CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202292Orig1s000

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	20-2292
Supporting document/s:	001
Applicant's letter date:	December 5, 2011
CDER stamp date:	December 5, 2011
Product:	Crofelemer Tablets
Indication:	Control and symptomatic relief of diarrhea in patients with HIV/AIDS on anti-retroviral therapy
Applicant:	Salix Pharmaceuticals
Review Division:	Division of Gastroenterology and Inborn Errors Products
Reviewer:	Sruthi Tallapragada King, PhD
Supervisor/Team Leader:	Sushanta Chakder, PhD
Division Director:	Donna Griebel, MD
Project Manager:	Kevin Bugin MS, RAC

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1 Executive Summary

1.1 Introduction

Crofelemer is a botanical agent which is a potent inhibitor of cAMP-stimulated cystic fibrosis transmembrane conductance regulator chloride channel and the calcium activated chloride channel in intestinal epithelial cells. In this submission, the applicant is proposing to market crofelemer for the control and symptomatic relief of diarrhea in patients with HIV/AIDS on anti-retroviral therapy. Secretory diarrhea is attributed to poor adherence to HAART (highly active antiretroviral therapy) and there is currently no approved product for this indication. The proposed dose for patients on HAART is 125 mg to be taken twice daily.

1.2 Brief Discussion of Nonclinical Findings

In support of the proposed NDA, the applicant conducted pharmacology, pharmacokinetics, general toxicology, genetic and reproductive toxicology studies. Briefly, crofelemer was shown to inhibit chloride ion secretion via cAMP/cGMP-mediated mechanism and decrease in fluid accumulation in an *in vivo* mouse model of secretory diarrhea. In safety pharmacology studies, crofelemer did not show any effects on cardiovascular, respiratory, neurobehavioral, or GI motility in rats at the highest dose tested (600 mg/kg).

Crofelemer was shown to be poorly absorbed after oral administration in rats, with ~1% bioavailability, while ~99% remained in the GI tract, suggesting that it acts locally in the GI tract. *In vitro*, crofelemer produced dose-dependent inhibition of hERG (human ether-a-go-go) K^+ current; however, because of its low oral bioavailability, the potential risk of QT prolongation due to <2% free crofelemer is likely minimal to none. Plasma concentrations of crofelemer were low even at the highest doses evaluated (up to 1200 mg/kg/day for 56 days in mice). Crofelemer was highly bound to human plasma proteins and precipitated pepsin but did not induce CYP metabolic enzymes.

In dogs, chronic oral administration of crofelemer for 9 months was not fatal but did produce dose-dependent gastrointestinal (GI) toxicity (emesis, abnormal excreta, diarrhea) and histological changes related to GI tract irritation and macrophage infiltration in the lymph nodes at doses greater than 175 mg/kg/day. At the highest dose tested (600 mg/kg/day), crofelemer produced significant decreases in body weight and food consumption and changes in clinical chemistry indicative of nutritional deficits. The NOAEL dose in dogs was 50 mg/kg/day, based on GI-related clinical signs at doses of $\geq$175 mg/kg/day. When administered chronically in mice and rats, crofelemer produced deaths at doses above 40 mg/kg/day, in some cases due to dosing-related injuries; however, the cause of death of many of these animals was not known. In rhesus monkeys, dose-dependent histopathological changes (increased presence of pigmented macrophages) in the small intestine and cecum were observed when crofelemer was administered orally at up to 100 mg/kg/day for 30 days. In an embryofetal development

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study in rabbits, there was an increase in the number of resorptions and abortions in animals treated with 400 mg/kg/day (8 abortions/resorptions) crofelemer, as compared to control treatment (3 abortions). Maternal toxicity, as indicated by decreases in body weight and food consumption, was observed in control and high dose animals. It is unclear whether the effects of crofelemer on the litters (resorptions and abortions) are secondary to maternal toxicity. Crofelemer was not teratogenic in rats and showed no evidence of impairment of fertility in male or female rats at oral doses of up to 738 mg/kg/day. In a rat pre- and postnatal development study, crofelemer at oral doses of up to 738 mg/kg/day did not affect F_0 pregnancy and lactation, and survival, sex ratio, physical and neurobehavioral development, or reproductive performance of F_1 animals. Maternal (F_0) exposure to crofelemer did not affect fertility parameters of F_1 animals or embryonic development of F_2 generation.

Crofelemer was also tested in juvenile animals. When administered daily by oral gavage to rats for 14 days (postnatal days 5 to 18) at doses of 50 and 100 mg/kg/day, there were 4 deaths (2 at high dose and 2 at low dose; none in control). There were also decreases in body weight at both doses tested and clinical chemistry changes, as compared to control. In juvenile monkeys (age 6-8 weeks), crofelemer administered by oral gavage at 10, 200 and 500 mg/kg/day for 2 weeks. Lymphoid depletion from the thymus was observed at 200 and 500 mg/kg/day and the no effect dose was 10 mg/kg/day.

The NOAEL dose in dogs provides a sufficient margin of safety for the recommended total daily dose of 250 mg/day (125 mg, twice daily). Thus, from a nonclinical standpoint, there are no significant safety concerns for the proposed dose for the proposed indication (i.e. control and symptomatic relief of diarrhea in patients with HIV/AIDS on anti-retroviral therapy).

1.3 Recommendations

1.3.1 Approvability

From a nonclinical standpoint, the NDA application is approvable for the indication proposed.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The following changes should be made prior to approval of the sponsor's proposed label.

Sponsor's proposed version:

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8. USE IN SPECIFIC POPULATIONS
8.1 Pregnancy

(b) (4)

Recommended Version:

8. USE IN SPECIFIC POPULATIONS
8.1 Pregnancy

Pregnancy Category C.
Reproduction studies performed with crofelemer in rats at oral doses up to 177 times the recommended daily human dose of 4.2 mg/kg revealed no evidence of impaired fertility or harm to the fetus. In pregnant rabbits, crofelemer at an oral dose of about 96 times the recommended daily human dose of 4.2 mg/kg, caused abortions and resorptions of fetuses. However, it is not clear whether these effects are related to the maternal toxicity observed. A pre- and postnatal development study performed with crofelemer in rats at oral doses of up to 177 times the recommended daily human dose of 4.2 mg/kg revealed no evidence of adverse pre- and postnatal effects in offspring. There are, however, no adequate, well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Sponsor's proposed version:

10.0 OVERDOSAGE

(b) (4)

Recommended version:

10.0 OVERDOSAGE

There has been no reported experience with overdosage of crofelemer (b) (4)

Sponsor's proposed version:

13.0 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Recommended Version:

13.0 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis
Long-term studies in animals have not been performed to evaluate the carcinogenic potential of crofelemer.

Mutagenesis
Crofelemer was negative in the bacterial reverse mutation assay, chromosomal aberration assay, and rat bone marrow micronucleus assay.

Impairment of Fertility
Crofelemer, at oral doses of up to 738 mg/kg/day (177 times the recommended human daily dose of 4.2 mg/kg) had no effects on fertility or reproductive performance of male and female rats.

13.2 Animal Toxicology and/or Pharmacology

Section 13.2 should be deleted.

2 Drug Information

2.1 Drug

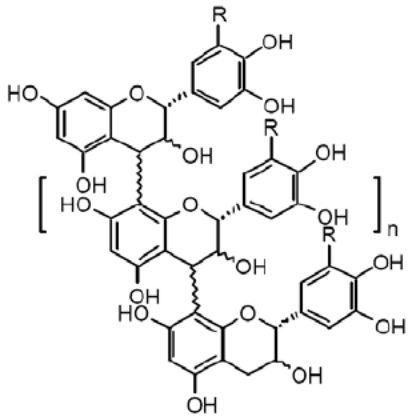
CAS Registry Number: 148465-45-6

Code Name: NP-303, SP-303, TRN-002, Provir™, Virend™

Chemical Name: Crofelemer (USAN)

Molecular Formula/Molecular Weight: $C_{15\cdot n}H_{12\cdot n+2}O_{6.5\cdot n}$, where n = number of monomer units/Avg Molecular Weight 1700 to 2500 Daltons

Structure or Biochemical Description



R = H (procyanidin) and/or R = OH (prodelphinidin)
n = 1-28; average n = 5

Pharmacologic Class: Anti diarrheal

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 51,818 (Crofelemer), IND [redacted] (b) (4) IND [redacted] (b) (4), DMF [redacted] (b) (4) DMF [redacted] (b) (4)

2.3 Drug Formulation

Crofelemer is formulated as white, oval, plain, film-coated tablets (125 mg each) for oral administration, as shown in the sponsor's table below:

Table 1: Clinical and Proposed Commercial Formulation for Crofelemer Tablets, 125 mg

Ingredient	Function	Theoretical Quantity mg/unit dose	Theoretical Quantity % w/w
Uncoated Tablet			
Crofelemer	Active	125.00[a]	25.00
Microcrystalline Cellulose (b) (4)	(b) (4)	(b) (4)	(b) (4)
Croscarmellose Sodium			
Colloidal Silicon Dioxide			
Magnesium Stearate			
Total Uncoated Tablet:			
Coating[c]			
Ethyl Acrylate and Methylacrylate Copolymer Dispersion – (b) (4)	(b) (4)	(b) (4)	
White Dispersion (b) (4)			
Talc (b) (4)			
Triethyl Citrate			
(b) (4)			
Total Coated Tablet:		550.0	NA

Abbreviations: NA = not applicable.

(b) (4)

The proposed commercial formulation was used in the Phase 3 (ADVENT) clinical trial.

2.4 Comments on Novel Excipients

The applicant stated that all excipients, except for White Dispersion (b) (4) meet USP/NF requirements. These include: microcrystalline cellulose, NF, croscarmellose sodium, NF, colloidal silicon dioxide, NF magnesium stearate NF, ethyl acrylate and methylacrylate copolymer dispersion (b) (4) NF, talc (b) (4), USP, triethyl citrate, NF. (b) (4) was prepared in house and met USP requirements. The sponsor confirmed that White Dispersion (b) (4) met specifications listed in the applicant's table below:

(b) (4)

2.5 Comments on Impurities/Degradants of Concern

The applicant has identified the following impurities (b) (4) The concentrations of these impurities in each lot of drug substance are shown in the applicant's table below:

Table 1: Related Substance / Impurity Profile: Crofelemer Validation Batches

Related Substances	Limit	Batch No. AA0068053	AA0068054	AA0068055	AA0068062	AA0068063	AA0068064	LOD %	LOQ %
(b) (4)									

The CDER Computational Toxicology Group was consulted regarding the genotoxic potential of taspine. Three software programs were used in the analysis: Derek Nexus 2.0.2, Leadscope Model Applier 1.3.3-3 and MC4PC 2.4.1.4. Based on the (Q)SAR analysis, taspine was predicted to be negative for genotoxicity on the Salmonella mutagenicity (Ames) endpoint. These predictions agree with the observation of crofelemer being negative in the Ames assay.

The applicant stated that heavy metal content was no more than (NMT) (b) (4) ppm, which is acceptable.

The applicant stated that no (b) (4) were used in the manufacturing process. (b) (4) were used in the manufacturing process and were NMT (b) (4) respectively, in the final drug substance. These levels are acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

The applicant is seeking approval for the control and symptomatic relief of diarrhea in patients with HIV/AIDS on anti-retroviral therapy. The proposed dosing regimen is 125 mg taken orally twice a day, with or without food.

2.7 Regulatory Background

Crofelemer was granted a fast track designation in April 1998 for the treatment of diarrhea in HIV/AIDS patients. An end of Phase 2 (EOP2) meeting was held with the review division on May 5, 2004 to discuss the clinical development plan for crofelemer for treating diarrhea in HIV-infected patients. At this meeting, the Agency requested the following additional studies from the sponsor: a 6-month oral toxicity study in rats with non-microencapsulated powdered crofelemer (in aqueous vehicle) and safety pharmacology studies to characterize CNS and respiratory effects of crofelemer. A pre-NDA meeting was held on January 19, 2011 to discuss submission of the NDA application. At this meeting, the Agency agreed that the nonclinical program was complete for NDA submission. Further, it was agreed that the 2-year carcinogenicity studies in rats and mice would be completed as a Phase 4 commitment. Another pre-NDA meeting was held on May 24, 2011 to discuss CMC-related issues and review the status of crofelemer manufacturing development processes.

3 Studies Submitted

3.1 Studies Reviewed

Table 2.6.3.1-1: Pharmacology Overview

Test Article: Crofelemer				
Type of Study	**Test System**	**Method of Administration**	**Testing Facility**	**Study or Report Number, publication reference, GLP status**
Primary Pharmacodynamics	In vitro cAMP-mediated Cl^- transport in Caco-2 cells (human intestinal epithelial cells).	Crofelemer in aqueous buffer, applied to cells.	University of North Carolina Cystic Fibrosis/Pulmonary Research and Treatment Center	SP303-E-067 and SP303-E-068 (publication: Gabriel et al, Am J Physiol Gastrointest Liver Physiol 1999;270:58-63), non-GLP study
	In vitro cAMP-mediated Cl^- transport in T-84 cells (human intestinal epithelial cells).	Crofelemer in aqueous buffer, applied to cells.	University of North Carolina Cystic Fibrosis/Pulmonary Research and Treatment Center	SP303-CL-001 (publication: Gabriel et al, Am J Physiol Gastrointest Liver Physiol 1999;270:58-63) , non-GLP study
	In vitro Cl^- transport by CFTR and by CaCC in T-84 cells (human intestinal epithelial cells).	Crofelemer in aqueous buffer, applied to cells.	University of California, San Francisco, Departments of Medicine and Physiology	Publication: Tradtrantip L, et al, Mol Pharmacol 2010;77:69-78, , non-GLP study
	In vivo cAMP-mediated Cl^- secretion and intestinal fluid accumulation in an adult mouse (C57Bl/6) model of secretory diarrhea.	Crofelemer in 7% $NaHCO_3$ (SP303-E-069 and SP303-E-070) or crofelemer formulated in enteric-coated beads (SP303-E-074), oral gavage.	University of North Carolina Cystic Fibrosis/Pulmonary Research and Treatment Center	SP303-E-069, SP303-E-070, and SP303-074 (publication: Gabriel et al, Am J Physiol Gastrointest Liver Physiol 1999;270:58-63), non-GLP study
	Gastric motility in mice (CD-1).	Crofelemer formulated in enteric-coated beads (SP303-E-074), oral gavage.	(b) (4)	SP303-E-075, non-GLP
Secondary Pharmacodynamics	No studies reported.			

Test Article: Crofelemer

Type of Study	Test System	Method of Administration	Testing Facility	Study or Report Number, publication reference, GLP status
Safety Pharmacology	Cardiovascular function in dogs.	Crofelemer in purified water, intravenous infusion.	(b) (4)	SP-303-F-015, non-GLP
	Cardiovascular function in dogs.	Crofelemer in purified water, intravenous infusion.		SP-303-F-016, non-GLP
	Neurobehavioral function in CD rats.	Crofelemer in purified water, oral gavage.		CRSP0201, GLP
	Cardiovascular function in beagle dogs.	Crofelemer tablets formulation in gelatin capsules, oral administration.		CRSP0300, GLP
	Gastrointestinal motility in CD rats.	Crofelemer in purified water, oral gavage.		CRSP0203, GLP
	Pulmonary function in CD rats.	Crofelemer in purified water, oral gavage.		CRSP0202, GLP
Pharmacodynamic Drug Interactions	No studies reported.			

Abbreviations: CFTR: cystic fibrosis transmembrane receptor; CaCC: calcium-activated chloride ion (Cl^-) channels; GLP: good laboratory practice.

