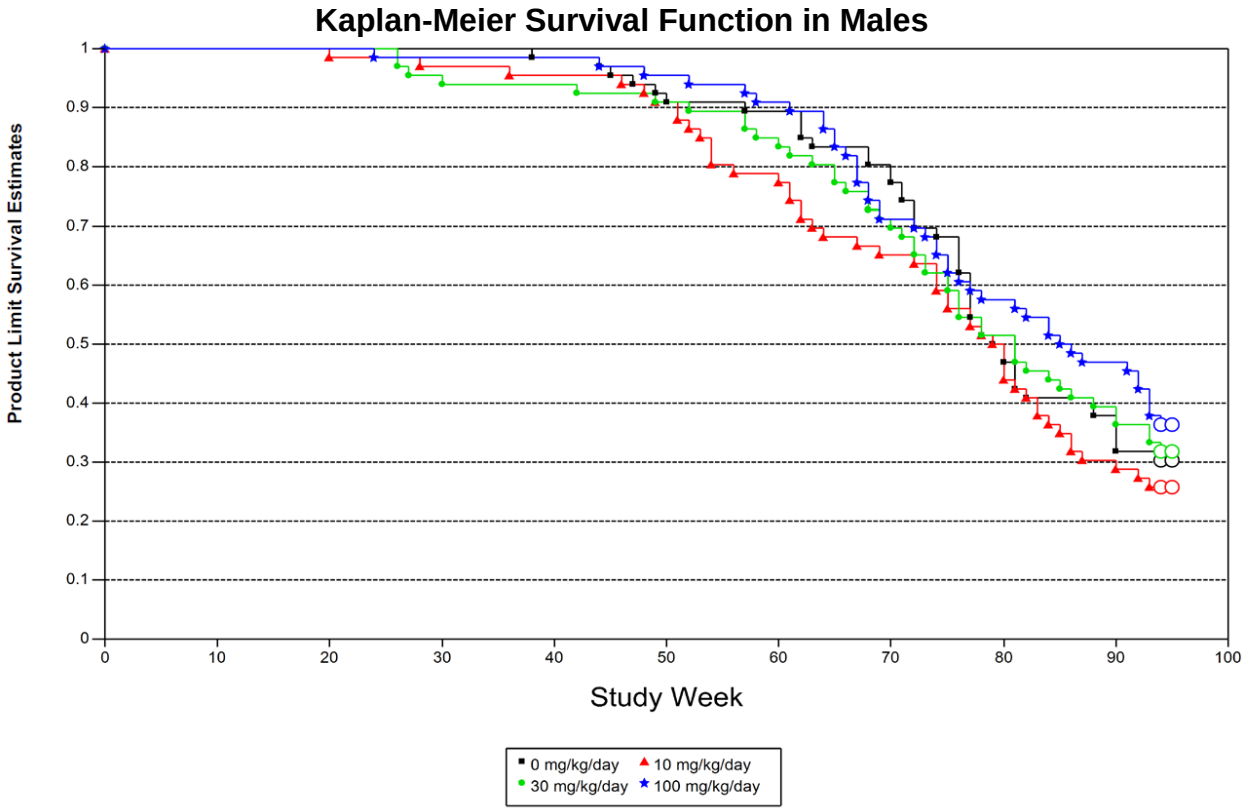
Group Assignments					
			Dose	Number of Animals	
Group Number	Dose Level (mg/kg/day)	Dose Volume (mL/kg/dose)	Concentration (mg/mL)	Male	Female
1	0	10	0	66	66
2	10	10	1	66	66
3	30	10	3	66	66
4	100	10	10	66	66

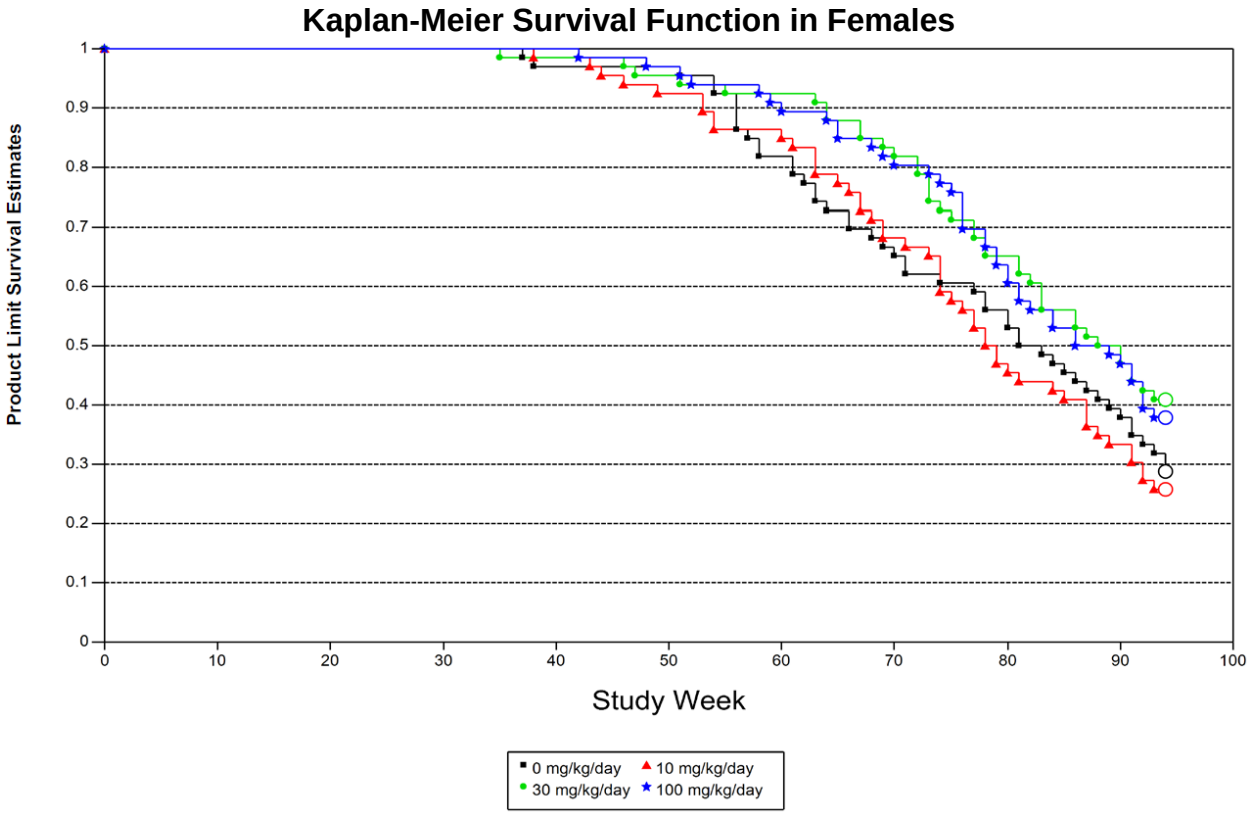
Observations and Results

Mortality

All animals were observed twice daily for morbidity and mortality, and beginning on week 53, a third mortality check in the evening was conducted. Due to low survival in the control groups, all surviving animals were sacrificed on week 94, based on the Executive CAC recommendations conveyed to the Sponsor on February 2, 2015.

At the end of the dosing period, there were no drug-related increases in the overall number of unscheduled deaths; survival rates in the plecanatide-treated groups exceeded that in the control groups in both sexes. The Sponsor indicated that survival rates for the control groups in this study are not atypical or dissimilar from those of recent/current historical control data for Sprague-Dawley rats in 2-year carcinogenicity studies conducted at (b) (4) The survival rates are summarized in the figures below taken from the Sponsor's study report.





The Sponsor indicated that all causes of death or moribundity were either commonly seen in rats of this strain and age or were considered incidental and unrelated to treatment due to the lack of a dose response and/or the lack of similar findings in both sexes. Infectious processes were observed in a very small minority of animals (e.g. bacterial urogenital tract infection, fungal rhinitis); however, the Sponsor indicated that these findings were consistent with those commonly observed in aging rats. The most common cause of death or moribundity in males was pituitary tumors, and unscheduled deaths of undetermined cause were also common in males. In females, the most common causes of death or moribundity were pituitary tumors and mammary tumors.

According to the FDA statistical review by Dr. Hepei Chen, dated 10/11/2016, there was no statistically significant difference in mortality rates in either male or female rats.

Clinical Signs

Animals were observed twice daily for abnormalities and general conditions, and beginning on week 53, a third mortality check in the evening was conducted. Detailed observations were performed once during the pre-dose period, and once weekly during the treatment period.

The table below summarizes the notable clinical signs.

Clinical Signs in the 104-Week Carcinogenicity Study in Rats

Clinical signs	Sex	Dose (mg/kg/day)			
		0	10	30	100
Number/sex/group		66	66	66	66
Appearance					
Impaired limb function	M	6	12	10	9
Red material around eye	M	3	2	8	6
	F	3	6	11	4
Hunched posture	M	4	2	5	8
Swelling	M	15	16	26	24
Thin	M	2	5	6	7
Behavior					
Inappetence	M	1	1	2	4
Ataxia	F	2	8	8	5
Excretion					
Few feces	M	0	1	2	5
Respiration					
Audible	M	4	6	3	10
Skin and Pelage					
Hair discolored, Tan	M	0	0	0	5
Hair discolored, Yellow	F	2	3	5	6
Scabbed area	M	14	9	24	16
Skin discolored, Blue	F	1	3	3	6

There were no drug-related clinical signs. The clinical signs were present in the control groups, showed no relationship to dose, occurred at low frequency, and/or occurred only in one sex; therefore, they were not considered as drug-related.

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

Summary of Notable Masses in the 104-Week Carcinogenicity Study in Rats **Masses**	**Sex**	**Doses (mg/kg/day)** **0**	**10**	**30**	**90**
Number/group/sex examined		66	66	66	66
Anogenital region	F	7	10	12	16
Axillary Region/left	M	1	0	0	5
	F	15	14	7	23
Inguinal region/left	F	6	15	8	10
Inguinal region/right	F	16	10	16	13

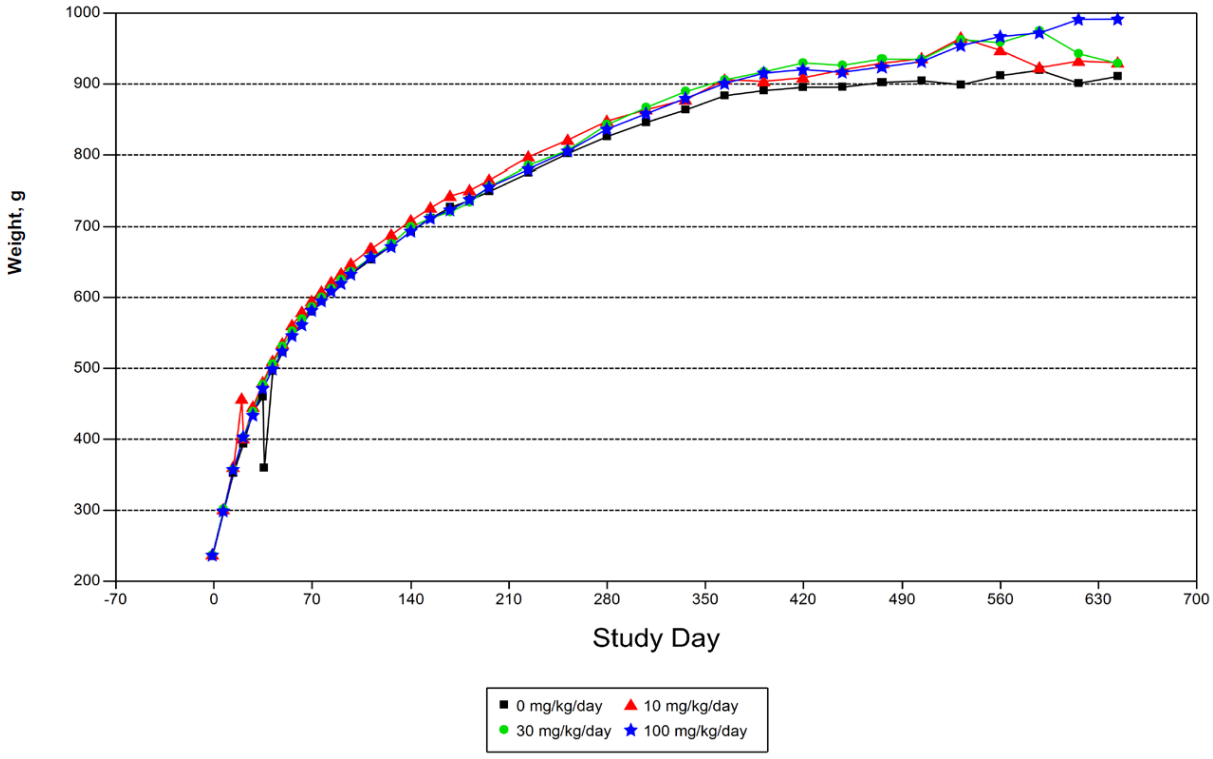
Lumber region	M	0	0	4	5
Thoracic region	F	15	16	12	22

Body Weights

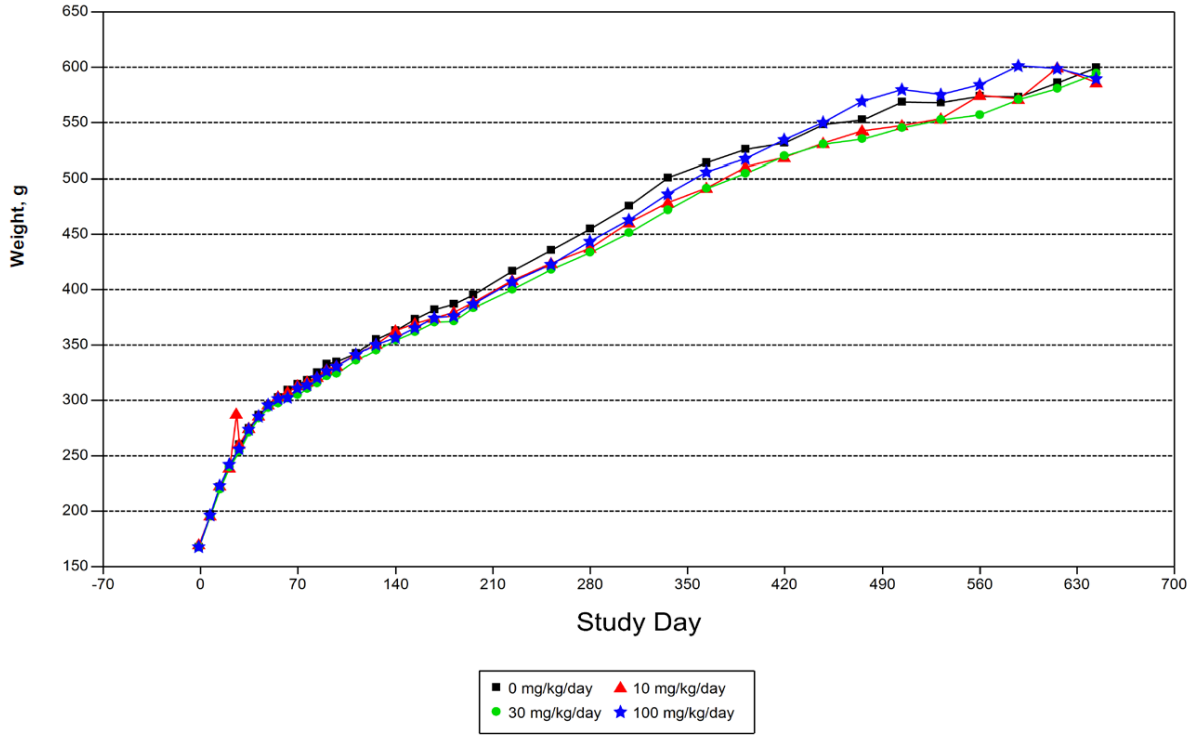
Animals were weighed twice during the pre-dose period, weekly for the first 14 weeks, bi-weekly for weeks 15 to 28, and every 4 weeks thereafter.

There were no meaningful drug-related changes in body weights throughout the treatment period, although sporadic and sometimes statistically significant changes were noted. These differences were not considered drug-related. The figures below, taken from the Sponsor's report, shows the effects of plecanatide on bodyweight.

Mean Bodyweight Data - Males



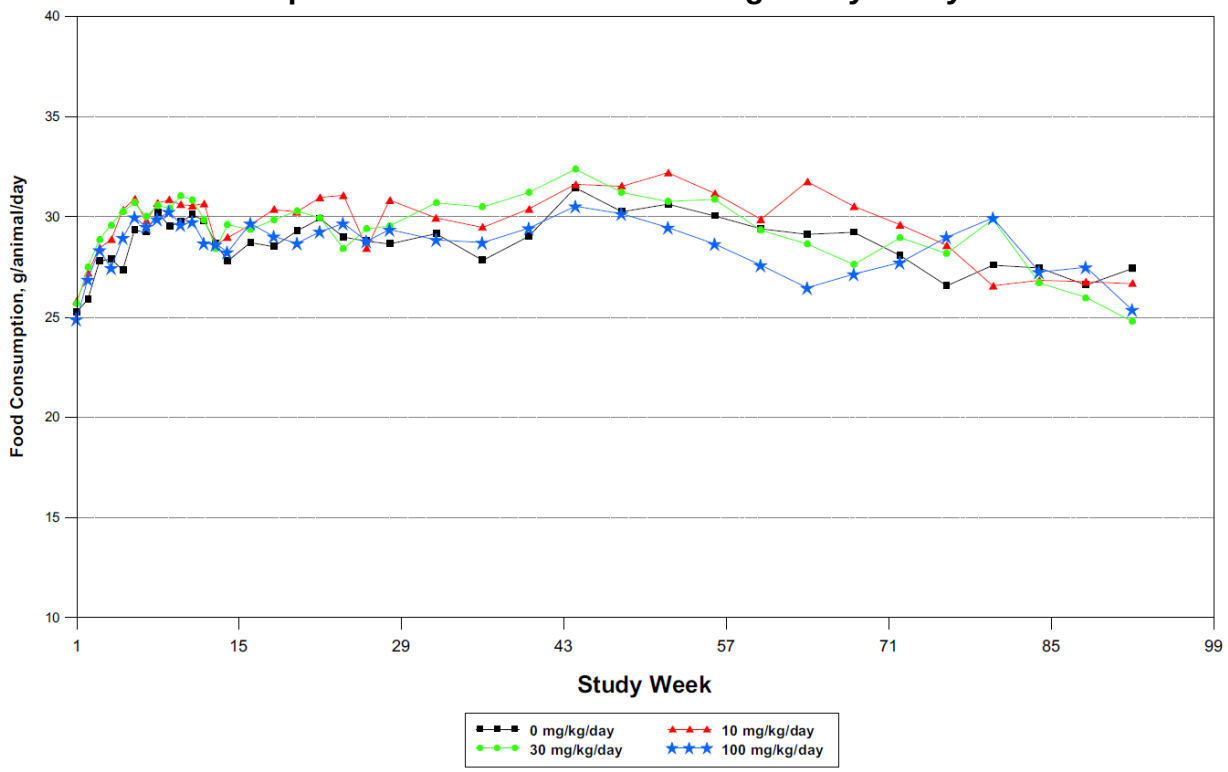
Mean Bodyweight Data - Females



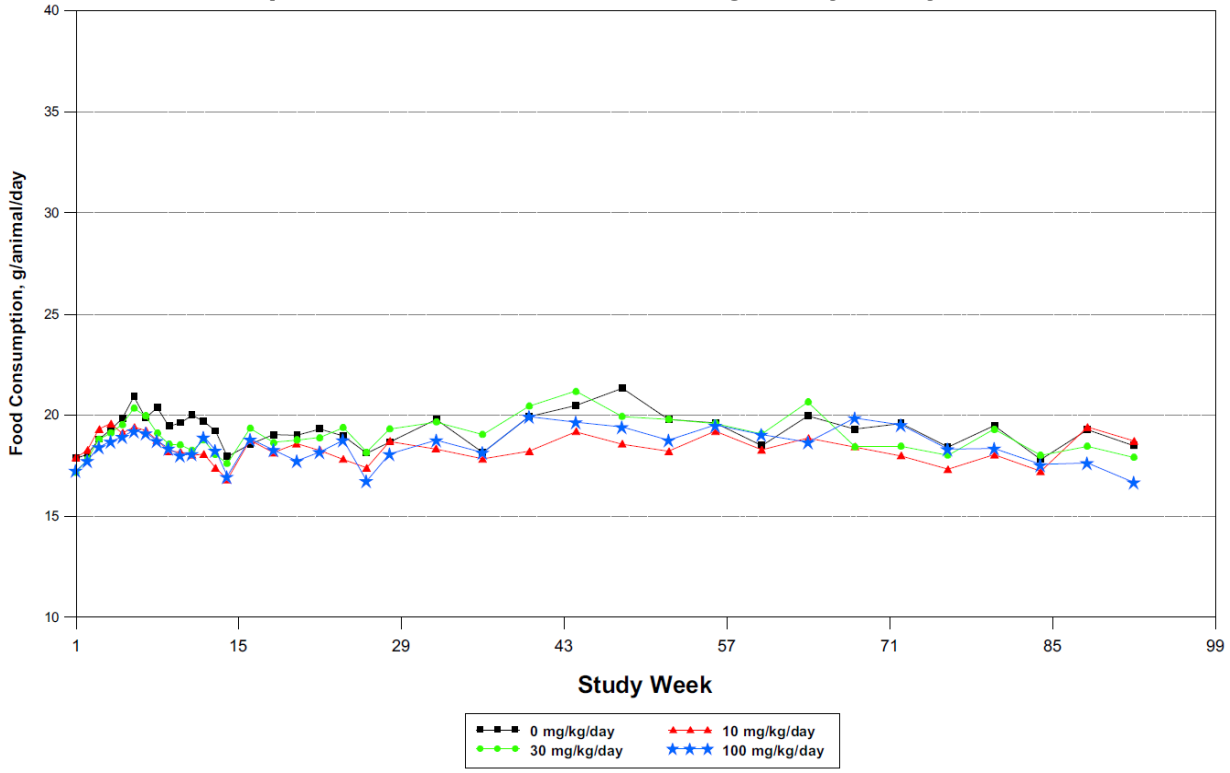
Feed Consumption

Food consumption was recorded weekly at weeks 1 through 14, bi-weekly at weeks 15 to 28, and every 4 weeks thereafter. As shown in the figures below (taken from the Sponsor's report), there were no meaningful drug-related changes in food consumption, although sporadic and sometimes statistically significant changes were noted.

Food Consumption in the 104-Week Carcinogenicity Study in Male Rats



Food Consumption in the 104-Week Carcinogenicity Study in Female Rats



Gross Pathology

Observations were made at sacrifice or immediately after premature death. Necropsies were performed on all animals euthanized *in extremis*, animals found dead, and all surviving animals sacrificed at scheduled termination. The animals were examined for palpable masses, and abnormalities were identified and correlated with postmortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities.

The noteworthy findings are shown in the table below.

Macroscopic Findings in the 104-Week Carcinogenicity Study in Rats

Organ/Tissue	Sex	Doses (mg/kg/day)			
		0	10	30	100
Number / group / sex examined		66	66	66	66
Liver *Discoloration, tan*	M	0	0	1	2
Lymph Node (Axillary) *Not identified*	F	1	1	2	4
Lymph Node (Inguinal) *Not identified*	M	1	1	2	3
Lymph Node (Mandibular) *Not identified*	M	0	0	0	2
Ovaries *Cysts*	F	3	7	8	6

Pituitary Gland *Enlarged*	M F	30 53	25 45	29 47	31 47
Prostate Gland *Enlarged*	M	0	3	0	3
Seminal vesicle *Large* *Small*	M M	1 0	0 0	0 0	3 2
Spleen *Focus/Foci, tan*	M	0	0	0	2
Stomach (Non-glandular) *Swollen/Thickened*	M	0	0	1	2
Uterus and Cervix *Enlarged*	F	1	2	4	3

Enlarged uterus and cervix is the only apparent drug-related macroscopic finding when scheduled sacrifice and premature death animals were both considered. However, since there were no microscopic findings that correlated with the macroscopic finding, this change is not considered as adverse. All other macroscopic findings were present in the control groups, showed no relationship to dose, and/or occurred only in one sex. Therefore, these macroscopic findings are not considered to be drug-related.

Histopathology

All study animals, including those euthanized in moribund condition or found dead before the end of the dosing period, were submitted to full necropsy. The following tissues and organs from all carcinogenicity animals were collected for microscopic examination during scheduled and unscheduled sacrifice, and from animals found dead during the study (table taken from Sponsor's study report).

Tissues and Organs Collected for Microscopic Examination

Tissue	Collected and Preserved	Microscopic Examination Groups 1, 4	Microscopic Examination Groups 2,3[a]
Adrenal glands	X	X	
Aorta	X	X	
Bone marrow smear	X		
Bone with bone marrow, femur	X	X	
Bone with bone marrow, sternum	X	X	
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	
Clitoral gland	X	X	
Coagulating glands	X	X	
Epididymides	X	X	
Esophagus	X	X	
Eyes (with optic nerve)	X	X	
GALT (Gut-Associated Lymphoid Tissue)	X	X	X

Harderian glands	X	X	
Heart	X	X	
Joint, tibiofemoral	X	X	
Kidneys	X	X	
Lacrimal glands, exorbital	X	X	
Large intestine, cecum	X	X	X
Large intestine, colon	X	X	X
Large intestine, rectum	X	X	X
Larynx	X	X	
Liver	X	X	
Lung with bronchi	X	X	
Lymph node, mandibular	X	X	
Lymph node, mesenteric	X	X	
Mammary gland (process females only)	X	X	
Nose (4 sections)	X	X	
Nerve, sciatic	X	X	
Ovaries	X	X	
Oviduct	X	X	
Pancreas	X	X	
Pharynx	X	X	
Pituitary gland	X	X	
Preputial gland	X	X	
Prostate gland	X	X	
Salivary gland, mandibular	X	X	
Salivary gland, parotid	X	X	
Salivary gland, sublingual	X	X	
Seminal vesicles	X	X	
Skeletal muscle, biceps femoris	X	X	
Skin	X	X	
Small intestine, duodenum	X	X	X
Small intestine, ileum	X	X	X

Small intestine, jejunum	X	X	X
Spinal cord, cervical	X	X	
Spinal cord, lumbar	X	X	
Spinal cord, thoracic	X	X	
Spleen	X	X	
Stomach, glandular	X	X	X
Stomach, nonglandular	X	X	X
Testes	X	X	
Thymus	X	X	
Thyroid gland (with parathyroid)	X	X	
Tongue	X	X	
Trachea	X	X	
Ureters	X	X	
Urinary bladder	X	X	
Uterus with cervix	X	X	
Vagina	X	X	
Zymbal's gland (auditory sebaceous gland)	X	X	
Potential Target organs	X	X	X
Gross lesions	X	X	X
Tissue masses with regional lymph node	X	X	X
[a]*Refer to the additional testing section above the table for specific examination requirements.*			

Peer Review - Yes

A peer review was performed on selected tissues at (b) (4) All tissues from at least 10% of the control and high-dose groups, all tumors from all groups for each type of tumor, and all target tissues that appear to have drug-related findings were examined.

Neoplastic

In the Sponsor's analysis, tumor incidence data were analyzed using both survival adjusted and unadjusted tests. The unadjusted tests were based on the incidence and number of sites examined for each tumor type. The Cochran-Armitage trend test was calculated, and Fisher's exact test was used to compare each treatment group with the control group. The survival adjusted test was conducted according to the prevalence/mortality methods described by Peto. One-sided positive trends in common (background incidence rate > 1%) and rare (background incidence < 1%) tumors as identified by the study pathologist were evaluated at the 0.005 and 0.025 significance levels, respectively. Pair-wise comparisons for common and rare tumors were evaluated at the 0.01 and 0.05 significance levels, respectively.

In the FDA statistical review, the tumor data were analyzed for dose-response relationships and pair-wise comparisons of control groups with each of the treatment

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

Notable neoplastic tumor findings are summarized in the table below.

Neoplastic Tumor Findings in the 104-Week Carcinogenicity Study in Rats

Organ/Tissue	Sex	Doses (mg/kg/day)			
		Control	10	30	100
Number/group/sex examined		66	66	66	66
Adrenals					
Benign pheochromocytoma	M	12	8	8	9
	F	1	4	3	3
Malignant pheochromocytoma	M	4	2	3	0
	F	0	1	1	0
Kidneys					
Benign renal tubule adenoma	M	0	1	3	2
	F	0	0	1	0
Malignant renal tubule carcinoma	M	1	0	0	0
	F	0	1	2	0
Mammary Gland					
Malignant adenocarcinoma	F	20	23	21	26
Benign adenoma	F	4	3	2	3
Malignant carcinosarcoma	F	0	0	0	2
Benign fibroadenoma	F	33	27	23	24
Malignant liposarcoma	F	1	0	0	0
Multicentric neoplasm					
Malignant lymphoma	M	0	2	3	2
Malignant hemangiosarcoma	F	0	0	2	0
Pancreas					
Benign islet cell adenoma	M	4/65	4	4	4
	F	0	0	1	0
Malignant islet cell carcinoma	M	0/65	1	1	4
	F	1	0	0	0
Parathyroid Glands					
Benign adenoma	M	1/57	1/56	0/59	2/57
	F	1	0	2	2
Pituitary Gland					

Benign pars distalis adenoma	M	47	42	44	46
	F	50	46	52	49
Malignant pars distalis carcinoma	M	0	2	0	1
	F	3	3	1	1
Skin and Subcutis					
Benign fibroma	M	2	1	3	5
	F	3	0	0	1
Malignant fibrosarcoma	M	1	2	2	1
	F	0	2	1	2
Benign squamous cell papilloma	M	0	2	2	0
Thyroid Gland					
Benign c-cell adenoma	M	4	7	5	9
	F	6	4	7	6
Malignant c-cell carcinoma	M	1	1	0	1
	F	0	1	1	0
Benign follicular cell adenoma	M	1	0	2	2
	F	0	1	0	1
Malignant follicular cell carcinoma	M	0	1	1	0
	F	0	0	1	0
Uterus and Cervix					
Malignant squamous cell carcinoma	F	0	0	1	0
Vagina					
Benign granular cell tumor	F	2	2	1	3

(Unless otherwise indicated, the # of animals/group/sex was 66)

The Sponsor reported that there were no drug-related increases in tumor incidence in either sex, and there were no statistically significant neoplastic findings. The most common tumor types in males were adenomas in the pars distalis of the pituitary glands and pheochromocytomas in the adrenal glands, while in females the most common tumor types were adenomas in the pars distalis of the pituitary glands and mammary gland tumors. The Sponsor indicated that the tumors noted were typical of those seen in rats of this strain and age, and were not considered as drug related.

According to the FDA statistical review by Dr. Hepei Chen, dated 10/11/2016, based on the criteria of adjustment for multiple testing, the analysis showed a statistically significant positive trend ($p = 0.0216$, significant for rare tumor criterion) for the incidence rates of carcinoma islet cell of pancreas in male rats. However, there were no statistically significant pairwise comparisons for this tumor. Therefore, it is this reviewer's conclusion that the finding is not considered as biologically significant. No other statistically significant findings were noted for male or female rats. The summary table below is from Dr. Chen's review.

Table 2. Summary Table of Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship and/or Pairwise Comparisons of Treated Groups and Vehicle Control Group in Rats

Organ name	Tumor name	0 mg Vehicle (C) P - Trend	10 mg Low (L) P - C vs. L	30 mg Mid (M) P - C vs. M	100 mg High (H) P - C vs. H
Male: Pancreas	Carcinoma, Islet Cell	0/32 (66)	1/29 (66)	1/31 (66)	4/36 (66)
		0.0216 $	0.4754	0.4921	0.0723

& X/YY (ZZ): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
$ = Statistically significant at 0.025 level in rare tumor for test of dose response relationship;
NC = Not calculable.

In addition, a request was made by this reviewer to Dr. Chen for a statistical analysis of the following tumor combinations:

1. adrenal benign and malignant pheochromocytoma
2. kidney renal tubule adenoma and carcinoma
3. mammary gland adenocarcinoma, adenoma, and carcinosarcoma
4. pancreas islet cell adenoma and carcinoma
5. pituitary gland pars distalis adenoma and carcinoma
6. skin/subcutis fibroma and fibrosarcoma
7. thyroid gland c-cell adenoma and carcinoma
8. thyroid follicular cell adenoma and carcinoma

No statistically significant changes were found in any of these combinations. The following are data from Dr. Chen's statistical analysis.

Table 2A: Tumor Rates and P-Values for Trend and Pairwise Comparisons of Tumor Combinations in Male Rats

Organ name	Tumor name	0 mg/kg Vehicle (C) P - Trend	10 mg/kg Low (L) P - C vs. L	30 mg/kg Mid (M) P - C vs. M	100 mg/kg High (H) P - C vs. H
Kidneys	Adenoma + Carcinoma, Renal Tubule, (Av) Type	1/33 (66)	1/30 (66)	3/33 (66)	2/35 (66)
		0.3330	0.7296	0.3066	0.5224
Mammary Gland	Adenocarcinoma + Adenoma	0/32 (66)	0/29 (66)	1/32 (66)	2/35 (66)
		0.0749	NC	0.5000	0.2691
Pancreas	Adenoma, Islet Cell + Carcinoma, Islet Cell	4/33 (66)	5/31 (66)	5/33 (66)	8/38 (66)
		0.1737	0.4589	0.5000	0.2486
Skin/Skin, Subcutis	Fibroma + Fibrosarcoma	3/33 (66)	3/30 (66)	5/33 (66)	5/36 (66)
		0.2875	0.6169	0.3542	0.4056
Thyroid Gland	Adenoma, C-Cell + Carcinoma, C-Cell	5/34 (66)	7/31 (66)	5/33 (66)	10/39 (66)
		0.1590	0.3094	0.6136	0.1947
	Adenoma, Follicular Cell + Carcinoma, Follicular Cell	1/33 (66)	1/29 (66)	3/32 (66)	2/35 (66)
		0.3404	0.7208	0.2949	0.5224

Organ name	Tumor name	0 mg/kg Vehicle (C) P - Trend	10 mg/kg Low (L) P - C vs. L	30 mg/kg Mid (M) P - C vs. M	100 mg/kg High (H) P - C vs. H
pituitary gland	Adenoma, Pars Distalis + Adenoma, Pars Intermedia + Carcinoma, Pars Distalis	48/55 (66) 0.6123	44/53 (66) 0.6372	45/52 (66) 0.4320	47/56 (66) 0.5909
	Adenoma, Pars Distalis + Carcinoma, Pars Distalis	47/55 (66) 0.5437	44/53 (66) 0.5335	44/51 (66) 0.5634	47/56 (66) 0.4837

& X/YY (ZZ): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed
$ = Statistically significant at 0.025 and 0.05 level for rare tumor for tests of dose response relationship and pairwise comparison, respectively
# = Statistically significant at 0.005 and 0.01 level for common tumor for tests of dose response relationship and pairwise comparison, respectively
NC = Not calculable

Table 2B: Tumor Rates and P-Values for Trend and Pairwise Comparisons of Tumor Combinations in Female Rats

Organ name	Tumor name	0 mg/kg Vehicle (C) P - Trend	10 mg/kg Low (L) P - L vs. C	30 mg/kg Mid (M) P - M vs. C	100 mg/kg High (H) P - H vs. C
Kidneys	Adenoma + Carcinoma, Renal Tubule, (Av) Type	0/31 (66) 0.6997	1/31 (66) 0.5000	3/37 (66) 0.1550	0/35 (66) NC
Pancreas	Adenoma, Islet Cell + Carcinoma, Islet Cell	1/32 (66) 0.6533	0/30 (66) 0.4839	1/36 (66) 0.2766	0/35 (66) 0.5224
Pituitary Gland	Adenoma, Pars Distalis + Carcinoma, Pars Distalis	53/59 (66) 0.6919	49/56 (66) 0.5404	53/60 (66) 0.4870	50/58 (66) 0.6248
Skin/Skin, Subcutis	Fibroma + Fibrosarcoma	3/32 (66) 0.4600	2/31 (66) 0.4846	1/36 (66) 0.7366	3/37 (66) 0.4090
Thyroid Gland	Adenoma, C-Cell + Carcinoma, C-Cell	6/33 (66) 0.5374	5/33 (66) 0.5000	8/39 (66) 0.5219	6/37 (66) 0.4621
	Adenoma, Follicular Cell + Carcinoma, Follicular Cell	0/31 (66) 0.3360	1/31 (66) 0.5000	1/36 (66) 0.5373	1/36 (66) 0.5373
Mammary gland	Adenocarcinoma + Adenoma + Carcinosarcoma	24/43 (66) 0.3341	26/44 (66) 0.4632	23/46 (66) 0.6316	29/48 (66) 0.4083

& X/YY (ZZ): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed
$ = Statistically significant at 0.025 and 0.05 level for rare tumor for tests of dose response relationship and pairwise comparison, respectively
# = Statistically significant at 0.005 and 0.01 level for common tumor for tests of dose response relationship and pairwise comparison, respectively
NC = Not calculable

Non-Neoplastic

Noteworthy non-neoplastic findings are summarized in the table below.