2.6.5.1 Pharmacokinetics Overview

Test Article: Crofelemer

Type of Study	Test System	Method of Administration	Testing Facility	Study or Report Number, GLP status
Absorption - in vitro studies	Permeability and P-glycoprotein transport of ^{14}C-methylated crofelemer in human Caco-2 intestinal epithelial cells.	**^{14}C-methylated crofelemer in aqueous buffer, applied to cells.**	(b) (4)	(b) (4) 21485, non-GLP
	Crofelemer effects on P-glycoprotein transporters in membrane vesicles isolated from P-glycoprotein overexpressing cells.	Crofelemer in aqueous buffer, incubated with membrane vesicles or applied to cells.		CRDM0103 (b) (4) non-GLP
Absorption - in vivo studies	Analysis of crofelemer in rat plasma following a single dose.	Intravenous and oral (gavage) administration of crofelemer dissolved in 5% dextrose.	Shaman Pharmaceuticals	SP-303-E-028, non-GLP
	Analysis of crofelemer in rat plasma following a single dose.	Intravenous and oral (gavage) administration of crofelemer dissolved in 5% dextrose.	Shaman Pharmaceuticals	SP-303-E-066, non-GLP
	Analysis of crofelemer in rat plasma following a single dose.	Intravenous and oral (gavage) administration of crofelemer dissolved in 5% dextrose. Oral (gavage) administration of crofelemer formulated in enteric-coated beads.	Shaman Pharmaceuticals	SP-303-E-073, non-GLP
	Analysis of crofelemer in rat plasma on Days 0 and 27 of the 28-day, repeat-dose toxicology study (b) (4) 88007.	Oral (gavage) administration of crofelemer formulated in enteric-coated beads.	(b) (4)	SP-303-F-039 - toxicokinetic analysis from toxicology study (b) (4) SP-303-F-045), GLP

Test Article: Crofelemer

Type of Study	Test System	Method of Administration	Testing Facility	Study or Report Number, GLP status
	Analysis of crofelemer in mice plasma on Days 1, 56, and 91 of the 13-week, repeat-dose toxicology study (b) (4) 1310-016	Oral (gavage) administration of crofelemer formulated in enteric-coated tablets.	(b) (4)	(b) (4) 1310-016 - toxicokinetic analysis from repeat-dose toxicology study (b) (4) 1310-016, GLP
	Analysis of crofelemer in rat plasma on Day 6 of the 7-day, repeat-dose toxicology study (b) (4)	Oral (gavage) administration of crofelemer formulated in enteric-coated beads.		SP-303-F-041 - toxicokinetic analysis from toxicology study (b) (4) SP-303-F-046), GLP
	Analysis of crofelemer in dog plasma on Days 0 and 6 of the 7-day, repeat-dose toxicology study (b) (4)	Oral administration of crofelemer formulated in enteric-coated tablets.		SP-303-F-040 - toxicokinetic analysis from study (b) (4) (SP-303-F-042), GLP
	Analysis of crofelemer in dog plasma on Days 0 and 6 of the 7-day, repeat-dose study (b) (4)	Oral administration of crofelemer formulated in enteric-coated tablets.		SP303-F-080 - toxicokinetic analysis from study (b) (4) (SP-303-F-047), GLP
	Analysis of crofelemer in dog plasma during the 30-day, repeat-dose study (b) (4)	Oral administration of crofelemer formulated in enteric-coated tablets.		SP303-E-081 - toxicokinetic analysis from (b) (4) SP-303-F-048) , GLP
Distribution	In vivo mass balance study of ^{14}C-methylated crofelemer in rats.	Oral gavage administration.		(b) (4) 1482-008, GLP
	In vitro study of protein binding to human plasma proteins.	^{14}C-methylated crofelemer dissolved in aqueous buffer, incubated with human plasma samples.		(b) (4) 021485, non-GLP
Metabolism	*In vitro* metabolism by CYP isozymes in human hepatocytes and human liver microsomes.	Crofelemer dissolved in aqueous buffer, applied to cells and incubated with microsomes.		CRDM0100 ((b) (4) , non-GLP
	In vitro study of induction of CYP-mediated metabolism in human hepatocytes.	Crofelemer dissolved in aqueous buffer, applied to cells.		CRDM0102 ((b) (4) non-GLP
	In vitro study of inhibition of CYP-mediated metabolism in human liver microsomes.	Crofelemer dissolved in aqueous buffer, incubated with microsomes.	(b) (4)	(b) (4) 021485, non-GLP
	In vitro study of inhibition of CYP-mediated metabolism in human liver microsomes and hepatocytes.	Crofelemer dissolved in aqueous buffer, applied to cells.		CRIV0101 (b) (4) non-GLP
	Analysis of crofelemer metabolites in urine samples from rats and humans.	Oral administration of crofelemer dissolved in water.	Shaman Pharmaceuticals	SP-303-E-059, non-GLP; human samples were from SP-303-I-04.

Abbreviations: CYP = cytochrome P450; GLP = Good Laboratory Practice.

Table 2.6.7.1-1

Type of Study	Species / Strain	Method of Admin.	Duration of Dosing	Test Article and Doses	Testing Facility	Study No. GLP Status
Single-Dose Toxicity	Mouse Crl:CD-1	IV or IP	Single Dose	Crofelemer in 5% dextrose for injection, USP IV: 0, 6.25, 12.5, 25.0 and 50.0 mg/kg IP: 0, 10, 20, 50 and 100 mg/kg	(b) (4)	SP-303-F-001 (b) (4) GLP
	Rat Sprague Dawley CD-VAF	Oral (gavage)	Single Dose	Crofelemer in Water 0, 30, 100 and 300 mg/kg		SP-303-F-003 (b) (4) GLP
	Rat Sprague Dawley Crl:CD(SD)	Oral (gavage)	Single Dose	Encapsulated Enteric Coated Tablets 600, 1200 and 2400 mg/kg		SP-303-F-046 (b) (4) GLP
	Rat Sprague Dawley CD-VAF	IV or IP	Single Dose	Crofelemer in 5% dextrose for injection, USP IV: 0, 3, 10, 20 and 50 mg/kg (vol. of 5 ml/kg) IP: 0, 10, 20, 50 and 100 mg/kg (vol. of 10 ml/kg)		SP-303-F-002 (b) (4) GLP
	Rat (Crl:CD [SD]IGS BR)	IP	Single Dose	Crofelemer in corn oil Study 1: 200, 500, 800, 1500 and 2000 mg/kg Study 2: 100 and 150 mg/kg		SP-303-AT-002 9288-0-4540ECD, dose range-finding studies for genotoxicity study) GLP
	Dog (Beagle)	Oral	Single Escalating Dose	Encapsulated Enteric Coated Tablets 100, 200, 400 and 800 mg/kg		SP-303-F-042 (b) (4) dose range-finding study for repeat-dose study) GLP
Single-Dose Toxicity con't	Dog (Beagle)	Oral	Single Escalating Dose	Encapsulated Enteric Coated Tablets 100, 300, 600 and 1200 mg/kg	(b) (4)	SP-303-F-047 (b) (4), dose range-finding study for repeat-dose study) GLP
	Dog (Beagle)	IV (infusion)	Single Infusion	10 and 18.9 mg/kg		SP-303-F-004 (b) (4) GLP
	Dog (Beagle)	IV (infusion)	Single Infusion	30 mg/kg		SP-303-F-015 (b) (4) Non-GLP
Repeat-Dose Toxicity	Mouse Crl:CD1®(ICR)	Oral (gavage)	4 weeks	Crofelemer in buffered saline 0, 40, 120, 400, and 1200 mg/kg/day		(b) (4) 1482-001 GLP
	Mouse Crl:CD1®(ICR)	Oral (gavage)	13 weeks (Note: due to mortalities, animals at 1200 mg/kg/day dose were administered drug for 8 weeks)	Crofelemer in purified water 0, 40, 400 and 1200 mg/kg/day		(b) (4) 1310-016 (CRSA0200) GLP
	Rat (Crl:CD)	Oral (gavage)	14 days	Crofelemer in Distilled Water 0, 10, 200 and 500 mg/kg/day		SP-303-F-007 (b) (4) GLP
	Rat (SD CD [VAF])	Oral (gavage)	30 days (with 30 days recovery)	Crofelemer in Distilled Water 0, 50, 200 and 500 mg/kg/day		SP-303-F-020 (b) (4) GLP
	Rat (Crl:CD [SD])	Oral (gavage)	7 days	Encapsulated Enteric Coated Tablets 0 and 1200 mg/kg/day		SP-303-F-046 (b) (4) GLP

Type of Study	Species / Strain	Method of Admin.	Duration of Dosing	Test Article and Doses	Testing Facility	Study No. GLP Status
	Rat (Crl:CD [SD]BR)	Oral (gavage)	32 days	Encapsulated Enteric Coated Tablets 0, 100 and 1000 (600) mg/kg/day (1000 mg/kg/day reduced to 600 mg/kg/day on Day 2)	(b) (4)	SP-303-F-045 (b) (4) GLP
Repeat-Dose Toxicity con't	Rat (neonatal SD Crl:CD BR)	Oral (gavage)	2 weeks	Crofelemer in Distilled Water 0, 10, 200, and 500 mg/kg/day		SP-303-F-030 (b) (4) GLP
	Rat (neonatal SD Crl:CD BR)	Oral (gavage)	2 weeks	Crofelemer in Distilled Water 0, 50, and 100 mg/kg/day		SP-303-F-035 (b) (4) GLP
	Rat Sprague-Dawley Crl:CD	Oral (gavage)	26 weeks (with 4 week recovery)	Crofelemer in buffered saline 0, 60, 200, and 600 mg/kg/day		(b) (4) 1482-002 GLP
	Rat (SD CD [VAF])	IV	5 days	Crofelemer in 5% dextrose 0, 5, 20, and 40 mg/kg/day		SP-303-F-005 (b) (4) GLP
	Dog (Beagle)	Oral (gavage)	5 days	Crofelemer in Sterile Water 0 and 100 mg/kg/day		(b) (4) 218-20-03-91 Non-GLP
	Dog (Beagle)	Oral	7 days	Encapsulated Enteric Coated Tablets 600 mg/kg/day		SP-303-F-042 (b) (4) GLP
	Dog (Beagle)	Oral	7 days	Encapsulated Enteric Coated Tablets 800 mg/kg/day		SP-303-F-047 (b) (4) GLP
	Dog (Beagle)	Oral	30 days	Encapsulated Enteric Coated Tablets 0, 50, 175 and 600 mg/kg/day		SP-303-F-048 (b) (4) GLP
	Dog (Beagle)	IV (bolus or infusion*)	5 days	Crofelemer in 5% dextrose 0, 5, 20 and 30 or 35 mg/kg/day		SP-303-F-009 (b) (4) GLP

Type of Study	Species / Strain	Method of Admin.	Duration of Dosing	Test Article and Doses	Testing Facility	Study No. GLP Status
	Dog (Beagle)	IV (infusion)	5 days (followed by 2-4 weeks recovery)	Crofelemer in 5% dextrose 0, 2.5, 5.0 and 10.0 mg/kg/day	(b) (4)	SP-303-F-010 (b) (4) GLP
Repeat-Dose Toxicity con't	Monkey (African Green)	Oral (gavage) IV (bolus and infusion)	5 days	Crofelemer in 5% dextrose Oral: 50 mg/kg/day IV: 5 mg/kg/day		SP-303-F-012 Non-GLP
	Monkey (Rhesus)	Oral (gavage)	5 days	Crofelemer in Sterile Water 100, 300 and 900 mg/kg/day		SP-303-F-013 Non-GLP
	Monkey (Rhesus)	Oral (gavage)	14 days	Crofelemer in Sterile Water 0, 10, 200 and 500 mg/kg/day		SP-303-F-022 (b) (4) GLP
	Monkey (Rhesus)	Oral (gavage)	14 days	Crofelemer in Sterile Water 0, 50 and 100 mg/kg/day		SP-303-F-014 (b) (4) GLP
	Monkey (Rhesus)	Oral (gavage)	30 days (followed by 30 days recovery in 2 animals each in control and high dose groups)	Crofelemer in Sterile Water 0, 30, 100 and 200 mg/kg/day		SP-303-F-019 (b) (4) GLP
	Monkey (Neonatal Rhesus)	Oral (gavage)	2 weeks	Crofelemer in Distilled Water 0, 10, 200 and 500 mg/kg/day		SP-303-F-034 (b) (4) GLP
	Rat (SD Albino)	Oral (gavage)	5 days	Crofelemer in Sterile Water 0, 100, 300 and 900 mg/kg/day	Shaman San Carlos, CA USA	--- Non-GLP

Type of Study	Species / Strain	Method of Admin.	Duration of Dosing	Test Article and Doses	Testing Facility	Study No. GLP Status
	Mouse (Swiss-Webster, Albino)	IP / Oral (gavage)	30 days	IP: Crofelemer in 5% dextrose 0, 2, 10 and 20 mg/kg/day Oral: Crofelemer in Sterile Water 0, 10, 30 and 90 mg/kg/day	Shaman San Carlos, CA USA	--- Non-GLP
	Dog (Beagle)	Oral	9 months	Encapsulated Enteric Coated Tablets 0, 50, 175 and 600 mg/kg/day	(b) (4)	(b) (4) GLP
Genotoxicity	Reverse Mutation (Ames) *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	*in vitro*	---	100 – 10,000 µg/plate (with and w/o metabolic activation)		SP-303-F-021 (T9636.501) GLP
	Chromosomal Aberration CHO cells, cultured	*in vitro*	---	Initial Test: 21.2, 43.0, 61.4, and 87.8 µg/mL (–S9); 125, 179, 256 and 366 µg/mL (+S9) Confirmatory Test: 10, 20, 35 and 50 µg/mL (–S9); 50, 100 and 300 µg/mL (+S9)		SP-303-AT-001 (19288-0-437-OECD) GLP
	Rat Micronucleus SD Rat	*in vivo* (IP)	single dose	Up to 200 mg/kg/day		SP-303-AT-002 (19288-0-454-OECD) GLP
Reproductive and Developmental Toxicity	Rat Sprague-Dawley Crl:CD(SD)IGS	Oral (gavage)	M: 28 days prior to mating to necropsy F: 14 days prior to mating to Day 7 gestation	Microencapsulated crofelemer beads 0, 123, 369, and 738 mg/kg/day		(b) (4) -288017 GLP
	Rat Sprague-Dawley Crl:CD(SD)IGS	Oral (gavage)	Days 6 to 17 of gestation	Microencapsulated crofelemer beads 0, 123, 307, 615, 922, and 1229 mg/kg/day		(b) (4) -288015 GLP
	Rat Sprague-Dawley Crl:CD(SD)IGS	Oral (gavage)	Days 6 to 17 of gestation	Microencapsulated crofelemer beads 0, 123, 369, and 738 mg/kg/day		(b) (4) -288019 GLP

Type of Study	Species / Strain	Method of Admin.	Duration of Dosing	Test Article and Doses	Testing Facility	Study No. GLP Status
	Rabbit New Zealand White	Oral (gavage)	Days 7 to 20 of gestation	Encapsulated crofelemer tablets 0, 100, 250, 500, 750, and 1000 mg/kg/day (given in two divided doses)	(b) (4)	(b) (4) -288016 GLP
	Rabbit New Zealand White	Oral (gavage)	Days 7 to 20 of gestation	Encapsulated crofelemer tablets 50, 200 and 400 mg/kg/day (given in two divided doses)		(b) (4) -288018 GLP
	Rat Sprague-Dawley Crl:CD(SD)IGS	Oral (gavage)	Day 6 gestation to day 20 lactation	Microencapsulated crofelemer beads 0, 123, 369, and 738 mg/kg		(b) (4) -288023 GLP
Other Toxicity	Rabbit New Zealand White	IV	Single dose	Crofelemer in 5% dextrose 3 mg/kg		SP-303-B-004 (ZB410.007) GLP
	Effects of crofelemer on hERG K+ currents in human cells	*In vitro*	---	The effect of crofelemer at concentrations of 0 (deionized water) and 1 nM to 30 µM was measured on the maximum amplitude of the tail current, a parameter determined from current traces obtained from voltage-clamped HEK-293/hERG cells using patch-clamp techniques in the whole cell configuration.		AA63535, non-GLP

Abbreviations: CHO = Chinese Hamster Ovary; GLP = Good Laboratory Practice; IP = intraperitoneal; HEK = human embryonic kidney; hERG = human ether-a-go-go related gene; IV = intravenous; SD rats = Sprague Dawley rats; USP = United States Pharmacopeia.

2.6.7.2 Overview of Toxicokinetics Studies

Table 2.6.7.2-1

Test Article: Crofelemer

Type of Study	Test System / Species	Method of Administration	Duration of Dosing	Doses	Study No. GLP Status
13-week repeat dose toxicity study	Mice (Crl:CD1 [ICR])	Oral (gavage)	13 weeks	0, 40, 400, and 1200 mg/kg/day	(b) (4) 1310-016 (CRSA0200) GLP
7-day repeat dose toxicity (range finding)	Rat (Crl:CD [SD])	Oral (gavage)	7 days	0 and 1200 mg/kg/day	SP-303-F-041 (TK of crofelemer from repeat-dose toxicology study SP-303-F-046 (b) (4) GLP
32-day repeat dose toxicity	Rat (Crl:CD [SD]BR)	Oral (gavage)	32 days	0, 100 and 1000 (600) mg/kg/day (1000 mg/kg/day reduced to 600 mg/kg/day on Day 2)	SP-303-F-045 (b) (4) GLP
7-day repeat dose toxicity	Dog (Beagle)	Oral	7 days	600 mg/kg/day	SP303-F-040 (TK of crofelemer from repeat-dose toxicology study SP-303-F-042 (b) (4) GLP
7-day escalating dose toxicity	Dog (Beagle)	Oral	7 days	800 mg/kg/day	SP-303-F-047 (b) (4) GLP
30-day repeat dose toxicity	Dog (Beagle)	Oral	30 days	0, 50, 175 and 600 mg/kg/day	SP-303-F-048 (b) (4) GLP

Abbreviations: GLP = Good Laboratory Practice; IP = intraperitoneal; IV = intravenous; SD rats = Sprague Dawley rats; TK = toxicokinetics.