Non-Neoplastic Findings in the 104-Week Carcinogenicity Study in Rats

Organ/Tissue	Sex	Doses (mg/kg/day)			
		Control	10	30	100
Number / group / sex examined		66	66	66	66
Adrenal Glands					
Focal cortical angiectasis/cystic degeneration	M	4	8	5	13
	F	55	53	53	61
Bone (Femur)					
Hyperostosis	M	0	3	0	2
	F	1	1	0	0
Bone (Sternum)					
Hyperostosis	M	0	0	0	2
Bone Marrow (Femur)					
Decreased cellularity	M	2	5	3	3
Cavity (Oral)					
Erosion/Ulcer	M	0	1	0	3
	F	3	0	0	2
Clitoral Glands					
Dilatation	F	0	0	2	2
Kidneys					
Bilateral hydronephrosis	M	1	2	0	3
	F	1	0	0	0
Cyst	M	6	0	1	0
	F	0	0	1	4
Mineralization	M	1	4	2	4
	F	41	44	47	47
Unilateral pyelonephritis	M	1	0	0	4
Large Intestine (Cecum)					
Polyarteritis	F	0	0	0	2
Liver					
Focal cystic degeneration	M	10	14	15	13
	F	1	5	4	0
Focal necrosis	M	3	5	4	6
	F	3	4	1	3
Ovaries					
Cyst	F	12	20	18	19
Pancreas					
Focal acinar cell hyperplasia	M	9	20	10	17
	F	3	3	3	4
Subacute/chronic inflammation	M	0	3	0	1
	F	0	4	2	2
Parathyroid Glands					
Focal hyperplasia	M	6	5	10	5
	F	3	2	2	6
Pituitary Gland					
Focal pars distalis hyperplasia	M	21	18	11	18
	F	11	17	11	13
Seminal Vesicles					
Subacute/chronic inflammation	M	3	2	3	5

Skeletal muscle (Biceps femoris)					
Myofiber degeneration/necrosis	M	5	7	14	11
	F	4	3	5	4
Mononuclear cell infiltration	M	1	3	7	5
	F	1	1	2	2
Skin					
Subacute/chronic inflammation	M	5	3	8	9
	F	7	13	7	6
Stomach (Glandular)					
Ulcer/erosion	M	1	1	2	3
	F	4	2	5	4
Subacute/chronic inflammation	M	4	3	6	9
	F	3	4	5	3
Stomach (Non-glandular)					
Ulcer/erosion	M	1	3	2	4
	F	2	0	4	0
Testes					
Bilateral seminiferous degeneration/atrophy	M	2	4	3	5
Polyarteritis	M	0	1	2	3
Urinary bladder					
Subacute/chronic inflammation	M	2	4	4	4
	F	0	0	2	1

The non-neoplastic findings lacked dose dependency, occurred in a single sex, were observed in the control and treatment groups, and/or were low in frequency. Therefore, there were no meaningful drug-related non-neoplastic findings.

Toxicokinetics

N/A

Dosing Solution Analysis

Stock assay solutions were tested appropriately for stability and drug concentration. Mean concentrations of the 1 mg/ml, 3 mg/ml, and 10 mg/ml dosing solutions ranged from 99.2% to 109.5% of nominal concentrations. Dose formulations are solutions at the concentrations used, therefore no homogeneity analysis was performed.

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

Executive CAC
Date of Meeting: April 9, 2013

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Karen Davis-Bruno, Ph.D., DMEP, Alternate Member
David Joseph, Ph.D., DGIEP, Team Leader
Yuk-Chow Ng, Ph.D., DGIEP, Presenting Reviewer

Author of Draft: Yuk-Chow Ng, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The Committee did not address the sponsor's proposed statistical evaluation for the carcinogenicity bioassay, as this does not affect the sponsor's ability to initiate the bioassay. 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 #74,883

Drug Name: plecanatide/SP-304

Sponsor: Synergy Pharmaceuticals, Inc.

Background:

The Sponsor is developing plecanatide for the treatment of chronic constipation and constipation-predominant irritable bowel syndrome. Plecanatide is a synthetic 16-amino acid peptide that is structurally related to uroguanylin, a member of the guanylin peptide family. It is a guanylate cyclase-C (GC-C) agonist. Binding of plecanatide to GC-C increases secretion of chloride, bicarbonate, and fluid, through local actions in the intestinal mucosa. Oral bioavailability of plecanatide is extremely limited.

Rat Carcinogenicity Study Protocol and Dose Selection:

The Sponsor proposes a 2-year carcinogenicity study in Sprague-Dawley CD® [Crl:CD®(SD)] rats (65/sex/group) at dose levels of 0 (distilled water as vehicle control), (b) (4) mg/kg/day plecanatide, administered by oral gavage.

The Sponsor's dose selection is based on high multiples of the anticipated high dose in humans adjusted for body surface area (BSA). The proposed high dose of (b) (4) (b) (4) than the predicted maximum human dose of 9 mg/day (5.55 mg/m^2 based on a 60-kg bodyweight), on a body surface area basis. It is noted that systemic exposure of plecanatide in the animal species tested has been found to be very low after oral administration, which is suggestive of limited oral bioavailability. In humans, plecanatide was not detectable in plasma (10 ng/ml limit of quantification) in any of the clinical studies. Thus, in this particular situation, where the human AUC can not be measured due to the absence of detectable plasma drug levels, the rodent to human AUC ratio should not be used for dose selection. The Sponsor provided no specific explanation for the proposed middle and low dose of (b) (4) mg/kg/day, respectively.

Dose selection in males cannot be based on an MTD, since no MTD was established due to the absence of adverse effects in the dose-ranging study.

Executive CAC Recommendations and Conclusions:

1. The Committee did not concur with the proposed doses for the 2-year carcinogenicity study in rats. The Committee recommended doses of 0, 10, 30, and 100 mg/kg/day for female rats, by oral gavage, based on reduced body weight gain in females at 300 mg/kg/day. The Committee recommended doses of 0, 10, 30, and 100 mg/kg/day for male rats based on an anticipated large rat to human margin of local drug concentration in the intestinal tract, and the expectation that the drug concentration in rats will achieve a maximum pharmacological effect. A rat to human local intestinal plecanatide *concentration* ratio was estimated according to the intestinal fluid volumes in rats and humans. The following table shows an estimation of the local exposure ratio based on the predicted maximum clinical dose of 9 mg/day and an oral dose of 100 mg/kg/day in rats.

Estimated Intestinal Plecanatide Concentration Based on GI Tract Volume

	Fluid volume-Fasting (ml)	Fluid volume-Fed (ml)	Dose (mg/day)	Estimated Drug Conc Fasting (mg/ml)	Estimated Drug Conc Fed (mg/ml)	Fold Human Conc
Human[a]	163	751	9	0.055	0.012	
Rat[b]	3.2	7.8	20*	6.25	2.56	47-520x

*Rat dose: 100 mg/kg/day; body weight 200 g
a: Aliment Pharmacol Ther 2005; 22: 971–979
b: J Pharmacy and Pharmacology 2008, 60: 63–70

2. If the sponsor plans histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathologic examination of other dose groups under any of the following circumstances:

(a) for any macroscopic findings in the low and mid dose groups for a given tissue, they will

need to look at that tissue for all of the dose groups
(b) for statistically significant or otherwise remarkable findings in the high dose group, the sponsor will need to look at the affected tissues in all of the dose groups.
(c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level,
(d) for an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

3. Cecum and stomach should be examined microscopically in all study groups, since these are likely to be major sites of pharmacological activity for plecanatide. The Sponsor may reconsider their proposed microscopic examination of larynx in all groups, given that this tissue is not expected to be a major site of pharmacological activity.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/IND 74,883/Division File, DGIEP
/David Joseph/Team leader, DGIEP
/Yuk-Chow Ng/Pharmacologist, DGIEP
/Matthew Scherer/PM, DGIEP
/ASeifried, OND IO

Executive CAC
Date of Meeting: January 29, 2013

Committee: Abby Jacobs, Ph.D., OND IO, Acting Chair
Paul Brown, Ph.D., OND IO, Member
Tom Papoian, Ph.D., DCRP, Alternate Member
David Joseph, Ph.D., DGIEP, Team Leader
Yuk-Chow Ng, Ph.D., DGIEP, Presenting Reviewer

Author of Draft: Yuk-Chow Ng, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

The Committee did not address the sponsor's proposed statistical evaluation for the carcinogenicity bioassay, as this does not affect the sponsor's ability to initiate the bioassay. 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 #74,883

Drug Name: plecanatide/SP-304

Sponsor: Synergy Pharmaceuticals, Inc.

Background:

The Sponsor is developing plecanatide for the treatment of chronic constipation and constipation-predominant irritable bowel syndrome. Plecanatide is a synthetic 16-amino acid peptide that is structurally related to the uroguanylin, a member of the guanylin peptide family. It is a guanylate cyclase-C (GC-C) agonist. Binding of plecanatide to GC-C increases secretion of chloride, bicarbonate, and fluid, through local actions in the intestinal mucosa. Oral bioavailability of plecanatide is extremely limited.

Mouse Carcinogenicity Study Protocol and Dose Selection:

The Sponsor proposes a 2-year carcinogenicity study in Albino Crl:CD-1® (ICR) mice (60/sex/group) at dose levels of 0 (distilled water as vehicle control), (b) (4)30, or 90 mg/kg/day plecanatide, administered by oral gavage.

The Sponsor's dose selection is based on high multiples of the anticipated high dose in humans adjusted for body surface area (BSA). The proposed high dose of 90 mg/kg, or 270 mg/m^2, is

49-fold higher than the predicted maximum human dose of 9 mg/day (5.55 mg/m^2 based on a 60-kg bodyweight), on a body surface area basis. Plecanatide was tolerated at doses of 20, 60, 150 and 400 mg/kg in a 26-week study in CD-1 mice. It is noted that systemic exposure of plecanatide in the animal species tested has been found to be very low after oral administration, which is suggestive of limited oral bioavailability. In the 26-week oral dose range-finding study in mice, plecanatide AUC could not be estimated in the 20 or 60 mg/kg/day mice on day 1, or in the 20 mg/kg/day mice at week 26, due to the absence of detectable drug levels at most time-points. In humans, plecanatide was not detectable in plasma (10 ng/ml limit of quantification) in any of the clinical studies. Thus, in this particular situation, where the human AUC can not be measured due to the absence of detectable plasma drug levels, the rodent to human AUC ratio can not be used for dose selection. The Sponsor provided no specific explanation for the proposed middle and low dose of 30 and (b) (4) mg/kg/day, respectively.

Executive CAC Recommendations and Conclusions:

1. The Committee recommended doses of 0, 10, 30, and 90 mg/kg/day, by oral gavage. The Committee notes that the high dose represents a sufficiently high multiple of local (intestinal) exposure relative to a pharmacologically active dose in a mouse model of DSS-induced colitis, based on a mg/kg comparison.

2. The Committee noted that the skin infection seen in several of the animals confounded interpretation of the 26-week study.

3. The Committee notes that the carcinogenicity study will be performed in a testing facility different from the facility that conducted the 26-week dose range-finding study, and a different animal vendor may be used as well. Therefore, concurrence on doses is contingent on comparable toxicity being achieved in the carcinogenicity study at similar time-points as that seen in the 26-week toxicity study.

4. In the event of adverse effects that raise a concern about increased mortality, the Sponsor should contact the FDA to obtain concurrence with any proposed change in dosing or termination of dosing in a study group prior to initiating the change.

5. If the sponsor plans histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathologic examination of other dose groups under any of the following circumstances:

(a) for any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups
(b) for statistically significant or otherwise remarkable findings in the high dose group, the sponsor will need to look at the affected tissues in all of the dose groups.
(c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level,

(d) for an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\
/IND 74,883/Division File, DGIEP
/David Joseph/Team leader, DGIEP
/Yuk-Chow Ng/Pharmacologist, DGIEP
/Matthew Scherer/PM, DGIEP
/ASeifried, OND IO

THE FOLLOWING IS THE EXECUTIVE CAC MEETING MINUTES FROM THE FINAL REPORT:

Executive CAC
Date of Meeting: July 26, 2016

Committee: Karen Davis Bruno, PhD, OND IO, Chair
Paul Brown, PhD, OND IO, Member
Tim McGovern, PhD, OND IO, Member
David B. Joseph, PhD, DGIEP, Lead Pharmacologist
Yuk-Chow Ng, PhD, Presenting Reviewer

Author of Draft: Yuk-Chow Ng, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA# 208,745

Drug Name: Plecanatide (SP-304)

Sponsor: Synergy Pharmaceutical Inc.

Background:

Plecanatide is a 16-amino acid peptide that binds to guanylate cyclase-C and stimulates the production of cyclic guanosine 3',5'-monophosphate. Plecanatide is a gastrointestinal prokinetic drug that acts through stimulation of guanylate cyclase C in the intestinal mucosa, leading to increased secretion of intestinal fluid, and accelerated gastrointestinal transit. Plecanatide is under development for treatment of chronic idiopathic constipation.

Plecanatide was negative in the Ames assay, the *in vitro* L5178Y/TK+/- mouse lymphoma mutation assay, and the *in vivo* mouse bone marrow micronucleus assay.

As part of the nonclinical program, the Sponsor conducted a 2-year oral gavage carcinogenicity study in mice and a 2-year oral gavage carcinogenicity study in rats.

Mouse Carcinogenicity Study:

The Executive CAC recommendations (see meeting minutes dated 1/29/2013) for both male and female mice, were doses of 0, 10, 30, and 90 mg/kg/day by oral gavage. These doses were based on a sufficiently high multiple of local (intestinal) drug concentration relative to a pharmacologically active dose in a mouse model of DSS-induced colitis, as predicted by a mg/kg comparison of the high dose to the pharmacological dose in mice.

In the 104-week oral carcinogenicity study in Crl:[CD-1(ICR)BR] mice, males and females were administered 0 (vehicle), 10, 30, or 90 mg/kg/day plecanatide by oral gavage. The vehicle was water. Due to low survival in the control males and 10 mg/kg/day females, all surviving male and female animals were sacrificed beginning on week 98 (males) and 104 (females), respectively, based on the Executive CAC recommendations conveyed on November 18, 2014.

There were no significant neoplasms in the mouse study.

Rat Carcinogenicity Study:

The doses tested were in accordance with the Executive CAC recommendations (see meeting minutes dated 4/9/2013). For male rats, the Committee recommended doses of 0, 10, 30, and 100 mg/kg/day based on the large estimated rat to human multiple of local drug concentration in the intestinal tract, and the expectation that the local drug concentration in rats will achieve a maximum pharmacological effect. For female rats, the Committee recommended doses of 0, 10, 30, and 100 mg/kg/day based on reduced bodyweight gain in females at 300 mg/kg/day in the dose-ranging study.

In the 104-week oral carcinogenicity study in CD[Crl:CD(SD)] rats, males and females were administered 0 (vehicle), 10, 30, or 100 mg/kg/day plecanatide by oral gavage. The vehicle was water. The study was terminated on week 94 for males and females due to low survival in the control groups, in accordance with Executive CAC recommendations conveyed on 2/2/2015.

There were no significant neoplasms in the rat study.

Executive CAC Recommendations and Conclusions:

Mouse:

1. The Committee concluded that the study was adequate, noting prior Exec CAC review of the protocol.

2. The Committee concluded that there were no treatment-related neoplasms.

Rat:

1. The Committee concluded that the study was adequate, noting prior Exec CAC review of the protocol.

2. The Committee concluded that there were no treatment-related neoplasms.

Karen Davis Bruno, PhD
Chair, Executive CAC

cc:\
/NDA 208,745/Division File, DGIEP
/David Joseph/Team leader, DGIEP
/Yuk-Chow Ng/Reviewer, DGIEP
/Maureen Dewey/PM, DGIEP
/ASeifried, OND IO

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Study of Fertility and Early Embryonic Development to Implantation of Plecanatide by Oral Gavage in Mice

Study no.:	20016090
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	7/5/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide (SP-304), batch #101221 (98.2%)

Key Study Findings

- Male mice (25/group) were administered 0 (vehicle), 20, 200, or 600 mg/kg/day plecanatide by oral gavage beginning 28 days prior to mating, during mating, and until necropsy on dosing days 63 to 66. Plecanatide-treated females were treated for at least 14 days prior to mating, during mating, and through gestation day (GD) 7. Twenty five mice/sex/group remained untreated throughout the study. Each drug-treated male was mated with an untreated female, and each female in the drug-treated group was assigned to mate with an untreated male. Females (untreated and treated) with a copulatory plug *in situ* or spermatozoa present in a smear of the vaginal contents were considered to be at GD 0, and assigned to individual housing. Males and females were sacrificed on dosing days 63 to 66 and GD 13, respectively.

- There were no meaningful drug-related changes in bodyweight or bodyweight gain in males or females.

- In males, there were no drug-related changes in sperm motility or density. In females, there were no drug-related effects on estrous cycle, percentage pregnancy rate (total pregnant/number mated), the number of corpora lutea, implantation sites, live or dead embryos, pre- or post-implantation losses, or dams with all nonviable embryos.

- The NOAEL for paternal and maternal toxicity is considered to be 600 mg/kg/day, based on the absence of drug-related paternal or maternal findings at this dose. The NOAEL for male and female fertility, and early embryonic development is considered to be 600 mg/kg/day.

Methods

Doses:	0 (vehicle), 20, 200, and 600 mg/kg/day
Frequency of dosing:	once daily
Dose volume:	10 ml/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	solution / sterile water
Species/Strain:	Mouse/Crl:CD1(ICR)
Number/Sex/Group:	25
Satellite groups:	None
Study design:	Plecanatide-treated males were dosed beginning 28 days prior to mating, during mating, and until necropsy (dosing days 63 to 66). Plecanatide-treated females were dosed for at least 14 days prior to mating, during mating, and through GD 7. There were also 25 untreated mice/sex/group (see Sponsor's table below). Each drug-treated male was mated with an untreated female. Males that failed to mate with an untreated female within the first 14 days of cohabitation were assigned an alternate untreated female, and remained in cohabitation for up to 3 additional days. Each drug-treated female was assigned to mate with an untreated male. Females (untreated and treated) with a copulatory plug *in situ* or spermatozoa present in a smear of the vaginal contents were considered to be at GD 0, and assigned to individual housing. Males and females were sacrificed on dosing days 63 to 66 and GD 13, respectively.
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

Study Design of the Fertility and Early Embryonic Development Study

Group No.	Test Material	Dosage Level[a] (mg/kg/day)	Concentration[a] (mg/mL)	Dosage Volume (mL/kg)	No. of Treated Mice		No. of Untreated Mice[b]	
					M	F	M	F
1	Sterile Water for Injection, USP	0 (Vehicle)	0	10	25	25	25	25
2	SP-304	20	2.0	10	25	25	25	25
3	SP-304	200	20.0	10	25	25	25	25
4	SP-304	600	60.0	10	25	25	25	25

On the day of arrival (5 days before start of treatment), the animals were approximately 63 days old, and their bodyweights ranged from 28.3 to 36.2 g in males and 21.8 to 28.2 g in females.

The Sponsor stated that the high dose selection was based on previous toxicity studies in mice, the solubility of plecanatide in water, and the expected range of dose levels in humans. In 4-week and 13-week oral toxicity studies of plecanatide in mice, the NOAELs were considered to be 200 and 20 mg/kg/day, respectively. In a 26-week oral toxicity study in mice, the NOAEL was considered to be 400 mg/kg/day, the highest dose tested (Note: This reviewer considered the NOAEL to be 150 mg/kg/day in that study based on a significant decrease in absolute reticulocyte count and sciatic nerve axonal/myelin degeneration at 400 mg/kg/day plecanatide). The oral gavage route of administration was selected because it is the intended route of administration in humans. The frequency of administration reflected possible clinical use, and the duration of administration was in compliance with the appropriate guideline.

Observations and Results

Mortality

All animals were checked at least twice daily for mortality. There were no drug-related deaths. A 600 mg/kg/day male was euthanized on day 40 of the study and a control female was euthanized on day 3 of presumed gestation due to moribund condition, which was attributed to intubation error.

Clinical Signs

Animals were observed at least weekly during the acclimation period, weekly during the pre-dose period, once daily before dose administration for all animals, and once daily post-dose for the treated females. Post-dose observations were made between 1 and 2 hours after dose administration.

There were no meaningful drug-related clinical signs. Sporadic clinical signs were observed; however, these are not considered to be drug-related because they were

related to an intubation error and/or lacked dose-dependency. Notable clinical observations are summarized in the Sponsor's tables below.

Clinical Observations (Drug-Treated Male Groups)

GROUP	1	2	3	4
TEST MATERIAL	STERILE WATER FOR INJECTION, USP	SP-304	SP-304	SP-304
DOSAGE (MG/KG/DAY)a	0 (VEHICLE)	20	200	600
MAXIMUM POSSIBLE INCIDENCE	1611/ 25	1612/ 25	1613/ 25	1588/ 25
UNSCHEDULED EUTHANASIA	0	0	0	1
DEHYDRATION - TOTAL	0/ 0	1/ 1	0/ 0	1/ 1
MILD	0/ 0	1/ 1	0/ 0	0/ 0
SEVERE	0/ 0	0/ 0	0/ 0	1/ 1b
PTOSIS	0/ 0	1/ 1	0/ 0	1/ 1b
COLD TO TOUCH	0/ 0	0/ 0	0/ 0	1/ 1b
URINE-STAINED ABDOMINAL FUR	0/ 0	0/ 0	0/ 0	1/ 1b
LEFT AND RIGHT AXILLARY REGION: SWOLLEN	0/ 0	0/ 0	0/ 0	1/ 1b
NECK: SWOLLEN	0/ 0	0/ 0	0/ 0	1/ 1b
HUNCHED POSTURE	0/ 0	0/ 0	0/ 0	1/ 1b
DECREASED MOTOR ACTIVITY	0/ 0	0/ 0	0/ 0	1/ 1b
DYSPNEA	0/ 0	0/ 0	0/ 0	1/ 1b
BRADYPNEA	0/ 0	0/ 0	0/ 0	1/ 1b
HYPERPNEA	0/ 0	0/ 0	0/ 0	1/ 1b
REDUCED FECES	0/ 0	0/ 0	0/ 0	1/ 1b
LEFT EYE: CORNEAL OPACITY	0/ 0	0/ 0	44/ 1	0/ 0
TAIL BENT	18/ 1	32/ 1	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF MICE WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x MICE)/NUMBER OF MICE EXAMINED PER GROUP
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
a. Dosage occurred once daily in the morning on Days 1 through 62, 63, 64 or 65 of the study.
b. Clinical sign observed in mouse 1493 prior to scheduled dosage on Day 40 of the study.

UNSCHEDULED EUTHANASIA	0	0	0	1
TIP OF TAIL: BLACK	0/ 0	32/ 1	0/ 0	0/ 0
TIP OF TAIL MISSING	0/ 0	8/ 1	0/ 0	0/ 0
ALL PAWS AND TAIL: PALE	0/ 0	4/ 1	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF MICE WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x MICE)/NUMBER OF MICE EXAMINED PER GROUP
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
a. Dosage occurred once daily in the morning on Days 1 through 62, 63, 64 or 65 of the study.

Clinical Observations (Drug-Treated Female Groups)

GROUP	1	2	3	4
TEST MATERIAL	STERILE WATER FOR INJECTION, USP	SP-304	SP-304	SP-304
DOSAGE (MG/KG/DAY)a	0 (VEHICLE)	20	200	600
UNSCHEDULED EUTHANASIA	1	0	0	0
PRECOHABITATION (DAY 1 OF STUDY TO THE DAY OF COHABITATION):				
MAXIMUM POSSIBLE INCIDENCE	350/ 25	350/ 25	350/ 25	350/ 25
		NO ADVERSE FINDINGS		
PRESUMED GESTATION:				
MAXIMUM POSSIBLE INCIDENCE	340/ 25	350/ 25	350/ 25	350/ 25
TAIL: SWOLLEN	0/ 0	0/ 0	0/ 0	2/ 1
TAIL: SCAB	0/ 0	0/ 0	0/ 0	1/ 1
DEHYDRATION - TOTAL	1/ 1	0/ 0	1/ 1	0/ 0
MILD	0/ 0	0/ 0	1/ 1	0/ 0
SLIGHT	1/ 1b	0/ 0	0/ 0	0/ 0
RED PERIVAGINAL SUBSTANCE	0/ 0	1/ 1	0/ 0	0/ 0
DECREASED MOTOR ACTIVITY	1/ 1b	0/ 0	0/ 0	0/ 0
LACRIMATION	1/ 1b	0/ 0	0/ 0	0/ 0
LIMITED USE OF RIGHT FORELIMB	1/ 1b	0/ 0	0/ 0	0/ 0
HUNCHED POSTURE	1/ 1b	0/ 0	0/ 0	0/ 0
RIGHT AXILLARY: SWOLLEN	1/ 1b	0/ 0	0/ 0	0/ 0
HEAD TILT TO THE RIGHT	1/ 1b	0/ 0	0/ 0	0/ 0
GASPING	1/ 1b	0/ 0	0/ 0	0/ 0
BRADYPNEA	1/ 1b	0/ 0	0/ 0	0/ 0

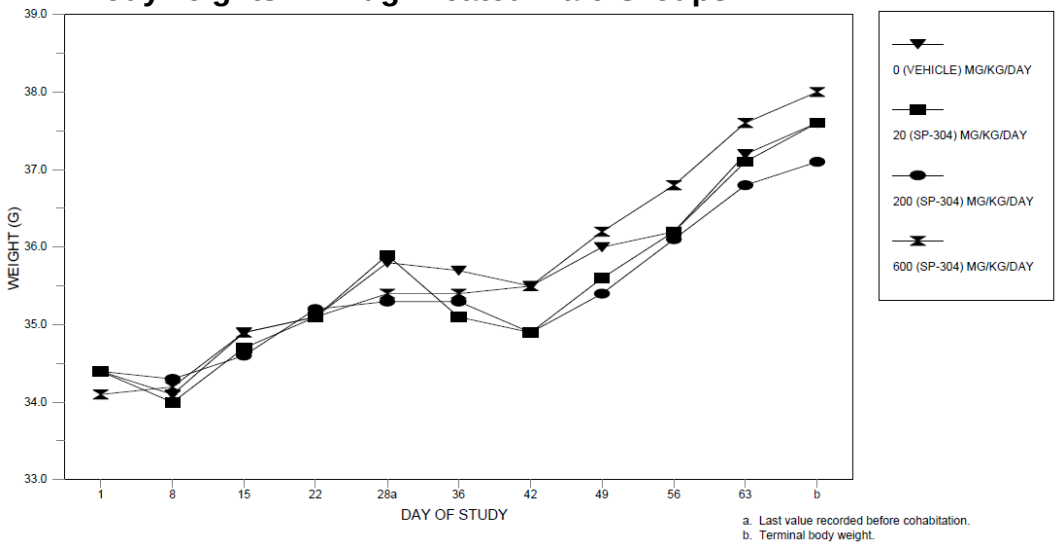
STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF MICE WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x MICE)/NUMBER OF MICE EXAMINED PER GROUP
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
a. Dosage occurred on Day 1 of study through Day 7 of presumed gestation.
b. Clinical sign observed in mouse 1717 prior to and/or after scheduled dosage on Day 3 of presumed gestation.

Body Weight

Bodyweights were recorded at least weekly during the acclimation period and pre-dose period (drug-treated female groups only), daily before each dose was administered, and once daily during the post-dose period (drug-treated female groups only). Bodyweights for untreated male and female mice were recorded at least once weekly. A terminal bodyweight was recorded prior to sacrifice.

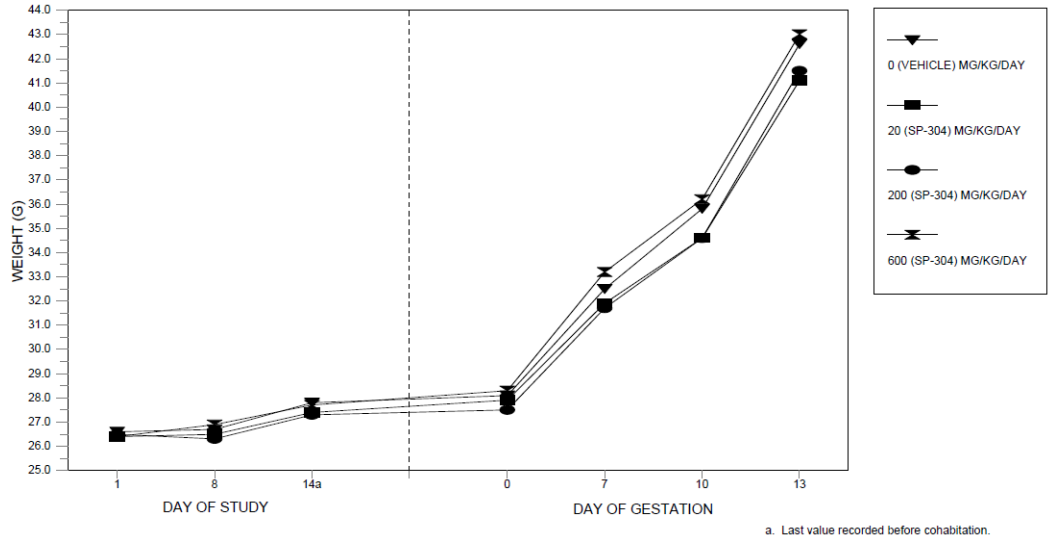
There were no meaningful drug-related changes in bodyweight or bodyweight gain in males or females. Sporadic statistically significant changes in bodyweight and bodyweight gain were noted. However, these changes are not considered to be drug-related because they were transient in nature and/or lacked dose-dependency. The Sponsor's figures below summarize the bodyweights in the plecanatide-treated males and females during the study period.

Bodyweights in Drug-Treated Male Groups



a. Last value recorded before cohabitation.
b. Terminal body weight.

Bodyweights in Drug-Treated Female Groups



a. Last value recorded before cohabitation.

Feed Consumption

Not measured.

Estrous Cycles

Estrous cycling was evaluated by examining vaginal cytology in the treated females for 15 days before the dosing period, 13 days during the dosing period, and then until a copulatory plug was observed *in situ* or spermatozoa was observed in a smear of the vaginal contents during the mating period.

There were no drug-related changes in estrous cycles. The Sponsor's table below summarizes the changes in estrous cycles in the plecanatide-treated females.

Effects of Plecanatide on Estrous Cycling (Drug-Treated Female Groups)

GROUP		1	2	3	4
TEST MATERIAL		STERILE WATER FOR INJECTION, USP	SP-304	SP-304	SP-304
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	600
ESTROUS CYCLING OBSERVATIONS					
MICE EVALUATED	N	25	25	25	25
PREDOSAGE ESTROUS CYCLING					
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	2.6 ± 0.8	2.2 ± 0.8	2.7 ± 0.6	2.4 ± 0.6
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N(%)	0(0.0)	1(4.0)	1(4.0)	0(0.0)
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N(%)	0(0.0)	0(0.0)	1(4.0)	1(4.0)
PRECOHABITATION ESTROUS CYCLING b					
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	2.6 ± 0.7	3.0 ± 0.8	2.6 ± 0.7	2.5 ± 0.8
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N(%)	3(12.0)	1(4.0)	5(20.0)	3(12.0)
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N(%)	1(4.0)	0(0.0)	0(0.0)	1(4.0)

a. Dosage occurred on Day 1 of study through Day 7 of presumed gestation.
b. Precohabitation period smears obtained on 27 July 2011 were obtained prior to dose administration.

Toxicokinetics

Not performed.

Dosing Solution Analysis

The concentration determination of plecanatide in the dosing formulation samples was performed in accordance with the validated method. Analyses conducted during the treatment period showed that the concentrations of dosing formulations at 2, 20, and 60 mg/ml were within ±10% of the nominal concentration (+1.1% to +6.5%).

Necropsy

Males and females were sacrificed on dosing day 63 to 66 and GD 13, respectively, and necropsies were performed. The tissues and organs that were collected and weighed are summarized in the Sponsor's table below.

Tissue and Organs Collected and Weighed

Tissue	Weigh	Collect	Microscopic Evaluation	Comment
Animal identification	-	X	-	All mice with gross lesions and/or retained tissues.
Cervix	-	X	-	All female mice at scheduled euthanasia. All female mice terminated early. Collected with uterus.
Epididymides	X	X	-	All treated male mice at scheduled euthanasia. All treated male mice terminated early. Individual weight.
Epididymis, left cauda	X	X	-	All treated male mice at scheduled euthanasia. All treated male mice terminated early.
Esophagus	-	X	-	Infused with 10% neutral buffered formalin. All treated mice terminated early.
Gland, prostate	X	X	-	All treated male mice at scheduled euthanasia. All treated male mice terminated early.
Gland, seminal vesicles	X	X	-	All treated male mice at scheduled euthanasia. All treated male mice terminated early. Paired weight with and without fluid.
Gross lesions/masses	-	X	-	All mice at scheduled euthanasia. All treated mice terminated early. Excluded confirmation of persistent adverse clinical signs.
Heart	-	X	-	All treated mice terminated early.
Kidney	-	X	-	All treated mice terminated early.
Liver	-	X	-	All treated mice terminated early.
Lung	-	X	-	Infused with 10% neutral buffered formalin. All treated mice terminated early.
Ovaries	-	X	-	All female mice at scheduled euthanasia. All treated female mice terminated early.
Oviducts	-	X	-	All female mice at scheduled euthanasia. All treated female mice terminated early.
Pituitary	-	X	-	All mice at scheduled euthanasia. All mice terminated early
Spleen	-	X	-	All treated mice terminated early.
Stomach	-	X	-	All treated mice terminated early.
Testes	X	X	-	All treated male mice at scheduled euthanasia. Treated male mouse terminated early. Individual weight; fixed in Bouin's solution for 48 to 96 hours and retained in neutral buffered 10% formalin.
Tibiofemoral joints	-	X	-	All mice at scheduled euthanasia. All mice terminated early.
Trachea	-	X	-	Infused with 10% neutral buffered formalin. All treated mice terminated early.
Uterus	-	X	-	All female mice at scheduled euthanasia. Treated female mice terminated early. Collected with cervix.

X = procedure to be conducted; - = not applicable.

There were no meaningful drug-related organ weight changes or macroscopic findings. The tables below (taken from the sponsor's study report) summarize organ weights and bodyweights in drug-treated males and notable macroscopic findings in drug-treated males and females.

Organ Weight and Terminal Body Weight (Drug-Treated Male Groups)

GROUP		1	2	3	4
TEST MATERIAL		STERILE WATER FOR INJECTION, USP	SP-304	SP-304	SP-304
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	600
MICE TESTED	N	25	25	25	25
INCLUDED IN ANALYSES	N	25	25	25	24b
TERMINAL BODY WEIGHT	MEAN±S.D.	37.6 ± 2.3	37.6 ± 1.7	37.1 ± 2.3	38.0 ± 1.9
EPIDIDYMIS LEFT c	MEAN±S.D.	149.075±13.812	150.576±19.657	146.057±14.776	147.931±15.322
CAUDA EPIDIDYMIS LEFT c	MEAN±S.D.	55.050 ± 9.884	51.277 ± 8.114	52.661 ± 6.366	51.338 ± 8.299
TESTIS LEFT c	MEAN±S.D.	329.470±29.691	339.522±53.846	320.550±46.555	332.283±51.886
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	1.082 ± 0.154	1.051 ± 0.165	1.088 ± 0.181	1.008 ± 0.176
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.576 ± 0.118	0.526 ± 0.101	0.574 ± 0.103	0.510 ± 0.094
EPIDIDYMIS RIGHT c	MEAN±S.D.	162.810±16.612	162.203±26.076	164.080±54.516	153.089±22.006
TESTIS RIGHT c	MEAN±S.D.	355.111±33.294	365.790±58.220	350.592±55.226	359.966±57.885
PROSTATE c	MEAN±S.D.	101.441±42.918	100.428±27.467	100.464±40.690	109.171±38.855

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.
a. Dosage occurred once daily in the morning on Days 1 through 62, 63, 64 or 65 of the study.
b. Excludes values for mouse 1493, which was euthanized on Day 40 of study due to adverse clinical observations.
c. Value scaled upward by 1000.

Summary of Parental Necropsy Findings (Drug-Treated Male Groups)

GROUP		1	2	3	4
TEST MATERIAL		STERILE WATER FOR INJECTION, USP	SP-304	SP-304	SP-304
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	600
MICE EXAMINED	N	25	25	25	25
UNSCHEDULED EUTHANASIA	N	0	0	0	1b
APPEARED NORMAL	N	25	25	25	24
CERVICAL AND AXILLARY REGIONS: BROWN GRANULAR MATERIAL PRESENT	N	0	0	0	1b
ESOPHAGUS: PERFORATION PRESENT	N	0	0	0	1b

a. Dosage occurred once daily in the morning on Days 1 through 62, 63, 64 or 65 of the study.
b. Mouse 1493 was euthanized on Day 40 of the study due to adverse clinical observations.

Summary of Parental Necropsy Findings (Drug-Treated Female Groups)

GROUP		1	2	3	4
TEST MATERIAL		STERILE WATER FOR INJECTION, USP	SP-304	SP-304	SP-304
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	600
MICE EXAMINED	N	25	25	25	25
UNSCHEDULED EUTHANASIA	N	1b	0	0	0
APPEARED NORMAL	N	24	25	24	25
ESOPHAGUS: ONE PERFORATION	N	1b	0	0	0
AXILLARY REGION: RIGHT, BROWN GRANULAR MATERIAL	N	1b	0	0	0
LIVER: ALL LOBES, THICK	N	0	0	1	0

a. Dosage occurred on Day 1 of study through Day 7 of presumed gestation.
b. Mouse 1717 was euthanized on Day 3 of presumed gestation.