3.2 Studies Not Reviewed

The following toxicology studies were not reviewed because they were non-GLP or dose range-finding studies.

Study #	Study Title
--	Non-GLP-30 day oral toxicity study in mice
(b) (4) 218-20-03-91	A 5-day study with test article SP-303 administered orally by gavage to beagle dogs

3.3 Previous Reviews Referenced

The following studies were reviewed previously under IND 51818. Full reviews are included in this review verbatim:

Study #	Study Title	Date of review	Reviewer
SP-303-F-045	A 32-day oral toxicity study of microencapsulated Provir in rats	September 8, 1998	Patrick G. Swann, Ph.D.
(b) (4) 288006	A 30-day oral toxicity study of enteric coated SP303 in dogs		
SP-303-E-073	Pharmacokinetics (PK) of intravenously and orally administered SP-303	September 30, 1997	Gerald A. Young, Ph.D.
SP303-E-028	Levels of S-303 in rat plasma following oral and intravenous administration	May 14, 1997	Tanveer Ahmad Ph.D.
SP303-E-066	PK and oral bioavailability of SP-303 in rats		
SP303-E-073	SP-303 absorption from enteric coated beads and		

	from aqueous solution in rats		
SP-303-F-001	Acute intravenous and intraperitoneal toxicity in mice	May 14, 1997	Tanveer Ahmad Ph.D.
SP303-F-002	Acute intravenous and intraperitoneal toxicity in rats		
SP303-F-003	Acute oral toxicity study in rats		
SP303-F-020	Thirty-day oral toxicity study of SP-303 with a thirty day recovery period in rats		
SP303-F-005	Dose range-finding intravenous toxicity study in rats		
SP303-F-010	A 5-day study with test article SP-303 administered intravenously to beagle dogs followed by a 2 and 4		
SP303-F-019	A 30-day study with test article SP-303 administered orally by gavage to rhesus monkeys followed by a 30-day recovery period		
SP303-F-030/035	2-week oral toxicity study of SP-303 in neonatal rats		
SP303-F-034	A 2-week oral toxicity study of SP-303 in neonatal rhesus monkeys		
SP303-F-021	Salmonella/Mammalian microsome plate incorporation mutagenicity assay (Ames test)		

4 Pharmacology

4.1 and 4.2 Primary and Secondary Pharmacology

In the non-GLP Study # SP-303-CL-001 (Primary pharmacodynamics), SP-303 inhibited *V. cholerae* toxin- and *E. coli* heat labile (HL) toxin-induced chloride ion secretion in vitro (human intestinal epithelial cell lines Caco-2 and T84 monolayers). The secretion of chloride ions is dependent on cAMP/cGMP-mediated pathways. SP-303 at a concentration of 40 μM inhibited chloride ion secretion by 58.5%, 53.5%, and 85.5%, when induced by cholera, *E.coli* HL, and *E. coli* STa toxins, respectively. SP-303-mediated inhibition does not affect the function of the chloride ion transporter on the basolateral side of the intestinal cell wall. This was demonstrated by a further decrease in chloride current induced by butamide, a specific inhibitor of the basolateral chloride transporter, in cells treated with SP-303.

The mechanism of action of SP-303 was described in two study reports: SP-303-E-67 (Effect of SP-303 Pretreatment on Caco-2 Short Circuit Current (SH1)) and SP-303-E-068 (Effect of SP-303 in Forskolin-Stimulated Isc Caco-2 cells (SH2)). Both studies were conducted at the University of North Carolina (Chapel Hill, NC). In these non-GLP studies, SP-303 was tested at a range of concentrations (1 - 610μM). Treatment of Caco-2 cells with SP-303 at 310 μM on the apical side produced the largest decrease (74%) in forskolin-induced chloride ion secretion when forskolin was applied bilaterally. This was also demonstrated in Caco-2 cells which were pre-treated with SP-303. SP-303 was not cytotoxic at the concentrations tested. Application of bumetanide (a specific inhibitor of Cl^- and Na^+ transport to the basolateral side produced a further decrease in chloride ion secretion, demonstrating that SP-303 affects chloride ion

secretion via a cAMP-mediated pathway, without disrupting the chloride transporter on the basolateral membrane.

Study title: Effect of SP-303 and Cholera Toxin Co-treatment on Fluid Accumulation in Adult Mouse Model of Secretory Diarrhea

Study no.:	SP-303-E-069
Study report location:	EDR
Conducting laboratory and location:	University of North Carolina Chapel Hill, NC
Date of study initiation:	Not provided
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	SP-303, Lot# 426I0893, Purity not specified

Methods

Doses:	25, 50, 100 mg/kg
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	Not provided
Formulation/Vehicle:	Solution in 7% NaHCO3
Species/Strain:	Mice/C57Bl/6 (repeated back-crossing of CFTR+/- heterozygotes)
Number/Sex/Group:	3-5/group
Age:	8-10 weeks of age
Weight:	12.1 – 36.0 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Key Study Findings

The purpose of this study was to determine the effect of SP-303 pretreatment on cholera toxin-induced fluid secretion in an adult mouse model of secretory diarrhea. In this model, mice were gavaged with test article or vehicle ($NaHCO_3$) and then administered a single dose of cholera toxin (CT) at 10 μg/15 g body weight. Cyano-acrylamide ester was then used as a sealant to inhibit the ileal-cecal reflex. The entire small intestine was isolated from the pylorus to the cecum and the weight of fluid accumulated was calculated. In this study, SP-303 significantly reduced cholera-toxin induced fluid accumulation in the small intestine of the sealed adult mouse model by 99.5% and 78% at 100 and 50 mg/kg, respectively, as compared to positive control (vehicle followed by single dose of cholera toxin).

Reference ID: 3168746

Study title: Effect of SP-303 Post-treatment on Cholera Toxin-Induced Fluid Accumulation in the Adult Mouse Model of Secretory Diarrhea

Study no.:	SP-303-E-070
Study report location:	EDR
Conducting laboratory and location:	University of North Carolina Chapel Hill, NC
Date of study initiation:	Not provided
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	SP-303, Lot# 426I0893, Purity not specified

Methods

Doses:	0.1, 0.3, 1, 3, 10, 25, 50, 100 mg/kg
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	Not provided
Formulation/Vehicle:	Solution in 7% $NaHCO_3$
Species/Strain:	Mice/C57Bl/6 (repeated back-crossing of CFTR+/- heterozygotes)
Number/Sex/Group:	9-12/group
Age:	8-10 weeks of age
Weight:	8.0 – 48.0 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Key Study Findings

Using the same adult mouse model of secretory diarrhea, the applicant demonstrated the efficacy of SP-303 on inhibiting fluid accumulation. In this study, SP-303 was administered at various doses 3 h after pre-treatment with cholera toxin (CT) at 10 μg/15 g body weight. SP-303 decreased fluid accumulation by 88%, 96%, and 74% at 100, 50 and 25 mg/kg, respectively, as compared to control. SP-303 did not produce statistically significant reductions in fluid accumulation at concentrations below 25 mg/kg.

Reference ID: 3168746

Study title: Effect of Enteric coated SP-303 on Intestinal Fluid Accumulation in Cholera Toxin-treated Mice

Study no.:	SP-303-E-074
Study report location:	EDR
Conducting laboratory and location:	Shaman Pharmaceuticals, Inc.
Date of study initiation:	Not provided
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Enteric coated SP-303, Lot # R10574, Purity not specified

Methods

Doses:	131 mg SP-303 in guar gum/kg
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	Not specified
Formulation/Vehicle:	Suspension in 0.75% gum guar solution (pH 2)/Water, Eudragit, gum guar
Species/Strain:	Mouse/C57Bl/6
Number/Sex/Group:	4 males/group
Age:	50-52 days old
Weight:	15.7 – 18.7 g
Satellite groups:	None
Unique study design:	Mice were pretreated with CT and dosed with SP-303 (or control) 3 hours later. Mice were sacrificed at 6 h and 7h post-dose and fluid accumulation was calculated.
Deviation from study protocol:	None

Key Study Findings

The purpose of this study was to determine the efficacy of enteric-coated SP-303 on fluid accumulation (FA) after cholera toxin (CT) treatment in the mouse model of secretory diarrhea. Enteric-coated SP-303 produced a marked decrease in FA at 7 hours post-dose, as compared to FA measured in SP-303-treated animals at 6 h post-dose, which was similar to H_2O (vehicle) treatment. The decrease in FA at 7 h post-dose observed with 131 mg/kg SP-303 was further compared to treatment with vehicle (water, Eudragit & sugar/gum guar). SP-303 treatment produced a statistically significant decrease in FA of 45% and 38%, as compared to treatment with water and Eudragit & sugar/gum guar solution, respectively.

Reference ID: 3168746

Study title: Gastrointestinal Propulsion Assay in Mice (0239XS19.001)

Study no.:	SP-303-E-075
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	May 20, 1997
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Provir beads (SP-303), Lot# R10765 Control beads, Lot# R10913 Purity not specified

Methods

Doses:	10, 100, 250 mg/kg Provir (SP-303), 250 mg/kg control beads, 10 mg/kg atropine sulfate (positive control)
Frequency of dosing:	Once (Provir at 10 mg/kg); Twice (Provir at 100 mg/kg); Four times (Provir at 250 mg/kg); Six times (Control beads)
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	Solution/0.25% (w/v) methylcellulose in water
Species/Strain:	Mouse/Crl:CD-1®(ICR)BR
Number/Sex/Group:	15 Males/Group
Age:	30 days
Weight:	18-22 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Key Study Findings

The purpose of this study was to determine the effect of Provir (SP-303) on gastrointestinal (GI) transit of a charcoal suspension in mice. A 10% suspension of activated charcoal in 0.25% methylcellulose was used to assess GI transit under various treatment conditions. Orally administered Provir produced a 6% decrease and a 29% and 14% increase in GI transit, as compared to control beads. These changes were not statistically significant. In contrast, atropine sulfate, the positive control, produced a statistically significant decrease of 27% in GI transit, as compared to control. Therefore, it was concluded that SP-303 does not affect GI transit.

The applicant also submitted two publications to further characterize the pharmacological properties of SP-303. In a study by Gabriel et al, (1999)[1], SP-303

[1] Gabriel SE, et al., (1999) Am J Physiol 276(39): G58-G63.

inhibited CT-induced FA in the small intestines of mice in a dose-dependent manner with an IC_{50} of 10 mg/kg. In a recent study by Tradtrantip et al.[2], (2010), crofelemer was shown to inhibit two separate intestinal calcium channels in vitro. Crofelemer inhibited the cystic fibrosis transmembrane regulator (CFTR) chloride channel with an IC_{50} of ~7 μM and the intestinal calcium-activated TMEM16A chloride channel by a voltage independent mechanism with an IC_{50} of ~6.5 μM.

4.3 Safety Pharmacology

Study title: Potential Cardiovascular Effects of Orally Administered Crofelemer in the Beagle Dog

Study no.:	1310-012
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 29, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Crofelemer, Lot # 3067356R Placebo, Lot # 3070578R Purity not specified

Methods

Doses:	0, 60, 200, 600 mg/kg Crofelemer
Frequency of dosing:	Once (single dose)
Route of administration:	Oral
Dose volume:	Single gelatin capsule
Formulation/Vehicle:	Whole tablets encapsulated into capsules/ #12 Torpac gelatin lock ring capsule
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4 males/group
Age:	1 yr 3 months to 1 yr 5 months
Weight:	9.63 to 12.93 kg
Satellite groups:	None
Unique study design:	The applicant employed a Latin-square design in this study with a 7-day washout period between each dose. The animals were instrumented for telemetry prior to initiation of dosing. ECG parameters assessed included RR interval, PR interval, QRS duration, QT interval, and corrected QT (QTc) interval.
Deviation from study protocol:	None

[2] Tradtrantip L, et al., (2010) Mol Pharmacol 77:69-78.

Key Study Findings

SP-303 did not affect cardiovascular parameters in conscious, freely moving dogs when administered as a gelatin capsule at doses of up to 600 mg/kg. Clinical signs were observed in animals treated with crofelemer at 200 and 600 mg/kg only, which included red or brown feces, soft and/or watery feces, and black material in cage (at 600 mg/kg only) at ~20-25 h post dose. In 3 of 4 placebo-treated animals, white fecal material was observed. There were no differences in body weight or body temperature related to treatment with crofelemer. There were no treatment-related changes in blood pressure (mean systolic, diastolic and mean arterial pressure), as compared to vehicle-treatment. There was no change in heart rate related to treatment with crofelemer during the course of the study. There were no treatment-related changes in RR interval, PR interval, QRS duration, QT and corrected QT interval. Overall, the NOAEL dose for cardiovascular adverse effects was 600 mg/kg.

Study title: Neurobehavioral Evaluation of Orally Administered Crofelemer in Rats

Study no.:	1310-013
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 29, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Crofelemer, Lot # 3072906R, Purity not specified (Sponsor provided a statement listing the 37 API lots which were (b) (4) to generate the test article. The sponsor stated that Lot 3072906R had an as-is potency value of 97.9% and (b) (4)

Methods

Doses:	Vehicle, 60, 200, 600 mg/kg Crofelemer, 20 mg/kg Chlorpromazine
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Solution in purified water/Purified water
Species/Strain:	Rat/CD [Crl:CD(SD)]
Number/Sex/Group:	6 males/group
Age:	6 weeks of age
Weight:	171-199 g
Satellite groups:	None
Unique Study Design:	None
Deviations:	None

The following evaluations (Functional Observational Battery, FOB) were conducted on all animals at 1 hour and 24 hours post-dose in each group:

FOB (Continuous Endpoints)	**FOB (Categorical Endpoints)**		
Thermal Response	Posture	Salivation	Bizarre Behavior
Mean Forelimb Grip Strength	Ease of Removal	Clonic Movements	Approach Response
Mean Hindlimb Grip Strength	Handling Reactivity	Tonic Movements	Touch Response
Body Weight	Lacrimation	Gait	Click Response
Body Temperature	Palpebral Closure	Mobility	Tail Pinch Response
Rearing	Piloerection	Arousal	Pupil Response
Defecation	Exophthalmus	Vocalizations	Righting Reflex
Urination		Respiration	
Mean Hindlimb Splay		Stereotypy	

Key Study Findings

Crofelemer, at oral doses of up to 600 mg/kg, did not produce any remarkable effects on neurobehavioral functions, as measured in the functional observational battery (FOB), in male rats. The 200 mg/kg crofelemer dose group showed an increase in mean grip strength at 24 h postdose; however, no dose response was observed and this effect was considered to be unrelated to treatment. The positive control chlorpromazine produced a decrease in general arousal and body temperature and an increase in palpebral closure, forelimb grip strength, and hindlimb splay. There were no other treatment-related changes with crofelemer, as compared to vehicle control.

Reference ID: 3168746

Study title: Potential Pulmonary Effects of Orally Administered Crofelemer in Rats

Study no.:	1310-014
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 29, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Crofelemer, Lot # 3072906R, Purity not specified (Sponsor provided a statement listing the 37 API lots which were (b) (4) to generate the test article. The sponsor stated that Lot 3072906R had an as-is potency value of 97.9% and (b) (4)

Methods

Doses:	Vehicle (0 mg/kg), 60, 200, 600 mg/kg Crofelemer, 100 mg/kg baclofen (positive control)
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	10 mL/kg (vehicle and crofelemer), 15 mL/kg (baclofen)
Formulation/Vehicle:	Aqueous solution/purified water
Species/Strain:	Rat/CD [Crl:CD(SD)]
Number/Sex/Group:	8/group
Age:	6 weeks
Weight:	227-258 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Key Study Findings

The purpose of the study was to determine whether orally administered crofelemer produced effects on respiratory parameters, as compared to vehicle (purified water) and positive control (baclofen). Pulmonary evaluation was conducted in a plethysmograph chamber, with at least 2 hours of monitoring pre-dose. After dosing, each animal was observed in the chamber for 4 h and the following parameters were monitored: respiratory rate, tidal volume, and minute volume.

Treatment with crofelemer at the highest dose (600 mg/kg) produced some sporadic decreases in respiratory rate and/or minute volume, as compared to control; however, these changes were not statistically significant. Clinical signs, such as difficulty breathing, brown material around mouth, and red material around nose, were observed in 1-3 animals treated with crofelemer at all doses. All baclofen-treated animals had

Reference ID: 3168746

decreased activity and slow breathing within 1 hour post dose. Overall, crofelemer at the highest dose produced some sporadic decreases in respiratory rate and/or minute volumes, as compared to control, but these changes were not statistically significant. Baclofen treatment produced a 70% decrease in respiratory rate and a ~350% and 130% increase in tidal and minute volumes, respectively. Therefore, based on the conditions of this study, crofelemer did not produce significant pulmonary effects after oral administration in rats.