There were no drug-related effects on sperm motility, count, or density in the drug-treated males. A statistically significant decrease in sperm count was observed in the 200 mg/kg/day group. However, the decrease was not dose-dependent and is not considered as drug-related. The data are summarized in the Sponsor's table below.

Sperm Motility, Count, and Density in Drug-Treated Male Groups

GROUP		1			2			3			4		
TEST MATERIAL		STERILE WATER FOR INJECTION, USP			SP-304			SP-304			SP-304		
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)			20			200			600		
MICE TESTED	N	25			25			25			24b		
VAS DEFERENS SPERM MOTILITY													
NUMBER MOTILE	MEAN±S.D.	640.1	±	312.8	615.4	±	410.0	621.8	±	313.9	482.6	±	293.9
MOTILE PERCENT	MEAN±S.D.	89.6	±	6.6	85.9	±	10.6	85.8	±	10.1	82.5	±	11.4
STATIC COUNT (NONMOTILE)	MEAN±S.D.	65.7	±	40.2	86.8	±	70.3	83.8	±	41.0	80.9	±	42.2
TOTAL COUNT c	MEAN±S.D.	705.8	±	331.1	702.2	±	442.3	705.5	±	320.9	563.5	±	295.2
CAUDA EPIDIDYMAL SPERM COUNT													
SPERM COUNT d	MEAN±S.D.	118.2	±	31.9	104.5	±	31.0	92.0	±	26.3*	112.6	±	40.1
SPERM DENSITY e	MEAN±S.D.	1510.48	±	389.06	1413.05	±	336.35	1251.69	±	392.79	1495.66	±	437.06

a. Dosage occurred once daily in the morning on Days 1 through 62, 63, 64 or 65 of the study.
b. Excludes vales for mouse 1493, which was euthanized on Day 40 of study due to adverse clinical observations.
c. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.
d. Sperm count used in the calculation of sperm density. Twenty fields were evaluated.
e. The sperm density was calculated by dividing the sperm count by the volume in the image area (76.6×10^{-6} mL), multiplying by 2 (dilution factor + 1) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 10 (volume) and divided by the weight of the left cauda epididymis (see Appendix 11 for the weight of the left cauda epididymis) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.
* Significantly different from the Group 1 value ($p \leq 0.05$).

Fertility Parameters

Each male in the drug-treated group was assigned to mate with a female in the untreated group. Males that failed to mate within the first 14 days of cohabitation were assigned an alternate untreated female, and remained in cohabitation for up to 3 additional days. Each female in the drug-treated group was assigned to mate with an untreated male. All treated females were mated within 5 days of cohabitation and assigned to individual housing. Females (untreated and treated) with a copulatory plug *in situ* or spermatozoa present in a smear of the vaginal contents were considered to be at DG 0 and assigned to individual housing.

There were no drug-related effects on any of the mating or fertility parameters in males or females. For treated males paired with untreated females, the mating index was 100% for all dose groups in males and females. The mean number of days needed to mate was 2.4, 2.1, 2.5, and 2.8 days in the control, 20, 200, and 600 mg/kg/day groups, respectively. The fertility indices were 88.0%, 84.0%, 92.0%, and 96.0% in the control,

20, 200, and 600 mg/kg/day groups, respectively. The reproductive indices are summarized in the tables below (taken from the Sponsor's study report).

Mating and Fertility in Males (Treated Males with Untreated Females)

GROUP		1	2	3	4
TEST MATERIAL		STERILE WATER FOR INJECTION, USP	SP-304	SP-304	SP-304
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	600
MICE IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION b,c	MEAN±S.D.	2.4 ± 1.1	2.1 ± 1.1	2.5 ± 1.2	2.8 ± 1.7
MICE THAT MATED c	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX d,e	N/N	22/ 25	21/ 25	23/ 25	24/ 25
	(%)	(88.0)	(84.0)	(92.0)	(96.0)
MICE WITH CONFIRMED MATING DATES	N	25	25	25	25
MATED WITH FEMALE					
DAYS 1-7	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
MICE PREGNANT/MICE IN COHABITATION e	N/N	22/ 25	21/ 25	23/ 25	24/ 25
	(%)	(88.0)	(84.0)	(92.0)	(96.0)

a. Dosage occurred once daily in the morning on Days 1 through 62, 63, 64 or 65 of the study.
b. Restricted to mice with a confirmed mating date.
c. Includes only one mating for each male mouse.
d. Number of pregnancies/number of mice that mated.
e. Includes only one pregnancy for each mouse that impregnated more than one female mouse.

Mating and Fertility in Females (Treated Males with Untreated Females)

UNTREATED GROUP		1	2	3	4
MICE IN COHABITATION	N	26	26	26	25
DAYS IN COHABITATION a	MEAN±S.D.	2.4 ± 1.1 [25]	2.1 ± 1.1 [25]	2.5 ± 1.2 [25]	2.8 ± 1.7
MICE THAT MATED	N(%)	26(100.0)	26(100.0)	26(100.0)	25(100.0)
FERTILITY INDEX b	N/N	23/26	22/26	24/26	24/25
	(%)	(88.5)	(84.6)	(92.3)	(96.0)
MICE WITH CONFIRMED MATING DATES	N	25	25	25	25
MATED BY FIRST MALE c					
DAYS 1-7	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
MICE PREGNANT/MICE IN COHABITATION	N/N	23/26	22/26	24/26	24/25
	(%)	(88.5)	(84.6)	(92.3)	(96.0)

[ ] = NUMBER OF VALUES AVERAGED
a. Restricted to mice with a confirmed mating date and mice that did not mate.
b. Number of pregnancies/number of mice that mated.
c. Restricted to mice with a confirmed mating date.

For untreated males paired with treated females, the mean number of days needed to mate was 2.0, 2.1, 2.4, and 1.8 in the control, 20, 200, and 600 mg/kg/day groups, respectively. The mating index was 100% in all dose groups. Fertility indices were 87.5%, 80.0%, 88.0%, and 88.0% in the control, 20, 200, and 600 mg/kg/day groups, respectively.

Mating and Fertility in Males (Untreated Males with Treated Females)

UNTREATED GROUP		1	2	3	4
MICE IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION a,b	MEAN±S.D.	2.0 ± 1.2	2.1 ± 1.2	2.4 ± 1.2	1.8 ± 0.9
MICE THAT MATED b	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX c,d	N/N	21/24e	20/25	22/25	22/25
	(%)	(87.5)	(80.0)	(88.0)	(88.0)
MICE WITH CONFIRMED MATING DATES	N	25	25	25	25
MATED WITH FEMALE					
DAYS 1-7	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
MICE PREGNANT/MICE IN COHABITATION d	N/N	21/24e	20/25	22/25	22/25
	(%)	(87.5)	(80.0)	(88.0)	(88.0)

a. Restricted to mice with a confirmed mating date and mice that did not mate.
b. Includes only one mating for each male mouse.
c. Number of pregnancies/number of mice that mated.
d. Includes only one pregnancy for each mouse that impregnated more than one female mouse.
e. Excludes values for mouse 1717, which pregnancy status could not be determined due to early gestational age.

Mating and Fertility in Females (Untreated Males with Treated Females)

GROUP		1	2	3	4
TEST MATERIAL		STERILE WATER FOR INJECTION, USP	SP-304	SP-304	SP-304
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	600
MATING OBSERVATIONS					
MICE IN COHABITATION	N	25	25	25	25
DAYS IN COHABITATION b	MEAN±S.D.	2.0 ± 1.2	2.1 ± 1.2	2.4 ± 1.2	1.8 ± 0.9
MICE THAT MATED	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX c	N/N	21/24d	20/25	22/25	22/25
	(%)	(87.5)	(80.0)	(88.0)	(88.0)
MICE WITH CONFIRMED MATING DATES	N	25	25	25	25
MATED BY FIRST MALE e					
DAYS 1-7	N(%)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
MICE PREGNANT/MICE IN COHABITATION	N/N	21/24d	20/25	22/25	22/25
	(%)	(87.5)	(80.0)	(88.0)	(88.0)

a. Dosage occurred on Day 1 of study through Day 7 of presumed gestation.
b. Restricted to mice with a confirmed mating date and mice that did not mate.
c. Number of pregnancies/number of mice that mated.
d. Excludes values for mouse 1717, whose pregnancy status could not be determined because it was euthanized at an early gestational age (Day 3 of presumed gestation).
e. Restricted to mice with a confirmed mating date.

Cesarean-Sectioning and Litter Observations

All surviving dams were sacrificed on GD 13, and a cesarean section was performed. The uterus from each gravid animal was excised, and examined for number and distribution of corpora lutea, implantation sites, placentae (size, color or shape), and live and dead embryos. The placentae were also examined.

In both the untreated and treated female groups, all parameters, including pregnancy rate, corpora lutea, implantation sites, number of live and dead embryos, the pre- and post-implantation losses, and dams with any or all nonviable embryos in each treatment

group were comparable to that of the control groups. All placentae appeared normal. The results are summarized in the Sponsor's tables below.

Summary of Cesarean Section Data in the Untreated Female Groups

UNTREATED GROUP		1	2	3	4
MICE TESTED	N	26	26	26	25
INCLUDED IN ANALYSES	N	25a	25a	25a	25
PREGNANT	N(%)	22(88.0)	21(84.0)	23(92.0)	24(96.0)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	22	21	23	24
CORPORA LUTEA	MEAN±S.D.	13.4 ± 2.8	13.6 ± 3.0	14.0 ± 2.6	14.0 ± 1.7
IMPLANTATIONS	MEAN±S.D.	13.0 ± 3.0	12.7 ± 3.3	13.1 ± 2.7	13.3 ± 1.6
% PREIMPLANTATION LOSS	MEAN±S.D.	4.1 ± 10.9	8.6 ± 15.5	7.2 ± 10.8	4.8 ± 7.6
LITTER SIZES	MEAN±S.D.	12.0 ± 3.2	11.4 ± 4.3	11.0 ± 4.2	12.3 ± 2.1
VIABLE EMBRYOS	N	264	240	254	295
	MEAN±S.D.	12.0 ± 3.2	11.4 ± 4.3	11.0 ± 4.2	12.3 ± 2.1
NONVIABLE EMBRYOS	N	23	27	48	24
	MEAN±S.D.	1.0 ± 1.2	1.3 ± 2.0	2.1 ± 3.3	1.0 ± 1.1
% POSTIMPLANTATION LOSS	MEAN±S.D.	8.1 ± 10.0	15.6 ± 27.6	16.0 ± 24.5	7.8 ± 8.6
DAMS WITH NONVIABLE EMBRYOS	N(%)	14(63.6)	12(57.1)	15(65.2)	16(66.7)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	1(4.8)	1(4.3)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	22(100.0)	20(95.2)	22(95.6)	24(100.0)
PLACENTAE APPEARED NORMAL b	N(%)	22(100.0)	20(100.0)	22(100.0)	24(100.0)
% NONVIABLE EMBRYOS/LITTER b	MEAN±S.D.	8.1 ± 10.0	11.4 ± 20.3	12.1 ± 16.7	7.8 ± 8.6

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Excludes values for mice that did not have a confirmed mating date.
b. Excludes values for dams with all nonviable embryos.

Summary of Cesarean Section Data in Treated Female Groups

GROUP		1	2	3	4
TEST MATERIAL		STERILE WATER FOR INJECTION, USP	SP-304	SP-304	SP-304
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	600
MICE TESTED	N	25	25	25	25
INCLUDED IN ANALYSES	N	24b	25	25	25
PREGNANT	N(%)	21(87.5)	20(80.0)	22(88.0)	22(88.0)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	21	20	22	22
CORPORA LUTEA	MEAN±S.D.	13.7 ± 2.6	13.6 ± 2.1	13.7 ± 2.0	13.6 ± 2.2
IMPLANTATIONS	MEAN±S.D.	13.0 ± 2.0	12.6 ± 2.2	13.0 ± 1.7	12.9 ± 1.8
% PREIMPLANTATION LOSS	MEAN±S.D.	4.6 ± 7.4	7.7 ± 11.3	4.6 ± 6.3	4.8 ± 5.7
LITTER SIZES	MEAN±S.D.	11.7 ± 2.0	12.0 ± 3.2	12.4 ± 1.5	12.0 ± 2.0
VIABLE EMBRYOS	N	246	239	274	263
	MEAN±S.D.	11.7 ± 2.0	12.0 ± 3.2	12.4 ± 1.5	12.0 ± 2.0
NONVIABLE EMBRYOS	N	26	14	13	21
	MEAN±S.D.	1.2 ± 1.2	0.7 ± 1.2	0.6 ± 0.7	1.0 ± 1.0
% POSTIMPLANTATION LOSS	MEAN±S.D.	9.5 ± 9.2	8.9 ± 22.5	4.3 ± 5.4	7.4 ± 8.0
DAMS WITH NONVIABLE EMBRYOS	N(%)	15(71.4)	7(35.0)	10(45.4)	13(59.1)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Dosage occurred on Day 1 of study through Day 7 of gestation.
b. Excludes values for mouse 1717, whose pregnancy status could not be determined because it was euthanized at an early gestational age (Day 3 of presumed gestation).

GROUP		1	2	3	4
TEST MATERIAL		STERILE WATER FOR INJECTION, USP	SP-304	SP-304	SP-304
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	600
MICE TESTED	N	25	25	25	25
INCLUDED IN ANALYSES	N	24b	25	25	25
PREGNANT	N(%)	21(87.5)	20(80.0)	22(88.0)	22(88.0)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	21(100.0)	19(95.0)	22(100.0)	22(100.0)
PLACENTAE APPEARED NORMAL c	N(%)	21(100.0)	19(100.0)	22(100.0)	22(100.0)
% NONVIABLE EMBRYOS/LITTER c	MEAN±S.D.	9.5 ± 9.2	4.2 ± 7.1	4.3 ± 5.4	7.4 ± 8.0

a. Dosage occurred on Day 1 of study through Day 7 of gestation.
b. Excludes values for mouse 1717, whose pregnancy status could not be determined because it was euthanized at an early gestational age (Day 3 of presumed gestation).
c. Excludes values for dams with all nonviable embryos.

In summary, the NOAEL for paternal and maternal toxicity is considered to be 600 mg/kg/day, based on the lack of adverse drug-related paternal or maternal findings at this dose. The NOAEL for male and female fertility and early embryonic development is considered to be 600 mg/kg/day.

9.2 Embryonic Fetal Development

Study title: Oral (Gavage) Developmental Toxicity Study of SP-304 in Mice

Study no.:	0020001133
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3/31/2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide (SP-304), FUROG0801A (96.5%) and 034E09 (96.4%)

Key Study Findings

- Pregnant mice, 25/group were administered 0 (vehicle), 20, 200, or 800 mg/kg/day plecanatide by oral gavage on GD 6-15. C-sections were performed on GD 18.

- There were no drug-related deaths, and there were no meaningful drug-related clinical signs or bodyweight changes during gestation.

- No drug-related macroscopic findings were noted when the dams were necropsied. All c-section parameters, including pregnancy rate, corpora lutea, implantation sites, resorptions (early or late), sex ratio, number of live fetuses, fetal weights, and the pre- and post-implantation losses in each treatment group, were comparable to that of the control group. There were no drug-related fetal external findings.

- There were no drug-related significant increases in soft tissue or skeletal alterations (variations or malformations), or fetal ossification sites per fetus.

- The NOEL for maternal toxicity is considered to be 800 mg/kg/day, based on the lack of adverse findings at the high dose. For embryo-fetal development, the NOEL is considered to be 800 mg/kg/day.

Methods

Doses:	0(vehicle), 20, 200, and 800 mg/kg/day
Frequency of dosing:	once daily
Dose volume:	10 ml/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	solution/sodium phosphate buffer
Species/Strain:	Mouse/Crl:CD1(ICR)
Number/Sex/Group:	25
Satellite groups:	N/A

Study design: Pregnant mice (25/group) were administered 0 (vehicle), 20, 200, or 800 mg/kg/day plecanatide by oral gavage on GD 6-15. Cesarean sections were performed on GD 18.

Deviation from study protocol: There were minor deviations that did not affect the quality or integrity of the study.

Study Design

Dosage Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Mice	Assigned Mouse Numbers
I	0 (Vehicle)	0	10	25	4101-4106, 1000[b], 4108-4120, 999[c], 4122-4125
II	20	2.0	10	25	4126-4150
III	200	20	10	25	4151-4175
IV	800	80	10	25	4176-4200

At the start of the mating period, the animals were approximately 70 days old, and their bodyweights ranged from 24.9 to 29.2 g at study assignment (GD 0).

The sponsor indicated that doses were selected on the basis of a dose range-finding study (Protocol VMF00028) and the expected doses in humans. In the dose range-finding study, 8 mice/group were treated orally with 0 (vehicle), 20, 200, or 1200 SP-304 during the period of organogenesis. There were no meaningful drug-related effects at the highest dose tested. The NOAEL was considered to be 1200 mg/kg/day. The Sponsor considered 800 mg/kg to be the maximum feasible dose based on the solubility of SP-304 and the recommended maximum dose volume (10 ml/kg) for mice. Other dose levels were selected at intervals which were expected to reveal any dose related trends. The oral gavage route of administration was selected because it is the intended route of administration in humans.

Observations and Results

Mortality

All animals were observed twice daily for mortality. There were no drug-related deaths during the course of the study. One mouse in the vehicle control group was euthanized on day 9 of presumed gestation because of an apparent gavage error, and another mouse in the control group was found dead on GD 3, before the initiation of dose administration. Both of these mice were replaced.

Clinical Signs

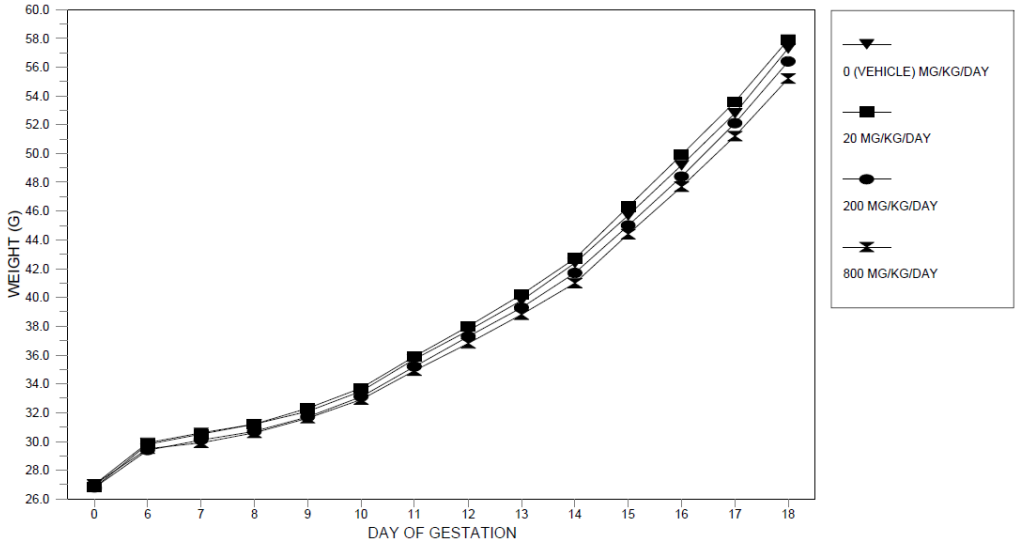
Animals were observed at least weekly during the acclimation period, on day 0 of presumed gestation, and once daily before and after dose administration during GD 1 to 18. Post-dose observations were made between 1 and 2 hours after dose administration.

There were no meaningful drug-related clinical signs. There were sporadic clinical signs that were not dose-dependent, and they are not considered as drug-related.

Body Weight

Animals were weighed on GD 0, and daily during the dosing phase (GD 6-15) and post-dose phase (GD15-18). There were no meaningful drug-related changes in bodyweight or bodyweight gain. The initial bodyweight (GD 0) of the control group was 27.0 g and the terminal bodyweight was 57.3 g (GD 18). Bodyweight gains during the dosing-period (calculated as GD 6 through 16) in the 20, 200, and 800 mg/kg/day groups were 105%, 99%, and 94%, respectively, of the control group. The bodyweights are shown in the Sponsor's figure below.

Maternal Bodyweight During Gestation



Feed Consumption

Not measured.

Toxicokinetics

Not performed.

Dosing Solution Analysis

The concentration determination of plecanatide in the dosing formulation samples was performed in accordance with the validated method. Analyses conducted during the treatment period showed that the concentrations of dosing formulations at 2, 20, and 80 mg/ml were within ±10% of the nominal concentration (-8.2% to +9.6%).

Necropsy

All surviving dams were sacrificed on GD 18. They were cesarean-sectioned and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed.

There were no drug-related macroscopic findings. There were sporadic findings, but these are not considered as drug-related because they were not dose-dependent. Notable necropsy observations are summarized in the Sponsor's table below.

Summary of Notable Necropsy Findings

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	800
MICE EXAMINED b	N	25	25	25	25
MORTALITY	N	0	0	0	0
APPEARED NORMAL	N	25	24	25	25
OVARIES:					
LEFT, CLEAR BURSAL CYST	N	0	1	0	0

a. Dosage occurred on days 6 through 15 of presumed gestation.
b. Refer to the individual clinical observations table (Table 12) for external observations confirmed at necropsy.

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

All surviving dams were sacrificed on GD 18, and a cesarean section was performed. The uterus from each gravid animal was excised, and examined for the number and placement of live and dead fetuses, the number of early or late resorptions, and any abnormalities. The right and left ovaries from each dam was examined for the number of corpora lutea. The placentae were also examined.

All parameters, including pregnancy rate, corpora lutea, implantation sites, resorptions (early or late), sex ratio, number of live fetuses, fetal weight, and the pre- and post-implantation losses in each treatment group, were comparable to that of the control group. All placentae appeared normal. The data are summarized in the Sponsor's tables below.

Summary of Cesarean Section Data

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	800
MICE TESTED	N	25	25	25	25
PREGNANT	N(%)	24(96.0)	22(88.0)	22(88.0)	22(88.0)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 18 OF GESTATION	N	24	22	22	22
CORPORA LUTEA	MEAN±S.D.	13.4 ± 2.7 [23]b	14.0 ± 2.0	13.2 ± 2.9	12.9 ± 3.1
IMPLANTATIONS	MEAN±S.D.	13.3 ± 2.8	13.9 ± 2.0	13.1 ± 2.8	12.9 ± 3.1
% PREIMPLANTATION LOSS	MEAN±S.D.	1.7 ± 7.0 [23]b	0.3 ± 1.5	0.6 ± 2.1	0.3 ± 1.4
LITTER SIZES	MEAN±S.D.	12.6 ± 3.1	13.0 ± 2.2	12.3 ± 3.3	12.0 ± 3.2
LIVE FETUSES	N	303	286	270	264
	MEAN±S.D.	12.6 ± 3.1	13.0 ± 2.2	12.3 ± 3.3	12.0 ± 3.2
DEAD FETUSES	N	0	0	1	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	0.7 ± 0.9	0.9 ± 1.0	0.8 ± 0.9	0.9 ± 1.4
EARLY RESORPTIONS	N	15	14	15	18
	MEAN±S.D.	0.6 ± 0.9	0.6 ± 0.9	0.7 ± 0.9	0.8 ± 1.3
LATE RESORPTIONS	N	1	6	3	1
	MEAN±S.D.	0.0 ± 0.2	0.3 ± 0.4	0.1 ± 0.4	0.0 ± 0.2
% POSTIMPLANTATION LOSS	MEAN±S.D.	6.8 ± 11.3	6.7 ± 6.9	8.4 ± 13.3	7.8 ± 12.0
MICE WITH ANY RESORPTIONS	N(%)	11(45.8)	13(59.1)	12(54.5)	9(40.9)
MICE WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
MICE WITH VIABLE FETUSES	N(%)	24(100.0)	22(100.0)	22(100.0)	22(100.0)
PLACENTAE APPEARED NORMAL	N(%)	24(100.0)	22(100.0)	22(100.0)	22(100.0)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 15 of gestation.
b. Excludes a value for mouse 4124 that appeared incorrectly recorded.

Summary of Litter Observations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	800
LITTERS WITH ONE OR MORE LIVE FETUSES	N	24	22	22	22
IMPLANTATIONS	MEAN±S.D.	13.3 ± 2.8	13.9 ± 2.0	13.1 ± 2.8	12.9 ± 3.1
LIVE FETUSES	N	303	286	270	264
	MEAN±S.D.	12.6 ± 3.1	13.0 ± 2.2	12.3 ± 3.3	12.0 ± 3.2
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	54.6 ± 19.7	48.9 ± 17.2	50.0 ± 19.4	50.7 ± 11.8
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	1.36 ± 0.15	1.30 ± 0.07	1.29 ± 0.12	1.32 ± 0.11
MALE FETUSES	MEAN±S.D.	1.37 ± 0.16	1.34 ± 0.07	1.30 ± 0.11 [21]b	1.34 ± 0.10
FEMALE FETUSES	MEAN±S.D.	1.32 ± 0.12 [23]c	1.27 ± 0.09	1.26 ± 0.13	1.29 ± 0.12
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	6.8 ± 11.3	6.7 ± 6.9	8.4 ± 13.3	7.8 ± 12.0

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 15 of gestation.
b. Litter 4152 had no male fetuses.
c. Litter 4106 had no female fetuses.

Offspring (Malformations, Variations, etc.)

Each fetus (live or dead) was weighed, and examined for external abnormalities. Live fetuses were sacrificed. The internal organs of the thoracic and abdominal cavities of

all fetuses were examined and the sex of each fetus (live or dead) was determined. Approximately one-half of the fetuses in each litter were fixed in Bouin's solution and examined for soft tissue alterations. In the remaining fetuses, viscera were removed, and carcasses were stained with Alizarin red S and examined for skeletal alterations.

There were no drug-related increases in fetal external findings. Sporadic gross external findings were observed, but were not considered as drug-related because they lacked dose-dependency. The incidence of total fetal and litter alterations was comparable across treatment groups. There were no drug-related increases in soft tissue alterations. Sporadic soft tissue alterations were observed, but these findings were not dose-dependent and/or occurred only in a single fetus. Therefore, these were not considered to be drug-related.

There were no drug-related significant increases in skeletal alterations. There were sporadic skeletal alterations, but these findings were not dose-dependent and/or occurred in a single fetus. Thus, there were no drug-related increases in skeletal variations or malformations.

There were no drug-related significant increases in fetal ossification sites per fetus.

Data for the gross external alterations, fetal soft tissue and skeletal alterations, and fetal ossification sites are summarized in the Sponsor's tables below.

Fetal Gross External Alterations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	800
LITTERS EVALUATED	N	24	22	22	22
FETUSES EVALUATED	N	303	286	271	264
LIVE	N	303	286	270	264
DEAD b	N	0	0	1	0
PALATE: CLEFT					
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	1(4.5)	1(4.5)
FETAL INCIDENCE	N(%)	1(0.3)	0(0.0)	1(0.4)	1(0.4)
EYE(S): LID(S) OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)c	0(0.0)
HEAD: EXENCEPHALY					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)c	0(0.0)
BODY: MENINGOCELE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)d	0(0.0)
FORE AND/OR HINDLIMB(S): ROTATED					
LITTER INCIDENCE	N(%)	4(16.7)	5(22.7)	3(13.6)	2(9.1)
FETAL INCIDENCE	N(%)	6(2.0)	6(2.1)	5(1.8)	2(0.8)
TAIL: PEDUNCULATED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)d	0(0.0)
TAIL: SHORT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)d	0(0.0)

a. Dosage occurred on days 6 through 15 of gestation.
b. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus are cited on Table 18.
c. Fetus 4159-4 had other gross external alterations.
d. Fetus 4159-7 had other gross external alterations.

Cesarean-Delivered Live Fetuses with Fetal Alterations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	800
LITTERS EVALUATED	N	24	22	22	22
FETUSES EVALUATED	N	303	286	271	264
LIVE	N	303	286	270	264
DEAD b	N	0	0	1	0
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	17(70.8)	20(90.9)	15(68.2)	17(77.3)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	49(16.2)	53(18.5)	36(13.3)	49(18.6)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	19.7 ± 23.4	18.0 ± 13.3	12.9 ± 13.5	19.0 ± 17.6

a. Dosage occurred on days 6 through 15 of gestation.
b. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus are cited on Table 18.

Fetal Soft Tissue Alterations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	800
LITTERS EVALUATED	N	23	22	22	22
FETUSES EVALUATED	N	146	137	131	127
LIVE	N	146	137	131	127
PALATE: CLEFT					
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.7)	0(0.0)	1(0.8)	0(0.0)
EYES: RETINA FOLDED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.5)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.8)c
ADRENALS: ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)b	0(0.0)
KIDNEYS: ABSENT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)b	0(0.0)
VESSELS: UMBILICAL ARTERY DESCENDS TO THE LEFT OF THE URINARY BLADDER					
LITTER INCIDENCE	N(%)	9(39.1)	12(54.5)	5(22.7)	10(45.4)
FETAL INCIDENCE	N(%)	11(7.5)	16(11.7)	7(5.3)	12(9.4)c
URETER: CONSTRICTED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)b	0(0.0)
GENITALIA: TESTIS MALPOSITIONED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.5)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.8)c

a. Dosage occurred on days 6 through 15 of gestation.
b. Fetus 4151-10 had other soft tissue alterations.
c. Fetus 4179-2 had other soft tissue alterations.

Fetal Skeletal Alterations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	800
LITTERS EVALUATED	N	24	22	22	22
FETUSES EVALUATED	N	157	149	140	137
LIVE	N	157	149	139	137
DEAD b	N	0	0	1	0
SKULL: FRONTALS, CONTAIN AN INTERFRONTAL					
LITTER INCIDENCE	N(%)	4(16.7)	4(18.2)	1(4.5)	3(13.6)
FETAL INCIDENCE	N(%)	9(5.7)c,d	4(2.7)e	3(2.2)j	6(4.4)k,L
SKULL: FRONTALS, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)g	0(0.0)
SKULL: PARIETALS, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)g	0(0.0)
SKULL: INTERPARIETALS, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)g	0(0.0)
SKULL: SUPRAOCCIPITALS, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)g	0(0.0)
SKULL: PALATE, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.5)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)n
CERVICAL VERTEBRAE: CERVICAL RIB PRESENT AT 7TH CERVICAL VERTEBRA					
LITTER INCIDENCE	N(%)	12(50.0)	13(59.1)	10(45.4)	10(45.4)
FETAL INCIDENCE	N(%)	25(15.9)c,d	26(17.4)e	17(12.2)h,i,j	30(21.9)k,L,m,n
LUMBAR VERTEBRAE: ARCH, OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)h	0(0.0)
SACRAL VERTEBRAE: ARCH, OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)h	0(0.0)
CAUDAL VERTEBRAE: ARCH, OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)h	0(0.0)
CAUDAL VERTEBRAE: ARCH, SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)h	0(0.0)
CAUDAL VERTEBRAE: ARCH, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)h	0(0.0)
RIBS: WAVY					
LITTER INCIDENCE	N(%)	1(4.2)	0(0.0)	0(0.0)	1(4.5)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)	0(0.0)	1(0.7)m
STERNAL CENTRA: SUMMARIZATION (SUMMARIZATION OF ASYMMETRIC, IRREGULARLY SHAPED AND FUSED)					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	3(13.6)	2(9.1)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)	3(2.2)	2(1.4)
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	2(9.1)	2(9.1)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)	2(1.4)	2(1.4)m
STERNAL CENTRA: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)i	0(0.0)
STERNAL CENTRA: FUSED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)i	0(0.0)

FORELIMB: METACARPAL, SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)f	0(0.0)	0(0.0)
FORELIMB: PHALANX, SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)f	0(0.0)	0(0.0)
FORELIMB: PHALANX, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)f	0(0.0)	0(0.0)
PELVIS: SUMMARIZATION (SUMMARIZATION OF ILIUM MISALIGNED, ISCHIUM MISALIGNED AND PUBIS MISALIGNED)					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)	0(0.0)
PELVIS: ILIUM, MISALIGNED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)h	0(0.0)
PELVIS: ISCHIUM, MISALIGNED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)h	0(0.0)
PELVIS: PUBIS, MISALIGNED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(4.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.7)h	0(0.0)

a. Dosage occurred on days 6 through 15 of gestation.
b. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus are cited on Table 18.
c. Fetus 4102-9 had other skeletal alterations.
d. Fetus 4102-11 had other skeletal alterations.
e. Fetus 4131-11 had other skeletal alterations.
f. Fetus 4150-6 had other skeletal alterations.
g. Fetus 4159-4 had other skeletal alterations.
h. Fetus 4159-7 had other skeletal alterations.
i. Fetus 4161-12 had other skeletal alterations.
j. Fetus 4170-9 had other skeletal alterations.
k. Fetus 4188-4 had other skeletal alterations.
L. Fetus 4189-13 had other skeletal alterations.
m. Fetus 4192-5 had other skeletal alterations.
n. Fetus 4197-1 had other skeletal alterations.

Summary of Fetal Ossification Sites

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	20	200	800
LITTERS EXAMINED	N	24	22	22	22
FETUSES EXAMINED	N	157	149	139	137
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	13.28 ± 0.28	13.29 ± 0.28	13.27 ± 0.31	13.19 ± 0.18
LUMBAR	MEAN±S.D.	5.71 ± 0.27	5.70 ± 0.28	5.72 ± 0.30	5.80 ± 0.18
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	8.79 ± 1.75	8.28 ± 0.95	8.34 ± 1.71	8.50 ± 1.22
RIBS (PAIRS)	MEAN±S.D.	13.23 ± 0.24	13.22 ± 0.22	13.22 ± 0.26	13.15 ± 0.15
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	4.02 ± 0.05	4.00 ± 0.00	4.03 ± 0.15	4.02 ± 0.11
XIPHOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	12.03 ± 0.40	11.97 ± 0.36	11.88 ± 0.45	11.71 ± 0.55
HINDLIMB b					
TARSALS	MEAN±S.D.	0.98 ± 0.41	0.95 ± 0.35	0.91 ± 0.46	0.98 ± 0.41
METATARSALS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	4.99 ± 0.04	5.00 ± 0.00
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	11.52 ± 1.06	11.18 ± 0.89	11.37 ± 1.30	11.03 ± 1.15

a. Dosage occurred on days 6 through 15 of gestation.
b. Calculated as average per limb.

In summary, the NOEL for maternal toxicity in mice is considered to be 800 mg/kg/day, based on the lack of dose-dependent findings. The NOEL for embryo-fetal development was 800 mg/kg/day.

Study title: Oral (Stomach Tube) Developmental Toxicity Study of SP-304 in Dutch Belted Rabbits

Study no.:	20003036
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/17/2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide (SP-304), FUROG1001 (96.1%)

Key Study Findings

- Pregnant female rabbits, 20/sex/group for controls and 21/sex/group for drug-treated animals, were administered 0 (vehicle), 15, 75, or 250 mg/kg/day plecanatide (SP-304) orally by stomach tube on GD 6-19. C-sections were performed on GD 29.

- There were no drug-related deaths. Drug-related clinical signs included soft feces and scant feces in groups receiving plecanatide at 15 mg/kg/day or higher, and liquid feces or both soft and liquid feces in the 250 mg/kg/day group. These clinical signs likely are related to the pharmacology of plecanatide, and they are not considered as adverse.

- There were no meaningful drug-related effects on bodyweight, bodyweight gain, or food consumption during gestation.

- Blood samples were obtained on GD 7 and 19. Maximum plasma levels of plecanatide were achieved around 0.5 hr after oral administration on GD 7 and GD 19. No quantifiable levels of plecanatide were detected prior to dosing on GD 19. Plasma exposure to plecanatide (AUC) was less than dose-proportional on GD 7 and GD 19. There was no or minimal drug accumulation in plasma after repeated dosing. Systemic exposure to SP-338, an active metabolite, was not determined.

- The only apparent drug-related macroscopic findings in the does were misshapen gallbladder observed in 3/21 and 2/21 animals in the 75 and 250 mg/kg/day groups, respectively.

- All c-section parameters, including pregnancy rate, corpora lutea, implantation sites, resorptions (early or late), sex ratio, number of live fetuses, fetal weight, and the pre- and post-implantation losses in each treatment group, were comparable to that of the control group. All placentae appeared normal and there were no dead fetuses. There were no drug-related fetal external findings.

- There were no drug-related significant increases in soft tissue or skeletal alterations (variations or malformations), or fetal ossification sites per fetus.

- The NOAEL for maternal toxicity is considered to be 250 mg/kg/day, based on the lack of adverse findings at the high dose. The NOEL for embryo-fetal development is considered to be 250 mg/kg/day based on the lack of drug-related findings.

Methods

Doses:	0 (vehicle control), 15, 75, and 250 mg/kg/day
Frequency of dosing:	once daily
Dose volume:	10 ml/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	solution/sodium phosphate buffer
Species/Strain:	rabbit/[MRI:(DB)SPF] Dutch Belted rabbit
Number/Sex/Group:	20/sex for controls and 21/sex/group for drug-treated groups

Satellite groups: N/A
Study design: Pregnant rabbits were administered 0 (vehicle), 15, 75, or 250 mg/kg/day plecanatide orally by stomach tube during GD 6-19. Blood samples for TK were obtained on GD 7 and 19. Cesarean sections were performed on GD 29.
Deviation from study protocol: There were minor deviations that did not affect the quality or integrity of the study.