Study title: Potential Effects of Orally Administered Crofelemer on Gut Motility Function in the Rat

Study no.:	1310-015
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 29, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Crofelemer, Lot # 3072906R, Purity not specified (Sponsor provided a statement listing the 37 API lots which were (b) (4) to generate the test article. The sponsor stated that Lot 3072906R had an as-is potency value of 97.9% and (b) (4)

Methods

Doses:	Vehicle control, 60, 200, 600 mg/kg crofelemer and 20 mg/kg morphine (positive control)
Frequency of dosing:	Once/day
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Aqueous solution/Purified water
Species/Strain:	Rat/CD [Crl:CD(SD)]
Number/Sex/Group:	8 males/group
Age:	8 weeks old
Weight:	280-306 g
Satellite groups:	None

Key Study Findings

The purpose of this study was to determine the acute effects of crofelemer on gastrointestinal (GI) motility in rats. One hour after drug administration (vehicle, crofelemer or the positive control morphine), all animals were administered a test meal of 5% charcoal suspension in 10% Acacia in deoinized water via oral gavage (dose volume = 10 mL/kg). Thirty minutes after administration of the test meal, all animals

were euthanized and the small intestine was removed. Total intestine length and distance traveled by charcoal were determined. Clinical observations (morbidity, mortality, injury, food/water availability) were observed twice daily, pre-dose and just prior to terminal sacrifice.

There were no remarkable changes in clinical signs or body weight in any dose groups. Treatment-related decrease in gastrointestinal motility, as measured by distance traveled by charcoal, was observed in rats administered Crofelemer at 200 and 600 mg/kg by oral gavage (summarized in table below).

Table: Percent change in GI motility in rats after oral administration of crofelemer (vehicle or positive control morphine)

	Dose (mg/kg)	% motility
Vehicle	0	81.12
Crofelemer	60	76.18
	200	73.83*
	600	68.88*
Morphine	20	56.34*

* $p < 0.01$ compared to vehicle control

Study title: The Determination of Pharmacotoxic Dose of SP-303A2 in a Dog

Study no.:	SP-303-F015
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 20, 1990
GLP compliance:	Not known
QA statement:	No
Drug, lot #, and % purity:	SP-0303A2, Batch 2, Purity not specified

Methods

Doses:	1 mg/kg/minute for 30 minutes
Frequency of dosing:	Once
Route of administration:	Intravenous infusion
Dose volume:	4 mL/minute
Formulation/Vehicle:	Aqueous solution at concentration of 2.0 mg/mL pH 6.4
Species/Strain:	Dog/Beagle
Number/Sex/Group:	1 female
Age:	Adult
Weight:	8 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Key Study Findings

The purpose of this study was to determine the pharmacotoxic dose of SP-303 in the dog after intravenous administration. SP-303 was administered at 1 mg/kg/minute and EKG (lead II), systolic blood pressure (tail cuff method) and heart rate were determined prior to and at 10 minute intervals for the duration of the infusion. In addition to monitoring cardiac parameters, the applicant also collected blood samples for determining total hemolytic complement activity *in vitro* with SP-303 at concentrations of 100, 50, 25, 10, 2, 1 and 0.25 μg/mL.

Administration of HP-303 at 1 mg/kg/minute produced a marked decrease in systolic blood pressure without affecting heart rate within the first 10 mins after dosing, as compared to pre-dose value. Systolic blood pressure continued to decrease over the next 10 min with a decrease in heart rate to 70 beats/min. At 30 min post dose, there was insufficient pulse pressure to determine systolic blood pressure. At 2 h post dose, the animal was euthanized after appearing moribund and lack of corneal reflex. There was a dose-dependent increase in intensity of observed clinical signs, which included pale mucosal membranes (capillary refill > 3.0 sec), shallow respiration and decreased general activity. Necropsy showed dark red coloration of gastrointestinal tract serosa, Peyers patches, spleen, kidneys and mesenteric lymph nodes. The spleen was enlarged and partially clotted blood was noted in the stomach, small intestine and cecum. Hemolysis was observed in all plasma samples collected after dosing, with the sample collected 165 min post-dose not clotting over a period of 20 h. The data are summarized in the sponsor's table below.

Effects of SP-0303A2 Upon
Systolic Blood Pressure and Heart Rate in a Dog

(b) (4)155-IN-001-90

Interval	Dose Administered	Systolic Blood Pressure (mmHg)	Heart Rate[b] (beats/min)	Heart Rate[c] (beats/min)
Predose	-	135	92	90
10 minutes of infusion	10 mg/kg	107	44	90
20 minutes of infusion	20 mg/kg	90	40	70
30 minutes of infusion	30 mg/kg	a	-	70

a = insufficient pulse pressure for evaluation
b = heart rate evaluated from tail cuff tracing
c = heart rate evaluated from ECG tracing

Study Title: A Cardiovascular Study in Conscious Beagle Dogs Administered SP-303 Intravenously
Study #: SP-303-F-016

In this telemetry study, 3 male beagle dogs were administered 3, 10, and 35 mg/kg SP-303 intravenously at 3.5 mL/kg (volume-to-body weight ratio), with a 30 min interval between doses. Two of the animals (#1001 and #1002) were administered SP-303 as bolus injections, while the third (#1003) received an infusion over 15 min. Animal #1002 also received a dose of atropine sulfate (0.2 mg/kg) 10 min prior to SP-303 injection.

Clinical signs related to treatment were observed at 35 mg/kg which included loss of consciousness due to hypotension, decreased activity, absent papillary and swallowing reflexes, excessive salivation, mydriasis, urination, absent pain sensation. At the high dose, animal #1001 showed a 60% and 78% decrease in mean arterial blood pressure (MABP) and 38% and 47% decrease in heart rate at 5 and 10 min post-dose, respectively. In animal #1002, which was pre-treated with atropine, there was an increase in heart rate pre-dose, immediately post-dose, followed by a 40% decrease at 20 min post-dose, and a 51-78% decrease in MABP at sacrifice. When infused over a 15 min period, heart rate and MABP decreased similarly (40% and 57%, respectively) and returned to pre-treatment values 15 min after the end of the infusion. There were no changes in ECG. There was a decrease in the absolute number of white blood cells and platelets at doses of SP-303 $\geq$ 10 mg/kg, regardless of whether SP-303 was administered as a bolus injection or as an infusion.

Study Title: Effects of Crofelemer on hERG K^+ currents in HEK-293 cells (non-GLP)
Study #: AA63535

Human embryonic kidney cells (HEK-293) stably expressing the hERG (human ether-a-go-go) K^+ channel were treated with crofelemer at concentrations ranging from 0.001 μM to 30 μM. Whole cell patch clamp technique was used to measure I_{Kr} (rapid potassium current) after crofelemer treatment. Crofelemer inhibited I_{Kr} in a dose-dependent manner with IC_{50} values of 1.79 μM and 1.75 μM for the first and second set of experiments, respectively. The positive control cisapride (10 μM) inhibited I_{Kr} by 99.67 and 100.47% in the first and second sets of experiments, respectively. Crofelemer is poorly absorbed (~1%) after oral administration and is highly bound (~98%) to human plasma protein. Because of its low oral bioavailability, the amount of free crofelemer available to produce I_{Kr} inhibition would be minimal.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

The following studies were reviewed previously by Dr. Tanveer Ahmad and are included here verbatim.

Levels of SP-303 in Rat Plasma Following Oral and Intravenous Administration
(Report #SP303-E-028)

Methods: Adult male Sprague-Dawley rats were given a single i.v. (5 mg/kg) or oral (gavage: 300 mg/kg) dose of SP-303. The vehicle was distilled water, and volume of administration was 10 ml/kg. Serial blood samples were taken from the rats which were given drug via i.v. route. The sampling times were 5, 25, 60, 180 and 360 min. after the drug administration. Transfusion of fresh whole blood (from a donor rat) equal to the sample volumes were given following the 25, 60 and 180 minute samples. In addition rats received periodic infusions of 0.9% NaCl to compensate for normal fluid loss (infusion amount was not given). Rats which received oral dose, were sacrificed at 3, 6, 12 and 24 hr after the drug administration and blood samples were collected immediately (3 rats/sampling times were used). Levels of drug in plasma samples were measured according to HPLC methods.

Results: SP-303 was not detected in plasma samples obtained from rats which received drug via oral route. Plasma concentrations of SP-303 decreased rapidly in rats which received the drug via i.v. route. Less than 5% of the initial plasma levels remained at the end of 360 min. (levels of SP-303: at 5 min.=99.7 μg/ml, and 360 min.: 4.5 μg/ml).

Pharmacokinetics and Oral Bioavailability of SP-303 in Rats
(Report #SP303-E-066)

Methods: Male rats (n=2-5/dose group) were given a single i.v. dose (0.3, 1.2, 3, 4.1, 9.6 or 10 mg/kg) of SP-303. The vehicle was 5% dextrose, and volume of administration was not reported. Blood samples were collected at 5, 15 min., 1, 2, 3, 4, 6, 12 and 24 hr after drug administration. Each time withdrawn blood was replaced by 2.5 ml of sterile 5% dextrose solution. Ten male rats were also given a single oral (gavage) dose of 200 or 500 mg/kg of SP-303. Blood samples were collected at 0.5, 1, 1.5, 3, 5, 7 and 12 hr after oral dose to monitor plasma levels of SP-303. Concentrations of SP-303 in plasma samples were measured by HPLC methods.

Reference ID: 3168746

Results: AUC values increased with increasing i.v. dosages. The plasma $t_{½}$ ranged from 1.5 ± 0.4 to 3.6 ± 1.1 hours.

Pharmacokinetic Parameters in Rats Post I.V. Dose

Dose (mg/kg)	AUC (μg.hr/ml)	$t_{½}$ (hr)	MRT (hr)	Cl (ml/kg·hr)
0.3	8.8±1.3	2.3±0.7	2.9±0.8	35±5
1.2	12.7	2.5	3.4	94
3.0	26.4±10.3	1.5±0.4	1.9±0.7	132±64
4.1	23.5±5.5	2.7±1.0	3.0±1.0	182±44
10.0	93.1±32.9	3.6±1.1	4.3±1.7	121±48

MRT = Mean residence time
Cl = total clearance

After oral dose (300 mg/kg), plasma levels of SP-303 were below or close to detection limit (limit of detection=0.1 μg/ml). Plasma level of SP-303 was 0.66 μg/ml at 11 hr (Tmax) after oral administration of 500 mg/kg of the drug. From this limited data, sponsor estimated absolute bioavailability to be less than 0.1%.

SP-303 Absorption From Enteric Coated Beads and From Aqueous Solution in Rats
(Report #SP303-E-073)

Methods: Groups of male rats (2-6/group) were given a single oral (gavage) dose of 500 mg/kg (in enteric coated bead solution or plain dissolve in distilled water) of SP-303. Another group of rats were given a single i.v. dose of 0.3 mg/kg of SP-303 (0.15 mg/ml in 5% dextrose solution). Blood samples were collected at 2, 4, 7 and 8 hr after oral dose and at 15 min., 1 and 3 hr after i.v. dose to measure SP-303 levels in plasma samples. SP-303 levels were analyzed by HPLC methods.

Results: After oral administration of 500 mg/kg of SP-303 as enteric-coated beads or as solution in water, SP-303 was not detected in rats plasma [only one out of 12 rats had SP-303 level higher than the detection limit (rat #F = 2.03 μg/ml; detection limit = 1.0 μg/ml). After i.v. dose of 0.3 mg/kg, plasma levels at 15 min. were 3.13±2.68 μg/ml (AUC_{0-3hr} = 3.28 μg·hr/ml).

Study Title: Metabolic stability of crofelemer in human liver microsomes
Study #: CRDM0100

The purpose of this study was to determine the metabolic stability of crofelemer (5 μM) in human liver microsomes in the presence/absence of NADPH. Crofelemer was incubated for up to 60 min (± NADPH-generating system) with human liver microsomes.

Reference ID: 3168746

At the end of 60 min, the percentage of crofelemer detected was 13% and 96% of the starting amount in the presence and absence of NADPH, respectively. These results indicated that crofelemer metabolism was enzymatic and dependent on NADPH. In human liver microsomes, crofelemer had an in vitro half life and in vitro clearance of 15.3 min and 90.4 μL/min/mg microsomal protein, respectively.

Study Title: *In vitro* evaluation of crofelemer as an inducer of cytochrome p450 expression in cultured human hepatocytes
Study #: CRDM0102

The purpose of the study was to determine the ability to induce cytochrome P450 (CYP) enzymes in cultured human hepatocytes. Crofelemer (10, 100 or 1000 nM), vehicle (0.1% v/v dimethylsulfoxide), or known CYP inducers (100 μM omeprazole, 750 μM phenobarbital, 10 μM rifampin) was added to primary cultures of human hepatocytes and cells were incubated. After incubation, cells were harvested and microsomes were isolated and analyzed for activation of specific CYP enzymes. Study results are summarized in the table below:

Table 3: CYP activity: The effects of treating cultured human hepatocytes with Crofelemer or prototypical inducers on microsomal cytochrome P450 (CYP) enzyme activity

Treatment	Concentration	Enzyme activity (pmol/mg protein/min) [a]		
		Phenacetin *O*-dealkylation (CYP1A2)	Bupropion hydroxylation (CYP2B6)	Testosterone 6β-hydroxylation (CYP3A4/5)
Dimethyl sulfoxide	0.1% (v/v)	29.0 ± 11.5	48.3 ± 45.8	2110 ± 1760
Crofelemer	10 nM	27.9 ± 10.0	45.2 ± 42.9	2130 ± 1790
Crofelemer	100 nM	28.6 ± 12.3	52.6 ± 54.0	1850 ± 1740
Crofelemer	1000 nM	29.1 ± 10.1	51.6 ± 49.9	2260 ± 1860
Omeprazole	100 nM	1100 ± 180	541 ± 769	4020 ± 1130
Phenobarbital	750 nM	56.0 ± 16.1	759 ± 1061	11800 ± 3400
Rifampin	10 nM	51.0 ± 24.5	353 ± 471	12100 ± 4100

a Values are the mean ± standard deviation of three determinations (human hepatocyte preparations H1001, H1005 and H1006).
Data are shown graphically in Figures 2 – 4. Individual data are shown in Appendix 3.

Under the conditions of this study, crofelemer at doses of up to 1000 nM failed to induce CYP enzymes CYP1A2, 2B6, and 3A4/5.

Study Title: *In vitro* interaction studies of crofelemer with human MRP2 (ABCC2) and MRP4 (ABCC4) ABC (efflux) transporters, and with human OATP2B1 (OATP-B), PEPT1, OATP1A2 and ASBT (SLC10A2) uptake transporters
Study #: CRDM0103

The ability of crofelemer to inhibit efflux and uptake transporters was investigated *in vitro*. Transport assays were performed using membrane vesicles which were prepared from CHO cells overexpressing the transporter of interest. Crofelemer was incubated with the membrane preparations and compared with the appropriate positive and negative controls for each assay. Study results showed that crofelemer inhibited transport of substrates specific for MRP2 and MRP4 with an IC_{50} of 15 and 7 μM, respectively. Crofelemer also potently inhibited OATP2B1, PEPT1, ASBT, and

Reference ID: 3168746

OATP1A2 transporters with an IC_{50} of 39, 50, 7 and 7 μM, respectively. These results are summarized in the table below.

Table: Inhibition of human efflux and uptake transporters by crofelemer

Assay	IC_{50} (μM)	Max inhibition (% control)
MRP2	15	100
MRP4	7	100
OATP2B1	39	95
PEPT1	50	91
ASBT	7	96
OATP1A2	7	99

Distribution

Study Title: A mass balance and tissue distribution study of radioactivity in Sprague-Dawley rats administered a single oral dose of [^{14}C]-methylated crofelemer
Study #: (b) (4) 1482-008

The purpose of this study was to determine the metabolic stability, absorption, and excretion of crofelemer after a single oral administration in rats. Male rats were administered a single dose of 2.8 mg/kg ^{14}C-methylated crofelemer (5 mL/kg dose volume). The majority of radioactivity was detected in the feces (88.2% of dosed radioactivity). The radioactivity recovered was 0.98% in urine and 0.88% in cage residues, indicating that crofelemer was poorly absorbed (1-2% of dosed radioactivity). No radioactivity was measured in expired air, pelt, tail skin, and carcass. In a second study, at 72 h post-dose, the mean recovery of radioactive crofelemer was 107.84 ± 1.64% of the dose, with majority of the radioactivity being detected in the feces. An examination of harvested tissues from treated animals revealed that the majority of crofelemer was excreted unabsorbed, with minimal urinary excretion within 24 h post-dose. At 24 h post-dose, the majority of crofelemer remained in the gastrointestinal tract. By 72 h post-dose, the majority of radioactivity was recovered.

Study Title: Protein binding of ^{14}C-methylated crofelemer
Study #: (b) (4) 021285-1

This study report references Study # 021485-1 (*In-vitro* studies of NP-303), which is reviewed below.

The purpose of this study was to determine: the intestinal permeability of NP-303 using Caco-2 cell monolayers; the percent of NP-303 that binds to proteins in human plasma; and the effect of NP-303 on the metabolic activity of CYP 1A2, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4 isozymes in pooled human liver microsomes. Crofelemer displayed high protein binding ability (95.4% and 98.8% at 30 and 50 μM [^{14}C] NP-303, respectively).

The average recovery of test article from the dialysis apparatus was 95% and 90% of total radioactive dose, at the low and high dose respectively, suggesting that there was minimal nonspecific binding. When tested for the ability to inhibit CYP enzymes, crofelemer did not show significant inhibitory activity in pooled human liver microsomes, as compared to positive controls. These data are shown in the sponsor's tables below:

Table 2: Summary of Inhibitory of NP-303 on CYP450 Activities in Human Liver Microsomes Non-Time Dependent

	Percent (%) Inhibition Non-Time Dependent Samples							
NP-303 Conc. (µM)	CYP1A2	CYP2B6	CYPC9	CYPC19	CYP2D6	CYP2E1	CYP3A4 (testosterone)	CYP3A4 (midazolam)
1	-16	-7	3	40	7	25	-7	3
10	35	29	20	53	51	33	33	20
50	87	79	94	92	100	53	94	94
75	87	83	94	92	100	53	94	94
Positive Control	87	74	94	92	98	39	90	94
Calculated IC_{50} (µM)	21.5	9.8	26.2	7.9	9.8	44.0	21.0	6.3

All values are the mean of duplicate determinations.

Table 3: Summary of Inhibitory of NP-303 on CYP450 Activities in Human Liver Microsomes Time Dependent

	Percent (%) Inhibition Time Dependent Samples							
NP-303 Conc. (µM)	CYP1A2	CYP2B6	CYPC9	CYPC19	CYP2D6	CYP2E1	CYP3A4 (testosterone)	CYP3A4 (midazolam)
10	33	22	-9	62	43	26	55	88
75	84	84	90	92	100	45	88	93
Calculated IC_{50} (µM)	31.7	39.4	48.7	8.1	18.0	83.3	9.1	5.7

All values are the mean of duplicate determinations.

In Caco-2 cell monolayers, crofelemer showed very low permeability in the apical to basolateral (A→B) direction and no permeability in the B→A direction, regardless of the presence of the P-glycoprotein inhibitor verapamil. These data are summarized in the sponsor's table below:

Table 12: Permeability with Caco-2 Cells

	A-B	B-A	B-A + Verapamil
Mannitol	0.6	0.8	ND
Caffeine	28.4	20.2	ND
Rhodamine	ND	7.0	0.0
NP 303 30 µM	0.0	0.04	0.04
NP 303 100 µM	0.0	0.02	0.02

ND = not determined

Study Title: Binding of SP-303 to proteins

Study #: SP-303-A-059

In a series of in vitro studies, the sponsor demonstrated that SP-303 binds to various proteins. In simulated gastric fluid (pH 1.05), SP-303 formed a reddish precipitate when added to a final concentration of 20 mg/mL. High performance capillary electrophoresis of the filtrates revealed that the peak area of pepsin was reduced by 93% with the addition of SP-303, which corresponds to a removal of 3.2 mg/mL SP-303 and 2.98 mg/mL pepsin from the reaction due to precipitation. In separate studies with insoluble

collagen, SP-303 at 0.2 mg/mL remained bound to collagen for 2-4 h, while binding continued for 24 h at a concentration of 2 mg/mL. The noncovalent binding between SP-303 and collagen was reversed with treatment with NaOH. When added to whole human plasma, SP-303 was shown to bind in a dose-dependent manner to plasma proteins. Precipitate formation was observed when SP-303 concentration exceeded 50 μg/mL. In an aqueous solutions of human or bovine serum albumin (concentration of up to 50 mg/mL), SP-303 at a concentration of 1 mg/mL was shown to be ~80% bound to protein, without evidence of precipitation. Similar dose-dependent binding was observed between SP-303 and rabbit gamma globulin fraction (IgG) and precipitation was observed. It was concluded that precipitation observed when SP-303 was incubated with whole human plasma was likely the result of SP-303 binding to serum gamma globulins. Therefore, these *in vitro* studies demonstrated that SP-303 binds to various proteins, including pepsin in simulated gastric fluid. Based on this observation, the sponsor suggested that enteric coating may be necessary to deliver the drug to the site of action (small intestine) and limit the effective dose administered.

Excretion

Study Title: Evidence of absorption and bioactivity of SP-303-derived metabolites following oral administration of SP-303
Study #: SP-303-E-059

In this study, urine samples from rats and humans dosed orally with SP-303 were analyzed for presence of SP-303-derived metabolites. Rats were maintained on flavonoid-free diet (boiled eggs, white bread, ascorbic acid, and powdered milk) to allow for detection of metabolites, which were undetectable when rats were maintained on rat chow. Solid phase extraction/HPLC-photodiode array methods were used to prepare and analyze urine samples. Three metabolites which were structurally related to catechin were detected. (b) (4) This finding was further confirmed in a repeat experiment.

Similar analytical methods were used to study the metabolite profile in human urine (clinical Study # SP303-1-04). Two human subjects, who received once daily dose of 2000 mg SP-303 for 7 days provided urine samples. SP-303 metabolites were detected in humans using the same methods which were used for rat urine. Accumulation of SP-303 derived compounds was detected in human urine after repeated oral dosing for 7 days.

Pharmacokinetic Drug Interactions

Study Title: *In vitro* evaluation of crofelemer as an inhibitor of cytochrome P450 (CYP) enzymes in human liver microsomes
Study #: CRIV0101

This study was conducted to determine whether crofelemer would inhibit the following CYP enzymes in human liver microsomes: CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5. Under the conditions of the experiment, crofelemer inhibited all CYP enzymes tested with IC_{50} of 0.28-3.5 μM (summarized in the sponsor's table below). Crofelemer showed time and metabolism dependent increase in inhibitory activity against CYP3A4/5 only.

Table 4: Summary of results: *In vitro* evaluation of Crofelemer as an inhibitor of human CYP enzymes

Test System: Human Liver Microsomes								
Enzyme	Enzyme reaction	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-minute preincubation		30-minute preincubation without NADPH		30-minute preincubation with NADPH		Potential for metabolism-dependent inhibition [c]
		IC_{50} (μM) [a]	Inhibition observed at 300 μM (%) [b]	IC_{50} (μM) [a]	Inhibition observed at 300 μM (%) [b]	IC_{50} (μM) [a]	Inhibition observed at 300 μM (%) [b]	
CYP1A2	Phenacetin *O*-dealkylation	2.9	100	2.7	100	2.9	100	No
CYP2B6	Efavirenz 8-hydroxylation	2.1	100	2.6	100	1.7	100	No
CYP2C8	Amodiaquine *N*-dealkylation	0.28	100	0.28	100	0.25	100	No
CYP2C9	Diclofenac 4'-hydroxylation	3.5	100	3.4	100	3.4	100	No
CYP2C19	*S*-Mephenytoin 4'-hydroxylation	3.1	100	3.1	100	3.0	100	No
CYP2D6	Dextromethorphan *O*-demethylation	2.9	100	2.6	100	2.9	100	No
CYP3A4/5	Testosterone 6β-hydroxylation	3.3	100	2.6	100	1.8	100	Yes
CYP3A4/5	Midazolam 1'-hydroxylation	1.1	100	1.1	100	1.0	100	No

Test System: Human Hepatocytes						
Enzyme	Enzyme reaction	Direct inhibition		Time-dependent inhibition		
		Zero-minute preincubation		30-minute preincubation		Potential for time-dependent inhibition [d]
		IC_{50} (μM) [a]	Inhibition observed at 300 μM (%) [b]	IC_{50} (μM) [a]	Inhibition observed at 300 μM (%) [b]	
CYP3A4/5	Midazolam 1'-hydroxylation	>300	28	>300	41	No

a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values.

b Inhibition observed (%) is calculated with the following formula (results are rounded to two significant figures):
Inhibition observed (%) = 100% − Percent solvent control.

c Metabolism-dependent inhibition was determined by comparison of IC_{50} values both with and without preincubation and with and without NADPH-generating system present in the preincubation, by comparison of the observed inhibition (%) for all preincubation conditions and by visual inspection of the IC_{50} plots.

d Time-dependent inhibition was determined by comparison of IC_{50} values both with and without preincubation, by comparison of the observed inhibition (%) for the preincubation conditions examined and by visual inspection of the IC_{50} plots.

5.2 Toxicokinetics

Study Title: Toxicokinetics of crofelemer from repeat-dose toxicology study (b) (4) 28809 in rats
Study #: SP-303-F-041

This study report was submitted in Appendix E, Study # SP-303-F-046 (An oral dose range-finding study of microencapsulated provir in rats ((b) (4) 288009), See review on page 39 of this document). Briefly, rats were administered 0 or 1200 mg/kg/day microencapsulated enteric coated SP-303 for 7 days and blood samples were collected on day 6 at 1, 2, 4, 6, and 24 h post-dose by the CRO. Samples were then shipped to the sponsor, who conducted the TK analysis. SP-303 was not detected in the plasma of placebo-treated animals. Animals treated with 1200 mg/kg/day SP-303 had plasma levels ranging from 1.3 to 4.0 μg/mL beginning at 2 h post-dose. SP-303 levels could be detected at up to 24 h post-dose. $AUC_{1\rightarrow24}$ was 43.7 μg·h/mL when rats were orally administered 1200 mg/kg/day microencapsulated enteric coated SP-303.

Study Title: Toxicokinetics of crofelemer from repeat-dose toxicology study SP-303-F-042 in dogs
Study #: SP-303-F-040

This study report was submitted as part of Study # SP-303-F-042 (An escalating dose oral toxicity study provir (enteric coated tablets) in dogs ((b) (4) 288008), which is reviewed on page 40 of this document). Briefly, dogs were administered enteric coated SP-303 tablets at a dose of 600 mg/kg/day for 7 days and blood samples were collected on Days 0 and 6. TK analysis indicated that SP-303 levels were ≤ 1.4 µg/mL in the plasma during the first 6 h and increased to 5 – 14.3 µg/mL within the first 24 h. Plasma levels were similar when measured on Day 6.

Study Title: Toxicokinetics of crofelemer from repeat-dose toxicology study SP-303-F-047 in dogs
Study #: SP-303-F-080

This study report was submitted as part of Study # SP-303-F-047 (An escalating dose oral toxicity study enteric coated SP-303 in dogs ((b) (4) 288012)) reviewed on page 39 of this document. Briefly, dogs were administered enteric coated SP-303 tablets at a dose of 800 mg/kg/day for 7 days and blood samples were collected on Days 0 and 6. TK analysis indicated that SP-303 levels were 1.1 – 4.7 µg/mL on Day 0 and plasma levels were higher on Day 6 (3.4 – 19.2 µg/mL). $AUC_{1\rightarrow24}$ on Day 0 and Day 6 ranged from 47.5 – 131.6 µg·h/mL and 140.6 µg·h/mL - 382.6 µg·h/mL, respectively.

Study Title: Toxicokinetics of crofelemer from repeat-dose toxicology study SP-303-F-048 in dogs
Study #: SP-303-E-081

Please refer to the review of Study # SP-303-F-048 (A 30-day oral toxicity study of enteric coated SP-303 in dogs ((b) (4) 288006) located on page 55 of this document.

Additional TK studies are reviewed with the associated general toxicity study.

6 General Toxicology

6.1 Single-Dose Toxicity

Single dose toxicity studies were conducted in mice (intravenous, IV, and intraperitoneal, IP), rats (oral and IP), and dogs (oral and IV). Full study reports were provided with the NDA and are summarized briefly here.

In a GLP study (Study # SP303-F-001), SP-303 (or vehicle, 5% dextrose) was injected into the tail vein of CD-1 mice (10/sex/dose) at 0, 6.25, 12.5, 25, and 50 mg/kg in a

Reference ID: 3168746

volume of 5 mL/kg. IP injections were administered at doses of 10, 20, 50, and 100 mg/kg in 10 mice/sex/dose. The LD_{50} of SP-303 was > 50 mg/kg and >100 mg/kg when administered IV and IP, respectively.

In rats, two oral single dose studies (Study # SP-303-F-046 and Study # SP-303-F-003), one IP study (Study # SP-303-AT-002), and one IV study (Study # SP-303-F-002) were conducted. Oral studies in Crl:CD(SD)BR rats (2/sex/dose) demonstrated that microencapsulated SP-303 at 600, 1200, and 2400 mg/kg by gavage followed by a water flush. All animals treated with 1200 mg/kg died within 4 h of dosing, even though the 2400 mg/kg was tolerated. Rales was the predominant treatment-related clinical sign. In an IV study, SP-303 (or vehicle, 5% dextrose) was injected into the tail vein (6/sex/dose) at 0, 0, 3, 10, 20, and 50 mg/kg in a volume of 5 mL/kg. IP injections were administered at doses of 10, 20, 50, and 100 mg/kg in a volume of 10 mL/kg. The LD_{50} of SP-303 was > 50 mg/kg and >100 mg/kg when administered IV and IP, respectively. IP-treated animals had increased liver size and spleen discoloration at the high dose.

In dogs, the toxicity of enteric-coated, gelatin encapsulated SP-303 was investigated in 2 studies (Study # SP-303-F-042 and Study # SP-303-F-047). SP-303 was tolerated at doses of up to 1200 mg/kg after oral administration. Treatment-related clinical signs included abnormal excreta, decreased food consumption, emesis and weight loss. Severity of GI-related adverse effects was greatest at the high dose of 1200 mg/kg. When administered intravenously (Study # SP-303-F-004), the MTD of SP-303 was 18.9 mg/kg.

6.2 Repeat-Dose Toxicity

In a dose range-finding (DRF) study, 1200 mg/kg/day microencapsulated test article (Study # SP-303-F-046) was orally administered for 7 days to Sprague-Dawley rats (6/sex/dose). There were 4 unscheduled deaths in SP-303-treated animals, with esophageal changes indicative of dosing errors in 2 of the animals. One animal receiving control treatment (slurry of 40-60 mesh sugar spheres in Eudragit L30D-55) also died prior to terminal sacrifice, although the cause of death was unclear. Clinical signs, which were observed in both SP-303 and control animals, included rales, dehydration, small feces, red material on body surfaces and yellow material on urogenital area. Histopathological changes in the esophagus (muscle degeneration, fibrosis and mononuclear cells infiltration) and stomach (moderate gastric necrosis and mild acute gastric inflammation) were noted in some of the 1200 mg/kg/day-treated animals. Based on the results of this DRF study, doses of 100 and 600 mg/kg/day SP-303 were chosen for the 1 month repeat-dose study along with a different control, since there was significant toxicity observed in control animals in this study.

Several DRF studies were conducted in beagle dogs (2 males/group) using different formulations of SP-303. In Study # (b) (4)218-20-03-91, test article was dissolved in sterile water and administered by oral gavage at a dose of 100 mg/kg/day for 5 days. As compared to control (sterile water, 4 mL/kg), SP-303 produced vomiting, a decrease

in food consumption, and dark discoloration of the proximal small intestine with no histopathological findings. Therefore, SP-303 was tolerated at 100 mg/kg/day, but produced mild gastrointestinal changes in dogs. When administered at 800 mg/kg/day for 7 days as enteric coated tablets, SP-303 produced body weight losses and significant signs of toxicity in the gastrointestinal system (dark mucoid/rust colored feces, sporadic emesis, microscopic changes indicative of intestinal tract damage). At a dose of 600 mg/kg/day as enteric coated tablets (Study # SP-303-F-042) for 7 days, SP-303 produced changes in excreta and minor body weight loss, and test article tablets were noted in the gastrointestinal tract at necropsy. Therefore, the 600 mg/kg/day oral dose administered as enteric coated tablets was tolerated. When administered intravenously for 5 days (Study # SP-303-F-009), SP-303 produced dose-dependent increases in CNS-related clinical signs, changes in clinical chemistry and hematology, gross and microscopic changes at doses > 20 mg/kg/day.