Study Design

Dosage Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rabbits	Assigned Rabbit Numbers
I	0 (Vehicle)	0	10	20	7801 - 7820
II	15	1.5	10	21	7821 - 7841
III	75	7.5	10	21	7842 - 7844, 7900[b], 7846 - 7862
IV	250	25.0	10	21	7863 - 7883

a. Calculations for formulations of SP-304 were corrected for both the peptide content and HPLC purity values specified in the C of A for the lot of test article used in the study. For lot FUROG1001, the combined correction factor was 1.11.
b. Rabbit 7845 was removed from study prior to dosing on DG 7 due to body weight loss and replaced with rabbit 7900.

The Sponsor indicated that the doses were selected on the basis of a dose range-finding study (Protocol VMF00029). In that study, two Dutch Belted female rabbits per group were treated orally with 100 mg/kg/day SP-304 (non-pregnant females), or 150 or 250 mg/kg/day SP-304 (pregnant females, dosing started on GD 7) for seven days. One 100 mg/kg/day rabbit showed soft or liquid feces, slight bodyweight loss, and declining feed consumption in the latter half of the dosing period. A 250 mg/kg/day pregnant rabbit showed soft or liquid feces, scant feces, or no feces in the cage pan on each day in the latter half of the dosing period. This rabbit also had substantial reductions in feed consumption (<10 g consumed per day). The necropsy findings in this animal included numerous red areas on the mucosal surface of the cardiac region of the stomach, and distension of intestines with gas. Similar areas of red discoloration were observed in the stomach of a 150 mg/kg/day pregnant rabbit. Based on these findings, 250 mg/kg/day was selected as the high dose because it was expected that some does given this dose will show substantial signs of toxicity. Doses of 15 and 75 mg/kg/day were selected in order to evaluate responses over an approximately 17-fold range.

Observations and Results

Mortality

All animals were observed twice daily for mortality. There were no drug-related deaths during the course of the study. A rabbit in the control group was euthanized on GD 25 due to adverse clinical observations, and a rabbit in the control group aborted on GD 24 and was euthanized subsequently.

Clinical Signs

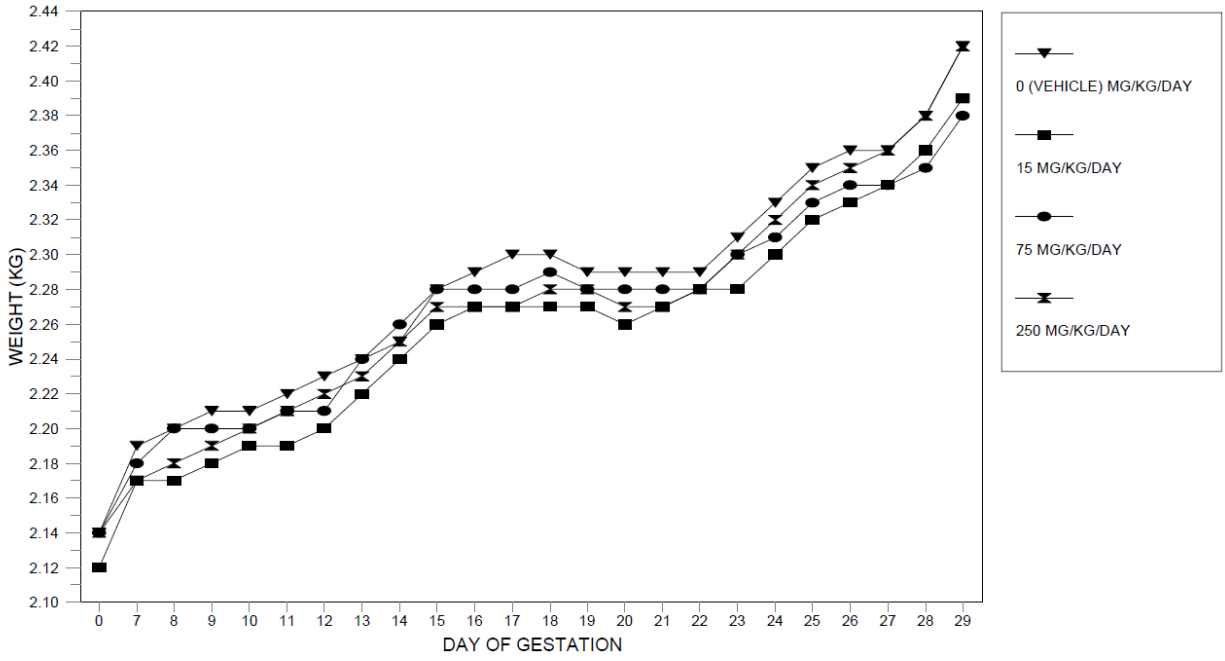
Animals were observed at least once daily during the pre-dose period. On the first four days of dosing, the animals were examined for clinical signs, abortions, premature deliveries, and deaths before dosing, between 3 to 4 hours post-dose, and at the end of a normal work day. On subsequent days of dosing, the animals were examined before dosing, between one and two hours after dosing, and at the end of a normal work day. Animals were examined once daily during the post-dose period.

Drug-related clinical signs included soft feces and scant feces in groups receiving plecanatide at 15 mg/kg/day or higher, and liquid feces or both soft and liquid feces in the 250 mg/kg/day group. These clinical signs are likely related to the pharmacology of plecanatide, and they are not considered as adverse.

Body Weight

Animals were weighed on GD 0, and daily during the dosing (GD 7-20) and post-dose period (GD 20-29). The initial bodyweight (GD 0) of the control group was 2.14 kg, and the terminal body weight was 2.42 kg (GD 29). There were no meaningful drug-related changes in bodyweight or bodyweight gain. Bodyweight gains during the dosing period (calculated as GD 7 through 20) in the 15, 75, and 250 mg/kg/day groups were 100%, 90%, and 90%, respectively, of the control group, and the differences were not statistically significant. The bodyweight and bodyweight gain data are shown in the Sponsor's figure and table below.

Maternal Bodyweight During Gestation



Maternal Bodyweight Gains During Gestation

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	15	75	250
RABBITS TESTED	N	20	21	21	21
PREGNANT	N	18	21	20	16
INCLUDED IN ANALYSES	N	17b	21	20	16
MATERNAL BODY WEIGHT CHANGE (KG)					
DAYS 0 - 7	MEAN±S.D.	+0.05 ± 0.06	+0.04 ± 0.06	+0.05 ± 0.05	+0.04 ± 0.09
DAYS 7 - 10	MEAN±S.D.	+0.02 ± 0.04	+0.02 ± 0.03	+0.01 ± 0.03	+0.03 ± 0.04
DAYS 10 - 13	MEAN±S.D.	+0.03 ± 0.02	+0.03 ± 0.02	+0.05 ± 0.04	+0.03 ± 0.03
DAYS 13 - 16	MEAN±S.D.	+0.05 ± 0.04	+0.05 ± 0.02	+0.04 ± 0.04	+0.04 ± 0.03
DAYS 16 - 20	MEAN±S.D.	+0.00 ± 0.04	-0.01 ± 0.03	-0.01 ± 0.04	-0.01 ± 0.03
DAYS 7 - 20	MEAN±S.D.	+0.10 ± 0.04	+0.10 ± 0.06	+0.09 ± 0.04	+0.09 ± 0.06
DAYS 20 - 24	MEAN±S.D.	+0.04 ± 0.03	+0.03 ± 0.04	+0.04 ± 0.02	+0.05 ± 0.02
DAYS 24 - 29	MEAN±S.D.	+0.09 ± 0.03 [16]c	+0.09 ± 0.03	+0.07 ± 0.05	+0.10 ± 0.03
DAYS 20 - 29	MEAN±S.D.	+0.14 ± 0.03 [16]c	+0.13 ± 0.06	+0.11 ± 0.06	+0.15 ± 0.04
DAYS 7 - 29	MEAN±S.D.	+0.24 ± 0.06 [16]c	+0.22 ± 0.10	+0.20 ± 0.06	+0.24 ± 0.07
DAYS 0 - 29	MEAN±S.D.	+0.29 ± 0.09 [16]c	+0.27 ± 0.12	+0.25 ± 0.08	+0.28 ± 0.09

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 19 of gestation.
b. Excludes values for doe 7801, which was euthanized on day 25 of gestation due to adverse clinical observations.
c. Excludes values for doe 7813, which aborted and was euthanized on day 24 of gestation.

Feed Consumption

Feed consumption was recorded daily. There were no meaningful drug-related effects on feed consumption. Absolute feed consumption (g/day) in the 15, 75, and 250 mg/kg/day groups for the dosing period (GD 7 to 20) were 100%, 100%, and 96% of the control group, respectively. Relative feed consumption (g/kg/day) in the 15, 75, and 250 mg/kg/day groups for the dosing period were 101%, 101%, and 97% of the control group, respectively.

Toxicokinetics

Blood samples were collected on GD 7 and 19. On GD 7, samples were collected at approximately 0.08, 0.25, 0.5, 1, 2, 4, and 8 hr post-dose. On GD 19, samples were collected pre-dose and at approximately 0.08, 0.25, 0.5, 1, 2, 4, and 8 hr post-dose. At least one blood sample, and no more than two, was collected from all animals on each of the collection days (three animals at each interval). When a second blood sample was required from some animals on the same collection day, those designated for the pre-dose collection were selected and the interval between the sample collections was at least one hour. The toxicokinetic data are shown in the Sponsor's table below.

Toxicokinetics of SP-304 in Pregnant Rabbits

Day 7 of Gestation

Group No.	Dose Level (mg/kg/day)	Tmax (h)	Cmax (ng/mL)	Cmax SE	AUC(0-t) (ng•h/mL)	AUC(0-t) SE	AUC(0-inf) (ng•h/mL)	T1/2 (h)	Cmax/D	AUC(0-t)/D	AUC(0-inf)/D
II	15	0.5	85.3	45.7	81.9	26.2	84.4	0.355	5.69	5.46	5.63
III	75	0.083	204	119	161	22.7	169	0.479	2.72	2.14	2.25
IV	250	0.5	306	13.4	321	15.0	343	0.466	1.23	1.28	1.37

Day 19 of Gestation

Group No.	Dose Level (mg/kg/day)	Tmax (h)	Cmax (ng/mL)	Cmax SE	AUC(0-t) (ng•h/mL)	AUC(0-t) SE	AUC(0-inf) (ng•h/mL)	T1/2 (h)	Cmax/D	AUC(0-t)/D	AUC(0-inf)/D
II	15	0.5	69.5	42.3	39.7	16.3	NE	NE	4.63	2.65	-
III	75	0.25	243	82.5	106	18.7	118	0.261	3.24	1.41	1.57
IV	250	0.5	513	99.0	449	70.2	RNR	RNR	2.05	1.79	-

RNR Result not reported because the AUC(0-inf) was extrapolated by more than 20% or Rsq was <0.800.
NE Parameter not estimable from data set.
- Not calculated.

Maximum plasma levels of SP-304 were achieved around 0.5 hr after oral administration on GD 7 and GD 19. No quantifiable levels of SP-304 were measured prior to dosage on GD 19. Plasma exposure to SP-304 (AUC) was less than dose-proportional on GD 7 and GD 19. There was no or minimal accumulation of SP-304 in plasma after repeated dosing; a modest increase (1.4 fold) in exposure between GD 7 and GD 19 was observed only at the 250 mg/kg/day dose level but not at lower dose levels. The C_{max} and AUC_{0-t} values in the 250 mg/kg/day group on GD 19 were 513 ng/mL and 499 ng·h/mL, respectively. Systemic exposure of the animals to SP-338, an active metabolite, was not determined.

Dosing Solution Analysis

The concentration determination of plecanatide in the dosing formulation samples was performed in accordance with the validated method. Analyses conducted during the treatment period showed that the concentrations of dose formulations at 1.5, 7.5, and 25 mg/ml were within ±10% of the nominal concentration (+1.2% to +9.2%).

Necropsy

All surviving does were sacrificed on GD 29. They were cesarean-sectioned, and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed.

Misshapen gallbladder was observed in 3/21 and 2/21 animals in the 75 and 250 mg/kg/day groups, respectively. There were other sporadic findings, but these are not considered as drug-related because they lacked dose-dependency and/or occurred only in a single animal in a dose group. Notable necropsy observations are summarized in the Sponsor's table below.

Summary of Necropsy Observations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	15	75	250
RABBITS EXAMINED b	N	20	21	21	21
UNSCHEDULED EUTHANASIA	N	1c	0	0	0
ABORTED AND EUTHANIZED	N	1d	0	0	0
APPEARED NORMAL	N	17	20	14	17
STOMACH:					
CARDIAC REGION, RED AREA	N	1c	0	0	0
UMBILICAL HERNIA	N	1c	0	0	0
LIVER:					
MEDIAN LOBE, DARK RED AREA	N	1d	0	0	0
ALL LOBES, MOTTLED TAN AND BROWN	N	0	0	0	1
SPLEEN:					
ACCESSORY SPLEEN	N	0	0	0	1
KIDNEYS:					
BILATERAL, NUMEROUS PITTED AREAS	N	1	0	0	1
GALLBLADDER:					
BILOBED	N	0	1	3	0
NUMEROUS TAN AREAS	N	0	0	1	0
MISSHAPEN	N	0	0	3	2

a. Dosage occurred on days 7 through 19 of presumed gestation.
b. Refer to the individual clinical observations table (Table 15) for external observations confirmed at necropsy.
c. Doe 7801 was euthanized on day 25 of gestation due to adverse clinical observations.
d. Doe 7813 aborted and was euthanized on day 24 of gestation.

The two control does that were sacrificed prior to the scheduled cesarean section were examined macroscopically for abnormalities of the thoracic, abdominal, and cervical viscera. The uterus and ovaries were examined for implantations and corpora lutea, respectively. An evaluation of the uterus was made to identify early- or late-resorbing fetuses, dead fetuses, or normally developed fetuses. One doe had a litter consisting of seven dead fetuses. All fetuses appeared normal for their developmental age on gross external and soft tissue examination. All fetuses had non-ossified pubes, which the Sponsor stated is normal for their developmental age. The litter of the other doe consisted of eight empty implantation sites; the conceptuses were presumed cannibalized.

Cesarean Section Data

All surviving does were euthanized on GD 29, and a cesarean section was performed. The uterus from each pregnant animal was excised, and examined for the number and placement of live and dead fetuses, the number of early or late resorptions, and any abnormalities. The right and left ovaries from each doe was examined for the number of corpora lutea. The placentas were also examined.

All c-section parameters, including pregnancy rate, corpora lutea, implantation sites, resorptions (early or late), sex ratio, number of live fetuses, fetal weight, and the pre- and post-implantation losses in each treatment group, were comparable to that of the control group. All placentae appeared normal, and there were no dead fetuses. The number of does with resorptions in the 75 mg/kg/day group was significantly reduced, compared to controls. This reduction was considered an incidental event, and not toxicologically relevant. The data are summarized in the Sponsor's tables below.

Summary of Cesarean Section Data

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	15	75	250
RABBITS TESTED	N	20	21	21	21
PREGNANT	N(%)	18(90.0)	21(100.0)	20(95.2)	16(76.2)
UNSCHEDULED EUTHANASIA	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
ABORTED AND EUTHANIZED	N(%)	1(5.6)	0(0.0)	0(0.0)	0(0.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	16	21	20	16
CORPORA LUTEA	MEAN±S.D.	7.4 ± 2.2	7.7 ± 1.6	7.4 ± 1.2	7.7 ± 1.6
IMPLANTATIONS	MEAN±S.D.	6.9 ± 2.2	7.1 ± 1.8	7.0 ± 1.4	7.4 ± 1.8
% PREIMPLANTATION LOSS	MEAN±S.D.	7.0 ± 8.9	7.1 ± 13.5	6.2 ± 10.1	3.8 ± 7.1
LITTER SIZES	MEAN±S.D.	6.0 ± 2.3	6.1 ± 2.6	6.4 ± 1.7	6.7 ± 1.9
LIVE FETUSES	N	96	129	129	107
	MEAN±S.D.	6.0 ± 2.3	6.1 ± 2.6	6.4 ± 1.7	6.7 ± 1.9
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.9 ± 1.1	1.0 ± 1.9	0.5 ± 1.2	0.8 ± 1.1
EARLY RESORPTIONS	N	12	17	10	6
	MEAN±S.D.	0.8 ± 0.9	0.8 ± 1.9	0.5 ± 1.2	0.4 ± 0.7
LATE RESORPTIONS	N	3	4	0	6
	MEAN±S.D.	0.2 ± 0.4	0.2 ± 0.5	0.0 ± 0.0	0.4 ± 1.0
% POSTIMPLANTATION LOSS	MEAN±S.D.	14.0 ± 16.0	14.5 ± 27.8	6.5 ± 16.0	10.3 ± 14.0
DOES WITH ANY RESORPTIONS	N(%)	10(62.5)	9(42.8)	3(15.0)**	7(43.8)
DOES WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	1(4.8)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	16(100.0)	20(95.2)	20(100.0)	16(100.0)
PLACENTAE APPEARED NORMAL	N(%)	16(100.0)	21(100.0)	20(100.0)	16(100.0)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Dosage occurred on days 7 through 19 of gestation.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Summary of Litter Observations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	15	75	250
LITTERS WITH ONE OR MORE LIVE FETUSES	N	16	20	20	16
IMPLANTATIONS	MEAN±S.D.	6.9 ± 2.2	7.1 ± 1.8	7.0 ± 1.4	7.4 ± 1.8
LIVE FETUSES	N	96	129	129	107
	MEAN±S.D.	6.0 ± 2.3	6.4 ± 2.2	6.4 ± 1.7	6.7 ± 1.9
LIVE MALE FETUSES	N	47	61	63	59
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	48.0 ± 19.3	51.6 ± 22.6	50.2 ± 24.2	54.9 ± 15.2
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	37.84 ± 4.39	37.63 ± 3.11	36.57 ± 3.83	37.04 ± 4.52
MALE FETUSES	MEAN±S.D.	39.12 ± 4.45	38.27 ± 3.59	37.74 ± 4.06	37.67 ± 4.26
FEMALE FETUSES	MEAN±S.D.	37.20 ± 4.62	36.94 ± 2.54 [18]b	35.89 ± 4.25 [18]b	36.40 ± 5.15
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	14.0 ± 16.0	10.2 ± 20.2	6.5 ± 16.0	10.3 ± 14.0

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 19 of gestation.
b. Excludes values for litters that had no female fetuses.

Offspring (Malformations, Variations, etc.)

Each fetus was weighed and examined for external abnormalities. The internal organs of the thoracic and abdominal cavities of all fetuses were examined and the sex of each fetus was determined. A single cross-section was made between the parietal and the frontal bones, and the brain was examined *in situ*. Viscera were removed and carcasses were stained with Alizarin red S and examined for skeletal alterations.

There were no drug-related increases in fetal external findings. Sporadic gross external findings were observed; however, these were not considered as drug-related because they lacked dose-dependency. The incidence of total fetal and litter alterations were comparable across treatment groups. Misshapen median lobe of the liver was noted in a 250 mg/kg/day fetus (0.9% fetal incidence), compared to controls (0% fetal incidence). This fetus also had a small 11th caudal vertebra and a misaligned 14th caudal vertebra. This variation is not considered drug-related due to the low incidence. There were other sporadic soft tissue variations; however, those findings were not dose-dependent, and were not considered to be drug-related. There were no drug-related significant increases in soft tissue malformations.

There were no drug-related significant increases in skeletal alterations (variations or malformations). Sporadic skeletal variations and malformations were observed; however, these findings were not dose-dependent and/or occurred in a single fetus at the high dose (250 mg/kg/day), and were not considered to be drug-related.

There were no drug-related changes in the number of fetal ossification sites per fetus.

Data for the gross external alterations, fetal soft tissue and skeletal alterations, and fetal ossification sites are summarized in the Sponsor's tables below.

Notable Fetal Gross External Alterations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	15	75	250
LITTERS EVALUATED	N	16	20	20	16
FETUSES EVALUATED	N	96	129	129	107
LIVE	N	96	129	129	107
PALATE: CLEFT					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.8)c	1(0.8)d	0(0.0)
HEAD: DOMED					
LITTER INCIDENCE	N(%)	1(6.2)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(1.0)b	1(0.8)c	1(0.8)d	0(0.0)
HEAD: EXENCEPHALY					
LITTER INCIDENCE	N(%)	1(6.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(1.0)	0(0.0)	0(0.0)	0(0.0)
EARS: LOW SET					
LITTER INCIDENCE	N(%)	1(6.2)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(1.0)b	0(0.0)	1(0.8)d	0(0.0)
FORE AND/OR HINDLIMBS: FLEXED					
LITTER INCIDENCE	N(%)	1(6.2)	0(0.0)	1(5.0)	1(6.2)
FETAL INCIDENCE	N(%)	1(1.0)	0(0.0)	1(0.8)d	1(0.9)
FORE AND/OR HINDLIMB(S): SHORT					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)d	0(0.0)

a. Dosage occurred on days 7 through 19 of gestation.
b. Fetus 7809-10 had other gross external alterations.
c. Fetus 7837-2 had other gross external alterations.
d. Fetus 7849-7 had other gross external alterations.

Cesarean-Delivered Live Fetuses with Alterations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	15	75	250
LITTERS EVALUATED	N	16	20	20	16
FETUSES EVALUATED	N	96	129	129	107
LIVE	N	96	129	129	107
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	11(68.8)	12(60.0)	14(70.0)	12(75.0)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	22(22.9)	27(20.9)	26(20.2)	23(21.5)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	25.8 ± 27.8	20.8 ± 23.1	20.7 ± 19.9	21.7 ± 19.1

a. Dosage occurred on days 7 through 19 of gestation.

Notable Fetal Soft Tissue Alterations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	15	75	250
LITTERS EVALUATED	N	16	20	20	16
FETUSES EVALUATED	N	96	129	129	107
LIVE	N	96	129	129	107
BRAIN: VENTRICULAR DILATION EXTREME					
LITTER INCIDENCE	N(%)	1(6.2)	0(0.0)	2(10.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(1.0)	0(0.0)	2(1.6)b	0(0.0)
BRAIN: VENTRICULAR DILATION MODERATE					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.8)	0(0.0)	0(0.0)
LUNGS: SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)b	0(0.0)
LIVER: MISSHAPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(6.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.9)
GALLBLADDER: BILOBED					
LITTER INCIDENCE	N(%)	1(6.2)	1(5.0)	2(10.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(1.0)	2(1.6)	3(2.3)	0(0.0)
GALLBLADDER: SMALL					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.8)	1(0.8)	0(0.0)
GALLBLADDER: MISSHAPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)	0(0.0)

a. Dosage occurred on days 7 through 19 of gestation.
b. Fetus 7849-7 had other soft tissue alterations.

Notable Fetal Skeletal Alterations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	15	75	250
LITTERS EVALUATED	N	16	20	20	16
FETUSES EVALUATED	N	96	129	129	107
LIVE	N	96	129	129	107
SKULL: IRREGULAR OSSIFICATION b (SUMMARIZATION OF ALL IRREGULAR OSSIFICATION OF THE SKULL c; INDIVIDUAL SUBCATEGORIES CITED BELOW)					
LITTER INCIDENCE	N(%)	1(6.2)	1(5.0)	0(0.0)	1(6.2)
FETAL INCIDENCE	N(%)	1(1.0)	1(0.8)	0(0.0)	1(0.9)
SKULL: FRONTALS, CONTAIN AN INTERFRONTAL					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(6.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.9)x
SKULL: FRONTAL, IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	1(6.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(1.0)	0(0.0)	0(0.0)	0(0.0)
SKULL: NASAL, CONTAINS AN INTRANASAL					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.8)h	0(0.0)	0(0.0)
SKULL - OTHER ALTERATIONS:					
SKULL: PARIETAL, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	2(12.5)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	3(3.1)	0(0.0)	1(0.8)m	0(0.0)
SKULL: PARIETAL, CONTAINS A HOLE					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(6.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.9)
SKULL: INTERPARIETALS, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	2(12.5)	0(0.0)	0(0.0)	1(6.2)
FETAL INCIDENCE	N(%)	4(4.2)	0(0.0)	0(0.0)	1(0.9)u
SKULL: INTERPARIETALS, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	1(6.2)	0(0.0)	1(5.0)	2(12.5)
FETAL INCIDENCE	N(%)	2(2.1)e	0(0.0)	1(0.8)	2(1.9)t
SKULL: SUPRAOCCIPITAL, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	1(6.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(1.0)	0(0.0)	0(0.0)	0(0.0)
SKULL: ANTERIOR FONTANELLE, LARGE					
LITTER INCIDENCE	N(%)	1(6.2)	1(5.0)	2(10.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(1.0)	1(0.8)	2(1.6)L,m	0(0.0)
SKULL: POSTERIOR FONTANELLE, LARGE					
LITTER INCIDENCE	N(%)	1(6.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(1.0)	0(0.0)	0(0.0)	0(0.0)
SKULL: PALATE, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.8)	1(0.8)L	0(0.0)
HYOID: ALA, SHORT					
LITTER INCIDENCE	N(%)	1(6.2)	2(10.0)	0(0.0)	1(6.2)
FETAL INCIDENCE	N(%)	1(1.0)	3(2.3)	0(0.0)	1(0.9)
HYOID: ALA, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.8)	0(0.0)	0(0.0)
HYOID: ALA, ANGULATED					
LITTER INCIDENCE	N(%)	0(0.0)	1(5.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.8)	2(1.6)	0(0.0)
CAUDAL VERTEBRAE: FUSED					
LITTER INCIDENCE	N(%)	2(12.5)	1(5.0)	1(5.0)	4(25.0)
FETAL INCIDENCE	N(%)	2(2.1)	1(0.8)	1(0.8)	4(3.7)w

CAUDAL VERTEBRAE: MISALIGNED					
LITTER INCIDENCE	N(%)	1(6.2)	4(20.0)	3(15.0)	3(18.8)
FETAL INCIDENCE	N(%)	1(1.0)f	5(3.9)g,i	3(2.3)j,o	6(5.6)p-s,v
CAUDAL VERTEBRAE: SMALL					
LITTER INCIDENCE	N(%)	1(6.2)	3(15.0)	5(25.0)	2(12.5)
FETAL INCIDENCE	N(%)	1(1.0)f	5(3.9)g,i	5(3.9)j,m,o	5(4.7)p-s,v
RIBS: THICKENED					
LITTER INCIDENCE	N(%)	1(6.2)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(1.0)d	0(0.0)	0(0.0)	0(0.0)
MANUBRIUM: IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(6.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.9)x
STERNAL CENTRA: FUSED					
LITTER INCIDENCE	N(%)	6(37.5)	4(20.0)	8(40.0)	7(43.8)
FETAL INCIDENCE	N(%)	9(9.4)d,e	9(7.0)	9(7.0)k,n	11(10.3)p,q,s,t,w
STERNAL CENTRA: INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	2(10.0)	3(15.0)	1(6.2)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.6)	3(2.3)j,k	2(1.9)t,u
STERNAL CENTRA: ASYMMETRIC					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)n	0(0.0)
STERNAL CENTRA: NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(6.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.9)x
SCAPULAE: ALA, IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)L	0(0.0)
FORELIMB: HUMERUS, IRREGULARLY SHAPED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)L	0(0.0)
PELVIS: PUBIS, INCOMPLETELY OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	2(10.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.6)h,i	1(0.8)	0(0.0)
PELVIS: PUBIS, NOT OSSIFIED					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.8)j	0(0.0)

a. Dosage occurred on days 7 through 19 of gestation.
b. Fetuses with alterations of the skull and/or hyoid are not separately identified in this summarization, except when alterations of other ossification sites were also present.
c. Includes all alterations noted for the skull except findings of the parietal, interparietals, supraoccipital, anterior fontanelle, posterior fontanelle, palate and hyoid. These categories are excluded because these alterations do not result from irregular ossification.
d. Fetus 7805-2 had other skeletal alterations.
e. Fetus 7809-7 had other skeletal alterations.
f. Fetus 7820-5 had other skeletal alterations.
g. Fetus 7828-4 had other skeletal alterations.
h. Fetus 7840-3 had other skeletal alterations.
i. Fetus 7841-6 had other skeletal alterations.
j. Fetus 7842-7 had other skeletal alterations.
k. Fetus 7843-2 had other skeletal alterations.
L. Fetus 7849-7 had other skeletal alterations.
m. Fetus 7852-2 had other skeletal alterations.
n. Fetus 7853-6 had other skeletal alterations.
o. Fetus 7859-2 had other skeletal alterations.
p. Fetus 7863-2 had other skeletal alterations.
q. Fetus 7863-3 had other skeletal alterations.
r. Fetus 7863-4 had other skeletal alterations.
s. Fetus 7863-8 had other skeletal alterations.
t. Fetus 7865-5 had other skeletal alterations.
u. Fetus 7865-8 had other skeletal alterations.
v. Fetus 7865-9 had other skeletal alterations.
w. Fetus 7877-1 had other skeletal alterations.
x. Fetus 7882-3 had other skeletal alterations.

Summary of Notable Fetal Ossification Sites

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	15	75	250
LITTERS EXAMINED	N	16	20	20	16
FETUSES EXAMINED	N	96	129	129	107
OSSIFICATION SITES PER FETUS PER LITTER					
HYOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.02	0.99 ± 0.05
VERTEBRAE					
CERVICAL	MEAN±S.D.	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00
THORACIC	MEAN±S.D.	12.56 ± 0.34	12.64 ± 0.26	12.64 ± 0.26	12.52 ± 0.40
LUMBAR	MEAN±S.D.	6.44 ± 0.34	6.36 ± 0.26	6.36 ± 0.27	6.48 ± 0.40
SACRAL	MEAN±S.D.	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
CAUDAL	MEAN±S.D.	16.78 ± 0.39	16.85 ± 0.37	16.66 ± 0.38	16.91 ± 0.58
RIBS (PAIRS)	MEAN±S.D.	12.47 ± 0.28	12.54 ± 0.28	12.55 ± 0.25	12.44 ± 0.36
STERNUM					
MANUBRIUM	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
STERNAL CENTERS	MEAN±S.D.	4.16 ± 0.22	4.24 ± 0.32	4.17 ± 0.16	4.24 ± 0.35
XIPHOID	MEAN±S.D.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
FORELIMB b					
CARPALS	MEAN±S.D.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
METACARPALS	MEAN±S.D.	4.99 ± 0.03	4.94 ± 0.12	4.92 ± 0.13	4.93 ± 0.09
DIGITS	MEAN±S.D.	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
PHALANGES	MEAN±S.D.	13.92 ± 0.25	13.92 ± 0.23	13.94 ± 0.09	13.96 ± 0.07
HINDLIMB b					
TARSALS	MEAN±S.D.	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
METATARSALS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
DIGITS	MEAN±S.D.	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
PHALANGES	MEAN±S.D.	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00

a. Dosage occurred on days 7 through 19 of gestation.
b. Calculated as average per limb.

In summary, the NOAEL for maternal toxicity is considered to be 250 mg/kg/day, based on the lack of adverse findings at the high dose. For embryo-fetal development, the NOEL is considered to be 250 mg/kg/day, based on the absence of drug-related findings. The C_{max} and AUC_{0-t} in the 250 mg/kg/day group on GD 19 were 513 ng/mL and 499 ng·h/mL, respectively.

9.3 Prenatal and Postnatal Development

Study title: A Developmental and Perinatal/Postnatal Reproduction Study of Plecanatide by Oral (Gavage) in Mice, Including a Postnatal Behavioral/Functional Evaluation

Study no.:	20053292
Study report location:	
Conducting laboratory and location:	(b) (4)
Date of study initiation:	1/28/2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide (SP-304), 121026B (97.1%)

Key Study Findings

- Mated female mice (25/group) were administered 0 (vehicle), 20, 200, or 600 mg/kg/day plecanatide by oral gavage from GD 6 to day 20 of lactation. F1

generation mice were allowed to mature untreated, and the effects of plecanatide on growth, development, and behavior were evaluated. At weaning, 25 males and females per group were randomly selected for continued evaluation. The selected F1 generation males and females from each dose group were mated at approximately 90 days of age. The F0 females were sacrificed on day 21 of lactation (weaning of litters). A necropsy was conducted on F1 generation pups prematurely killed, found dead during lactation, and unselected pups after weaning. Mated F1 generation adult males were necropsied after completion of the mating period. Mated F1 generation females were sacrificed on GD 18.

- Plecanatide had no effects on overall bodyweight or bodyweight gain in F0 females during gestation or lactation. The transient decreases in bodyweight and bodyweight gain during gestation were not considered as drug-related because they lacked dose-dependency.

- No drug-related maternal necropsy findings in the F0 females and no drug-related effects on pregnancy or litter parameters were observed.

- There were no drug-related effects on clinical signs, bodyweight, or bodyweight gain in the F1 generation animals either during lactation or post-weaning.

- Plecanatide had no effects on physical development of the F1 generation pups during lactation, or learning and memory, auditory startle reflex, or motor activity post-weaning.
- Plecanatide had no effects on bodyweight or testes and epididymides weights in the F1 generation adult males. No drug-related macroscopic findings in the F1 generation adult males were observed.

- Plecanatide had no effects on mating performance, fertility, or pregnancy parameters in the F1 generation animals.

- The NOAEL for maternal effects (F0 females) and their offspring (F1 generation) was 600 mg/kg/day.

Methods

Doses:	0 (vehicle), 20, 200, and 600 mg/kg/day
Frequency of dosing:	once daily
Dose volume:	10 ml/kg
Route of administration:	oral (gavage)
Formulation/Vehicle:	solution/sterile water for injection
Species/Strain:	Mouse/Crl:CD1(ICR)
Number/Sex/Group:	25
Satellite groups:	None
Study design:	Mated females received plecanatide from GD 6 to day 20 of lactation for mice that delivered a litter, or to GD 22 for mice that did not deliver a litter. Selected F1 generation mice were allowed to mature untreated, and the effects of plecanatide on growth, development, behavior, and reproductive performance were evaluated. The design of the study is summarized in the Sponsor's table below.
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

Study Design

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals
1	Control Article	0	10	0	25
2	SP-304	20	10	2	25
3	SP-304	200	10	20	25
4	SP-304	600	10	60	25

[a] The test article was considered 92.1% pure for the purpose of dose calculations.

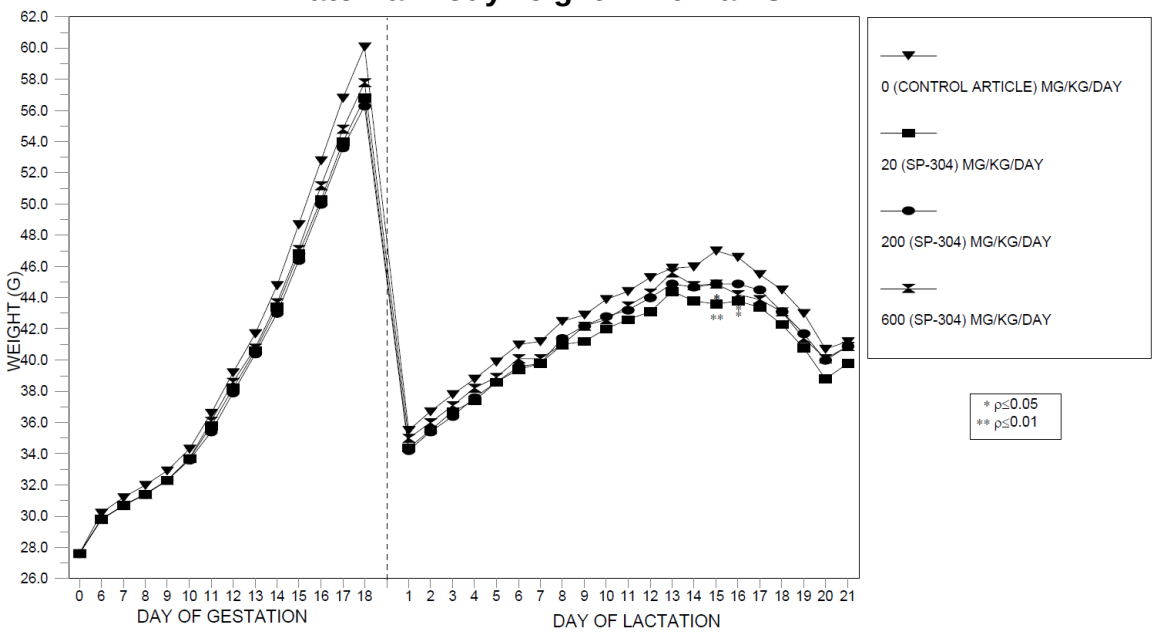
The Sponsor stated that the high dose selection was based on previous toxicity studies in mice, the solubility of plecanatide in water, and the expected range of dose levels in humans. In the 4-week and 13-week oral toxicity studies of plecanatide in mice, the NOAELs were considered to be 200 and 20 mg/kg/day, respectively. In a 26-week oral toxicity study in mice, the NOAEL was considered to be 400 mg/kg/day, the highest dose tested (Note: This reviewer considered the NOAEL to be 150 mg/kg/day based on a significant decrease in absolute reticulocyte count and sciatic nerve axonal/myelin degeneration at 400 mg/kg/day plecanatide). The Sponsor considered 600 mg/kg/day to be the maximum feasible dose for a solution in water based on the solubility data for plecanatide and the recommended maximum dose volume of 10 ml/kg for mice. The oral gavage route of administration was selected because it is the intended route of administration in humans. The frequency of administration reflected possible clinical use, and the duration of administration was in compliance with the appropriate guideline.

Observations and Results

F_0 Dams

Survival:	Animals were observed twice daily for mortality. There were no deaths.
Clinical signs:	All F0 animals were observed daily after the initiation of dosing. There were no drug-related clinical signs.
Body weight:	Bodyweights were recorded on GD 0 and daily until scheduled sacrifice. There were no meaningful drug-related effects on bodyweight or bodyweight gain during gestation or lactation. During GD 0 to 18, a statistically significant decrease in bodyweight gain in the 20 (-10.1%) and 200 (-11.7%) mg/kg/day groups, compared to controls, was noted. However, during GD 6 to 18, there were no significant changes in bodyweight gain. During lactation day 15 and 16, small but statistically significant reductions in bodyweight in the 20, 200, and 600 mg/kg/day groups were noted. The effects were transient and/or not dose-dependent, and they were not considered as drug-related. The data on bodyweight and bodyweight gain are summarized in the Sponsor's figure and table below.
Feed consumption:	Not measured.
Uterine content:	There were no drug-related effects on pregnancy or litter parameters, including pregnancy rate, duration of gestation, implantation sites per delivered litter, mice with stillborn pups, and gestation index. The data are summarized in the Sponsor's table below.
Necropsy observation:	F0 females were sacrificed on lactation day 21. Females that were not pregnant were sacrificed on day 23 post-mating. Females were sacrificed if all pups in their litter died prior to day 21 of lactation. There were no drug-related maternal necropsy findings.
Toxicokinetics:	Not performed.
Dosing Solution Analysis:	The analysis confirmed the plecanatide concentrations in the formulations were within the range of 97.2% to 106% of the nominal concentration.
Other:	N/A

Maternal Bodyweight in F0 Dams



Bodyweight Changes in F0 Females During Gestation

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a		0	20	200	600
MICE TESTED	N	25	25	25	25
PREGNANT	N	24	22	22	25
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 6	MEAN±S.D.	+2.5 ± 1.3	+2.2 ± 1.0	+2.2 ± 1.0	+2.2 ± 1.0
DAYS 6 - 9	MEAN±S.D.	+2.7 ± 1.3	+2.5 ± 0.6	+2.4 ± 0.6	+2.5 ± 0.6
DAYS 9 - 12	MEAN±S.D.	+6.3 ± 0.9	+5.8 ± 1.5	+5.6 ± 1.4	+6.3 ± 1.0
DAYS 12 - 15	MEAN±S.D.	+9.5 ± 1.2	+8.6 ± 1.9	+8.5 ± 1.7	+8.5 ± 1.4
DAYS 15 - 18	MEAN±S.D.	+11.7 ± 1.9 [23]b	+10.4 ± 2.5 [19]b	+10.5 ± 2.0 [20]b	+11.0 ± 2.0 [22]b
DAYS 6 - 18	MEAN±S.D.	+30.0 ± 4.0 [23]b	+26.9 ± 6.0 [19]b	+26.6 ± 4.8 [20]b	+28.1 ± 4.3 [22]b
DAYS 0 - 18	MEAN±S.D.	+32.5 ± 4.0 [23]b	+29.2 ± 5.9* [19]b	+28.7 ± 4.6* [20]b	+30.2 ± 4.6 [22]b

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Day 6 of gestation through Day 20 of lactation or Day 22 of gestation (mice that did not deliver a litter).
b. Excludes values for mice that were in the process of delivering or had delivered.
* Significantly different from the control group value (p≤0.05).

Bodyweights in F0 Females During Lactation

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a		0	20	200	600
MICE TESTED	N	25	25	25	25
PREGNANT	N	24	22	22	25
DELIVERED A LITTER	N	24	22	22	25
MATERNAL BODY WEIGHT (G)					
DAY 1	MEAN±S.D.	35.5 ± 1.9	34.4 ± 2.3	34.2 ± 1.8	35.0 ± 1.7
DAY 2	MEAN±S.D.	36.7 ± 1.9	35.5 ± 2.6	35.4 ± 1.8	36.0 ± 1.5
DAY 3	MEAN±S.D.	37.8 ± 1.7	36.7 ± 3.2	36.4 ± 1.9	37.1 ± 1.8
DAY 4	MEAN±S.D.	38.8 ± 2.0	37.4 ± 3.1 [21]b	37.6 ± 2.6	38.2 ± 1.9
DAY 5	MEAN±S.D.	39.9 ± 2.2	38.6 ± 2.9 [21]b	38.6 ± 2.4	38.9 ± 2.0
DAY 6	MEAN±S.D.	41.0 ± 2.3	39.4 ± 3.2 [21]b	39.6 ± 2.3	40.1 ± 1.8
DAY 7	MEAN±S.D.	41.2 ± 2.4	39.8 ± 3.4 [21]b	39.8 ± 2.6	40.1 ± 2.0
DAY 8	MEAN±S.D.	42.5 ± 2.1	41.0 ± 3.5 [21]b	41.4 ± 2.7	41.0 ± 2.2
DAY 9	MEAN±S.D.	42.9 ± 2.4	41.2 ± 3.2 [21]b	42.2 ± 2.8	42.2 ± 2.0
DAY 10	MEAN±S.D.	43.9 ± 2.5	42.0 ± 4.0 [21]b	42.8 ± 3.1	42.6 ± 2.3
DAY 11	MEAN±S.D.	44.4 ± 2.6	42.6 ± 4.0 [21]b	43.2 ± 2.8	43.5 ± 2.1
DAY 12	MEAN±S.D.	45.3 ± 2.6	43.1 ± 4.6 [21]b	44.0 ± 3.1	44.3 ± 2.1

DAY = DAY OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Day 6 of gestation through Day 20 of lactation or Day 22 of gestation (mice that did not deliver a litter).
b. Excludes values for mouse 5432, which was euthanized on Day 3 of lactation due to no surviving pups.

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a		0	20	200	600
MICE TESTED	N	25	25	25	25
PREGNANT	N	24	22	22	25
DELIVERED A LITTER	N	24	22	22	25
MATERNAL BODY WEIGHT (G)					
DAY 13	MEAN±S.D.	45.9 ± 2.1	44.4 ± 4.3 [21]b	44.9 ± 3.2	45.6 ± 2.2
DAY 14	MEAN±S.D.	46.0 ± 2.5	43.8 ± 4.2 [21]b	44.7 ± 3.4	44.8 ± 1.9
DAY 15	MEAN±S.D.	47.0 ± 2.4	43.6 ± 3.9** [21]b	44.9 ± 2.8*	44.9 ± 2.3*
DAY 16	MEAN±S.D.	46.6 ± 2.9	43.8 ± 5.2* [21]b	44.9 ± 3.2	44.2 ± 2.4*
DAY 17	MEAN±S.D.	45.5 ± 2.4	43.4 ± 5.2 [21]b	44.5 ± 3.4	43.9 ± 2.4
DAY 18	MEAN±S.D.	44.5 ± 2.2	42.3 ± 4.4 [21]b	43.1 ± 3.0	43.1 ± 2.5
DAY 19	MEAN±S.D.	43.0 ± 2.7	40.8 ± 3.8 [21]b	41.7 ± 2.8	41.4 ± 2.5
DAY 20	MEAN±S.D.	40.7 ± 3.0	38.8 ± 3.2 [21]b	40.0 ± 3.2	40.1 ± 2.9
DAY 21	MEAN±S.D.	41.2 ± 3.7	39.8 ± 3.2 [21]b	40.9 ± 3.8	40.9 ± 3.9

DAY = DAY OF LACTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Day 6 of gestation through Day 20 of lactation or Day 22 of gestation (mice that did not deliver a litter).
b. Excludes values for mouse 5432, which was euthanized on Day 3 of lactation due to no surviving pups.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

Pregnancy and Litter Data in F0 Generation Females

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a		0	20	200	600
MICE TESTED	N	25	25	25	25
PREGNANT	N(%)	24(96.0)	22(88.0)	22(88.0)	25(100.0)
DELIVERED A LITTER	N(%)	24(100.0)	22(100.0)	22(100.0)	25(100.0)
DURATION OF GESTATION b	MEAN±S.D.	19.9 ± 0.3	19.8 ± 0.4	19.9 ± 0.4	19.8 ± 0.4
IMPLANTATION SITES	N	312	300	305	352
PER DELIVERED LITTER	MEAN±S.D.	14.8 ± 2.1 [21]c	13.6 ± 3.0	13.9 ± 2.3	14.1 ± 2.1
MICE WITH STILLBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
MICE WITH NO LIVEBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GESTATION INDEX d	%	100.0	100.0	100.0	100.0
	N/N	24/ 24	22/ 22	22/ 22	25/ 25
MICE WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	1(4.5)	0(0.0)	0(0.0)
MICE WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

[ ] = NUMBER OF VALUES AVERAGED

a. Dose administration occurred on Day 6 of gestation through Day 20 of lactation or Day 22 of gestation (mice that did not deliver a litter).

b. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as Day 0 of gestation) and the day the first pup was delivered.

c. Excludes values that were not recorded or appeared incorrectly recorded.

d. Number of mice with live offspring/number of pregnant mice.

F_1 Generation

Survival: On day 1 of lactation, the pups were examined for malformations, sexed, and the numbers of live and dead pups were recorded. Mortality was examined twice daily. In the F1 generation pups, there were no drug-related effects on pup viability, survival, or lactation indices. A statistically significant decrease in the viability index was noted in the 20 mg/kg/day group, compared to the controls. The decrease was the result of a statistically significant increase in the number of pups found dead or presumed cannibalized between postnatal days (PNDs) 2 to 4. The change was not considered as drug-related because it lacked dose-dependency. There was also a statistically significant decrease in the number of pups found dead or presumed cannibalized in the 20, 200 and 600 mg/kg/day dose groups between PNDs 15 and 21. This decrease resulted in a statistically significant increase in the lactation index in the 20, 200 and 600 mg/kg/day dose group. The changes are not considered to be toxicologically relevant because they reflected an increase in the number of pups found dead or presumed cannibalized in the control group between PNDs 15 and 21. There were no drug-related effects on surviving pups/litter, sex ratios, or litter size. F1 generation litter observations are summarized in the Sponsor's tables below.

Clinical signs: The pups were examined daily during lactation for clinical signs. There were no drug-related clinical signs during lactation.

Post-weaning F1 generation animals were examined daily for clinical signs. There were no drug-related clinical signs post-weaning.

Body weight: Pups were weighed on days 1, 4, 7, 14 and 21 of lactation. There were no drug-related bodyweight changes during lactation. Bodyweight data are summarized in the Sponsor's tables below.

Bodyweights in the post-weaning F1 generation males were recorded once weekly and on the day of

scheduled sacrifice. Female bodyweights were recorded once weekly until paring with males, and then on days 0, 7, 10, 14, and 17 of gestation, and on the day of scheduled sacrifice. There were no drug-related effects on bodyweight or bodyweight gain in males or females post-weaning. The bodyweight data are summarized in the Sponsor's tables below.

Feed consumption: Not measured

Physical development: Preputial separation or vaginal patency was evaluated daily on day 28-32 or day 28 postpartum in males and females, respectively, until the criterion was achieved. There were no drug-related effects on the average age (postnatal day) when preputial separation or vaginal patency occurred in the F1 generation mice. The data are summarized in the Sponsor's table below.

Neurological assessment: Learning test: Between postpartum days 66 and 90, one F1 generation male and female from each litter were evaluated for learning and memory in a water-filled Morris maze system. Time to reach the platform or cue was recorded. There were no drug-related effects in the Morris watermaze parameters in the F1 generation males or females.

Auditory startle reflex: On postpartum day 60 (± 2 days), one F1 generation male and female from each litter were tested for their reactivity to auditory stimuli and habituation of responses with repeated presentation of stimuli. There were no significant drug-related effects on reactivity to auditory stimuli or auditory startle habituation response in the F1 generation males or females.

Motor activity: On postpartum day 64 (± 2 days), one F1 generation male and female from each litter were evaluated for fine movement and ambulation during a 90-minute session using an automated infra-red beam activity monitoring system. There were no significant drug-related effects on motor activity in the F1 generation males or females.

Overall, there were no meaningful drug-related effects on learning and memory, auditory startle reflex, or motor activity in the F1 generation mice.

Reproduction: At weaning on day 21 postpartum, 25 male and female pups per group were randomly selected. At least one male and one female pup were selected from each litter, when possible. At approximately 90 days of age, mice were randomly assigned to mating within each dose group (one male with one female). Sibling paring was excluded. There was a 9-day mating period. Females with a copulatory plug

observed *in situ* were considered to be at GD 0, and assigned to individual housing. Female mice not observed to have a copulatory plug *in situ* were returned to cohabitation in the evening.

The time course of mating, fertility indices, and maternal bodyweights were assessed. There were no drug-related effects on mating performance, fertility indices, or maternal bodyweight in the maternal drug-treated F1 generation mice. The results are summarized in the Sponsor's tables below.

Mated F1 generation females were sacrificed on GD 18. There were 23, 23, 20 and 20 pregnant females at scheduled necropsy in the control, 20, 200, and 600 mg/kg/day groups, respectively. Cesarean section parameters and litter observations, including number of corpora lutea, implantations, live fetuses, incidence of pre- and post-implantation losses, litter size, early and late resorptions, and placenta appearance were assessed. Fetal gross external alterations were evaluated. There were no drug-related effects on any of the c-section parameters or litter observations. There were no drug-related fetal gross external alterations. The data on c-section parameters and litter observations are summarized in the Sponsor's tables below.

Other: F1 generation pre-weaning pups that were found dead or euthanized before scheduled termination were examined for gross lesions and the cause of death. Post-weaning mice that were sacrificed before scheduled termination were examined for gross lesions, and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. All pups that were not selected for continued observation were sacrificed on PPD 21. They were examined for gross lesions, and a necropsy was conducted that included examination of cross-sectioned brains for apparent hydrocephaly. There were no drug-related findings in any of the parameters.

F1 generation adult males were sacrificed after completion of cohabitation. Necropsy was conducted, and bodyweights, testes and epididymides weights were recorded. There were no drug-related necropsy findings, or changes in testes or epididymides weights. The data are summarized in the Sponsor's table below.

Therefore, there were no macroscopic findings in the F1 generation pups or adults that are related to maternal administration of plecanatide.

Litter Observations in F1 Generation Mice

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a		0	20	200	600
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	22	22	25
PUPS DELIVERED (TOTAL)	N	336	282	277	335
	MEAN±S.D.	14.0 ± 2.3	12.8 ± 3.4	12.6 ± 2.6	13.4 ± 2.2
LIVEBORN	MEAN±S.D.	14.0 ± 2.3	12.8 ± 3.4	12.5 ± 2.6	13.4 ± 2.2
	N(%)	336(100.0)	281(99.6)	276(99.6)	335(100.0)
STILLBORN	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
UNKNOWN VITAL STATUS b	N	0	1	1	0
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED					
DAY 1	N/N(%)	0/336(0.0)	0/281(0.0)	0/276(0.0)	0/335(0.0)
DAYS 2- 4	N/N(%)	9/336(2.7)	21/281(7.5)**	5/276(1.8)	15/335(4.5)
DAYS 5- 7	N/N(%)	3/327(0.9)	2/260(0.8)	1/271(0.4)	4/320(1.2)
DAYS 8-14	N/N(%)	1/324(0.3)	0/258(0.0)	2/270(0.7)	2/316(0.6)
DAYS 15-21	N/N(%)	14/323(4.3)	0/257(0.0)**c	3/268(1.1)**	1/314(0.3)**
VIABILITY INDEX d	%	97.3	92.5**	98.2	95.5
	N/N	327/336	260/281	271/276	320/335
LACTATION INDEX e	%	94.5	99.2**c	97.8**	97.8**
	N/N	309/327	257/259c	265/271	313/320

DAY(S) = DAY(S) POSTPARTUM
a. Dose administration occurred on Day 6 of gestation through Day 20 of lactation or Day 22 of gestation (mice that did not deliver a litter).
b. Autolysis precluded identification of vital status at birth.
c. Excludes one pup from litter 5433, which was found outside of nesting box on Day 20 postpartum and was euthanized.
d. Number of live pups on Day 4 postpartum/number of liveborn pups on Day 1 postpartum.
e. Number of live pups on Day 21 (weaning) postpartum/number of live pups on Day 4 postpartum.
** Significantly different from the control group value (p≤0.01).

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a		0	20	200	600
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	22	22	25
SURVIVING PUPS/LITTER b					
DAY 1c	MEAN±S.D.	14.0 ± 2.3	12.8 ± 3.4	12.5 ± 2.6	13.4 ± 2.2
DAY 4	MEAN±S.D.	13.6 ± 2.4	11.8 ± 4.2	12.3 ± 2.5	12.8 ± 2.6
DAY 7	MEAN±S.D.	13.5 ± 2.3	11.7 ± 4.2	12.3 ± 2.4	12.6 ± 2.5
DAY 14	MEAN±S.D.	13.4 ± 2.3	11.7 ± 4.2	12.2 ± 2.4	12.6 ± 2.4
DAY 21	MEAN±S.D.	12.9 ± 2.8	11.7 ± 4.2	12.0 ± 2.4	12.5 ± 2.4
PERCENT MALE PUPS PER NUMBER OF PUPS SEXED					
DAY 1c	MEAN±S.D.	50.3 ± 16.3	51.7 ± 13.5	51.1 ± 14.8	52.4 ± 14.1
DAY 4	MEAN±S.D.	50.8 ± 16.0	54.0 ± 12.7 [21]d	51.2 ± 15.3	51.9 ± 13.7
DAY 7	MEAN±S.D.	50.7 ± 15.8	54.0 ± 12.9 [21]d	51.4 ± 15.6	52.2 ± 13.5
DAY 14	MEAN±S.D.	50.9 ± 15.8	54.0 ± 12.9 [21]d	51.3 ± 15.5	52.3 ± 14.0
DAY 21	MEAN±S.D.	52.5 ± 17.8	54.2 ± 13.3 [21]d	51.5 ± 16.3	52.1 ± 14.1

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Day 6 of gestation through Day 20 of lactation or Day 22 of gestation (mice that did not deliver a litter).
b. Average number of live pups per litter, including litters with no surviving pups.
c. Includes liveborn pups and pups that died before weighing on Day 1 postpartum.
d. Excludes values for litter 5432, which had no surviving pups on Day 3 postpartum.

Litter Size and Bodyweights in F1 Generation Pups During Lactation

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a		0	20	200	600
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	22	22	25
LIVE LITTER SIZE AT WEIGHING					
DAY 1	MEAN±S.D.	14.0 ± 2.3	12.7 ± 3.6	12.5 ± 2.6	13.4 ± 2.2
DAY 4	MEAN±S.D.	13.6 ± 2.4	12.4 ± 3.4 [21]b	12.3 ± 2.5	12.8 ± 2.6
DAY 7	MEAN±S.D.	13.5 ± 2.3	12.3 ± 3.4 [21]b	12.3 ± 2.4	12.6 ± 2.5
DAY 14	MEAN±S.D.	13.4 ± 2.3	12.3 ± 3.4 [21]b	12.2 ± 2.4	12.6 ± 2.4
DAY 21	MEAN±S.D.	12.9 ± 2.8	12.2 ± 3.4 [21]b	12.0 ± 2.4	12.5 ± 2.4
PUP WEIGHT/LITTER (GRAMS)					
DAY 1	MEAN±S.D.	1.6 ± 0.1	1.6 ± 0.2	1.6 ± 0.2	1.5 ± 0.1
DAY 4	MEAN±S.D.	2.5 ± 0.3	2.5 ± 0.4 [21]b	2.6 ± 0.4	2.5 ± 0.3
DAY 7	MEAN±S.D.	3.8 ± 0.6	4.0 ± 0.6 [21]b	3.8 ± 0.6	3.8 ± 0.5
DAY 14	MEAN±S.D.	5.8 ± 1.1	6.2 ± 1.4 [21]b	5.9 ± 1.2	5.8 ± 1.0
DAY 21	MEAN±S.D.	9.0 ± 2.5	10.0 ± 2.4 [21]b	9.3 ± 2.5	9.4 ± 1.9

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Day 6 of gestation through Day 20 of lactation or Day 22 of gestation (mice that did not deliver a litter).
b. Excludes values for litter 5432, which had no surviving pups on Day 3 postpartum.

Bodyweights in F1 Generation Post-Weaning Males

MATERNAL GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
MATERNAL DOSE LEVEL (MG/KG/DAY)		0	20	200	600
MICE TESTED	N	25	25	25	25
BODY WEIGHT (G)					
DAY 22	MEAN±S.D.	10.8 ± 2.4	11.5 ± 2.5	10.6 ± 2.3	11.2 ± 2.1
DAY 29	MEAN±S.D.	21.1 ± 3.6	21.8 ± 3.1	20.3 ± 2.8	21.2 ± 3.1
DAY 36	MEAN±S.D.	28.4 ± 2.4	29.0 ± 2.4	27.6 ± 1.8	28.6 ± 2.2
DAY 43	MEAN±S.D.	31.1 ± 2.3	31.2 ± 2.8	30.1 ± 1.7	30.7 ± 2.3
DAY 50	MEAN±S.D.	33.0 ± 2.2	32.8 ± 2.7	32.0 ± 1.8	32.7 ± 2.6
DAY 57	MEAN±S.D.	33.7 ± 2.1	33.8 ± 2.9	33.0 ± 2.2	33.5 ± 2.8
DAY 64	MEAN±S.D.	34.6 ± 2.1	34.6 ± 3.0	33.9 ± 2.4	34.4 ± 3.0
DAY 71	MEAN±S.D.	34.6 ± 2.3	34.6 ± 3.0	33.7 ± 2.6	34.3 ± 3.0
DAY 78	MEAN±S.D.	35.0 ± 2.4	35.5 ± 3.1	34.2 ± 2.5	34.9 ± 3.3
DAY 85a	MEAN±S.D.	36.2 ± 1.9	36.2 ± 3.1	35.1 ± 3.0	36.1 ± 3.3
PRECOHABITATION b	MEAN±S.D.	37.4 ± 2.1	37.5 ± 3.6	36.7 ± 2.9	37.4 ± 3.9

DAY = DAY POSTPARTUM
a. Because body weight values were recorded at weekly intervals, based on each mouse's day postpartum, Day 85 postpartum was the last day in which the youngest mice had a body weight value recorded before cohabitation.
b. Precohabitation body weights were recorded on the day cohabitation began for the F1 generation mice; at that time these mice were 90 to 93 days of age.

DAY 99a	MEAN±S.D.	36.9 ± 2.0	37.0 ± 3.5	36.6 ± 2.5 [24]b	37.4 ± 3.9
DAY 106	MEAN±S.D.	37.5 ± 2.2	37.9 ± 3.9	37.1 ± 2.8	38.1 ± 4.2
DAY 113	MEAN±S.D.	38.0 ± 2.6	38.0 ± 3.9	37.4 ± 3.0	38.8 ± 4.9

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. First value recorded after the majority of the mice were removed from cohabitation.
b. Excludes a value for mouse 6658, which was in cohabitation.

Bodyweights in F1 Generation Post-Weaning Females

MATERNAL GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
MATERNAL DOSE LEVEL (MG/KG/DAY)		0	20	200	600
MICE TESTED	N	25	25	25	25
BODY WEIGHT (G)					
DAY 22	MEAN±S.D.	10.2 ± 2.2	11.2 ± 2.5	10.8 ± 2.3	10.9 ± 1.9
DAY 29	MEAN±S.D.	17.8 ± 3.0	18.6 ± 2.9	18.2 ± 2.4	18.2 ± 2.5
DAY 36	MEAN±S.D.	23.0 ± 1.9	23.5 ± 2.1 [24]a	23.2 ± 1.8	22.9 ± 2.0
DAY 43	MEAN±S.D.	23.7 ± 1.9	23.8 ± 2.2 [24]a	24.3 ± 1.7	23.6 ± 2.0
DAY 50	MEAN±S.D.	25.2 ± 1.9	24.5 ± 2.0 [24]a	25.3 ± 1.9	24.8 ± 2.0
DAY 57	MEAN±S.D.	25.9 ± 1.9	25.3 ± 2.0 [24]a	25.8 ± 2.0	25.3 ± 2.0
DAY 64	MEAN±S.D.	26.7 ± 2.0	26.4 ± 1.9 [24]a	26.4 ± 1.9	25.9 ± 1.9
DAY 71	MEAN±S.D.	27.0 ± 2.0	26.3 ± 2.1 [24]a	26.9 ± 1.9	26.2 ± 1.7
DAY 78	MEAN±S.D.	27.7 ± 2.2	27.4 ± 2.5 [24]a	27.2 ± 2.0	27.0 ± 2.1
DAY 85b	MEAN±S.D.	28.5 ± 1.6	28.1 ± 2.3 [24]a	28.2 ± 1.9	27.9 ± 2.0
PRECOHABITATION c	MEAN±S.D.	29.0 ± 1.8	28.2 ± 2.3 [24]a	28.6 ± 2.2	28.1 ± 2.1

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for mouse 6735, which was euthanized on Day 30 postpartum when found outside of nesting box.
b. Because body weight values were recorded at weekly intervals, based on each mouse's day postpartum, Day 85 postpartum was the last day in which the youngest mice had a body weight value recorded before cohabitation.
c. Precohabitation body weights were recorded on the day cohabitation began for the F1 generation mice; at that time these mice were 90 to 93 days of age.

Summary of Sexual Maturation Data in F1 Generation Mice

MATERNAL GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
MATERNAL DOSE LEVEL (MG/KG/DAY)		0	20	200	600
MALE MICE	N	9a	8a	4a	8a
PREPUTIAL SEPARATION b	MEAN±S.D.	31.2 ± 1.7	29.9 ± 0.8	30.2 ± 0.5	32.0 ± 1.5
BODY WEIGHT AT SEPARATION (G)c	MEAN±S.D.	22.14 ± 2.84	22.69 ± 3.51	20.02 ± 1.55	23.96 ± 3.93
FEMALE MICE	N	16a	13a	16a	15a
VAGINAL PATENCY d	MEAN±S.D.	30.9 ± 2.4	30.6 ± 1.8	30.9 ± 1.7	30.7 ± 2.0
BODY WEIGHT AT VAGINAL PATENCY (G)e	MEAN±S.D.	18.79 ± 2.13	18.92 ± 1.41	19.20 ± 2.02	19.17 ± 1.87

a. Excludes values for mice of which the exact day of maturity could not be determined.
b. Average day postpartum that the prepuce was observed to be separated.
c. Average body weight on day prepuce was first observed to be separated.
d. Average day postpartum that the vagina was observed to be patent.
e. Average body weight on day vagina was first observed to be patent.

Mating and Fertility Data in F1 Generation Males

MATERNAL GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
MATERNAL DOSE LEVEL (MG/KG/DAY)		0	20	200	600
MICE IN COHABITATION	N	25	24a	25	25
DAYS IN COHABITATION b	MEAN±S.D.	1.6 ± 0.8	1.9 ± 1.5	2.4 ± 1.8	1.8 ± 1.3
MICE THAT MATED	N(%)	25(100.0)	24(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX c	N/N	23/25	23/24	20/25	20/25
	(%)	(92.0)	(95.8)	(80.0)	(80.0)
MICE WITH CONFIRMED MATING DATES	N	25	24	25	25
MATED WITH FEMALE d					
DAYS 1-7	N(%)	25(100.0)	24(100.0)	24(96.0)	25(100.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
MICE PREGNANT/MICE IN COHABITATION	N/N	23/25	23/24	20/25	20/25
	(%)	(92.0)	(95.8)	(80.0)	(80.0)

a. Excludes values for mouse 6648, which was not assigned to cohabitation because there were no available female mice.
b. Restricted to mice with a confirmed mating date and mice that did not mate.
c. Number of pregnancies/number of mice that mated.
d. Restricted to mice with a confirmed mating date.

Mating and Fertility Data in F1 Generation Females

MATERNAL GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
MATERNAL DOSE LEVEL (MG/KG/DAY)		0	20	200	600
MICE IN COHABITATION	N	25	24a	25	25
DAYS IN COHABITATION b	MEAN±S.D.	1.6 ± 0.8	1.9 ± 1.5	2.4 ± 1.8	1.8 ± 1.3
MICE THAT MATED	N(%)	25(100.0)	24(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX c	N/N	23/25	23/24	20/25	20/25
	(%)	(92.0)	(95.8)	(80.0)	(80.0)
MICE WITH CONFIRMED MATING DATES	N	25	24	25	25
MATED BY MALE d					
DAYS 1-7	N(%)	25(100.0)	24(100.0)	24(96.0)	25(100.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)
MICE PREGNANT/MICE IN COHABITATION	N/N	23/25	23/24	20/25	20/25
	(%)	(92.0)	(95.8)	(80.0)	(80.0)

a. Excludes values for mouse 6735, which was euthanized on Day 30 postpartum when found outside of nesting box.
b. Restricted to mice with a confirmed mating date and mice that did not mate.
c. Number of pregnancies/number of mice that mated.
d. Restricted to mice with a confirmed mating date.

Maternal Bodyweights in F1 Females

MATERNAL GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
MATERNAL DOSE LEVEL (MG/KG/DAY)		0	20	200	600
MICE TESTED	N	25	24a	25	25
PREGNANT	N	23	23	20	20
MATERNAL BODY WEIGHT (G)					
DAY 0	MEAN±S.D.	29.4 ± 1.7	28.6 ± 2.4	29.4 ± 2.1	28.6 ± 1.6
DAY 7	MEAN±S.D.	33.2 ± 2.0	32.9 ± 2.9	33.6 ± 2.7	32.5 ± 2.0
DAY 10	MEAN±S.D.	36.9 ± 2.2	36.3 ± 3.2	37.1 ± 2.7	35.9 ± 2.2
DAY 14	MEAN±S.D.	46.8 ± 4.1	45.2 ± 5.1	47.0 ± 3.7	45.8 ± 4.2
DAY 17	MEAN±S.D.	58.1 ± 6.3	55.7 ± 8.0	58.8 ± 5.5	57.6 ± 6.8
DAY 18	MEAN±S.D.	62.4 ± 7.3	59.2 ± 9.4	62.2 ± 6.0	60.7 ± 7.6

DAY = DAY OF GESTATION
a. Excludes values for mouse 6735, which was euthanized on Day 30 postpartum when found outside of nesting box.

Cesarean Section Data in F1 Generation Females

MATERNAL GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
MATERNAL DOSE LEVEL (MG/KG/DAY)		0	20	200	600
MICE TESTED	N	25	24a	25	25
PREGNANT	N(%)	23(92.0)	23(95.8)	20(80.0)	20(80.0)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 18 OF GESTATION	N	23	23	20	20
CORPORA LUTEA	MEAN±S.D.	15.3 ± 3.0	14.0 ± 2.9	15.4 ± 2.8	14.1 ± 3.7
IMPLANTATIONS	MEAN±S.D.	14.9 ± 3.3	13.4 ± 3.5	14.8 ± 2.9	13.8 ± 3.6
% PREIMPLANTATION LOSS	MEAN±S.D.	4.0 ± 10.7	5.3 ± 13.3	3.6 ± 6.3	2.3 ± 4.6
LITTER SIZES	MEAN±S.D.	12.9 ± 3.5	11.6 ± 4.4	13.0 ± 3.0	12.4 ± 3.7
LIVE FETUSES	N	295	265	259	249
	MEAN±S.D.	12.8 ± 3.5	11.5 ± 4.6	13.0 ± 3.0	12.4 ± 3.7
DEAD FETUSES	N	2	2	0	0
	MEAN±S.D.	0.1 ± 0.3	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	2.0 ± 2.1	1.8 ± 2.5	1.8 ± 1.9	1.3 ± 1.3
EARLY RESORPTIONS	N	42	41	34	23
	MEAN±S.D.	1.8 ± 2.2	1.8 ± 2.6	1.7 ± 1.9	1.2 ± 1.3
LATE RESORPTIONS	N	4	1	3	3
	MEAN±S.D.	0.2 ± 0.4	0.0 ± 0.2	0.2 ± 0.5	0.2 ± 0.4
% POSTIMPLANTATION LOSS	MEAN±S.D.	13.7 ± 12.9	15.3 ± 22.8	12.2 ± 11.7	10.4 ± 10.4

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Excludes values for mouse 6735, which was euthanized on Day 30 postpartum when found outside of nesting box.

MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 18 OF GESTATION	N	23	23	20	20
DAMS WITH ANY RESORPTIONS	N(%)	18(78.3)	16(69.6)	15(75.0)	14(70.0)
DAMS WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	23(100.0)	23(100.0)	20(100.0)	20(100.0)
PLACENTAE APPEARED NORMAL	N(%)	23(100.0)	23(100.0)	20(100.0)	20(100.0)

a. Excludes values for mouse 6735, which was euthanized on Day 30 postpartum when found outside of nesting box.

Litter Observations in F1 Generation Females

MATERNAL GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
MATERNAL DOSE LEVEL (MG/KG/DAY)		0	20	200	600
LITTERS WITH ONE OR MORE LIVE FETUSES	N	23	23	20	20
IMPLANTATIONS	MEAN±S.D.	14.9 ± 3.3	13.4 ± 3.5	14.8 ± 2.9	13.8 ± 3.6
LIVE FETUSES	N	295	265	259	249
	MEAN±S.D.	12.8 ± 3.5	11.5 ± 4.6	13.0 ± 3.0	12.4 ± 3.7
LIVE MALE FETUSES	N	163	128	146	141
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	57.6 ± 14.8	44.8 ± 18.9*	54.9 ± 16.4	60.2 ± 15.5
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	1.34 ± 0.14	1.30 ± 0.22	1.32 ± 0.12	1.29 ± 0.10
MALE FETUSES	MEAN±S.D.	1.37 ± 0.14	1.33 ± 0.22 [21]a	1.36 ± 0.14	1.32 ± 0.11
FEMALE FETUSES	MEAN±S.D.	1.29 ± 0.12 [22]b	1.28 ± 0.22	1.29 ± 0.13	1.25 ± 0.10
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	13.7 ± 12.9	15.3 ± 22.8	12.2 ± 11.7	10.4 ± 10.4

[ ] = NUMBER OF VALUES AVERAGED
a. Litters 6739 and 6741 had no male fetuses.
b. Litter 6712 had no female fetuses.
* Significantly different from the control group value ($p \leq 0.05$).

Fetal Gross External Observations in Fetuses from F1 Generation Females

MATERNAL GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
MATERNAL DOSE LEVEL (MG/KG/DAY)		0	20	200	600
LITTERS EVALUATED	N	23	23	20	20
LITTERS WITH LIVE FETUS(ES)	N	23	23	20	20
FETUSES EVALUATED	N	297	267	259	249
LIVE	N	295	265	259	249
DEAD a	N	2	2	0	0
PALATE: CLEFT					
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)	0(0.0)	0(0.0)	0(0.0)
BODY: UMBILICAL HERNIA					
LITTER INCIDENCE	N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.3)	0(0.0)	0(0.0)	0(0.0)
HEAD: EXENCEPHALY					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.4)
EYELID(S): OPEN					
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.4)	1(0.4)

a. Dead fetuses were excluded from summarization and statistical analyses. These conceptuses appeared normal for developmental ages.

Bodyweights, Testes and Epididymides Weights in F1 Generation Adult Males

MATERNAL GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
MATERNAL DOSE LEVEL (MG/KG/DAY)		0	20	200	600
MICE TESTED	N	25	25	25	25
TERMINAL BODY WEIGHT	MEAN±S.D.	38.9 ± 2.8	38.9 ± 4.1	38.9 ± 3.3	40.2 ± 5.0
EPIDIDYMIDES PAIRED	MEAN±S.D.	0.126 ± 0.022	0.122 ± 0.019	0.124 ± 0.019	0.120 ± 0.009
EPIDIDYMIDES PAIRED (%)	MEAN±S.D.	0.326 ± 0.053	0.313 ± 0.050	0.320 ± 0.053	0.302 ± 0.040
TESTES PAIRED	MEAN±S.D.	0.276 ± 0.042	0.261 ± 0.036	0.267 ± 0.041	0.265 ± 0.028
TESTES PAIRED (%)	MEAN±S.D.	0.712 ± 0.112	0.676 ± 0.106	0.687 ± 0.098	0.666 ± 0.090

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

F_2 Generation

Survival: N/A
Body weight: N/A
External evaluation: N/A
Male/Female ratio: N/A
Other: N/A

In summary, maternal administration of plecanatide at up to 600 mg/kg/day did not affect the growth, development, or reproductive performance of the offspring. The NOAEL for maternal effects and F1 generation offspring is considered to be 600 mg/kg/day.

Juvenile Animal Studies

Acute- and short-term non-GLP juvenile toxicity studies were conducted in mice. These included single dose studies with dosing on PND (post-natal day) 7, 14, 21, or 28, and a 7-day repeated dose study with dosing performed from PND 7 through 13. Results of the single dose study with dosing on PND 7 and 28 are summarized below.

In study 20034218 (b) (4) plecanatide was administered by oral gavage at 0.01, 0.05, 0.1, or 0.25 mg/kg to mice (10/sex/group) on PND 7. The pups were observed at least twice daily for mortality and morbidity. They were observed for general appearance once daily during pre-dose and recovery periods, before and after dose administration, and on the day of scheduled sacrifice. Clinical signs and bodyweights were recorded. The pups were sacrificed on PND 10. All mice survived the treatment, and there were no meaningful drug-related effects on clinical signs, bodyweight, or bodyweight gain at doses up to 0.25 mg/kg.