In Study # SP-303-F-012, intravenous administration of 5 mg/kg/day (1 h infusion) of SP-303 for 5 days to African Green monkeys did not produce any changes in hematological parameters. Likewise, when administered orally at 50 mg/kg/day for 5 days, SP-303 did not affect hematological parameters. In a 5-day repeat-dose oral toxicity study (Study # SP-303-F-013), SP-303 was tolerated at doses of up to 900 mg/kg/day with no significant treatment-related adverse effects. The NOAEL dose of SP-303 after oral administration for 14 days was greater 10 mg/kg but lower than 200 mg/kg/day (Study # SP-303-F-022), since doses of 200 and 500 mg/kg/day produced dose-dependent increases in GI-related adverse effects and histopathological changes consistent with thymic lymphoid involution. This was further confirmed in another 14-day study (Study # SP-303-F-014) where oral administration of SP-303 at doses up to 100 mg/kg/day failed to produce significant treatment-related toxicities.

The following studies were previously reviewed by Dr. Tanveer Ahmad and are included here verbatim.

30-Day Oral Toxicity Study in Rats
(Report #SF303-F-020)

Testing Laboratories: [redacted] (b) (4)

Study Started: July 25, 1991

Study Completed: December 23, 1991

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Animals: Six weeks old Sprague-Dawley rats of both sexes (males: 130-154.8 g, females: 109-137.1 g).

Drug Batch No.: Not given

Methods: Groups of rats (12/sex/group) were given daily oral (gavage) doses of vehicle (distilled water), 50, 200 and 500 mg/kg/day of SP-303 (pH of the drug solution was not adjusted). The volume of administration was fixed at 20 ml/kg. Additionally 5 rats/sex each were included in control and high dose groups and used for 30 day recovery study. All rats were observed daily for clinical signs and mortality daily. Body weights were recorded every 3rd or 4th day during study period, food intakes were recorded twice weekly. Ophthalmoscopic examinations were performed on all rats during pretest, on days 9, 23 and 51 of the

study. Blood samples were collected from orbital sinus on days 5 and 10 (5 rats/sex from control and high dose groups), at the end of treatment period (12 rats/sex/group) and at the end of recovery period (5 rats/sex from control and high dose groups) for hematology and serum chemistry tests. Overnight urine samples were collected from 5 rats/sex/group on days 9 and 10, at the end of treatment/recovery period for urinalysis. Just before sacrifice blood samples were also collected for monitoring drug plasma levels, however, no such report was submitted. All rats were subjected to complete necropsy. Only tissue from control and high dose groups were examined microscopically.

Results:

1. **Observed Effects:** Rough haircoat was seen in some of mid and high dose treated rats. Additionally, one high dose treated rat of each sex had hunched posture on a few occasions.

2. **Mortality:** One high dose treated male was found dead on day 21 of the study. The cause of death could not be established.

3. **Body Weights/Food Consumptions/Water Consumptions:** In males, at the end of treatment period, body weight gains were reduced by 8.2%, 14.5% and 12.4% at low, mid and high dose respectively, compared to control values. In females body weight gains were reduced by 5.2% and 8.9% at mid and high dose respectively, compared to control values. At the end of 30-day recovery period body weight gains in high dose treated males were comparable to that seen in control males. However, in high dose treated females, at the end of recovery period, body weight gains were still 19.2% lower than that seen in control females. Food intakes were no affected by the treatment.

4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen.

5. **Blood Chemistry/Urinalysis:** No treatment related effects were seen.

6. **Vital Signs/Physical Examination/Ophthalmic Examination:** No treatment related effects were seen.

7. **Organ Weights:** No treatment related effects were seen.

8. **Gross Pathology:** No treatment related effects were seen.

9. **Histopathology:** No treatment related effects were seen.

Reference ID: 3168746

In this study, highest tested dose produces lethality. Mid dose level produced decrease in body weight gains (males: 14.5% and females: 5.2%) and rough haircoats were seen in some of the mid dose treated rats. This dose level can be considered as well tolerated dose.

I.V. Dose Ranging Study in Rats
(Report #SP303-F-005)

Testing Laboratories: (b) (4)

Study Started: November 7, 1990

Study Completed: March 6, 1991

GLP Requirements: A Statement of Compliance with the GLP regulations were included.

Drug Batch No.: SP-0303C

Animals: Six weeks old Sprague-Dawley rats of both sexes (80-100 g).

Methods: Groups of Sprague-Dawley rats (5/sex/group) were given i.v. daily doses of vehicle (5% dextrose), 5, 20 and 35 mg/kg/day of SP-303 for 5 consecutive days. The volume of administration was fixed at 5 ml/kg (1 ml/kg/min). All rats were observed daily for clinical signs and mortality. Body weights were recorded daily. Food consumptions were recorded at pretest, 4th and 5th days of the study. Just before sacrifice (i.e. 24 hr. after the last dose) blood samples were collected from vena cava for hematology and serum chemistry tests. At the end of treatment period rats were sacrificed and necropsied. Only tissues from control and high dose groups were examined microscopically.

Results: No clinical sign or mortality was seen during the study period, except discoloration at the injection site (tail) was evident in treated rats. In males, body weight gains were reduced by 21%, 82% and 88% at low, mid and high dose respectively, when compared to the control values. In males food consumptions were also decreased by 7%, 27% and 32% at low, mid and high dose respectively, when compared to control values. In females, only at high dose, body weight gains were reduced by 54% along with 16% reduction in food intakes, when compared to their respective control values. Due to "technical problem" hematology values in males were not reported. In treated females, significant increases in white blood cell, segmented neutrophil and platelet counts and decreases in red blood cell counts, hemiglobin concentrations and hematocrit were seen.

Reference ID: 3168746

Effects on Hematological Parameters in Female Rats

Parameters	Control	Low Dose	Mid Dose	High Dose
RBC ($10^6/\mu l$)	5.94±0.13	5.82±0.20	4.97±0.44	4.54±0.36
Hemoglobin (g/dl)	13.35±0.21	12.58±0.73	10.88±1.08	9.94±0.60
Hematocrit (%)	36.7±1.13	35.50±1.79	30.0±2.36	26.9±1.70
Platelet ($10^3/\mu l$)	927±448	1498±125	1450±109	1324±214
WBC ($10^3/\mu l$)	9.2±2.4	12.35±0.9	18.48±2.66	25.30±6.26
Segmented Neutrophils ($10^3/\mu l$)	1.0±0.1	0.09±0.3	5.6±1.2	9.0±2.7
NRBC ($10^3/\mu l$/100 WBC)	0±0	0±0	0.4±0.5	2.4±2.8

Only in females, liver weights were increased by 15%, 28% and 33% in low, mid and high dose groups respectively, when compared to control values. In females, spleen weights were also increased by 35%, 71% and 131% in low, mid and high dose groups respectively, compared to control values. No treatment related histological changes were evident in this study, except histological changes at the injection sites (tail: hemorrhage, necrosis, inflammation and/or thrombosis) were seen in mid and high dose treated rats.

In this study, lowest tested dose (5 mg/kg/day) can be considered as well tolerated dose, since it only produced slight reduction in body weight gains in males (21%) and decrease in food intakes (7%) along with some minor changes in hematological parameters in females [increases in platelet counts (61%) and WBC count (34%)] (hematological parameters in males were not reported).

5 Day I.V. Toxicity Study in Dogs
(Report #303-F-010)

Testing Laboratories: (b) (4)

Study Started: January 22, 1991

Study Completed: April 9, 1991

GLP Requirements: A State of Compliance with GLP regulations was included.

Drug Batch No.: SP-303C

Animals: Five months old beagle dogs (males: 6.0-7.5 kg and females 5.9-8.3 kg).

Methods: Groups of dogs (3/sex/group) were given I.V. (infused over 30 min.) doses of vehicle (5% dextrose), 2.5, 5.0 and 10 mg/kg/day of SP-303 for 5 consecutive days. The volume of administration was fixed at 5 ml/kg. Additionally, two dogs/sex were also included in high dose group for recovery study (1 dog/sex each was used for 2-week and 4-week recovery periods respectively). All dogs were observed daily for clinical signs and mortality. Body weights were recorded daily during treatment period and weekly during recovery period. Food consumptions were recorded daily. Blood pressure and heart rate were recorded daily, just prior to dosing and at 5, 15, 30, 60 and 120 min. after the start of drug administration. Blood samples were collected from jugular/saphenous vein daily at pretest, 15, 30, 60 and 120 minutes after drug administration for hematological tests. Samples obtained 120 min. post dosing were also used for blood chemistry tests. Additionally, hematology and serum chemistry tests were also monitored on recovery days 1, 3, 5, 7, 14, 21 and 28. Blood samples collected from high dose group at end of dosing (i.e. end of 30 min. infusion) and 15 min. post dosing on days 1 and 5 were also used to monitor plasma drug levels. All dogs at the end of treatment/recovery period were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects:** No treatment related effects were seen.

2. **Mortality:** None.

3. **Body Weights/Food Consumptions/Water Consumptions:** No biologically significant effects were seen in treated dogs.

4. **Hematology/Coagulation/Bone Marrow:** Dose related decreases in platelet count (up to 50%) were seen in treated dogs, and this effect disappeared at the end of 2-weeks of recovery period.

5. **Blood Chemistry/Urinalysis:** No treatment related effects were seen.

6. **Vital Signs/Physical Examinations/Blood Pressure/Heart Rates:** No treatment related effects were seen.

7. **Organ Weight:** No treatment related effects were seen.

8. **Gross Pathology:** Dark discoloration was seen in small intestine of treated dogs (males: control=0/3, low dose=1/3, mid dose=1/3 and high dose=3/3).

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9. **Histopathology:** Pigmentation in the hepatic sinusoidal cells and in the small intestine were seen in mid and high dose treated dogs. Histiocytic foci in the villi of proximal small intestine were seen in dogs of all treated groups. The incidences of adverse histopathological findings were as follows:

Histopathological Findings in Dogs

Tissue/Findings	Sex (M/F)	Control	Low Dose	Mid Dose	High Dose
Liver					
Pigmentation	M	0/3	0/3	2/3	3/3
	F	0/3	0/3	2/3	3/3
Small Intestine					
Histiocytic Foci	M	0/3	1/3	3/3	3/3
	F	0/3	1/3	2/3	3/3
Pigmentation	M	0/3	0/3	2/3	3/3
	F	0/3	0/3	2/3	3/3

Additionally, only at high dose, hepatic sinusoidal histiocytosis (males: 2/3 and females: 3/3), cytoplasmic vacuoles in the spleen (males: 3/3 and females: 2/3), enlarged and vacuolated histiocytes in the mesenteric lymph nodes (males: 3/3 and females: 1/3) and focal thrombosis in the lungs (males: 2/3 and females: 1/3) were seen. At high dose, neutral lipids (increased oil red 0 positive cells) were also seen in the vacuoles of spleen (males: 3/3 and females: 2/3) and mesenteric lymph node (males: 3/3 and females: 1/3). At the injection sites thrombosis were seen in some of the treated dogs (none in the control groups).

At the end of 4-weeks of recovery period some of the above findings were still present. Furthermore, pigmentation was also seen in the pancreatic and mesenteric lymph nodes (only 1/sex of high dose group dog was used for recovery study).

30-Day Oral Toxicity Study in Rhesus Monkeys
(Report #SP303-F-019)

Testing Laboratories: (b) (4)

Study Started: August 9, 1991

Study Completed: January 6, 1992

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Drug Batch No.: SP-3032

Animals: Young adult rhesus monkey (males: 2.7-4.2 kg and females 2.8-4.3 kg).

Methods: Groups of rhesus monkeys (4/sex/group) were given orally (gavage) daily doses of vehicle (sterile water), 30, 100 and 200 mg/kg/day of SP-303 for 30 days. The volume of administration was fixed at 5 ml/kg. Additionally, 2 monkeys/sex were included in control and high dose groups and treated in similar fashion and used for 30-day recovery study. All animals were observed daily for clinical signs and mortality. Body weights were recorded twice weekly and food and water intakes were monitored daily. Ophthalmoscopic examinations were conducted once pre-test and at the end of treatment/recovery period on all monkeys. EKG were performed on all monkeys before sacrifice. Blood samples were collected from femoral vein following overnight starvation at pretest, days 15 and 30 of the study and at the end of recovery period for hematological and serum chemistry tests. Blood samples were also collected at 2 hr. post drug/vehicle administration on days 2, 15 and 30 of the study for measuring plasma drug levels. At the end of treatment/ recovery period all monkeys were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects:** Liquid/soft feces were seen in high dose treated monkeys. Occasional vomiting was also seen in some of the mid and high dose treated monkeys.

2. **Mortality:** One monkey from low dose group died on day 29 of the study. The cause of death was accidental.

3. **Body Weights/Food Consumptions/Water Consumptions:** No treatment related effects were seen.

4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen.

5. **Blood Chemistry/Urinalysis:** No treatment related effects were seen.

6. **Vital Signs/Physical Examinations/Ophthalmic Examination/EKG:** No treatment related effects were seen.

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7. **Organ Weights:** In males, prostate weights were increased by 64%, 120% and 142% at low, mid and high dose respectively, when compared to control values. In high dose treated males spleen weights were increased by 84% (relative weight: 78%) compared to control values.

8. **Gross Pathology:** Grey discoloration of the mucosa of the proximal small intestine was seen in all high dose treated monkeys. This finding was not evident at the end of recovery period.

9. **Histopathology:** Increased incidence of pigmented histiocytes in small intestine and cecum were seen in mid and high dose treated monkeys. Increased incidences of pigmented histiocytes were also seen in colon and liver of high dose treated monkeys. The incidence of above findings were as follows:

Histopathological Findings in Monkeys

Tissue/Findings	Sex (M/F)	Control	Low Dose	Mid Dose	High Dose
# Examined		4	4	4	4
Small Intestine: Pigmented Histiocytes	M F	0 0	0 0	3 2	4 4
Cecum: Pigmented Histiocytes	M F	0 0	0 0	1 0	3 3
Colon: Pigmented Histiocytes	M F	0 0	0 0	0 0	1 2
Liver: Pigmented Sinusoidal Cells	M F	0 0	0 0	0 0	1 3

At the end of recovery period most of these findings disappeared or significantly reduced.

In this study, the low dose (30 mg/kg/day) was the no effect dose. Mid (100 mg/kg/day) and high dose (200 mg/kg/day) produced pigmentation of histiocytes in the liver (sinusoidal cells), small intestine, cecum and colon. These histological findings most likely related to the colored nature of the drug substance. Hence 200 mg/kg/day can be considered as well tolerated dose in this study.

2-Week Oral Toxicity Study in Neonatal Rats
(Report SP303-F-030)

Testing Laboratories: (b) (4)

Study Started: June 10, 1993

Study Completed: December 8, 1993

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Comments: In this study neonatal Sprague-Dawley rats (10/sex/group) were given daily oral (gavage) doses of vehicle (distilled water), 10, 200 and 500 mg/kg/day of SP-303 for 2 weeks (from day 5-18 of lactation). This study was not very informative, because mid and high doses were lethal (mortality rates: males: 40% and 100% and females: 50% and 80% respectively). The cause of death could not be established clearly. According to sponsor, drug induced toxicity (e.g. dehydration and emaciation) and error in dosing were the causes of death. However, at low dose (10 mg/kg/day) no observable toxicity was evident. Sponsor repeated the above study with different dose levels (see below).

2-Week Oral Toxicity Study in Neonatal Rats
(Report #SP303-F-035)

Testing Laboratories: (b) (4)

Study Started: September 13, 1993

Study Completed: December 9, 1993

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Drug Batch No.: PL-026

Methods: Groups of neonatal sprague-Dawley rats (15/sex/group) were given daily oral (gavage) doses of vehicle (distilled water), 50 and 100 mg/kg/day of SP-303 for 2 weeks (from days 5 to 18 of lactation). The volume of administration was fixed at 5 mg/kg. All dams and pups were observed twice daily for mortality. Pups were observed for clinical signs on days 1, 5, 7, 9, 10, 12, 14, 16 and 18 of lactation. Body weights were also

recorded on lactation days 5, 7, 9, 10, 12, 14, 16 and 18. On day 19 of lactation blood samples were collected from orbital sinus for hematology and serum chemistry tests. At the end of study period all animals were sacrificed, subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects:** One male and one female from low dose group, and 2 females from high dose group had no visible milk in their stomach on days 9/10/5 of the lactation period.

2. **Mortality:** Three pups (2 females from low dose group and one male from high dose group) died during study period. The cause of death was accident (dosing error).

3. **Body Weights/Food Consumptions/Water Consumptions:** At the end of treatment period, body weight gains in males were reduced by 23% and 29% at low and high dose respectively, when compared to control values, the corresponding decreases in females were 20% and 28% respectively.

4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen.