In study 20039567 (b) (4) plecanatide was administered by oral gavage at 1, 3, 10, 30, 50, 100, 200, or 300 mg/kg to mice (10/sex/group) on PND 28. The pups were observed at least twice daily for mortality and morbidity. They were observed for general appearance once daily during pre-dose and recovery periods, before and after dose administration, and on the day of scheduled sacrifice. Clinical signs and bodyweights were recorded. The animals were sacrificed on PND 35. All

mice survived the treatment, and there were no meaningful drug-related effects on clinical signs, bodyweight, or bodyweight gain at does up to 300 mg/kg in PND 28 mice.

Study title: Plecanatide: An Acute Oral Toxicity Study in Pre-Weaning and Weaning CD1 Mice

Study no.:	20046300
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/6/2013
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Plecanatide (SP-340), #FUROG1002A (91.2%)

Key Study Findings

- Pups in cohort 1 were administered a single dose of 0 (vehicle), 1, 3, 10, or 20 mg/kg plecanatide on PND 14. Pups in cohort 2 were given 0 (vehicle control), 25, 50, 100, or 300 mg/kg plecanatide on PND 21. All pups were sacrificed 3 days after plecanatide administration. PND 1 was defined as the day of birth.

- Deaths occurred at 10 and 20 mg/kg in pups administered plecanatide on PND 14. Clinical signs included loss of righting reflex, decreased motor activity, ataxia, cold to the touch, and moderate dehydration (based on skin turgor). No drug-related gross lesions were identified at necropsy of the mice that died prior to scheduled sacrifice. There were no deaths or clinical signs in pups administered plecanatide on PND 21.

- In cohort 1, a slight reduction in bodyweight gain after dosing (PNDs 14 to 15) was observed in the 3 mg/kg female pups. Decreased bodyweight gain and/or a bodyweight loss were noted in the 10 and 20 mg/kg pups following dose administration. Bodyweight gains were 89%, 100%, 56%, and 56% of the controls in 1, 3, 10 and 20 mg/kg males, respectively, for the study period (PNDs 14 to 17). Bodyweight gains were 100%, 70% and 50% of the controls in the 1, 3, and 10 mg/kg female pups, respectively. Bodyweight loss occurred in the 20 mg/kg female pups.

- In male and female pups administered plecanatide on PND 21 (cohort 2), there were no deaths, no drug-related clinical signs, and no changes in bodyweight or bodyweight gain.

- The dose levels of 3 mg/kg and 300 mg/kg were well tolerated when treatment was initiated on PNDs 14 and 21, respectively.

Methods

Doses:	PND 14 mice: 0 (vehicle), 1, 3, 10, or 20 mg/kg PND 21 mice: 0 (vehicle), 25, 50, 100, or 300 mg/kg
Frequency of dosing:	single dose
Route of administration:	oral (gavage)
Dose volume:	5 ml/kg
Formulation/Vehicle:	solution/sterile water
Species/Strain:	Mouse/Crl:CD1(ICR)
Number/Sex/Group:	15
Age:	PND 14 or 21
Weight:	PND 14: Males: 7.0 g Females; 6.8 g PND 21: Males: 10.6 g Females: 9.9 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

Pups in cohort 1 were administered 0 (vehicle control), 1, 3, 10, or 20 mg/kg plecanatide on PND 14, and pups in cohort 2 were administered 0 (vehicle control), 25, 50, 100, or 300 mg/kg plecanatide on PND 21. All pups were sacrificed three days after plecanatide administration. PND 1 was defined as the day of birth. The design of the studies is summarized in the Sponsor's tables below.

Study Design

Group	Treatment	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)	Number per Sex	
					Males	Females
Cohort 1 – PND 14 Administration						
1	Vehicle	0	5	0	15	15
2	Plecanatide	1	5	0.2	15	15
3	Plecanatide	3	5	0.6	15	15
4	Plecanatide	10	5	2	15	15
5	Plecanatide	20	5	4	15	15
Cohort 2 – PND 21 Administration						
1	Vehicle	0	5	0	15	15
2	Plecanatide	25	5	5	15	15
3	Plecanatide	50	5	10	15	15
4	Plecanatide	100	5	20	15	15
5	Plecanatide	300	5	60	15	15

Observations and Results

Mortality

All pups were observed at least twice daily for mortality and morbidity. In pups treated with plecanatide on PND 14, six of the 10 mg/kg mice (4/15 males and 2/15 females) and twenty of the 20 mg/kg mice (10/15 males and 10/15 females) were found dead on the day of dosing or on the following day. No drug-related gross lesions were identified at necropsy of the mice that died prior to scheduled euthanasia.

In pups administered plecanatide on PND 21, all animals survived until scheduled sacrifice on PND 24.

Clinical Signs

All pups were observed at least daily for general appearance. Observations were made approximately one to two hours post-dose and at the end of each working day. In pups treated with plecanatide on PND 14 and subsequently found dead, drug-related clinical signs included loss of righting reflex, decreased motor activity, ataxia, cold to the touch, and moderate dehydration (based on skin turgor). Mild dehydration was also observed in pups that survived until scheduled sacrifice. A 20 mg/kg female pup that survived until scheduled sacrifice was observed with limited use of the limbs, cold to the touch, decreased motor activity, ataxia, moderate dehydration, hunched posture, and thin body condition. The clinical signs are summarized in the Sponsor's tables below.

Clinical Signs in Male Pups Administered Plecanatide on PND 14

GROUP	1	2	3	4	5
TEST MATERIAL	CONTROL ARTICLE	SP-304	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG)a	0	1	3	10	20
MAXIMUM POSSIBLE INCIDENCE	60/ 15	60/ 15	60/ 15	49/ 15	32/ 15
FOUND DEAD	0	0	0	4b	10b
DEHYDRATION - TOTAL	0/ 0	2/ 1	0/ 0	5/ 3	3/ 1
MILD	0/ 0	1/ 1	0/ 0	4/ 2	3/ 1
MODERATE	0/ 0	1/ 1	0/ 0	1/ 1	0/ 0
DECREASED MOTOR ACTIVITY	0/ 0	0/ 0	0/ 0	3/ 3	1/ 1
ATAXIA	0/ 0	0/ 0	0/ 0	2/ 2	0/ 0
HUNCHED POSTURE	0/ 0	2/ 1	0/ 0	0/ 0	0/ 0
LOST RIGHTING REFLEX	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
COLD TO TOUCH	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
THIN BODY CONDITION	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0

MAXIMUM POSSIBLE INCIDENCE = (DAYS x MICE)/NUMBER OF MICE EXAMINED PER GROUP
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
a. Dose administration occurred on Postnatal Day 14.
b. Refer to the individual clinical observations appendix (Appendix 3) for observations of mice that were found dead.

Clinical Signs in Female Pups Administered Plecanatide on PND 14

GROUP	1	2	3	4	5
TEST MATERIAL	CONTROL ARTICLE	SP-304	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG)a	0	1	3	10	20
MAXIMUM POSSIBLE INCIDENCE	60/ 15	60/ 15	60/ 15	51/ 15	30/ 15
FOUND DEAD	0	0	0	2b	10b
DEHYDRATION - TOTAL	0/ 0	0/ 0	3/ 1	15/ 5	12/ 4
MILD	0/ 0	0/ 0	3/ 1	15/ 5	6/ 2
MODERATE	0/ 0	0/ 0	0/ 0	0/ 0	6/ 2
DECREASED MOTOR ACTIVITY	0/ 0	0/ 0	0/ 0	2/ 2	5/ 4
ATAXIA	0/ 0	0/ 0	0/ 0	1/ 1	5/ 3
COLD TO TOUCH	0/ 0	0/ 0	0/ 0	1/ 1	2/ 2
HUNCHED POSTURE	0/ 0	0/ 0	0/ 0	0/ 0	2/ 1
THIN BODY CONDITION	0/ 0	0/ 0	0/ 0	0/ 0	2/ 1
LIMITED USE OF BOTH REAR LIMBS	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1

MAXIMUM POSSIBLE INCIDENCE = (DAYS x MICE)/NUMBER OF MICE EXAMINED PER GROUP
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
a. Dose administration occurred on Postnatal Day 14.
b. Refer to the individual clinical observations appendix (Appendix 4) for observations of mice that were found dead.

In pups administered plecanatide on PND 21, there were no drug-related clinical signs.

Body Weights

In cohort 1, bodyweights were measured on PNDs 1, 4, 13, on the day of dosing, and daily during the post-dose period. In cohort 2, bodyweights were measured on PNDs 1, 4, 13, 20, on the day of dosing, and daily during the post-dose period. In cohort 1, there was a slight reduction in bodyweight gain after dose administration (PNDs 14 to 15) in the 3 mg/kg female pups. Decreased bodyweight gain was noted in the 10 and 20 mg/kg male and female pups following dose administration (PNDs 14-15). Bodyweight loss occurred in the 20 mg/kg female pups. In the surviving pups in cohort 1, bodyweight gains were 89%, 100%, 56%, and 56% of the control group in the 1, 3, 10 and 20 mg/kg male pups, respectively, for the study period (PNDs 14 to 17). Bodyweight gains were 100%, 70% and 50% of the control group in the 1, 3, and 10 mg/kg female pups, respectively. The surviving 20 mg/kg/day female pups lost 0.2 g during this period. Bodyweights and bodyweight changes are summarized in the Sponsor's tables below.

Bodyweight and Bodyweight Changes in Male Pups Administered Plecanatide on PND 14

GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG)a		0	1	3	10	20
MICE TESTED	N	15	15	15	15	15
BODY WEIGHT (G)						
DAY 14	MEAN±S.D.	7.0 ± 0.6	6.8 ± 0.7	6.8 ± 0.5	7.0 ± 0.6	6.8 ± 0.7
DAY 15	MEAN±S.D.	7.5 ± 0.8	7.5 ± 0.7	7.4 ± 0.7	7.3 ± 0.6 [11]b	7.5 ± 0.7 [5]b
DAY 16	MEAN±S.D.	7.6 ± 0.6	7.4 ± 0.6	7.4 ± 0.7	7.3 ± 0.6 [11]b	7.8 ± 0.8 [5]b
DAY 17	MEAN±S.D.	7.9 ± 0.6	7.6 ± 0.8	7.7 ± 0.6	7.7 ± 0.6 [11]b	7.9 ± 0.9 [5]b
BODY WEIGHT CHANGE (G)						
DAYS 14 - 15	MEAN±S.D.	+0.5 ± 0.5	+0.7 ± 0.4	+0.5 ± 0.4	+0.1 ± 0.5 [11]b	+0.0 ± 0.6 [5]b
DAYS 15 - 16	MEAN±S.D.	+0.1 ± 0.4	-0.1 ± 0.4	+0.0 ± 0.4	+0.0 ± 0.5 [11]b	+0.3 ± 0.4 [5]b
DAYS 16 - 17	MEAN±S.D.	+0.3 ± 0.2	+0.2 ± 0.5	+0.3 ± 0.1	+0.4 ± 0.2 [11]b	+0.2 ± 0.2 [5]b
DAYS 14 - 17	MEAN±S.D.	+0.9 ± 0.3	+0.8 ± 0.5	+0.9 ± 0.3	+0.5 ± 0.3 [11]b	+0.5 ± 0.5 [5]b

DAY(S) = POSTNATAL DAY(S)
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Postnatal Day 14.
b. Excludes values for mice that were found dead.

Bodyweight and Bodyweight Changes in Female Pups Administered Plecanatide on PND 14

GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG)a		0	1	3	10	20
MICE TESTED	N	15	15	15	15	15
BODY WEIGHT (G)						
DAY 14	MEAN±S.D.	6.8 ± 0.8	6.8 ± 0.8	6.7 ± 0.8	6.8 ± 0.8	6.8 ± 0.9
DAY 15	MEAN±S.D.	7.3 ± 0.6	7.4 ± 0.8	6.9 ± 0.7	7.1 ± 0.6 [13]b	7.2 ± 1.1 [5]b
DAY 16	MEAN±S.D.	7.5 ± 0.6	7.5 ± 0.7	7.1 ± 0.7	7.2 ± 0.7 [13]b	7.2 ± 1.0 [5]b
DAY 17	MEAN±S.D.	7.9 ± 0.6	7.8 ± 0.7	7.4 ± 0.8	7.5 ± 0.6 [13]b	7.4 ± 1.0 [5]b
BODY WEIGHT CHANGE (G)						
DAYS 14 - 15	MEAN±S.D.	+0.4 ± 0.4	+0.5 ± 0.3	+0.2 ± 0.4	+0.1 ± 0.3 [13]b	-0.4 ± 0.6 [5]b
DAYS 15 - 16	MEAN±S.D.	+0.3 ± 0.4	+0.2 ± 0.2	+0.1 ± 0.2	+0.1 ± 0.2 [13]b	+0.0 ± 0.5 [5]b
DAYS 16 - 17	MEAN±S.D.	+0.3 ± 0.2	+0.3 ± 0.1	+0.3 ± 0.2	+0.3 ± 0.2 [13]b	+0.1 ± 0.3 [5]b
DAYS 14 - 17	MEAN±S.D.	+1.0 ± 0.7	+1.0 ± 0.3	+0.7 ± 0.4	+0.5 ± 0.4 [13]b	-0.2 ± 0.7 [5]b

DAY(S) = POSTNATAL DAY(S)
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Postnatal Day 14.
b. Excludes values for mice that were found dead.

In male and female pups administered plecanatide on PND 21 (cohort 2), there were no drug-related changes in bodyweight or bodyweight gain.

Dose levels up to 3 mg/kg and 300 mg/kg were well tolerated when treatment was initiated on PNDs 14 and 21, respectively.

Study title: Plecanatide: A Multiple Dose Oral Toxicity Study in Juvenile CD 1 Mice

Study no.:	20035794
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/26/2012
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Plecanatide, #110111 (97.5%)

Key Study Findings

- Plecanatide was administered by oral gavage at 0.05, 0.1, 0.5, or 1.0 mg/kg/day to PND 7 mice for 7 days, followed by a 2-day observation period.
- Doses of 0.5 and 1.0 mg/kg/day resulted in mortality within the first or second day after dosing. Clinical signs included dehydration in the 0.5 and 1.0 mg/kg/day groups, and decreased motor activity in the 1.0 mg/kg/day group.
- Decreases in bodyweight and bodyweight gain, compared to controls, were noted in the 0.5 and 1.0 mg/kg/day groups. No gross lesions were noted at necropsy.
- Administration of 0.1 mg/kg/day plecanatide was well tolerated with treatment initiated on PND 7.

Methods

Doses:	0 (vehicle), 0.05, 0.1, 0.5, and 1.0 mg/kg/day
Frequency of dosing:	once daily
Route of administration:	oral (gavage)
Dose volume:	5 ml/kg
Formulation/Vehicle:	solution/sterile water
Species/Strain:	mouse/Crl:CD1(ICR)
Number/Sex/Group:	15
Age:	PND 7
Weight:	Males: 4.3 g Females: 4.2 g
Satellite groups:	none
Unique study design:	none
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

Study Design

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals	
					Males	Females
1	Control Article	0	5	0	15	15
2	SP-304	0.05	5	0.01	15	15
3	SP-304	0.1	5	0.02	15	15
4	SP-304	0.5	5	0.1	15	15
5	SP-304	1.0	5	0.2	15	15

Plecanatide was administered (via oral gavage) to juvenile mice for 7 days starting on PND 7, followed by a 2-day observation period. All surviving pups were sacrificed on PND 16.

Observations and Results

Mortality

Pups were observed at least twice daily for mortality and morbidity. One male in the 0.05 mg/kg/day group was found dead on PND 13. This death was not considered to be drug-related because it occurred at the lowest dose. Three (3/15) males in the 0.5 mg/kg/day group were found dead after a single dose of plecanatide. Thirteen (13/30) mice in the 1.0 mg/kg/day group (6/15 males and 7/15 females) were found dead after either one or two doses of plecanatide.

Dose administration was terminated for male and female mice in the 1.0 mg/kg/day dose group after completion of dosing on PND 8 due to high mortality.

Clinical Signs

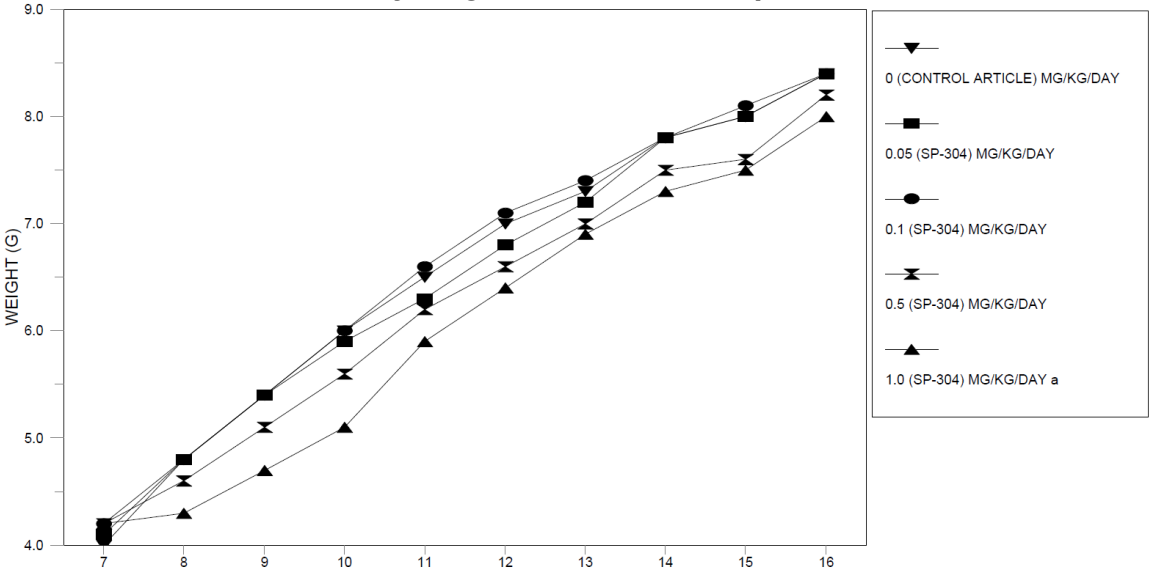
Pups were observed at least daily for general appearance. Post-dose observations were made approximately one to two hours post dose and at the end of each working day. Mild dehydration was noted in one male and one female at 0.5 mg/kg/day. Increased incidence of mild or moderate dehydration was observed in the 1.0 mg/kg/day males (7/15) and females (4/15). Dehydration was still evident in some mice in this group after dosing was discontinued. Decreased motor activity was noted in the 1.0 mg/kg/day males (2/15) and females (2/15). No gross lesions were identified at necropsy.

Body Weights

Bodyweights were measured daily from PND 7 to 16. Decreases in bodyweight, compared to controls, were noted by PND 9 in the 0.5 mg/kg/day males (-5.6%) and females (-12.7%), and in the 1.0 mg/kg/day males (-13.0%) and females (-18.2%). Decreases in bodyweight gain were noted in the 0.5 and 1.0 mg/kg/day males and females between PND 7 and 9, compared to controls. The gains in these groups were comparable to control values between PND 10 and 16. Bodyweight gains for the study period (PND 7 to 16) were reduced by 9.5% and 11.9% in the 0.5 mg/kg/day males and females, respectively, and by 9.5% and 21.6% in the 1.0 mg/kg/day males and females,

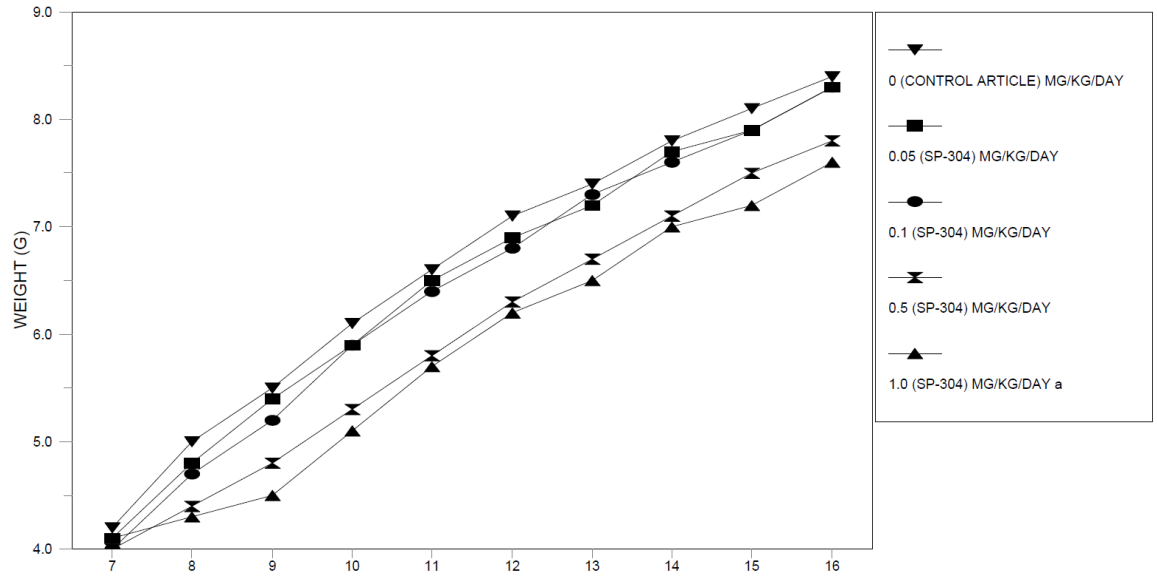
respectively. The bodyweight data is presented in the figures below (taken from the study report).

Bodyweights in the Male Pups



a. Dose administration was terminated for Group 5 (1.0 mg/kg/day) after completion of dosing on Postnatal Day 8.

Bodyweights in the Female Pups



a. Dose administration was terminated for Group 5 (1.0 mg/kg/day) after completion of dosing on Postnatal Day 8.

Dose up to 0.1 mg/kg/day in neonatal mice (PND 7) were well tolerated, based on mortality, clinical signs, and decreased bodyweight gain observed at higher doses.

Study title: Oral (Gavage) Dose Range-Finding Toxicity and Toxicokinetic Study of Plecanatide in Preweaning and Weaning CD-1 Mice

Study no.:	20049883
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/28/2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide, #110111 (97.2%)

Key Study Findings

- Pups in cohort 1 were administered with 0 (vehicle), 1, 3, 10, or 20 mg/kg/day plecanatide starting on PND 14, through PND 27. Pups in cohort 2 were administered with 0 (vehicle), 30, 100, or 300 mg/kg/day plecanatide starting on PND 21, through PND 34. Cohort 1 pups were sacrificed on PND 14 or 27, and cohort 2 pups were sacrificed on PND 21 or 34.

- In pups administered daily with plecanatide starting on PND 14, three 10 mg/kg/day mice were found dead on the day following the first dose administration. Decreased motor activity was observed in these animals prior to death. Mild dehydration was observed in three 10 mg/kg/day pups that survived until scheduled sacrifice on PND 27.

- Small decreases in bodyweight gain, compared to controls, were noted between PND 14 and 16. After the initial decreases, bodyweight gains were generally comparable across the dose groups for the remainder of the study period.

- The weight of the content in small intestines increased in the 1, 3, and 10 mg/kg/day male and female pups; the increase started at 0.25 hour after dose administration, and continued until 4 hours post-dose. There were no drug-related effects on small intestine weight in male and female mice on PND 27 after repeated dosing. Small intestine length and the terminal body weights were comparable among groups and different measuring time points on PND 14 and PND 27.

- In pups administered daily with plecanatide starting on PND 21, all animals survived until scheduled sacrifice on PND 34. There were no drug-related clinical signs, or effects on bodyweight or bodyweight gain during the study period. On PND 21, the weight of the intestinal contents at 0.5 and 1 hr post-dose was increased in male and female pups at one or more doses, and there was a small increase in the average content weight from all time points in all treatment groups.

- Dose levels up to 3 and 300 mg/kg/day plecanatide were well tolerated when treatment was initiated on PND 14 and 21, respectively.

Methods

Doses:	PND 14 mice: 0 (vehicle), 1, 3, and 10 mg/kg/day PND 21 mice: 0 (vehicle), 30, 100, and 300 mg/kg/day
Frequency of dosing:	once daily
Route of administration:	oral (gavage)
Dose volume:	5 ml/kg
Formulation/Vehicle:	solution/sterile water
Species/Strain:	mouse/ Crl:CD1(ICR)
Number/Sex/Group:	39
Age:	PND 14 or 21
Weight:	PND 14: males: 7.5 g females: 7.3 g PND 21: males: 12.0 g females: 11.4 g
Satellite groups:	Toxicokinetic groups: 39/sex/group The mice in these groups were also used for intestinal weight measurements.
Unique study design:	None
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

The following study review is taken from the Pharmacology/Toxicology review of IND 74,883 dated 6/11/2014.

In the oral range-finding study in juvenile mice [redacted] (b) (4) Study No. 20049883; GLP compliant) submitted in this amendment, PND 14 mice (Cohort 1) (39/sex/group) were administered 0 (vehicle), 1, 3, or 10 mg/kg/day plecanatide for 14 days, and PND 21 mice (Cohort 2) (39/sex/group) were administered 0 (vehicle), 30, 100, or 300 mg/kg/day plecanatide for 14 days. The dose volume was 5 mL/kg. The following parameters were evaluated: viability, clinical signs, body weights, bodyweight changes, toxicokinetic parameters, gross necropsy findings, and small intestine length and weight. In each cohort, approximately one-half of the mice were sacrificed and blood was collected at intervals prior to or after the first and last dose for toxicokinetic evaluation. The small intestine was removed, and the length and weight (with and without contents) were measured.

In the PND 14 group (Cohort 1), two males and one female in the 10 mg/kg/day group were found dead on the day following the first dose administration. All three pups were reported to show decreased motor activity following dose administration the previous day. All the mice in the PND 21 group (Cohort 2) survived until scheduled sacrifice. The mortality and clinical observation data are shown in the tables below (taken from the Sponsor's study report).

Mortality and Clinical Observations in Cohort 1 Males

GROUP	1	2	3	4
TEST MATERIAL	CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a	0	1	3	10
MAXIMUM POSSIBLE INCIDENCE	312/ 39	312/ 39	312/ 39	288/ 39
FOUND DEAD	0	0	0	2b,c
DECREASED MOTOR ACTIVITY	0/ 0	0/ 0	0/ 0	2/ 2b,c
DEHYDRATION - MILD	0/ 0	0/ 0	0/ 0	8/ 1

MAXIMUM POSSIBLE INCIDENCE = (DAYS x MICE)/NUMBER OF MICE EXAMINED PER GROUP
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
a. Dose administration occurred on Postnatal Days 14 through 27.
b. Mouse 904 was found dead on Postnatal Day 15.
c. Mouse 1104 was found dead on Postnatal Day 15.

Mortality and Clinical Observations in Cohort 1 Females

GROUP	1	2	3	4
TEST MATERIAL	CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a	0	1	3	10
MAXIMUM POSSIBLE INCIDENCE	312/ 39	312/ 39	312/ 39	300/ 39
FOUND DEAD	0	0	0	1b
DEHYDRATION - MILD	9/ 2	14/ 3	0/ 0	9/ 2
DECREASED MOTOR ACTIVITY	0/ 0	0/ 0	0/ 0	1/ 1b

MAXIMUM POSSIBLE INCIDENCE = (DAYS x MICE)/NUMBER OF MICE EXAMINED PER GROUP
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
a. Dose administration occurred on Postnatal Days 14 through 27.
b. Mouse 1108 was found dead on Postnatal Day 15.

Mortality and Clinical Observations in Cohort 2 Males

GROUP	5	6	7	8
TEST MATERIAL	CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a	0	30	100	300
MAXIMUM POSSIBLE INCIDENCE	312/ 39	312/ 39	312/ 39	312/ 39
MORTALITY	0	0	0	0
NO ADVERSE FINDINGS				

MAXIMUM POSSIBLE INCIDENCE = (DAYS x MICE)/NUMBER OF MICE EXAMINED PER GROUP
a. Dose administration occurred on Postnatal Days 21 through 34.

Mortality and Clinical Observations in Cohort 2 Females

GROUP	5	6	7	8
TEST MATERIAL	CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a	0	30	100	300
MAXIMUM POSSIBLE INCIDENCE	312/ 39	312/ 39	312/ 39	312/ 39
MORTALITY	0	0	0	0
		NO ADVERSE FINDINGS		

MAXIMUM POSSIBLE INCIDENCE = (DAYS x MICE)/NUMBER OF MICE EXAMINED PER GROUP
a. Dose administration occurred on Postnatal Days 21 through 34.

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In Cohort 1, mild dehydration, not clearly defined by the Sponsor, was observed in one male and two females in the 10 mg/kg/day group that survived until scheduled sacrifice. The 10 mg/kg/day male and female pups showed a transient slight decrease in bodyweight gain on the day following drug administration (0.0±0.2 g vs. 0.3±0.2 g in male controls, and 0.1±0.2 g vs 0.3±0.1 g in female controls). In Cohort 2, doses of plecanatide as high as 300 mg/kg/day were not associated with any adverse clinical signs, and the bodyweights were comparable among all groups. Data for bodyweight gains in Cohort 1 are summarized in the table below (taken from the Sponsor's study report)

Changes in Bodyweight Gain in Male Mice in Cohort 1

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a		0	1	3	10
MICE TESTED	N	21b	21b	21b	19b,c
BODY WEIGHT CHANGE (G)					
DAYS 14 - 15	MEAN±S.D.	+0.3 ± 0.2	+0.2 ± 0.2	+0.2 ± 0.2	+0.0 ± 0.2
DAYS 15 - 16	MEAN±S.D.	+0.2 ± 0.3	+0.2 ± 0.4	+0.2 ± 0.2	+0.1 ± 0.2
DAYS 16 - 17	MEAN±S.D.	+0.3 ± 0.3	+0.2 ± 0.3	+0.2 ± 0.2	+0.2 ± 0.2
DAYS 17 - 18	MEAN±S.D.	+0.6 ± 0.4	+0.6 ± 0.4	+0.6 ± 0.4	+0.6 ± 0.4
DAYS 18 - 19	MEAN±S.D.	+0.9 ± 0.2	+1.1 ± 0.3	+0.9 ± 0.2	+0.8 ± 0.2
DAYS 19 - 20	MEAN±S.D.	+0.9 ± 0.3	+1.0 ± 0.2	+1.1 ± 0.3	+1.0 ± 0.3
DAYS 20 - 21	MEAN±S.D.	+1.0 ± 0.4	+1.0 ± 0.3	+1.0 ± 0.4	+0.9 ± 0.4
DAYS 21 - 22	MEAN±S.D.	+1.0 ± 0.3	+1.1 ± 0.3	+0.9 ± 0.4	+0.8 ± 0.5
DAYS 22 - 23	MEAN±S.D.	+1.3 ± 0.4	+1.2 ± 0.5	+1.3 ± 0.5	+1.2 ± 0.4
DAYS 23 - 24	MEAN±S.D.	+1.5 ± 0.3	+1.7 ± 0.4	+1.5 ± 0.4	+1.4 ± 0.3
DAYS 24 - 25	MEAN±S.D.	+1.5 ± 0.4	+1.6 ± 0.4	+1.4 ± 0.4	+1.4 ± 0.4
DAYS 25 - 26	MEAN±S.D.	+1.4 ± 0.4	+1.4 ± 0.4	+1.4 ± 0.5	+1.2 ± 0.4
DAYS 26 - 27	MEAN±S.D.	+1.6 ± 0.8	+1.7 ± 0.4	+1.6 ± 0.6	+1.6 ± 0.6
DAYS 14 - 27	MEAN±S.D.	+12.5 ± 1.4	+13.0 ± 1.8	+12.3 ± 2.3	+11.4 ± 1.8

DAYS = POSTNATAL DAYS
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Postnatal Days 14 through 27.
b. Excludes values for mice that were euthanized on Postnatal Day 14.
c. Excludes values for mice that were found dead.

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Changes in Bodyweight Gain in Female Mice in Cohort 1

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)a		0	1	3	10
MICE TESTED	N	21b	21b	21b	20b,c
BODY WEIGHT CHANGE (G)					
DAYS 14 - 15	MEAN±S.D.	+0.3 ± 0.1	+0.2 ± 0.2	+0.2 ± 0.2	+0.1 ± 0.2
DAYS 15 - 16	MEAN±S.D.	+0.2 ± 0.2	+0.2 ± 0.2	+0.2 ± 0.2	+0.1 ± 0.2
DAYS 16 - 17	MEAN±S.D.	+0.2 ± 0.2	+0.3 ± 0.3	+0.3 ± 0.2	+0.2 ± 0.3
DAYS 17 - 18	MEAN±S.D.	+0.6 ± 0.5	+0.8 ± 0.4	+0.7 ± 0.4	+0.7 ± 0.4
DAYS 18 - 19	MEAN±S.D.	+0.9 ± 0.2	+0.9 ± 0.2	+0.8 ± 0.2	+0.9 ± 0.2
DAYS 19 - 20	MEAN±S.D.	+1.0 ± 0.3	+0.9 ± 0.2	+0.9 ± 0.4	+1.0 ± 0.2
DAYS 20 - 21	MEAN±S.D.	+0.9 ± 0.3	+0.9 ± 0.3	+0.9 ± 0.3	+0.8 ± 0.3
DAYS 21 - 22	MEAN±S.D.	+0.9 ± 0.3	+0.9 ± 0.3	+0.8 ± 0.3	+0.8 ± 0.4
DAYS 22 - 23	MEAN±S.D.	+1.0 ± 0.4	+1.1 ± 0.3	+1.0 ± 0.2	+1.1 ± 0.3
DAYS 23 - 24	MEAN±S.D.	+1.3 ± 0.3	+1.2 ± 0.3	+1.3 ± 0.4	+1.1 ± 0.4
DAYS 24 - 25	MEAN±S.D.	+1.2 ± 0.2	+1.2 ± 0.3	+1.1 ± 0.2	+1.3 ± 0.4
DAYS 25 - 26	MEAN±S.D.	+0.9 ± 0.5	+0.9 ± 0.5	+0.9 ± 0.3	+1.1 ± 0.3
DAYS 26 - 27	MEAN±S.D.	+1.3 ± 0.6	+1.2 ± 0.7	+0.9 ± 0.6	+1.2 ± 0.4
DAYS 14 - 27	MEAN±S.D.	+10.8 ± 1.3	+10.7 ± 1.8	+10.0 ± 1.6	+10.4 ± 1.0

DAYS = POSTNATAL DAYS
[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Postnatal Days 14 through 27.
b. Excludes values for mice that were euthanized on Postnatal Day 14.
c. Excludes values for mouse 1108, which was found dead on Postnatal Day 15.

Cohort 1 exhibited an increase in the weight of small intestine with contents and the calculated weight of the intestinal contents in both sexes at all doses, compared to that of the control values. The changes occurred at the 15-minute interval after plecanatide administration and continued until the last evaluation at 4 hours after drug administration. The weights generally were comparable among dose groups for both male and female mice on PND 27, after repeated exposure to plecanatide. The intestine and intestinal content weights at different time intervals (0, 5, 15, 30, 60, 120, and 240 minutes) during the 4-hour period were averaged and summarized in the table below (taken from the Sponsor's study report). The increases in intestinal content weight were substantially higher on PND 14 than PND 27 in both male and female mice.

Small Intestine Length and Weight in Male Mice in Cohort 1 on day 14

GROUP	TIMEPOINT		SMALL INTESTINE LENGTH (CM)	SMALL INTESTINES WITH CONTENTS ABS. WT (G)	SMALL INTESTINES WITHOUT CONTENTS ABS. WT (G)	WEIGHT OF CONTENTS (G)
1	All PND 14	MEAN±	21.92	0.3266	0.2688	0.0577
		S.D.	1.75	0.0331	0.0382	0.0347
2	All PND 14	MEAN±	22.82	0.4443	0.2749	0.1694
		S.D.	1.97	0.0924	0.0410	0.0764
3	All PND 14	MEAN±	22.34	0.4387	0.2583	0.1804
		S.D.	1.76	0.1122	0.0498	0.0857
4	All PND 14	MEAN±	22.36	0.4713	0.2705	0.2008
		S.D.	2.09	0.1375	0.0553	0.1128

Small Intestine Length and Weight in Male Mice in Cohort 1 on day 27

GROUP	TIMEPOINT		SMALL INTESTINE LENGTH (CM)	SMALL INTESTINES WITH CONTENTS ABS. WT (G)	SMALL INTESTINES WITHOUT CONTENTS ABS. WT (G)	WEIGHT OF CONTENTS (G)
1	All PND 27	MEAN±	38.53	1.7115	1.1458	0.5657
		S.D.	3.12	0.2188	0.1131	0.1814
2	All PND 27	MEAN±	40.21	1.9249	1.3206	0.6043
		S.D.	3.24	0.3651	0.2314	0.2075
3	All PND 27	MEAN±	40.67	1.8430	1.2506	0.5925
		S.D.	3.25	0.2739	0.1996	0.1086
4	All PND 27	MEAN±	39.29	1.7247	1.1735	0.5512
		S.D.	3.72	0.2720	0.1962	0.1544

Small Intestine Length and Weight in Female Mice in Cohort 1 on day 14

GROUP	TIMEPOINT		SMALL INTESTINE LENGTH (CM)	SMALL INTESTINES WITH CONTENTS ABS. WT (G)	SMALL INTESTINES WITHOUT CONTENTS ABS. WT (G)	WEIGHT OF CONTENTS (G)
1	All PND 14	MEAN±	22.37	0.3264	0.2643	0.0621
		S.D.	1.90	0.0456	0.0420	0.0295
2	All PND 14	MEAN±	23.12	0.4210	0.2477	0.1733
		S.D.	1.42	0.0780	0.0324	0.0704
3	All PND 14	MEAN±	22.01	0.4339	0.2543	0.1797
		S.D.	1.50	0.1116	0.0568	0.0929
4	All PND 14	MEAN±	22.35	0.4637	0.2684	0.1953
		S.D.	1.80	0.1041	0.0456	0.0772

Small Intestine Length and Weight in Female Mice in Cohort 1 on day 27

GROUP	TIMEPOINT		SMALL INTESTINE LENGTH (CM)	SMALL INTESTINES WITH CONTENTS ABS. WT (G)	SMALL INTESTINES WITHOUT CONTENTS ABS. WT (G)	WEIGHT OF CONTENTS (G)
1	All PND 27	MEAN±	38.99	1.5098	1.0567	0.4531
		S.D.	3.58	0.2006	0.1226	0.1202
2	All PND 27	MEAN±	40.07	1.6268	1.0903	0.5365
		S.D.	3.42	0.2720	0.1673	0.1947
3	All PND 27	MEAN±	39.86	1.5461	1.0512	0.4949
		S.D.	3.46	0.2352	0.1544	0.1224
4	All PND 27	MEAN±	40.25	1.6794	1.1259	0.5535
		S.D.	2.74	0.2287	0.1863	0.1554

Groups: 1) control; 2) 1 mg/kg/day; 3) 3 mg/kg/day; 4) 10 mg/kg/day

Plecanatide administration starting on PND 21 increased the weight of the small intestine with contents and the calculated weights of the intestinal contents in male and female pups at one or more doses at 30 minutes and 1 hour after drug administration. On PND 34, these weights were generally comparable among the dose groups with the exception of increases in pups at one or more doses at 15 minutes, 1 hour and 2 hours post-dose. The intestine and intestinal content weights at different time intervals (0, 5, 15, 30, 60, 120, and 240 minutes) during the 4-hour period were averaged and summarized in the table below (taken from the Sponsor's study report). Only very modest increases in the averaged intestine and intestinal content weights were noted on PND 21 and PND 34.

Small Intestine Length and Weight in Male Mice in Cohort 2 on day 21

GROUP	TIMEPOINT		SMALL INTESTINE LENGTH (CM)	SMALL INTESTINES WITH CONTENTS ABS. WT (G)	SMALL INTESTINES WITHOUT CONTENTS ABS. WT (G)	WEIGHT OF CONTENTS (G)
1	All PND 21	MEAN±	31.01	1.0673	0.7366	0.3308
		S.D.	3.29	0.3287	0.2440	0.1925
2	All PND 21	MEAN±	31.72	1.0333	0.6540	0.3793
		S.D.	3.29	0.1713	0.1283	0.1049
3	All PND 21	MEAN±	31.94	1.0191	0.6295	0.3896
		S.D.	3.10	0.2168	0.1079	0.1716
4	All PND 21	MEAN±	30.56	1.0206	0.6226	0.3980
		S.D.	2.67	0.2603	0.1194	0.1784

Small Intestine Length and Weight in Male Mice in Cohort 2 on day 34

GROUP	TIMEPOINT		SMALL INTESTINE LENGTH (CM)	SMALL INTESTINES WITH CONTENTS ABS. WT (G)	SMALL INTESTINES WITHOUT CONTENTS ABS. WT (G)	WEIGHT OF CONTENTS (G)
1	All PND 34	MEAN±	45.09	2.1857	1.6263	0.5594
		S.D.	2.61	0.2684	0.1564	0.1337
2	All PND 34	MEAN±	44.07	2.2699	1.6459	0.6240
		S.D.	2.30	0.2509	0.1494	0.1544
3	All PND 34	MEAN±	44.60	2.2986	1.6339	0.6647
		S.D.	3.11	0.3169	0.1846	0.1794
4	All PND 34	MEAN±	44.88	2.3939	1.6579	0.7360
		S.D.	3.39	0.3112	0.1951	0.1919

Small Intestine Length and Weight in Female Mice in Cohort 2 on day 21

GROUP	TIMEPOINT		SMALL INTESTINE LENGTH (CM)	SMALL INTESTINES WITH CONTENTS ABS. WT (G)	SMALL INTESTINES WITHOUT CONTENTS ABS. WT (G)	WEIGHT OF CONTENTS (G)
1	All PND 21	MEAN±	32.27	0.8734	0.5776	0.2959
		S.D.	2.47	0.1334	0.1086	0.0986
2	All PND 21	MEAN±	31.72	0.9652	0.5924	0.3728
		S.D.	2.44	0.1470	0.1194	0.0899
3	All PND 21	MEAN±	31.90	0.9304	0.5743	0.3561
		S.D.	3.79	0.2242	0.1229	0.1261
4	All PND 21	MEAN±	31.88	0.8738	0.5471	0.3268
		S.D.	2.06	0.1478	0.0880	0.1059

Small Intestine Length and Weight in Female Mice in Cohort 2 on day 34

GROUP	TIMEPOINT		SMALL INTESTINE LENGTH (CM)	SMALL INTESTINES WITH CONTENTS ABS. WT (G)	SMALL INTESTINES WITHOUT CONTENTS ABS. WT (G)	WEIGHT OF CONTENTS (G)
1	All PND 34	MEAN±	42.98	1.8461	1.3327	0.5135
		S.D.	2.76	0.2325	0.1643	0.1273
2	All PND 34	MEAN±	43.59	1.9158	1.3687	0.5470
		S.D.	3.70	0.2814	0.1747	0.1443
3	All PND 34	MEAN±	43.25	1.9637	1.4107	0.5530
		S.D.	3.75	0.2507	0.1296	0.1617
4	All PND 34	MEAN±	44.63	1.9738	1.4434	0.5304
		S.D.	4.45	0.3136	0.1969	0.1430

Groups: 1) control; 2) 30 mg/kg/day; 3) 100 mg/kg/day; 4) 300 mg/kg/day

The increase in intestine weight after plecanatide administration appears to be consistent with the pharmacological action of plecanatide.

Maximum plasma levels of plecanatide were achieved between 5 minutes and 1 hour post-dose on PNDs 14 and 27, and PNDs 21 and 34. When there were sufficient data

points to calculate half-lives, the $t_{1/2}$ ranged between 0.3 and 1.8 hours. Plasma exposure to plecanatide was not dose-proportional; plasma C_{max} and AUC values appear to be highly variable. The exposure to plecanatide on PND 27 for the 1, 3, and 10 mg/kg/day dose groups was decreased after 14 daily doses, compared to the values on PND 14, and a similar trend was also noted in the 30 mg/kg/day group in Cohort 2 when comparing exposure on PND 34 to that on PND 21. By contrast, in the 100 mg/kg/day group, plecanatide exposure increased on PND 34 after 14 daily doses compared to the values on PND 21, and in the 300 mg/kg/day group, the trends were different for males and females. For the 30, 100, and 300 mg/kg/day groups, plecanatide exposure was generally higher for males than females. The data are summarized in the Sponsor's table below.

Study 20049883: Toxicokinetic Parameters for Juvenile Mice in a Repeated-dose Evaluation of Plecanatide Beginning on Postnatal Day 14 or 21

Dose	Sex	Tmax		Cmax		AUC(0-t)		T½	
Cohort 1 (Dosing from PND 14-27)									
		14	27	14	27	14	27	14	27
1	Female	0.0833	0.250	12.7 ± 1.86	4.32 ± 3.09	13.4 ± 8.31	NR	1.78	NR
	Male	0.0833	NR	21.3 ± 3.86	NR	4.14 ± 0.984	NR	NR	NR
3	Female	0.0833	0.250	60.4 ± 42.4	15.4 ± 12.7	41.6 ± 14.0	3.82 ± 2.68	1.01	NR
	Male	0.0833	1.00	775. ± 748.	4.00 ± 4.00	116. ± 94.3	1.39 ± 1.03	NR	NR
10	Female	0.250	0.250	40.0 ± 28.5	19.6 ± 18.5	59.0 ± 30.2	10.5 ± 5.86	0.914	NR
	Male	0.0833	0.0833	110. ± 71.8	3.20 ± 0.995	129. ± 75.9	NR	NR	NR
Cohort 2 (Dosing from PND 21-34)									
		21	34	21	34	21	34	21	34
30	Female	1.00	0.0833	50.1 ± 48.9	28.9 ± 12.9	21.8 ± 12.9	11.6 ± 4.17	NR	NR
	Male	0.0833	0.250	240 ± 226	77.0 ± 69.5	34.3 ± 28.2	23.6 ± 14.8	0.499	NR
100	Female	0.250	00833	20.8 ± 7.00	35.9 ± 16.4	8.58 ± 1.63	17.9 ± 3.44	0.319	0.325
	Male	0.0833	0.250	54.4 ± 36.3	457 ± 234	23.3 ± 5.56	121. ± 49.3	NR	0.701
300	Female	0.250	0.0833	258 ± 121	75.0 ± 10.3	87.0 ± 25.8	39.0 ± 2.72	NR	0.447
	Male	0.0833	0.250	405 ± 297	489 ± 391	101. ± 39.5	283. ± 126.	NR	NR

NR = Not Reported; ±*n* = ±SEM
C_{max} = ng/ml; AUC = ng•hr/ml

Study title: Plecanatide: Oral (Gavage) Toxicity Study in Juvenile CD-1 Mice

Study no.:	20059246
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/1/2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Plecanatide (SP-304), #140109 (97.5%) and #140402 (98.1%)

Key Study Findings

- PND 14 pups were administered 0 (vehicle), 3, 10, 0/100, and 0/300 mg/kg/day plecanatide through PND 111. In the 0/100 and 0/300 mg/kg/day groups, pups received only vehicle from PND 14 to 20.

- In the 10 mg/kg/day group, 7/34 male pups and 5/34 female pups were either found dead or missing, and presumed cannibalized on the day following the first dose of plecanatide on PND 14. A male pup that was presumed cannibalized was observed with decreased motor activity and moderate dehydration after dosing on PND 14; no clinical signs were observed on PND 14 in the other pups that died subsequently.

- There were no drug-related effects on bodyweight or bodyweight gain at doses up to 10 mg/kg/day in males or females that received daily plecanatide starting on PND 14, or doses up to 300 mg/kg/day in pups that received daily plecanatide starting on PND 21. In the fertility evaluation phase of the study, no drug-related effects on bodyweight or bodyweight gain were observed in females during the gestation period (GD 0 to 13).

- There were no drug-related effects on the ophthalmic evaluation, sexual maturation, open field observation parameters, functional observational battery parameters, hematology and clinical chemistry parameters, or gross pathology.

- Significant decreases in pituitary weight (absolute, %bodyweight, and/or %brain weight) were noted at scheduled sacrificed (PND111) in the 3, 10, 0/100, and 0/300 males (up to -35.1% in absolute weight) and 10, 0/100, and 0/300 mg/kg/day females (up to -55.3% in absolute weight), compared to controls. There were no changes in pituitary weight in animals sacrificed on PND 135 or 137, 24 or 26 days after the last dose. No microscopic findings correlated with the pituitary weight changes.

- There were no drug-related microscopic findings, or effects on any of the bone parameters evaluated.

- There were no drug-related effects on mating and fertility, cesarean section, or litter parameters.

- The NOAEL for treatment initiated on PND 14 was 3 mg/kg/day, based on deaths at 10 mg/kg/day. The NOAEL for treatment initiated on PND 21 was 300 mg/kg/day, the highest dose tested.

Methods

Doses:	0 (vehicle control), 3, 10, 0/100, and 0/300 mg/kg/day
Frequency of dosing:	once daily from PND 14 to 111
Route of administration:	oral (gavage)
Dose volume:	5 ml/kg
Formulation/Vehicle:	solution/sterile water
Species/Strain:	mouse/Crl:CD1(ICR)
Number/Sex/Group:	Cohort 1: 12/sex/group; Cohort 2: 22/sex/group
Age:	PND 14 and 21
Weight:	PND 14: 7.6 g for males and 7.4 g for females PND 21: 12.0 g for males and 11.5 for females
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	There were minor deviations that did not affect the quality or integrity of the study.

Study Design

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals[b]			
					Cohort 1		Cohort 2	
					Males	Females	Males	Females
1	Control Article	0	5	0	12	12	22	22
2	SP-304	3	5	0.6	12	12	22	22
3	SP-304	10	5	2	12	12	22	22
4	SP-304	0/100[a]	5	0/20[a]	12	12	22	22
5	SP-304	0/300[a]	5	0/60[a]	12	12	22	22

[a] The dose level for the pups was 0 mg/kg/day from PNDs 14 to 20. Starting on PND 21, the dose level was 100 mg/kg/day (Group 4) or 300 mg/kg/day (Group 5) for the remainder of the dose period.

[b] F1 generation mice were administered the test article or the control article formulations once daily starting on PND 14 continuing through PND 111 (see Appendix 1, Protocol, Amendments, and Deviations).

The following parameters were evaluated:

In both cohorts: viability, clinical signs, bodyweights and bodyweight changes, sexual maturation, gross necropsy findings, and organ weights,

In cohort 1 only: open field observations, functional observational battery (FOB) testing, ophthalmology, clinical pathology (hematology and clinical chemistry), bone evaluations (femur densitometry, geometry, and length), and histopathology,

In cohort 2 only: estrous cycles, mating and fertility, and cesarean section.

Observations and Results

Mortality

Pups were observed at least twice daily for mortality and morbidity. In the 10 mg/kg/day group, 7 males and 5 females were either found dead or were missing and presumed cannibalized on the day following the first dose of plecanatide on PND 14. A male pup that was presumed cannibalized was observed with decreased motor activity and moderate dehydration after dosing on PND 14; no clinical signs were observed on PND 14 in the other pups that subsequently died. The deaths are considered as drug-related. At necropsy, all tissues that could be evaluated appeared normal in relation to the slight or moderate degree of autolysis that was observed. The tissues were not evaluated microscopically. More pups at PND 14 of age were added to the 10 mg/kg/day group. No additional drug-related mortalities occurred in the study. Two mice at 5 weeks of age or older in the 0/300 mg/kg/day group were euthanized due to apparent accidental injuries.

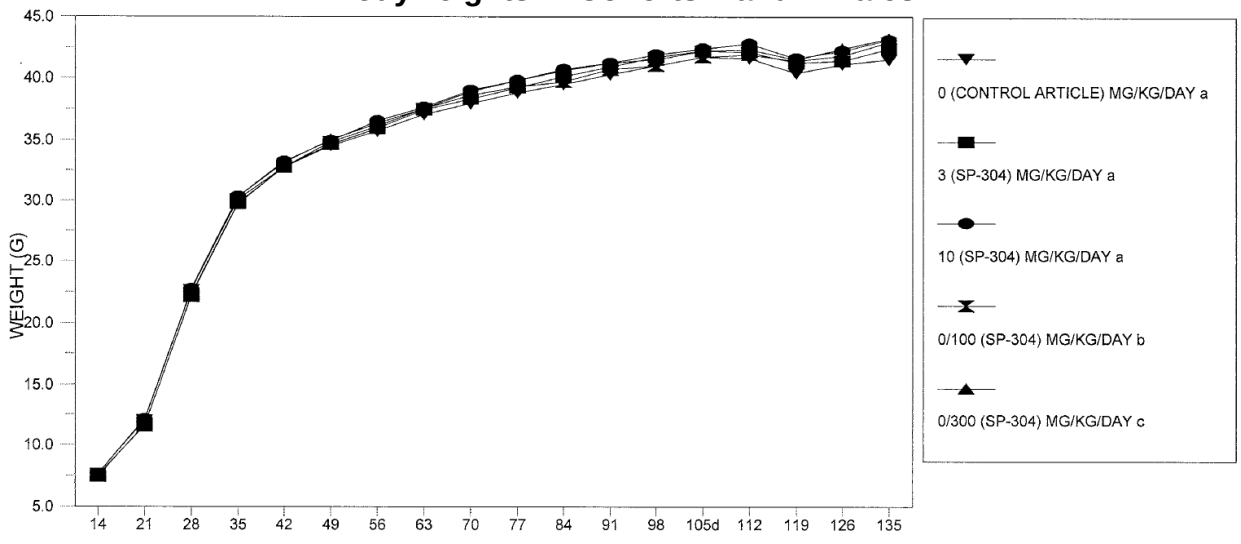
Clinical Signs

Pups were observed at least daily for general appearance. Post-dose observations were made approximately one to two hours post-dose and at the end of each working day. Starting with the twelfth dose, post-dose observations were recorded only between one to two hours post-dose. Sporadic clinical signs were observed, which included the following: swollen right forelimb and/or forepaw; limited use of right forelimb and forepaw, red right forelimb and forepaw; mass on the right inguinal area and/or mouth; scab on the neck, head, back and/or right and left ears; sparse hair coat and/or abrasion on the neck and/or head; torn right and left ears; abdominal distention; bent tail; mild or moderate dehydration; localized alopecia on the neck; lacrimation; discoloration (black or purple) on the tail or tip of tail; tip of tail missing; laceration on the distal third of the tail; and/or swollen tail. These signs are not considered to be drug-related because they lacked dose-dependency, occurred in a single sex, and/or were observed at very low frequencies.

Body Weights

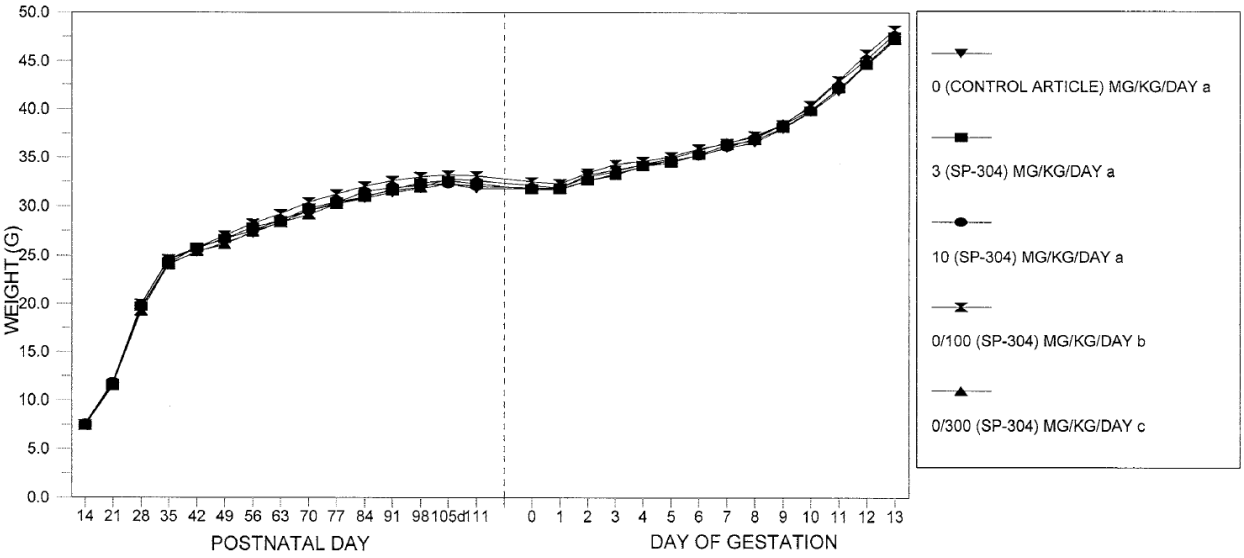
Bodyweights were measured daily during the dosing period. In pups treated with plecanatide starting on PND 14 (3 or 10 mg/kg/day), there were no drug-related effects on bodyweight or bodyweight gain. In pups treated with plecanatide starting on PND 21 (100 or 300 mg/kg/day), there were no drug-related effects on bodyweight or bodyweight gain. Bodyweight gains for the study period (calculated as PNDs 14 to 111) in males were 102%, 103%, 101%, and 101% of the controls in the 3, 10, 0/100 and 0/300 mg/kg/day groups, respectively. Bodyweight gains for the study period in females were 102%, 100%, 105%, and 102% of the controls in the 3, 10, 0/100 and 0/300 mg/kg/day dose groups, respectively. Bodyweight gains for the gestation period, calculated as GD 0 to 13, were 99%, 98%, 101%, and 100% of the controls in the 3, 10, 0/100 and 0/300 mg/kg/day groups, respectively. The bodyweights in males and females in cohorts 1 and 2 are summarized in Sponsor's figures below.

Bodyweights in Cohorts 1 and 2 Males



a. Dose administration occurred on Postnatal Days 14 through 111.
b. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 100 mg/kg/day on Postnatal Days 21 through 111).
c. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 300 mg/kg/day on Postnatal Days 21 through 111).
d. Last weekly value recorded before cohabitation for cohort 2 mice.

Bodyweights in Cohorts 1 and 2 Females



a. Dose administration occurred on Postnatal Days 14 through 111.
b. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 100 mg/kg/day on Postnatal Days 21 through 111).
c. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 300 mg/kg/day on Postnatal Days 21 through 111).
d. Last weekly value recorded before cohabitation for cohort 2 mice.

Feed Consumption

Not measured.

Ophthalmoscopy

Ophthalmology examinations were conducted in cohort 1 males and females. Evaluations were performed after a minimum of 87 and 80 doses in the pups administered plecanatide starting on PND 14 and PND 21, respectively. The ophthalmic findings are summarized in the Sponsor's table below.

Ophthalmic Findings in Cohort 1 Males and Females

	Sex	Males					Females				
	Group No.	1	2	3	4	5	1	2	3	4	5
	Dose Level[a]	0	3	10	0/100[b]	0/300[b]	0	3	10	0/100[b]	0/300[b]
Observation	No. Examined	12	12	12	12	11	12	12	12	12	12
Retinal Degeneration		0	0	0	0	0	0	1R	0	0	1R
Scleral Coloboma		0	0	0	0	0	0	0	1L, 1R	0	0

Value recorded is the total no. of animals with that observation.
L = Left eye; R = Right eye
[a] (mg/kg/day)
[b] The dose level for the pups was 0 mg/kg/day from PNDs 14 to 20. Starting on PND 21, the dose level was 100 mg/kg/day (Group 4) or 300 mg/kg/day (Group 5) for the remainder of the dose period.

The findings are not considered as drug-related because they lacked dose-dependency, occurred in a single sex and/or occurred at low frequencies. The Sponsor also noted that the retinal degeneration observed was of the nature commonly found in this strain and age of mice. The Sponsor noted that the finding of scleral coloboma in two 10 mg/kg/day females was considered to be a developmental anomaly which may have been secondary to changes in the globe as the animal matured and, therefore, was considered unrelated to administration of plecanatide.

ECG

Not measured.

Sexual Maturation

Sexual maturation was examined in cohorts 1 and 2. There were no drug-related effects in preputial separation in male pups or vaginal patency in female pups.

Open Field Observations

Open field observations were performed once on PND 28 ± 1 in cohort 1 animals, prior to dose administration. The observations were conducted by an observer blinded to the group assignments of the animals. Mice were observed for at least 1 minute while in the open field. The observer examined each mouse either while handling the animal, and/or in an open field to assess parameters including, but not limited to the following: lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to light, piloerection, respiration (autonomic functions), behavior in the open field (excitability), gait pattern in the open field, and severity of gait abnormalities. Abnormal clinical signs, including but not limited to convulsions, tremors and other unusual

behavior, hypotonia or hypertonia, emaciation, dehydration, unkempt appearance, and deposits around the eyes, nose or mouth were evaluated.

There were no drug-related effects in any of the parameters evaluated.

Functional Observational Battery (FOB) Evaluations

The FOB testing (including detailed clinical observations) was recorded on PND 49 ± 2 in cohort 1 animals, prior to dose administration. The FOB was conducted by an observer blinded to the group assignments of the animals. The observer examined the mouse in its home cage, while handling the animal, and/or in an open field to assess parameters including, but not limited to the following: lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to light, piloerection, respiration, and urination and defecation (autonomic functions); sensorimotor responses to visual, acoustic, tactile, and painful stimuli (reactivity and sensitivity); reactions to handling and behavior in the open field (excitability); gait pattern in the open field, severity of gait abnormalities, air righting reaction, visual placing response, and landing foot splay (gait and sensorimotor coordination); forelimb and hindlimb grip strength; abnormal clinical observations including, but not be limited to: general appearance, body position and posture, neuromuscular system, and behavior. Body temperature was measured at the completion of the FOB evaluation using a rectal probe.

There were no meaningful drug-related effects in any of the parameters evaluated. A statistically significant decrease in the number of 0/100 and 0/300 mg/kg/day males observed to be awake but immobile in the home cage behavior assessment, as compared to controls, was noted. The Sponsor considered these differences as not biologically important because: 1) the mice were observed either sleeping or displaying normal movement which are considered to be normal behavior when a mouse is in the home cage; and 2) the groups were comparable to controls in other assessments of activity in the FOB tests.

Hematology

Blood samples for hematology evaluations were collected on the day of scheduled sacrifice (PND 112) in cohort 1 animals. The following parameters were evaluated (table taken from the Sponsor's study report).

Red blood cell (erythrocyte) count	White blood cell (leukocyte) count
Hemoglobin concentration	Neutrophil count
Hematocrit	Lymphocyte count
Mean corpuscular volume	Monocyte count
Red blood cell distribution width	Eosinophil count
Mean corpuscular hemoglobin concentration	Basophil count
Mean corpuscular hemoglobin	Large unstained cells
Reticulocyte count (absolute)	Other cells (as appropriate)
Platelet count	

There were no meaningful drug-related effects in any of the parameters examined. A statistically significant increase in platelets (+25.0%) was noted in the 0/300 mg/kg/day

females. This finding is not considered as drug-related because the magnitude of change was small and it occurred only in one sex.

Clinical Chemistry

Blood samples for clinical chemistry evaluations were collected on the day of scheduled euthanasia (PND 112) in cohort 1 animals. The following parameters were evaluated (table taken from the Sponsor's study report).

Calcium	Amylase
Phosphorus	Lipase
Sodium	Alkaline phosphatase
Potassium	Gamma-glutamyltransferase
Chloride	Total bilirubin[a]
Alanine aminotransferase	Total protein
Urea nitrogen	Albumin/globulin ratio
Albumin	Creatine Kinase
Aspartate aminotransferase	Cholesterol
Creatinine	Triglycerides
Glucose	Globulin

[a] When total bilirubin was >0.5 mg/dL, indirect and direct bilirubin was also measured.

Statistically significant decreases in glucose levels in the 0/100 mg/kg/day males (-20%) and 0/300 mg/kg/day males (-16%) were noted. However, the decreases are not considered as drug-related due to the lack of dose-response, small magnitude of change, and absence of correlating observations.

Urinalysis

Not performed.

Gross Pathology

Observations were made in cohort 1 and 2 animals at scheduled sacrifice. Cohort 1 animals were sacrificed on PND 112. Cohort 2 males were sacrificed on PND 135 or 137 (24 or 26 days after the last dose), and mated females were sacrificed on GD 13 (14 to 27 days after the last dose).

There were no meaningful drug-related macroscopic findings.

Organ Weights

Tissues and organs were collected in cohorts 1 and 2 males and females on the day of scheduled sacrifice. Cohort 1 animals were sacrificed on PND 112. Cohort 2 males were sacrificed on PND 135 and 137 (24 or 26 days after the last dose) and mated females were sacrificed on GD 13 (14 to 27 days after the last dose). The following organs were collected and their weights were measured (table taken from the Sponsor's study report).

Tissues and Organs collected in Cohort 1 and 2 Mice

Tissue	Weighed	Collected	Microscopic Evaluation	Comment
Animal identification	-	X	-	-
Artery, aorta	-	X	X	-
Body cavity, nasal	-	X	-	-
Bone marrow smear	-	X	-	Two bone marrow smears were collected from the femur. Smears were not collected from animals that were found dead. Bone marrow smears were allowed to air dry and were not fixed in formalin.
Bone marrow, femur	-	X	X	Right Femur
Bone marrow, sternum	-	X	X	-
Bone, femur	-	X	X	Left Femur: Retained whole, wrapped in saline soaked gauze and plastic wrap and placed in appropriately sized pouches on wet ice until stored in a freezer set to maintain -20°C within 2 hours of collection (scheduled euthanized animals only).
Bone, sternum	-	X	X	-
Brain (including the olfactory bulb and nasal cavity, if possible)	X	X	X	Seven brain levels examined to include olfactory bulb.
Cervix	-	X	X	Collected with uterus and oviduct.
Epididymis	X	X	X	Paired weight and examination
Esophagus	-	X	X	Infused with 10% neutral buffered formalin.
Eye	-	X	X	Collected with harderian gland and optic nerve. Paired examination; Preserved in Davidson's fixative; rinsed per Testing Facility SOP (scheduled euthanized animals only).
Gallbladder	-	X	X	-
Gland, adrenal	X	X	X	Paired weight and examination.
Gland, harderian	-	X	X	Collected with optic nerve and eye.
Gland, lacrimal	-	X	X	Only 1 required for examination.
Gland, mammary	-	X	X	All animals. For males: examined only if present in routine section of skin.
Gland, parathyroid	-	X	X	Examined only if present in the routine section of thyroid.
Gland, pituitary	X	X	X	Fixed weight[a]
Gland, prostate	X	X	X	-
Gland, salivary	-	X	X	Only 1 required for examination.
Gland, seminal vesicle	-	X	X	Paired examination.
Gland, thyroid	X	X	X	Paired fixed weight[a] and examination; weight includes parathyroid.
Gross lesions/masses	-	X	X	-
Gut-associated lymphoid tissue	-	X	X	Examined only if present in routine section of small intestine.
Heart	X	X	X	-

Tissue	Weighed	Collected	Microscopic Evaluation	Comment
Kidney	X	X	X	Paired weight and examination.
Large intestine, cecum[b]	-	X	X	-
Large intestine, colon[b]	-	X	X	-
Large intestine, rectum[b]	-	X	X	-
Liver	X	X	X	Gallbladder was drained before weighing
Lung	X	X	X	Infused with 10% neutral buffered formalin.
Lymph node, mandibular	-	X	X	Only 1 required for examination.
Lymph node, mesenteric	-	X	X	-
Muscle, skeletal	-	X	X	-
Nerve, optic	-	X	X	Collected with harderian gland and eye. Examined only if present in the routine section of the eye
Nerve, sciatic	-	X	X	Only 1 required for examination.
Ovary	X	X	X	Paired weight and examination.
Oviduct	-	X	X	Only 1 required for examination. Collected with uterus and cervix.
Pancreas	-	X	X	-
Skin	-	X	X	-
Small intestine, duodenum[a]	-	X	X	-
Small intestine, ileum[b]	-	X	X	-
Small intestine, jejunum[b]	-	X	X	-
Spinal cord	-	X	X	-
Spleen	X	X	X	-
Stomach[b]	-	X	X	-
Testis	X	X	X	Paired weight and examination; Preserved in Modified Davidson's fixative as per Testing Facility SOP (scheduled euthanized animals only).
Thymus	X	X	X	-
Tongue	-	X	X	-
Trachea	-	X	X	Infused with 10% neutral buffered formalin.
Ureter	-	X	X	Only 1 required for examination.
Urinary bladder	-	X	X	-
Uterus	X	X	X	Collected with oviduct and cervix.
Vagina	-	X	X	-

X = Procedure conducted; - = Not applicable; SOP = Standard Operating Procedures.

[a] The pituitary gland and thyroid/parathyroid glands for the Cohort 1 male and female mice were weighed after 8 to 13 days of fixation. These tissues were weighed in animal order by sex and dose group; ie, beginning with male mice in Group 1, then males in Group 2, …, and finishing with females in Group 5.

[b] Target tissues.

In cohort 1, statistically significant decreases in pituitary weight (absolute, %bodyweight, and/or %brain weight) were noted in the 3, 10, 0/100, and 0/300 males (up to -35.1% in absolute weight) and in the 10, 0/100, and 0/300 mg/kg/day females (up to -55.3% in absolute weight). The data are summarized in the Sponsor's table below.

Effects of Plecanatide in Pituitary Weight in Cohort 1 Mice on PND 112

	Males				
Group	**1**	**2**	**3**	**4**	**5**
Dose (mg/kg/day)	**0**	**3**	**10**	**0/100**	**0/300**
No. Animals Examined	**12**	**12**	**12**	**12**	**12**
Pituitary (No. Weighed)	12	12	12	12	10
Absolute value	0.0037	**0.0027****	0.0031	**0.0024****	**0.0024****
% of body weight[a]	8.928	**6.389****	**7.212***	**5.948****	**5.663****
% of brain weight	0.7	**0.5****	0.6	**0.5****	**0.4****

	Females				
Group	**1**	**2**	**3**	**4**	**5**
Dose (mg/kg/day)	**0**	**3**	**10**	**0/100**	**0/300**
No. Animals Examined	**12**	**12**	**12**	**12**	**12**
Pituitary (No. Weighed)	12	11	12	12	12
Absolute value	0.0038	0.0037	**0.0031***	**0.0021****	**0.0017****
% of body weight[a]	12.202	11.448	**9.586****	**6.705****	**5.303****
% of brain weight	0.7	0.7	**0.6***	**0.4****	**0.3****

Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – *P ≤ 0.05, ** P ≤ 0.01.

a. Value was multiplied by 1000.

The decreases in pituitary weight were not observed in the cohort 2 males and females. Cohort 2 males were sacrificed on PND 135 or 137 (24 or 26 days after the last dose) and mated females were sacrificed on GD 13 (14 to 27 days after the last dose).

Effects of Plecanatide in Pituitary Weight in Cohort 2 Mice on PND 135 or 137

	Males				
Group	**1**	**2**	**3**	**4**	**5**
Dose (mg/kg/day)	**0**	**3**	**10**	**0/100**	**0/300**
No. Animals Available	10	10	10	10	10
Pituitary (No. Weighed)	10	10	10	10	10
Absolute value	0.0037	0.0038	0.0036	0.0038	0.0038
% of body weight[a]	9.206	8.877	8.001	8.857	8.713

	Females				
Group	**1**	**2**	**3**	**4**	**5**
Dose (mg/kg/day)	**0**	**3**	**10**	**0/100**	**0/300**
No. Animals Available	10	10	10	10	10
No. Pregnant Animals	7	10	9	7	7
Pituitary (No. Weighed)	10	10	10	8	10
Absolute value	0.0052	0.0050	0.0052	0.0054	0.0049
% of body weight[a]	12.455	10.986	11.592	11.729	11.492

a. Value was multiplied by 1000.

No pituitary microscopic findings correlated with the organ weight changes.

There were other sporadic statistically significant organ weight changes. However, they lacked dose-dependency and are not considered as drug-related.

Histopathology

Tissues and organs were collected in cohort 1 males and females on the day of scheduled sacrifice (PND 112). The organs/tissues that were examined are listed in the table above (taken from Sponsor's study report).

Adequate Battery - Yes

Peer Review - No

Histological Findings

Notable microscopic findings are summarized in the table below.

Notable Microscopic Findings

Organ/Tissue	**Sex**	**Dose (mg/kg/day)**				
		0	3	10	0/100	0/300
Number/group/sex examined (M/F)		12/12	12/12	12/12	12/12	11/12
Adrenal Gland						
Subcapsular cell hyperplasia	M	2	-	0	-	1
	F	1	-	3	-	6
Harderian gland						
Mononuclear cell infiltration	M	0	-	2	-	0
	F	3	-	4	-	7
Lacrimal Gland						
Mononuclear cell infiltration	M	4	-	7	-	6
	F	1	-	3	-	2
Salivary Gland						
Mononuclear cell infiltration	M	2	-	5	-	2
	F	0	-	3	-	1
Kidney						
Mononuclear cell infiltration	M	0	-	2	-	0

	F	1	-	0	-	3
Spleen *Increased hematopoiesis*	M F	4 4	- -	6 8	- -	6 7

The findings are not considered as drug-related because they lacked dose-dependency.

Bone Evaluations

The left femur from all scheduled sacrificed mice in cohort 1 was cleaned of soft tissue at necropsy for bone evaluations. Bone densitometry was measured by *ex vivo* peripheral Quantitative Computed Tomography. Bone parameters, including total area, bone mineral content (BMC) and bone mineral density (BMD), trabecular BMC and BMD, cortical/subcortical BMC and BMD, cortical area and thickness, periosteal and endosteal circumference, and bone length, were evaluated.

No drug-related effects were observed for bone densitometry parameters at the distal femur metaphysis or femur diaphysis, bone geometry at the femur diaphysis, or femur bone length. The data are summarized in the Sponsor's tables below.

Bone Densitometry Values in Metaphysis Femur – Males

				Control	SP-304 3 mg/kg/day		SP-304 10 mg/kg/day		SP-304 0/100 mg/kg/day		SP-304 0/300 mg/kg/day	
		Units	Group	Value	Value	% vs C	Value	% vs C	Value	% vs C	Value	% vs C
Total	Area	mm^2	Mean	4.85	4.89	0.9	4.85	0.0	4.70	-3.2	4.76	-1.8
			SD	0.47	0.44		0.76		0.44		0.42	
	BMC	mg/mm	Mean	2.36	2.46	4.5	2.38	1.0	2.24	-4.8	2.46	4.2
			SD	0.29	0.22		0.50		0.19		0.23	
	BMD	mg/cm^3	Mean	486.15	504.49	3.8	489.39	0.7	478.59	-1.6	517.67	6.5
			SD	39.45	33.50		54.95		35.79		44.37	
Trabecular	BMC	mg/mm	Mean	0.75	0.81	9.1	0.77	3.6	0.68	-9.1	0.78	4.2
			SD	0.14	0.15		0.28		0.12		0.15	
	BMD	mg/cm^3	Mean	256.43	277.32	8.1	262.49	2.4	240.55	-6.2	272.33	6.2
			SD	41.57	44.23		68.52		36.68		44.00	
Cortical/ Subcortical	BMC	mg/mm	Mean	1.61	1.65	2.4	1.60	-0.3	1.56	-2.8	1.68	4.4
			SD	0.19	0.13		0.24		0.10		0.11	
	BMD	mg/cm^3	Mean	830.85	845.97	1.8	830.22	-0.1	836.18	0.6	885.49	6.6
			SD	62.88	48.10		50.58		59.25		63.06	

C = Control.