5. **Blood Chemistry/Urinalysis:** At high dose, serum aspartate aminotransferase and alanine aminotransferase were significantly increased in males (34% and 28% respectively) and females (41% and 37% respectively) when compared to control values. A/G rates in all treated males and females were decreased by 21% and 15% respectively.

6. **Vital Signs/Physical Examinations/Ophthalmic Examinations:** Not reported.

7. **Organ Weights:** Not reported.

8. **Gross Pathology:** No treatment related effects were seen.

9. **Histopathology:** No treatment related effects were seen.

In this study no effect dose was not established. Body weight gains in males were reduced by 23% and 29% at low and high dose respectively, when compared to control values, the corresponding decreases in females were 20% and 28% respectively. At high dose, serum aspartate aminotransferase and alanine aminotransferase were significantly increased in males (34% and 28% respectively) and females (41% and 37% respectively) when compared to control values. A/G rates in all treated males and females were decreased by 21% and 15% respectively.

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2-Week Oral Toxicity Study in Neonatal Rhesus Monkeys
(Report #SP-303-F-034)

Testing Laboratories: [redacted] (b) (4)

Study Started: May 19, 1993

Study Completed: September 13, 1993

GLP Requirements: A Statement of Compliance with GLP regulations was included.

Animals: 6-8 weeks old infant monkeys of both sexes (males: 565-868 g and females: 606-808 g).

Drug Batch No.: PL-027

Methods: Groups of neonatal rhesus monkeys (3/sex/group) were given daily oral (gavage) doses of vehicle (distilled water), 10, 200 and 500 mg/kg/day of SP-303 for 2 weeks. The volume of administration was fixed at 6.7 ml/kg. All monkeys were observed daily for clinical signs and mortality. Body weights were recorded daily. Blood samples were collected from the femoral vein from all monkeys at pretest and on days 8 and 12 of the study for hematology and serum chemistry tests. At the end of treatment period all monkeys were sacrificed, necropsied and subjected to complete histopathological examinations.

Results:

1. **Observed Effects:** Sporadic emesis were seen in mid and high dose treated monkeys.

2. **Mortality:** None.

3. **Body Weights/Food Consumptions/Water Consumptions:** At the end of treatment period, in males body weight gains were decreased by 43%, 19% and 26% at low, mid and high dose respectively, when compared to control values. In high dose treated females, body weight gains were reduced by 10.5%, compared to control values. Food intakes were not monitored in this study.

4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen.

5. **Blood Chemistry/Urinalysis:** No treatment related effects were seen.

6. **Organ Weights:** No treatment related effects were seen.

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7. **Gross Pathology:** No treatment related effects were seen.

8. **Histopathology:** Lymphoid depletion from thymus was seen in mid and high dose treated males and high dose treated females (males: control=0/3, low dose=0/3, mid dose=1/3 and high dose=2/3; females: control=0/3, low dose=0/3, mid dose=0/3 and high dose=1/3).

In this study, lymphoid depletion from thymus was seen in mid and high dose treated males and high dose treated females. The lowest tested dose (10 mg/kg/day) was the no effect dose.

The following studies were reviewed previously by Dr. Patrick Swann and are included here verbatim.

1. A 32-Day Oral Toxicity Study of Microencapsulated Provir in Rats (b) (4) SP-303-F-045)

Testing Laboratories: (b) (4)

Study Started: December 6, 1996

Study Completed: August 25, 1997

GLP Requirement: A statement of compliance with the GLP regulations and the quality assurance unit was included.

Animals: Eighty male and eighty female Crl:CD®(SD)BR rats were approximately 9 weeks and 13 weeks old, respectively when dosing was initiated. Body weights ranged from 252 g to 289 g for males and 240 g to 300 g for females.

Drug Lot No.: SP-303 enteric coated beads, Batch No. R10934

Methods: SP-303 was administered orally via wide-bore gavage using a water flush to two toxicology groups for a period of 32 or 33 days and to two toxicokinetic groups for a period of 28 days. The dosage levels were chosen based on the results of a dose range finding study which consisted of an acute phase and a repeated dose phase. For the acute phase, dosages of 600, 1200, and 2400 mg/kg were investigated. For the repeated dose phase, SP-303 was administered for seven days at a dose of 1200 mg/kg/day to one group of six males and six females. In the acute phase study, two of two males in the 1200 mg/kg group died on study day 0. All other animals (dosed with either 600 mg/kg or 2400 mg/kg) in the acute phase survived to day 6 euthanization. In the repeated dose phase, one control animal and four 1200 mg/kg/day group animals (two males and two females) were found dead prior to the scheduled necropsy. In this subacute study, dosage levels of 100 (low) and 1000 (high) mg/kg/day were initially selected; however, as the SP-303 beads behaved differently from that provided for the preceding dose range-finding study, the 1000 mg/kg/day dosage level was reduced to 600 mg/kg/day on the second day of dosing. A control group received enteric coated beads placebo. Toxicity studies utilized 15 animals per sex per group. Toxicokinetic studies were conducted on two satellite groups (12 animals per sex per group) that received either the low or high dose of SP-303.

The physical nature of the SP-303 enteric coated beads required a novel dosing technique. Specifically, the placebo or SP-303 were administered by wide-bore, flexible gavage tubing. Once the gavage tube was seated in the stomach, the contents of a capsule containing the appropriate amount of SP-303 or placebo beads were poured into a syringe attached to the tubing. A water flush of 2 ml was added to the syringe prior to the capsule contents. When necessary, an additional water flush of 2.5-3 mls was used.

Animals were observed twice daily for mortality and moribundity. Detailed physical examinations were performed weekly. Body weights and food consumption were recorded weekly. Blood samples for hematology and serum chemistry evaluation were taken from all surviving rats at necropsy. Urine samples were collected using metabolism cages overnight prior to collection of blood samples. Ophthalmic examinations were conducted prior to initiation of dosing and during study week 3. Blood samples for the determination of plasma concentrations of SP-303 were collected on study days 0 and 27. Blood samples were collected from three rats/ sex/group at 1-, 3-, and 12-hours postdosing. No animal was sampled more than once on a collection day. A necropsy examination was conducted on all animals. The following tissues and organs were collected and examined for control and high dose treated animals: adrenals, aorta, bone, brain, eyes, gallbladder, GI tract, heart, kidneys, liver, lungs, lymph node (mesenteric and suprapharyngeal), ovaries, pancreas, pituitary, prostate, salivary gland, skeletal muscle (vastus medialis), skin, spinal cord, spleen, testes, thymus, thyroids, trachea, urinary

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bladder, uterus, gross lesions. Microscopic examinations were not performed on 100 mg/kg/day group animals that were found dead or died *in extremis*. The following organs from all animals were weighed: adrenals, brain, heart, kidneys, liver, ovaries, pancreas, spleen, testes, thymus, and thyroid.

Results:

Mortality: Seven control group animals, eight 100 mg/kg/day group animals and one 600 mg/kg/day group animal were found dead or were euthanized *in extremis* during the study. The cause of death in five control animals, two 100 mg/kg/day group animals and the one 600 mg/kg/day group animal was attributed to intubation trauma.

Clinical Observations: Clinical findings in the animals that died during the dosing phase of the study included rales, labored respiration, gasping, dehydration, decreased defecation and decreased urination. SP-303 related clinical findings were noted in the 600 mg/kg/day group and consisted of black or small feces. Black feces were observed in all 600 mg/kg/day group animals from approximately day 4 until the end of the study. No other SP-303 related clinical findings were observed. Rales and red material around the mouth were noted throughout the study in all groups. The incidence of these findings were increased 1-hour postdosing suggesting the rales and red material around the mouth were related to the method of administration.

Body Weights: No drug related effects were noted on mean body weights or body weight gains.

Food Consumption: No drug related effects were noted on food consumption.

Hematology: No drug related effects were noted on hematology parameters.

Serum Chemistry: No drug related effects were noted on serum chemistry parameters.

Urinalysis: Males dosed with 600 mg/kg/day of SP-303 had a statistically significant decrease in mean urinary pH value. Specifically, mean urinary pH for placebo treated animals was 7.1 ± 0.23 units while the mean urinary pH for animals treated with 600 mg/kg/day SP-303 was 6.6 ± 0.48 units.

Ophthalmic Observations: No oculopathic lesions indicative of toxic effect were observed at either dose level.

Pathology: Seven control group animals, eight 100 mg/kg/day group animals and one 600 mg/kg/day group animal were found dead or were euthanized *in extremis* during the study. Findings indicative of intubation trauma were found in five control animals and the one 600 mg/kg/day that were found dead or died *in extremis*. No SP-303 related gross findings were apparent. At necropsy, local irritation was observed in the 600 mg/kg/day group and consisted of reddened mucosa in the duodenum in 11/15 males and 6/14 females.

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Organ Weights: Organ weights were unaffected by SP-303 administration. The mean absolute heart weight was greater in the 100 mg/kg/day group when compared to control ($p < 0.05$) but a dose-related response was not observed in the 600 mg/kg/day group.

Microscopic Examination: Seven control group animals, eight 100 mg/kg/day group animals and one 600 mg/kg/day group animal were found dead or were euthanized *in extremis* during the study. Findings indicative of intubation trauma were found in five control animals and the one 600 mg/kg/day that were found dead or died *in extremis*. This findings included suppurative (i.e. pus producing) inflammation, muscle degeneration and/or perforation. The cause of death for the remaining control group animals was not determined. Microscopic examination was not performed on the 100 mg/kg/day group animals.

In conclusion, seven control group animals, eight 100 mg/kg/day group animals and one 600 mg/kg/day group animal were found dead or were euthanized *in extremis* during the study. Findings indicative of intubation trauma were found in five control animals and the one 600 mg/kg/day that were found dead or died *in extremis*. A dose-related trend was not observed in the mortality data which is consistent with trauma associated with the dosing technique rather than the test article. It is difficult to assess the potential toxicity of the test compound given the high baseline toxicity/mortality seen as a result of the dosing technique.

2. A 30-Day Oral Toxicity Study of Enteric Coated SP303 in Dogs (b) (4)-288006).

Testing Laboratories: (b) (4)

Study Started: May 15, 1997

Study Completed: February 26, 1998

GLP Requirement: A statement of compliance with the GLP regulations and the quality assurance unit was included.

Animals: Four groups of Beagle dogs with four dogs per sex per group for a total of 32 dogs. Dogs were approximately 27 weeks old when dosing was initiated. Body weight values ranged from 7.0 to 9.3 kg for the males and 5.7 to 7.9 kg for females.

Drug Lot No.: SP-303 enteric coated tablets (125 mg delayed release tablets) , Lot No. 970096

Methods: SP-303 was administered orally to three groups of beagle dogs for a minimum of 30 days. Dosage levels were based on the results of a previous dose-range finding study. Specifically, a dose-range finding study was conducted in two phases, an escalating dose study and a repeated doses study. In the escalating dose study, SP-303 (125 mg tablets placed in capsules) was administered in a step-wise fashion (each dose was different from the previous

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dose) to four dogs (two males and two females) at single doses of 100, 300, 600, and 1200 mg/kg. Each dose was separated by 2-3 nondosing days. After completion of the escalating dose phase, animals were given a 7-day rest period prior to the initiation of the repeated dose phase. During the repeated dose phase, all dogs were dosed once daily with 800 mg/kg for seven consecutive days.

In the escalating dose study at the 1200 mg/kg dose level, all animals had decreased food consumption and abnormal excreta. Emesis were noted for both males and one female. Decreased food consumption and decreased defecation were noted for all animals at two, three, four, and/or five days following administration of SP-303 at 1200 mg/kg. In the repeated dose phase, the predominant observations noted at the time of dosing with 800 mg/kg were decreased food consumption and abnormal excreta. All dogs had sporadic occurrences of emesis at the time of dosing.

In a separate dose-range finding study using the same experimental design and different doses, it was found that, during the repeated dose phase, a dose of 600 mg/kg/day resulted in decreased food consumption and emesis for only a few animals.

Dosage levels for the subacute study were set at 50, 175, or 600 mg/kg/day. A concurrent control group received 125 mg delayed release placebo tablets in a number equivalent to the high dose group.

Dogs were observed twice daily for mortality and moribundity. All animals received a clinical examination at the time of dosing and 2-3 hours following dosing. Detailed physical examinations were performed on all animals weekly. Individual food consumption was recorded daily. Individual body weights were recorded weekly throughout the study. Blood samples were collected prior to treatment and on study day 26 from which hematology and serum chemistry values were obtained. The animals were placed in metabolism cages for urine collection overnight prior to blood collection. On day 0 and day 29 of the study, blood samples were collected at 2, 6, 12, 18 and 24 hours for toxicokinetic determinations. Ocular examinations were performed prior to the initiation of dosing and during study week 4. Electrocardiograms were recorded for all animals prior to initiation of dosing and on study day 29. Complete necropsies were conducted on all animals. The following tissues and organs were collected: adrenals, aorta, bone, brain, eyes, gallbladder, GI tract, heart, kidneys, liver, lungs, lymph node (mesenteric and suprapharyngeal), ovaries, pancreas, pituitary, prostate, salivary gland, skeletal muscle (vastus medialis), skin, spinal cord, spleen, testes, thymus, thyroids, trachea, urinary bladder, uterus with vagina, and gross lesions. Microscopic examination was performed on all above listed tissues from all animals. The following organs from all animals were weighed: adrenals, brain, heart, kidneys, liver, ovaries, pancreas, spleen, testes, thymus, and thyroid.

Results:

Mortality: All animals survived to the scheduled necropsy.

Clinical Observations: Dose-related findings relating to changes in feces were observed in the 175 and 600 mg/kg/day groups. These were predominantly diarrhea and mucoid feces and their frequency was dose-related. There were no other treatment related findings at any dose level.

Body Weights: There were no definite test article-related statistically significant effects on absolute body weight at any dose level. At 600 mg/kg/day, slight mean body weight lôsses were noted for the first and third weeks of dosing. Differences from control values were statistically significant for males during the first week and females during the third week. During the last week of dosing, there was some accommodation as males gained more weight than controls. Summary data on body weight changes are presented in the table below.

Time Period	Control	50 mg/kg/day	175 mg/kg/day	600 mg/kg/day
	MALES			
Week 0-1	0.2	0.1	0.1	-0.2*
Week 0-3	0.9	0.5	0.9	0.1*
Week 0-4	1.1	0.8	1.2	0.7
	FEMALES			
Week 0-1	0.1	0.1	0.0	-0.2
Week 0-3	0.4	0.6	0.3	-0.1
Week 0-4	0.5	0.7	0.4	0.1

* values statistically significantly different from controls ($p < 0.05$)

Food Consumption: There were no treatment-related effects on food consumption at any dose level. Transient (not statistically significant) reductions in food consumption were noted in 600 mg/kg/day group animals. For example, mean weekly food consumption for control animals during the first week of dosing was 221 grams/animal/day for males and 202 grams/animal/day for females. For the 600 mg/kg/day group, these values were 196 grams/animal/day and 188 grams/animal/day, respectively.

Hematology: A decreased hematocrit was observed for females in the 600 mg/kg/day group at study week 3. Specifically, the hematocrit for control females during week 3 was 42.1% while the hematocrit for females dosed with 600 mg/kg/day during week 3 was 36.2%. No other test article-related effects were noted on hematology parameters at any dose level.

Serum Chemistry: Statistically significant decreases in serum albumin were noted in the 600 mg/kg/day group at study day 26. Specifically, control serum albumin concentrations were 3.6 mg/dL on study day 26. In the 600 mg/kg/day group, mean serum albumin concentrations were 3.1 mg/dL for males and 2.9 mg/dL for females. However, these values were within ranges

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for this parameter from historical control data and were similar to pretest values (which were also significantly less than control values for the females in the 600 mg/kg/day group). In addition, in the 600 mg/kg/day group, statistically significant decreases were noted in sodium for males and gamma glutamyltransferase and calcium for females. Only the serum calcium levels in females of the 600 mg/kg/day group were outside of historical norms. Specifically, control serum calcium levels were 10.4 mg/dL for females while females in the 600 mg/kg/day group had values of 9.8 mg/dL on study day 26. Historical values for females (mean ± two standard deviations) range from 10.06 to 11.54 mg/dL. No other test article-related effects on serum chemistry parameters were noted at any dose level.

Urinalysis: Urinalysis parameters were unaffected by test article administration.

Ophthalmology: No oculopathic lesions indicative of toxic effect were observed in the treated groups.

Electrocardiography: No test-article effects were noted in the electrocardiographic data at the study week 4 evaluation.

Pathology: Treatment-related gross findings included dark red intestinal contents in the colon or rectum in several animals from the 600 mg/kg/day group. In addition, reddened duodenal mucosa in three of four males and all females and reddened mesenteric lymph nodes in all dogs in the 600 mg/kg/day group. Reddening of the duodenal mucosa or mesenteric lymph nodes were also seen in the 175 mg/kg/day group but at a lower frequency (2 animals for each observation). No gross findings were noted in the 50 mg/kg/day group.