Bone Densitometry Values in Metaphysis Femur – Females

				Control	SP-304 3 mg/kg/day		SP-304 10 mg/kg/day		SP-304 0/100 mg/kg/day		SP-304 0/300 mg/kg/day	
		Units	Group	Value	Value	% vs C	Value	% vs C	Value	% vs C	Value	% vs C
Total	Area	mm^2	Mean	4.25	4.35	2.3	4.51	6.1	4.32	1.6	4.32	1.6
			SD	0.28	0.46		0.36		0.44		0.32	
	BMC	mg/mm	Mean	2.40	2.41	0.2	2.46	2.4	2.24	-6.5	2.33	-2.9
			SD	0.31	0.28		0.45		0.19		0.24	
	BMD	mg/cm^3	Mean	564.21	555.96	-1.5	542.63	-3.8	522.24	-7.4	540.08	-4.3
			SD	57.95	61.09		70.49		46.29		42.08	
Trabecular	BMC	mg/mm	Mean	0.80	0.76	-4.5	0.74	-6.9	0.65	-18.4	0.71	-11.2
			SD	0.19	0.16		0.23		0.11		0.13	
	BMD	mg/cm^3	Mean	312.56	294.34	-5.8	271.48	-13.1	252.53	-19.2	273.25	-12.6
			SD	70.06	68.17		72.36		47.68		50.65	
Cortical/ Subcortical	BMC	mg/mm	Mean	1.60	1.65	2.7	1.72	7.1	1.59	-0.6	1.62	1.3
			SD	0.17	0.18		0.25		0.12		0.15	
	BMD	mg/cm^3	Mean	941.48	947.92	0.7	949.45	0.8	926.29	-1.6	940.23	-0.1
			SD	59.49	60.79		85.43		57.46		50.28	

C = Control.

Bone Densitometry Values in Diaphysis Femur - Males

		Units	Group	Control Value	SP-304 3 mg/kg/day Value	SP-304 3 mg/kg/day % vs C	SP-304 10 mg/kg/day Value	SP-304 10 mg/kg/day % vs C	SP-304 0/100 mg/kg/day Value	SP-304 0/100 mg/kg/day % vs C	SP-304 0/300 mg/kg/day Value	SP-304 0/300 mg/kg/day % vs C
Length		mm	Mean	17.05	16.64	-2.4	16.61	-2.6	16.59	-2.7	16.91	-0.8
			SD	0.28	0.41		0.33		0.60		0.81	
Total Area		mm^2	Mean	2.70	2.73	0.8	2.72	0.6	2.72	0.6	2.77	2.3
			SD	0.23	0.27		0.35		0.19		0.25	
Cortical	Area	mm^2	Mean	1.69	1.77	4.7	1.66	-1.8	1.64	-3.0	1.76	4.1
			SD	0.20	0.22		0.18		0.14		0.19	
	BMC	mg/mm	Mean	2.04	2.15	5.6	1.99	-2.6	1.95	-4.4	2.15	5.3
			SD	0.27	0.29		0.24		0.19		0.27	
	BMD	mg/cm^3	Mean	1206.00	1214.50	0.7	1195.60	-0.9	1188.11	-1.5	1218.93	1.1
			SD	25.14	23.42		24.98		17.63		23.21	
	Thickness	mm	Mean	0.36	0.38	5.9	0.35	-2.4	0.34	-4.2	0.37	3.7
			SD	0.04	0.04		0.03		0.03		0.03	
Periosteal Circumference		mm	Mean	5.82	5.85	0.4	5.83	0.2	5.84	0.3	5.89	1.1
			SD	0.25	0.29		0.38		0.20		0.26	
Endosteal Circumference		mm	Mean	3.57	3.45	-3.1	3.63	1.8	3.68	3.2	3.55	-0.5
			SD	0.22	0.28		0.39		0.22		0.20	

C = Control.

Bone Densitometry Values in Diaphysis Femur - Females

		Units	Group	Control Value	SP-304 3 mg/kg/day Value	SP-304 3 mg/kg/day % vs C	SP-304 10 mg/kg/day Value	SP-304 10 mg/kg/day % vs C	SP-304 0/100 mg/kg/day Value	SP-304 0/100 mg/kg/day % vs C	SP-304 0/300 mg/kg/day Value	SP-304 0/300 mg/kg/day % vs C
Length		mm	Mean	16.68	16.52	-0.9	16.28	-2.4	16.24	-2.6	16.46	-1.3
			SD	0.42	0.66		0.50		0.57		0.41	
Total Area		mm^2	Mean	2.15	2.24	4.2	2.22	3.5	2.21	2.9	2.20	2.4
			SD	0.19	0.19		0.35		0.23		0.23	
Cortical	Area	mm^2	Mean	1.32	1.34	1.2	1.38	4.6	1.31	-0.8	1.34	1.1
			SD	0.14	0.09		0.28		0.13		0.15	
	BMC	mg/mm	Mean	1.56	1.57	0.6	1.63	4.9	1.52	-2.2	1.57	0.9
			SD	0.18	0.13		0.39		0.16		0.20	
	BMD	mg/cm^3	Mean	1177.93	1172.96	-0.4	1175.48	-0.2	1163.60	-1.2	1173.53	-0.4
			SD	21.96	24.80		41.33		18.66		20.73	
	Thickness	mm	Mean	0.31	0.31	-1.2	0.32	3.0	0.30	-3.3	0.31	-0.4
			SD	0.02	0.02		0.05		0.02		0.02	
Periosteal Circumference		mm	Mean	5.19	5.30	2.0	5.27	1.5	5.26	1.4	5.25	1.2
			SD	0.23	0.23		0.40		0.27		0.27	
Endosteal Circumference		mm	Mean	3.22	3.35	4.1	3.24	0.7	3.36	4.3	3.29	2.2
			SD	0.17	0.31		0.27		0.24		0.23	

C = Control.

Fertility Parameters

In cohort 2 females, estrous cycles were evaluated by examining the vaginal cytology of samples obtained by vaginal lavage. Samples were collected for 15 consecutive days (beginning at PND 97-102) before initiation of the cohabitation period, and until spermatozoa were observed in a smear of the vaginal contents and/or a copulatory plug was observed during the mating period. Within each dose group, a male was assigned randomly to mate with a female starting on PNDs 111 to 116 (after completion of the dosing period). The mating period was up to 10 days. Females with a copulatory plug were considered to be at GD 0, and assigned to individual housing.

There were no drug-related effects on estrous cycles or any of the mating and fertility parameters in males or females. The data are summarized in the Sponsor's tables below.

Mating and Fertility in Males

GROUP		1	2	3	4	5
TEST MATERIAL		CONTROL ARTICLE	SP-304	SP-304	SP-304	SP-304
DOSE LEVEL (MG/KG/DAY)		0a	3a	10a	0/100b	0/300c
MICE IN COHABITATION	N	22	22	22	22	21d
DAYS IN COHABITATION	MEAN±S.D.	2.2 ± 1.0	2.7 ± 2.2	3.4 ± 2.4	2.5 ± 2.2	2.8 ± 2.0
ANIMALS THAT MATED	N(%)	22(100.0)	22(100.0)	22(100.0)	22(100.0)	21(100.0)
FERTILITY INDEX e	N/N	19/ 22	21/ 22	19/ 22	18/ 22	18/ 21
	(%)	(86.4)	(95.4)	(86.4)	(81.8)	(85.7)
MICE WITH CONFIRMED MATING DATES	N	22	22	22	22	21
MATED WITH FEMALE						
DAYS 1- 7	N(%)	22(100.0)	21(95.4)	20(90.9)	21(95.4)	21(100.0)
DAYS 8-14	N(%)	0(0.0)	1(4.5)	2(9.1)	1(4.5)	0(0.0)
MICE PREGNANT/MICE IN COHABITATION	N/N	19/ 22	21/ 22	19/ 22	18/ 22	18/ 21
	(%)	(86.4)	(95.4)	(86.4)	(81.8)	(85.7)

a. Dose administration occurred on Postnatal Days 14 through 111.
b. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 100 mg/kg/day on Postnatal Days 21 through 111).
c. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 300 mg/kg/day on Postnatal Days 21 through 111).
d. Excludes values for male 4303, which was not assigned to cohabitation because there were no available female mice.
e. Number of pregnancies/number of males that mated.

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Mating and Fertility in Females

GROUP TEST MATERIAL DOSE LEVEL (MG/KG/DAY)		1 CONTROL ARTICLE 0a	2 SP-304 3a	3 SP-304 10a	4 SP-304 0/100b	5 SP-304 0/300c
ESTROUS CYCLING OBSERVATIONS						
MICE TESTED	N	22	22	22	22	21d
PRECOHABITATION ESTROUS CYCLING						
ESTROUS STAGES/ 15 DAYS	MEAN±S.D.	2.5 ± 0.8	2.1 ± 0.7	2.2 ± 0.7	1.9 ± 0.9	2.1 ± 0.8
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N(%)	10(45.4)	10(45.4)	11(50.0)	13(59.1)	12(57.1)
MICE WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

a. Dose administration occurred on Postnatal Days 14 through 111.
b. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 100 mg/kg/day on Postnatal Days 21 through 111).
c. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 300 mg/kg/day on Postnatal Days 21 through 111).
d. Excludes values for mouse 2108, which was euthanized on Postnatal Day 36 due to adverse clinical observations.

GROUP TEST MATERIAL DOSE LEVEL (MG/KG/DAY)		1 CONTROL ARTICLE 0a	2 SP-304 3a	3 SP-304 10a	4 SP-304 0/100b	5 SP-304 0/300c
MATING OBSERVATIONS						
FEMALES IN COHABITATION	N	22	22	22	22	21d
DAYS IN COHABITATION	MEAN±S.D.	2.2 ± 1.0	2.7 ± 2.2	3.4 ± 2.4	2.5 ± 2.2	2.8 ± 2.0
FEMALES THAT MATED	N(%)	22(100.0)	22(100.0)	22(100.0)	22(100.0)	21(100.0)
FERTILITY INDEX e	N/N (%)	19/ 22 (86.4)	21/ 22 (95.4)	19/ 22 (86.4)	18/ 22 (81.8)	18/ 21 (85.7)
FEMALES WITH CONFIRMED MATING DATES	N	22	22	22	22	21
MATED BY FIRST MALE						
DAYS 1-7	N(%)	22(100.0)	21(95.4)	20(90.9)	21(95.4)	21(100.0)
DAYS 8-14	N(%)	0(0.0)	1(4.5)	2(9.1)	1(4.5)	0(0.0)
MATED BY SECOND MALE	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
FEMALES PREGNANT/FEMALES IN COHABITATION	N/N (%)	19/ 22 (86.4)	21/ 22 (95.4)	19/ 22 (86.4)	18/ 22 (81.8)	18/ 21 (85.7)

a. Dose administration occurred on Postnatal Days 14 through 111.
b. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 100 mg/kg/day on Postnatal Days 21 through 111).
c. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 300 mg/kg/day on Postnatal Days 21 through 111).
d. Excludes values for mouse 2108, which was euthanized on Postnatal Day 36 due to adverse clinical observations.
e. Number of pregnancies/number of females that mated.

Cesarean-Sectioning and Litter observations

All surviving dams were sacrificed on GD 13, and a cesarean section was performed. The uterus from each gravid animal was excised, and examined for the number and distribution of corpora lutea, implantation sites, placentae (size, color, or shape), and viable and nonviable fetuses.

There were no drug-related effects on any of the parameters examined. All placentae appeared normal. The data are summarized in the Sponsor's tables below.

Summary of Cesarean Section Data

GROUP TEST MATERIAL DOSE LEVEL (MG/KG/DAY)		1 CONTROL ARTICLE 0a	2 SP-304 3a	3 SP-304 10a	4 SP-304 0/100b	5 SP-304 0/300c
MICE TESTED	N	22	22	22	22	22
INCLUDED IN ANALYSES	N	22	22	22	22	21d
PREGNANT	N(%)	19(86.4)	21(95.4)	19(86.4)	18(81.8)	18(85.7)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	19	21	19	18	18
CORPORA LUTEA	MEAN±S.D.	16.2 ± 1.8	14.2 ± 2.9	15.1 ± 1.7	16.0 ± 2.5	15.5 ± 2.0
IMPLANTATIONS	MEAN±S.D.	15.5 ± 1.8	13.8 ± 3.1	14.8 ± 1.7	15.7 ± 2.2	15.0 ± 1.9
% PREIMPLANTATION LOSS	MEAN±S.D.	4.4 ± 5.4	3.2 ± 6.9	1.7 ± 3.0	2.2 ± 3.3	3.1 ± 4.3
VIABLE EMBRYOS	N MEAN±S.D.	269 14.2 ± 2.8	272 13.0 ± 3.4	273 14.4 ± 1.5	261 14.5 ± 2.8	261 14.5 ± 2.1
NONVIABLE EMBRYOS	N MEAN±S.D.	25 1.3 ± 2.4	19 0.9 ± 1.3	9 0.5 ± 1.0	21 1.2 ± 1.9	9 0.5 ± 0.8
% POSTIMPLANTATION LOSS	MEAN±S.D.	8.3 ± 15.7	7.3 ± 9.5	2.9 ± 5.8	7.4 ± 11.3	3.4 ± 5.8
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	8(42.1)	9(42.8)	5(26.3)	9(50.0)	6(33.3)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	19(100.0)	21(100.0)	19(100.0)	18(100.0)	18(100.0)
PLACENTAE APPEARED NORMAL	N(%)	19(100.0)	21(100.0)	19(100.0)	18(100.0)	18(100.0)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100

a. Dose administration occurred on Postnatal Days 14 through 111.
b. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 100 mg/kg/day on Postnatal Days 21 through 111).
c. Dose administration occurred on Postnatal Days 14 through 111 (at a dose level of 0 mg/kg/day on Postnatal Days 14 through 20 and at a dose level of 300 mg/kg/day on Postnatal Days 21 through 111).
d. Excludes values for mouse 2108, which was euthanized on Postnatal Day 36 due to adverse clinical observations.

Special Evaluation

N/A

Toxicokinetics

N/A

Dosing Solution Analysis

Stock assay solutions were tested appropriately for stability and drug concentration. Mean concentrations of the 0.6, 2.0, 20, and 60 mg/ml dosing solutions ranged from 96.2% to 107% of nominal concentrations. The homogeneity values were 0.4%, 0.4%, 0.6%, and 0.7% RSD for the 0.6, 2.0, 20, and 60 mg/ml formulations, respectively.

In summary, the NOAEL for treatment initiated on PND 14 was 3 mg/kg/day, based on deaths at 10 mg/kg/day. The NOAEL for treatment initiated on PND 21 was 300 mg/kg/day, the highest dose tested.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Plecanatide (SP-304) is a synthetic analog of the endogenous human hexadecapeptide uroguanylin (UG). It differs from UG in the substitution of aspartate at the third position with glutamate. Plecanatide and its metabolite SP-338 bind with high affinity to GC-C (guanylate cyclase C) and stimulate cGMP production in T84 human colon carcinoma cells and mice epithelial cells in vitro. In vitro studies demonstrated that receptor binding affinities and cGMP-stimulatory activities of plecanatide were enhanced at pH 5, as compared to pH 8. By contrast, GC-C binding affinity of linaclotide, an approved drug from the same pharmacological class, was not appreciably different at pH 5 and 8. In addition, a study in T84 cells and an *in situ* study in proximal intestine and colon tissues from mice demonstrated that the activity of plecanatide to stimulate the short circuit *Isc* current was higher at pH 5, as compared to that at pH 8. By contrast, the stimulation of *Isc* by STp and linaclotide were comparable at pH 5 and 8 in either of the tissue segments. The charged N-terminal amino acids of plecanatide produced an increase in GC-C binding affinity and cGMP-stimulatory activity at pH 5, as compared to pH 8. In secondary pharmacology screening studies, plecanatide did not exhibit any off-target binding to a large number of targets.

Plecanatide and SP-338 accelerated intestinal transit and stimulated fluid secretion in rat ligated duodenal and/or ileal loops. In animal models of visceral hypersensitivity, plecanatide reduced abdominal contractions induced by inflammation (TNBS-induced colitis) or stress (partial restraint). In addition, in mouse models of colitis using TNBS or DSS induction, plecanatide attenuated some of the adverse pathology and clinical signs associated with these animal models.

In safety pharmacology studies, plecanatide had no significant effects on hERG channel current at concentrations up to 1000 µM. Plecanatide produced a small but dose-dependent increase in heart rate in monkeys at oral doses of 0.1, 25, and 2000 mg/kg. A transient slight decrease in systolic and diastolic pressure occurred in the 2000 mg/kg group. No other ECG parameters, including QT_c, were affected by plecanatide. In the respiratory safety study in mice, single oral doses up to 2000 mg/kg did not affect respiratory function under the study conditions. However, the results were considered to be inconclusive due to the use of an extremely high dose volume (30 ml/kg). Similarly, in the gastrointestinal safety study in mice, no conclusions about the effects of plecanatide can be made because of the extremely high dose volume. Plecanatide had no effects on behavioral or neurological parameters in mice treated orally with up to 2000 mg/kg.

Plecanatide is rapidly absorbed and degraded/metabolized following oral administration. Oral bioavailability is extremely limited; in mice and monkeys it is <0.1%. SP-338, a 15-mer C-terminal des-leucine metabolite of plecanatide, is found to be the only active metabolite. A putative metabolic degradation pathway for plecanatide was proposed based on peptide degradants that were identified in ligated intestinal loops. The major route of elimination of ^{14}C-derived radioactivity following a single oral administration of ^{14}C-plecanatide to rats is from expired air, with lesser amounts in feces and urine. Catabolism of ^{14}C-plecanatide followed by incorporation of ^{14}C into other proteins or molecules may account for the presence of radioactivity in plasma, tissues, and carcass at 72 hours post-dose. Plecanatide did not bind to human serum albumin (HSA) or human α1-acid glycoprotein (AGP). Neither plecanatide nor SP-338 is an inhibitor of CYP2C9 or CYP3A4, or an inducer of CYP3A4 cytochrome P450 enzymes. Furthermore, they are not substrates or inhibitors of the P-gp or BCRP transporters.

The toxicity of plecanatide was evaluated in single- and repeated-dose studies in rats, mice, and monkeys. In all three species, plecanatide was generally well tolerated. In the 13-week oral toxicity study in mice, animals were treated with 0 (vehicle), 20, 200, or 800 mg/kg/day plecanatide for 13 weeks. Recovery animals were treated with 0 or 800 mg/kg/day plecanatide for 13 weeks, followed by a 2-week recovery period. There were no clear drug-related clinical signs, although some of the 800 mg/kg/day animals showed an unkempt appearance. Microscopic findings were observed in the following tissues: tibiofemoral joint (synovial hyperplasia/hypertrophy, subacute/chronic inflammation), liver (mononuclear cell infiltration), ovary (follicular cyst), and pituitary gland (focal necrosis). It is noted that these findings were not observed in the subsequent 26-week oral toxicity study in mice at doses up to 150 mg/kg/day. At the end of the 2-week recovery period, liver mononuclear cell infiltration, synovial hyperplasia/hypertrophy, and ovary follicular cyst were still evident and did not appear to be completely reversible. Systemic exposure levels increased in approximate proportion to dose between 200 and 800 mg/kg/day, and there was no significant drug accumulation after repeated dosing. The NOAEL is considered to be 20 mg/kg/day based on the microscopic findings at 200 and 800 mg/kg/day.

In the 13-week oral toxicity study in rats, animals were treated with 0 (vehicle), 30, 100, or 300 mg/kg/day plecanatide for 13 weeks. There were no deaths during the study. A dose-dependent decrease in bodyweight gain in the females, although not statistically significant, was noted. This decrease in bodyweight gain appears to correlate with significant decreases in food consumption in the 300 mg/kg/day females. There were no meaningful drug-related clinical signs, and no changes in hematology or clinical chemistry were observed. There were no drug-related macroscopic or microscopic findings. The NOAEL in males is considered to be 300 mg/kg/day. The NOAEL in females is considered to be 100 mg/kg/day based on the decrease in bodyweight gain. The maximum tolerated dose (MTD) is considered to be 100 mg/kg/day in females. Plecanatide was detected in plasma at all dose levels. There was some drug accumulation between day 1 and day 84 after repeated dosing.

In the 13-week oral toxicity study in monkeys, animals were treated with 0 (vehicle), 1, 10, or 100 mg/kg/day plecanatide for 13 weeks. Recovery animals were treated with 0 or 100 mg/kg/day plecanatide for 13 weeks, followed by a 2-week recovery period. There were no deaths during the study. Red discolored skin and dry skin were noted in the 100 mg/kg/day males and females. A dose-related increase in the incidence of loose/liquid stools occurred in males and females. This is considered a pharmacologic effect of plecanatide, and is not considered as adverse. The incidence of sinus tachycardia was greater in the drug-treated groups as compared to controls, although there was no clear dose relationship. The effect was not considered adverse because sinus tachycardia was considered to be a normal variant in monkeys. There were no significant changes in RR, PR, QT_c intervals, or QRS duration. There were no drug-related effects on hematology, clinical chemistry, or urinalysis parameters, and no meaningful organ weight changes, macroscopic findings, or microscopic findings. The NOAEL was considered to be 100 mg/kg/day, the highest dose studied.

In the 26-week oral toxicity study in mice, animals were treated with 0 (vehicle), 20, 60, 150, or 400 mg/kg/day plecanatide. Recovery animals were treated with 0, 150, or 400 mg/kg/day plecanatide for 26 weeks, followed by a 4-week recovery period. Eighteen animals were found dead or euthanized *in extremis.* The deaths are not considered drug-related because there were no clinical observations which indicated toxicity, no consistent macroscopic or microscopic findings that support a particular cause of death, and there was no dose relationship. Significant decreases in absolute reticulocyte count were observed in the 400 mg/kg/day males (-26%) and females (-25%) during the treatment period, and the effect was reversible after the recovery period. Sciatic nerve axonal/myelin degeneration was observed in the 400 mg/kg/day males and females. There was no significant drug accumulation between day 1 and week 26 after repeated dosing. The average C_{max} and $AUC_{0\text{-}tlast}$ values for both sexes in the 400 mg/kg/day group were 150 ng/mL and 30.7 ng·h/mL, respectively. The NOAEL is considered to be 150 mg/kg/day based on a significant decrease in absolute reticulocyte count and sciatic nerve axonal/myelin degeneration at 400 mg/kg/day plecanatide.

In the 39-week orally toxicity study in monkeys, animals were treated with 0 (vehicle), 2, 10, or 100 mg/kg/day plecanatide. There were no deaths during the study. A drug-related increase in the incidence of loose/watery stools occurred in males and females at doses ≥ 2 mg/kg/day. This is considered a pharmacologic effect of plecanatide, and is not considered as adverse. A slightly higher incidence of sinus tachycardia was observed in the 100 mg/kg/day group. This effect was not considered adverse because sinus tachycardia was considered to be a normal variant in monkeys. There were no significant drug-related changes in RR, PR, QT_c intervals, or QRS duration. There were no drug-related effects on hematology, clinical chemistry, or urinalysis parameters, and no meaningful organ weight changes, macroscopic findings, or microscopic findings. The NOAEL was considered to be 100 mg/kg/day, the highest dose studied. Systemic exposure (AUC) increased with dose between 2 to 100 mg/kg/day, and exposures during weeks 1 and 39 were approximately equal.

In the reproductive toxicity study in mice, males were administered 0 (vehicle), 20, 200, or 600 mg/kg/day plecanatide by oral gavage beginning 28 days prior to mating, during mating, and until necropsy on dosing days 63 to 66. Females were treated with plecanatide for at least 14 days prior to mating, during mating, and through gestation day (GD) 7. Twenty-five mice/sex/group remained untreated throughout the study. Each drug-treated male was mated with an untreated female, and each female in the drug-treated group was assigned to mate with an untreated male. Females (untreated and treated) with a copulatory plug *in situ* or spermatozoa present in a smear of the vaginal contents were considered to be at GD 0, and assigned to individual housing. Males and females were sacrificed on dosing days 63 to 66 and GD 13, respectively. There were no meaningful drug-related changes in bodyweight or bodyweight gain in males or females. In males, there were no drug-related changes in sperm motility or density. In females, there were no drug-related effects on estrous cycle, percentage pregnancy rate (total pregnant/number mated), the number of corpora lutea, implantation sites, live or dead embryos, pre- or post-implantation losses, or dams with all nonviable embryos. The NOAEL for paternal and maternal toxicity is considered to be 600 mg/kg/day, based on the absence of drug-related paternal or maternal findings at this dose. The NOAEL for male and female fertility, and early embryonic development is considered to be 600 mg/kg/day.

The developmental toxicity of plecanatide was studied in mice and rabbits. In the embryo-fetal developmental study in mice, pregnant mice were administered 0 (vehicle), 20, 200, or 800 mg/kg/day plecanatide by oral gavage on GD 6-15. C-sections were performed on GD 18. There were no drug-related deaths, and there were no meaningful drug-related clinical signs or bodyweight changes during gestation. No drug-related macroscopic findings were noted when the dams were necropsied. All c-section parameters, including pregnancy rate, corpora lutea, implantation sites, resorptions (early or late), sex ratio, number of live fetuses, fetal weights, and the pre- and post-implantation losses in each treatment group, were comparable to that of the control group. There were no drug-related fetal external findings, significant increases in soft tissue or skeletal alterations (variations or malformations), or fetal ossification sites per fetus. The NOEL for maternal toxicity is considered to be 800 mg/kg/day, based on the lack of adverse findings at the high dose. For embryo-fetal development, the NOEL is considered to be 800 mg/kg/day.

In the embryo-fetal developmental study in rabbits, pregnant animals were administered 0 (vehicle), 15, 75, or 250 mg/kg/day plecanatide (SP-304) orally by stomach tube on GD 6-19. C-sections were performed on GD 29. There were no drug-related deaths. Drug-related clinical signs included soft feces and scant feces in groups receiving plecanatide at 15 mg/kg/day or higher, and liquid feces or both soft and liquid feces in the 250 mg/kg/day group. These clinical signs were likely related to the pharmacology of plecanatide, and they are not considered as adverse. There were no meaningful drug-related effects on bodyweight, bodyweight gain, or food consumption during gestation. No quantifiable levels of plecanatide were detected prior to dosing on GD 19. Plasma exposure to plecanatide (AUC) was less than dose-proportional on GD 7 and GD 19. There was no or minimal drug accumulation in plasma after repeated dosing.

All c-section parameters in each treatment group, were comparable to that of the control group. All placentae appeared normal and there were no dead fetuses. There were no drug-related fetal external findings, significant increases in soft tissue or skeletal alterations (variations or malformations), or fetal ossification sites per fetus. The NOAEL for maternal toxicity is considered to be 250 mg/kg/day, based on the lack of adverse findings at the high dose. The NOEL for embryo-fetal development is considered to be 250 mg/kg/day based on the lack of drug-related findings.

In the pre- and postnatal developmental study in mice, mated female mice were administered 0 (vehicle), 20, 200, or 600 mg/kg/day plecanatide by oral gavage from GD 6 to day 20 of lactation. F1 generation mice were allowed to mature untreated, and the effects of plecanatide on growth, development, and behavior were evaluated. At weaning, males and females were randomly selected for continued evaluation. The selected F1 generation males and females from each dose group were mated at approximately 90 days of age. The F0 females were sacrificed on day 21 of lactation (weaning of litters). A necropsy was conducted on F1 generation pups prematurely killed, found dead during lactation, or unselected pups after weaning. Mated F1 generation adult males were necropsied after completion of the mating period. Mated F1 generation females were sacrificed on GD 18. Plecanatide had no effects on pregnancy or litter parameters in F0 females. There were no effects on physical development of the F1 generation pups during lactation, or learning and memory, auditory startle reflex, or motor activity post-weaning. No effects on mating performance, fertility, or pregnancy parameters in the F1 generation animals were observed. The NOAEL for maternal effects (F0 females) and their offspring (F1 generation) was 600 mg/kg/day.

Neonatal/juvenile mice have been shown to be particularly sensitive to plecanatide toxicity. In the single-dose oral toxicity study in neonatal/juvenile mice, pups were administered a single dose of 0 (vehicle), 1, 3, 10, or 20 mg/kg plecanatide on PND 14, or a single dose of 0 (vehicle control), 25, 50, 100, or 300 mg/kg plecanatide on PND 21. The pups were sacrificed 3 days after plecanatide administration. Deaths occurred at 10 and 20 mg/kg in pups administered plecanatide on PND 14. Clinical signs included loss of righting reflex, decreased motor activity, ataxia, cold to the touch, and moderate dehydration (based on skin turgor). No drug-related gross lesions were identified at necropsy of the mice that died prior to scheduled sacrifice. There were no deaths or clinical signs in pups administered plecanatide on PND 21.

In the repeated-dose oral toxicity study in neonatal mice, plecanatide was administered by oral gavage at 0.05, 0.1, 0.5, or 1.0 mg/kg/day to PND 7 mice for 7 days, followed by a 2-day observation period. Doses of 0.5 and 1.0 mg/kg/day resulted in mortality within the first or second day after dosing. Clinical signs included dehydration in the 0.5 and 1.0 mg/kg/day groups, and decreased motor activity in the 1.0 mg/kg/day group. Decreases in bodyweight and bodyweight gain, compared to controls, were noted in the 0.5 and 1.0 mg/kg/day groups. No gross lesions were noted at necropsy. There were no deaths or clinical signs in PND 7 mice administered 0.1 mg/kg/day plecanatide.

In the oral range-finding study in juvenile mice, PND 14 mice were administered 0 (vehicle), 1, 3, or 10 mg/kg/day plecanatide for 14 days, and PND 21 mice were administered 0 (vehicle), 30, 100, or 300 mg/kg/day plecanatide for 14 days. In the PND 14 group, two males and one female in the 10 mg/kg/day group were found dead on the day following the first dose. The pups showed decreased motor activity following dose administration the previous day. Mild dehydration was reported in one male and two females in the 10 mg/kg/day group that survived until scheduled sacrifice. Animals in the PND 14 group showed increase in the weight of small intestine (with contents) and intestinal contents at all doses. The changes occurred at the 15-minute interval after plecanatide administration and continued until the last evaluation at 4 hours after drug administration. The weights generally were comparable among the control and drug-treated groups after 2 weeks of repeated dosing on PND 27. All the mice in the PND 21 group survived until scheduled sacrifice, and doses of plecanatide as high as 300 mg/kg/day were not associated with any adverse clinical signs. Plecanatide administration starting on PND 21 produced a modest increase in the weight of the small intestine (with contents) and intestinal content at one or more doses. On PND 34 after repeated dosing, these weights were generally comparable among the control and drug-treated groups. The increase in intestine weight after plecanatide administration appears to be consistent with the pharmacological action of plecanatide.

In the repeated-dose toxicity study in juvenile mice, PND 14 pups were administered 0 (vehicle), 3, 10, 0/100, and 0/300 mg/kg/day plecanatide through PND 111. In the 0/100 and 0/300 mg/kg/day groups, pups received only vehicle from PND 14 to 20. In the 10 mg/kg/day group, 12 of 64 pups were either found dead or missing on the day following the first dose of plecanatide on PND 14. No consistent clinical signs were associated with the deaths. In the fertility evaluation phase of the study, no drug-related effects on bodyweight or bodyweight gain were observed in females during the gestation period (GD 0 to 13). There were no drug-related effects on mating and fertility, cesarean section parameters, or litter parameters. The NOAEL for treatment initiated on PND 14 was 3 mg/kg/day, based on deaths at 10 mg/kg/day. The NOAEL for treatment initiated on PND 21 was 300 mg/kg/day, the highest dose tested.

Therefore, lethality of plecanatide was found to be highly age-dependent; the minimum lethal doses in PND 7 and PND 14 mice were 0.5 and 10 mg/kg/day, respectively. There were no deaths at up to 300 mg/kg/day in juvenile mice administered plecanatide starting on PND 21. The minimum lethal dose of 0.5 mg/kg/day at PND 7 is 10 times the recommended human dose of 3 mg/day (0.05 mg/kg/day based on a 60-kg bodyweight). It is noted that the levels of expression of the receptor for heat-stable enterotoxin (i.e. GC-C) in the small and large intestine of children is age dependent; a greater number of receptors are present in infants and the number decreases with increasing age (Cohen et al., Gastroenterology, 94: 367-373, 1988).

It is not known whether plecanatide is excreted in milk. It is noted that neither plecanatide nor its active metabolite were detected in human plasma after administration of the recommended dose level. Therefore, based on the low oral

bioavailability, it appears unlikely that measurable levels of plecanatide or its active metabolite would be present in the milk of lactating women.

Plecanatide does not appear to be mutagenic or clastogenic; it tested negative in the Ames assay, the *in vitro* L5178Y/TK$^{+/-}$ mouse lymphoma mutation assay, and the in vivo mouse bone marrow micronucleus assay.

Plecanatide was not tumorigenic in rats or mice. In the 104-week oral carcinogenicity studies, plecanatide at doses up to 100 mg/kg/day in rats and 90 mg/kg/day in mice had no significant effects on survival rates, clinical signs, incidence of masses detected in clinical observations, body weights, food consumption, hematology, macroscopic observations, or microscopic findings (neoplastic and non-neoplastic) in the males or females.

There are no novel excipients used in the manufacture of plecanatide tablets. The excipients to be used in the plecanatide formulation appear to be safe. FDA Inactive Ingredients Database confirms that all the excipients are present in approved oral formulations at levels (e.g., mg/tablet) that exceed the estimated maximum daily dose (mg) in plecanatide tablets.

In a pre-NDA meeting held on October 30, 2014, the CMC and nonclinical teams agreed with the Sponsor that the reporting, identification, and qualification thresholds for potential impurities should be (b) (4) respectively. The Sponsor has reported and identified five drug product impurities (b) (4) (b) (4) (b) (4) Taken together, the process impurities/ degradation products (b) (4) (b) (4) are considered qualified.

In summary, plecanatide acts locally in the intestine. The molecular target of plecanatide is the membrane guanylate cyclase-C (GC-C) receptor, located on the luminal surface of the intestine. The oral bioavailability of plecanatide is extremely

limited in the animal species studied. The metabolite, SP-338, has the same pharmacological activity as plecanatide, and likely contributes to the therapeutic effects. Oral toxicity studies in rats, mice, and monkeys showed that plecanatide is well tolerated in all three species at dose levels markedly higher than the proposed recommended clinical dose on a bodyweight basis (see table below). However, neonatal/juvenile mice are particularly sensitive to the toxicity of plecanatide. The minimum lethal dose of plecanatide in mice on PND 7 was 10 times the recommended human on a bodyweight basis. Plecanatide had no effects on mating or fertility in mice. No developmental toxicity was observed in the segment 2 studies in mice and rabbits, or in the segment 3 study in mice. Plecanatide is neither mutagenic nor tumorigenic. The table below summarizes dose multiples of the maximum recommended clinical dose at the NOAELs. Plecanatide and its active metabolite are not measurable in human plasma following administration of the recommended clinical dose. Therefore, animal and human doses should not be compared directly for evaluating relative plecanatide exposure.

Study	Species	NOAEL (mg/kg/day) M/F	Multiples of Human Dose
13-week oral	Rat	M: 300; F: 100	6,000; 2,000
	Mouse	M & F: 20	400
	Monkey	M & F: 100	2,000
26-week oral	Mouse	M & F: 150	3,000
39-week oral	Monkey	M & F: 100	2,000
Segment 1 Reproductive	Mouse	M & F: 600	12,000
Segment 2 Reproductive	Mouse	F: 800	16,000
	Rabbit	F: 250	5,000
Segment 3 Reproductive	Mice	M & F: 600	12,000
Neonatal PND 7	Mice	M & F: 0.1	2
Juvenile PND 14 and 21	Mice	M&F: 3 (PND 14) M&F: 300 (PND 21)	60 6,000
Juvenile PND 14 and 21	Mice	M&F: 3 (PND 14) M&F: 300 (PND 21)	60 6,000

Recommended Human Dose: 3 mg/day or 0.05 mg/kg/day based on a 60-kg bodyweight

cc:
Orig NDA 208,745
DGIEP
DGIEP/PM
DGIEP/D. Joseph
DGIEP/Y.-C. Ng
DGIEP/L. Hanes
DGIEP/L. Muldowney
OND IO/A. Jacobs
R/D INIT.: D. Joseph 10/17/2016

12 Appendix/Attachments

None

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/s/

YUK-CHOW NG
10/18/2016
From a nonclinical standpoint, there are no approvability issues.

DAVID B JOSEPH
10/18/2016
I concur.

Comments from A. Jacobs, AD

For NDA 208745

Date: 10/13/16

I concur that there are no pharm-tox related approval issues.

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/s/

ABIGAIL C JACOBS
10/14/2016