Organ Weights: Testes weights for males in the 600 mg/kg/day group were lower than controls. This difference was not statistically significant. Thyroid weights relative to final body weights were significantly increased in females in the 175 mg/kg/day group. However, this effect was not seen in the 600 mg/kg/day group. Therefore no relationship to the test article was apparent.

Microscopic Examinations: In the 600 mg/kg/day group, grossly observed dark red intestinal contents occasionally appeared to contain red and white cells which is suggestive of gastrointestinal hemorrhage. Other treatment-related microscopic changes were limited to congestion in varying segments of the small and large intestines, an associated incidence of congestion in the mesenteric lymph nodes suggestive of intestinal irritation of males and females at all dose levels. These findings were most prominent in the 600 mg/kg/day group. These findings occurred less frequently in the 175 mg/kg/day group and were limited to single instance in one male and one female in the 50 mg/kg/day group. Microscopic examination of the testes did not reveal test-article specific changes that could explain the reduction in weights. All other findings in the treated groups were considered incidental and/or spontaneous and unrelated to test article.

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Toxicokinetics: On the first and last days of dosing, blood samples were collected at 2, 6, 12, 18 and 24 hours for toxicokinetic determinations. The samples were analyzed for their content of SP-303 with an HPLC method. The lower limit of detection of this method is 0.1 µg/ml. It was found that SP-303 at doses of 50 mg/kg/day and higher is absorbed by dogs. On day 0, SP-303 was detected in the plasma of most dogs by 12 hours with a Tmax of between 12 and 18 hours. A statistically significant difference in Cmax and $AUC_{0\text{-}24\ hrs}$ was observed between males and females in the 50 and 175 mg/kg/day groups on day 29. No gender related differences were noted on Day 0 or at the 600 mg/kg/day dose level on day 29. Day 29 toxicokinetic parameters are summarized in the following table.

Dose (mg/kg/day)	Parameter	Male	Female
50	Cmax (µg/ml)	1.02	2.97
50	$AUC_{0\text{-}24\ hr}$ (µg · hr/ml)	17.89	45.59
175	Cmax (µg/ml)	4.10	8.35
175	$AUC_{0\text{-}24\ hr}$ (µg · hr/ml)	60.04	131.88
600	Cmax (µg/ml)	23.36	17.45
600	$AUC_{0\text{-}24\ hr}$ (µg · hr/ml)	390.55	322.37

In conclusion, test article-related clinical findings consisted of changes to feces (rust and/or black diarrhea and/or mucoid feces). The rate of incidence was dose-responsive; there were no treatment-related clinical findings at a dose level of 50 mg/kg/day. For the 600 mg/kg/day group, body weight gains and food consumption were slightly reduced during study weeks 0 to 1 and/or 2 to 3. Test-article related decrease in hematocrit of females during study week 3 was not statistically significant but may be related to possible gastrointestinal hemorrhage noted upon microscopic examination. The observed decreases in serum albumin could also be secondary to GI hemorrhage. Decreased testes weight may be secondary to decreased body weight and retarded sexual maturity. Microscopic examination revealed dose-related incidence of intestinal irritation. Thus, the primary toxicity appears to be related to localized gastrointestinal irritation. This effect was noted at doses of 175 mg/kg/day and above. The no observed adverse effect level (NOAEL) for SP-303 tablets in this 30 day subchronic toxicity study in beagle dogs was 50 mg/kg/day.

Study title: 4-week oral toxicity study of crofelemer administered to mice

Study no.:	(b) (4) 1482-001
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 1, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Crofelemer, Lot # AA0067003, Purity not specified

Key Study Findings

Twice daily oral dosing with crofelemer at doses of up to 1200 mg/kg/day in mice produced several deaths. Two males and 3 females at 1200 mg/kg/day and 1 female at 120 mg/kg/day died prior to scheduled termination. Clinical signs observed in these animals included audible/difficult breathing, brown/red material around the mouth, hunched posture, decreased activity, thin, and/or cold to touch; however, a clear cause of death was not identified. In surviving animals, clinical signs, which included unkempt appearance, decreased/absent feces, discolored/sparse/absent hair and/or scabbing, were not dose dependent. Minor changes in hematology and clinical chemistry were observed at 400 and 1200 mg/kg/day. Crofelemer was tolerated at up to 400 mg/kg/day in mice.

Methods

Doses:	40, 120, 400, 1200 mg/kg/day
Frequency of dosing:	Twice daily, 8 h apart
Route of administration:	Oral gavage
Dose volume:	10 mL/kg/dose
Formulation/Vehicle:	Solution in phosphate buffered saline (PBS) pH 7.4/PBS
Species/Strain:	Mice/Crl:CD1(Icr)
Number/Sex/Group:	10/sex/group
Age:	6 weeks old
Weight:	26.7 – 32.7 g (males), 22.0 – 25.9 g (females)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

All animals were observed twice daily during the treatment period for morbidity, mortality, injury and availability of food and water.

There were several deaths during the course of treatment with SP-303: 2 males and 3 females at 1200 mg/kg/day and one female at 120 mg/kg/day. There were 2 deaths on Study Days 21 and 23 in males at 1200 mg/kg/day. The 120 mg/kg/day female died on Study Day 28. The 1200 mg/kg/day females died on Study Days 6, 9, and on 28.

Clinical Signs

Assessment of clinical signs was conducted daily for all animals.

Clinical signs observed in animals that died prior to scheduled sacrifice included audible/difficult breathing, brown/red material around the mouth, hunched posture, decreased activity, thin, and/or cold to touch. Clinical signs in surviving animals included unkempt appearance, decreased/absent feces, discolored/sparse/absent hair

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and/or scabbing. A clear treatment-related effect could not be identified. Clinical signs in animals while on study did not indicate a clear cause of death.

Body Weights

Body weights were measured at receipt, prior to randomization, and weekly during the course of the study.

There were no changes in body weight related to treatment.

Feed Consumption

Food consumption was recorded weekly during the course of the study.

There were no changes in food consumption related to treatment.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted on animals prior to initiation of SP-303 dosing and just before study termination.

One high dose male had cataract and phthisis bulbi in the right eye. One 120 mg/kg/day female had a cataract and retinal atrophy in the left eye. These were considered to be unrelated to treatment. No other treatment-related findings were reported in any other animals.

ECG

ECG was not monitored.

Hematology

Sampling for hematological evaluation was obtained from 5 animals/sex/dose prior to terminal necropsy. The following parameters were measured: leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, absolute and percent reticulocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and large unstained cells.

Minor decreases (< 10%) in hemoglobin and hematocrit were observed in 400 (Hct only) and 1200 mg/kg/day females, as compared to control females. No other treatment-related changes were reported.

Clinical Chemistry

Sampling for clinical chemistry evaluation was obtained from 5 animals/sex/dose prior to terminal necropsy. The following parameters were measured: calcium, phosphorus,

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alkaline phosphatase, total bilirubin, aspartate aminotransferase, alanine aminotransferase, urea nitrogen, total protein, albumin, globulin, albumin/globulin ratio, and glucose.

At the high dose of 1200 mg/kg/day, there was an increase in globulins (~9%) and total protein (~13%) in males and an increase in phosphorus in females, as compared to control animals. No other significant treatment-related findings were reported. Therefore, crofelemer at up to 400 mg/kg/day did not produce any changes in clinical chemistry parameters.

Urinalysis

Urinalysis was not conducted.

Gross Pathology

Necropsy was performed on all animals. In addition to examining the external surface, the thoracic, abdominal and cranial cavities were also examined.

Discoloration (black) of duodenum at the highest dose was observed in males and females (1-2 animals/10). No other treatment-related findings were reported.

Organ Weights

Organ weights were determined at necropsy with paired organs being weighed together. The thyroid and bilateral parathyroid were weighed together, as were the right mandibular/sublingual salivary glands.

There were no significant treatment-related changes in organ weights in either sex.

Histopathology

Adequate Battery: Yes

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- Adrenal (2)*
- Aorta
- Bone with marrow [femur]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*
- Epididymis (2)*
- Eye including optic nerve (2)
- Gallbladder
- Gastrointestinal tract:
  - esophagus
  - stomach [glandular and nonglandular]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2)* with oviduct (2)
  - testis (2)*
- Gross lesions
- Heart*
- Joint, tibiofemoral
- Kidney (2)*
- Lacrimal gland, exorbital (2)
- Larynx
- Liver [collected whole; 2 examined]*
- Lung with bronchi [collected whole; 1 section examined]*
- Lymph nodes: mandibular and mesenteric
- Mammary gland [process females only]
- Pancreas
- Peyer's patch
- Pituitary*
- Prostate and seminal vesicle (2)
- Salivary gland, mandibular/sublingual [2 collected; 1 examined]#*
- Salivary gland, parotid [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen*
- Thymus*
- Thyroid/parathyroid (2)*
- Tongue
- Trachea
- Ureter (2)
- Urinary bladder
- Uterus [both horns]/Cervix
- Vagina

[a]Bone marrow smears were collected at the scheduled necropsy and held.
#The combined weight of the right mandibular/sublingual salivary gland weight was obtained.
*Organ weighed
(2) Paired organ

Peer Review: No

Histological Findings: Microscopic examination did not reveal significant treatment-related changes in either sex.

Special Evaluation

None

Toxicokinetics

None

Dosing Solution Analysis

Samples were collected but not analyzed by the CRO, under the direction of the sponsor.

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Study title: 13-week oral toxicity study of crofelemer administered to mice

Study no.:	(b) (4) 1310-016
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 25, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Crofelemer, Batch # 3072906R, Purity not specified

Key Study Findings

There were 20, 5, and 2 deaths in the 1200, 400 and 40 mg/kg/day dose groups, respectively. Microscopic evaluation revealed lesions in lungs, trachea, and/or larynx, likely due to accidental gavage injury, aspiration and/or presence of test article in 7 of these animals. The cause of death of the remaining 20 animals was not determined. There was a statistically significant decrease (~ 10%) in body weight only in males treated with doses ≥ 400 mg/kg/day crofelemer. When compared to control, crofelemer treatment at 400 mg/kg/day was associated with a decrease in lymphocytes, eosinophils, basophils and an increase in other cells (not specified) in females only. There was a slight increase (14.5%) in urea nitrogen in 400 mg/kg/day females, as compared to control. Necropsy revealed gas distention of the cecum, colon, ileum, jejunum, and stomach (females only) in high dose animals. Microscopic evaluations revealed mild inflammation in the esophagus of high dose males, lymphoid depletion in the thymus at 400 and 1200 mg/kg/day, and necrosis in the lymphoid cortex of the thymus in high dose females. The sponsor concluded that NOAEL dose was 40 mg/kg/day in females. However, there were deaths of two 40 mg/kg/day animals, one with an undetermined cause of death. Therefore, a NOAEL dose was not established.

Methods

Doses:	0, 40, 400, 1200 mg/kg/day
Frequency of dosing:	Twice daily
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	Aqueous solution/Purified water
Species/Strain:	Mice/Crl:CD1(ICR)
Number/Sex/Group:	Main Study: 15/sex/group TK Study: 8/sex (Control) and 39/sex/group (crofelemer)
Age:	6 weeks old
Weight:	27.5-33.9 g (Males), 22.4-27.6 g (Females)
Satellite groups:	Yes
Unique study design:	The TK high dose group was terminated on Day 56.
Deviation from study protocol:	None which affected the outcome of the study

Observations and Results

Mortality

All animals were examined twice daily for signs of morbidity and mortality.

Eight animals were replaced within the first week of the study because of mortalities in all three crofelemer dose groups: 1 animal in control group, 3 animals at 400 mg/kg/day, and 4 animals at 1200 mg/kg/day. There were several unscheduled deaths during the course of the study. There were 20 deaths (13 females, 7 males) in the 1200 mg/kg/day dosing group, with 15 animals declared as an undetermined cause of death and 5 as accidental injury. There were 5 deaths (1 due to accidental injury, 4 due to undetermined causes) in the mid-dose group and 2 deaths (accidental injury and undetermined cause, one each) in the low dose group. There were also 2 deaths in the control group (cause of death was undetermined). Animals labeled as “undetermined cause of death” represent those in which microscopic examination failed to reveal a cause of death. These results are summarized in the sponsor’s table below:

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Animal Number	Sex	Cause of Death	Day of Death
Group 1 (1009)	Male	Undetermined	21
Group 1 (1015)	Male	Undetermined	73
Group 2 (1025)	Male	Undetermined	32
Group 2 (1215)	Female	Accidental Injury	83
Group 3 (1036)	Male	Accidental Injury	15
Group 3 (1035)	Male	Undetermined	53
Group 3 (1221)	Female	Undetermined	62
Group 3 (1040)	Male	Undetermined	89
Group 3 (1225)	Female	Undetermined	89
Group 4 (641)	Female	Undetermined	5
Group 4 (445)	Male	Accidental Injury	7
Group 4 (1051)	Male	Accidental Injury	9
Group 4 (1236)	Female	Undetermined	9
Group 4 (1058)	Male	Accidental Injury	13
Group 4 (1241)	Female	Undetermined	14
Group 4 (1055)	Male	Undetermined	15
Group 4 (1243[r])	Female	Undetermined	15
Group 4 (1231)	Female	Undetermined	17
Group 4 (1238)	Female	Undetermined	21
Group 4 (1053)	Male	Undetermined	27
Group 4 (1054)	Male	Undetermined	27
Group 4 (1235)	Female	Undetermined	27
Group 4 (1060)	Male	Undetermined	31
Group 4 (1233)	Female	Undetermined	36
Group 4 (1240)	Female	Accidental Injury	42
Group 4 (1244)	Female	Undetermined	44
Group 4 (1234)	Female	Undetermined	48
Group 4 (1239)	Female	Accidental Injury	51
Group 4 (1242)	Female	Undetermined	53

Clinical Signs

All animals were examined twice daily for signs of injury. All main study animals underwent a detailed clinical examination prior to randomization and weekly thereafter during the course of the study. Detailed clinical examination included an evaluation of skin, fur, eyes, ears, nose, oral cavity, thorax, abdominal cavity, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects, and nervous system effects.

Clinical signs observed in animals of both sexes treated with 400 and 1200 mg/kg/day crofelemer included audible, difficult, shallow/slow breathing, decreased activity, pale body and/or skin cold to the tough, unkempt appearance, and/or distended abdomen. In some cases, these animals also had few/absent feces, partially closed eyelids. The number of animals experiencing these adverse effects was greater at the high dose. There was greater morbidity observed in animals at the high dose.

Body Weights

Body weights were recorded prior to randomization and weekly during the course of the study.

There was a statistically significant decrease (~ 10%) in body weight in males treated with doses ≥ 400 mg/kg/day crofelemer. Body weight changes were not significant in females in any dose group.

Feed Consumption

Food consumption was measured weekly during the course of the study for main study animals only.

Overall, food consumption was similar throughout the duration of the study. There were, however, sporadic, statistically significant decreases in food consumption at all doses of crofelemer, as compared to placebo.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted on all main study pretest and at terminal necropsy by one veterinary ophthalmologist. Another veterinary ophthalmologist conducted examinations on Day 55 for 1200 mg/kg/day main study animals.

There were no treatment-related ophthalmological findings in the crofelemer dosing groups.

ECG

No ECG studies were conducted.

Hematology

Samples for hematological analysis were collected from all main study animals prior to terminal necropsy on Day 56 (for 1200 mg/kg/day dose group) or on day 92 (all other dose groups). The following parameters were assessed: leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, absolute reticulocytes, neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, basophils, and other cells.

Hematological changes from the 1200 mg/kg/day dose group cannot be compared to the control group since sampling was conducted at 2 separate times (Day 56 for high dose and Day 92 for all other dose groups). When compared to control, crofelemer treatment at 400 mg/kg/day produced a decrease in lymphocytes, eosinophils, basophils and an increase in other cells (not specified) in females only. No such changes were observed in males.

Clinical Chemistry

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Sampling for clinical chemistry analysis was conducted on all main study animals prior to necropsy on Day 56 (for 1200 mg/kg/day dose group) or on day 92 (all other dose groups). The following parameters were assessed: calcium, phosphorus, alkaline phosphatase, total bilirubin, aspartate amino transferase, alanine amino transferase, urea nitrogen, total protein, albumin, globulin, albumin/globulin ratio, and glucose.

Clinical chemistry changes from the 1200 mg/kg/day dose group cannot be compared to the control group since sampling was conducted at 2 separate times (Day 56 for high dose and Day 92 for all other dose groups).

When compared to control, crofelemer did not produce significant changes in clinical chemistry parameters at any dose. There was a slight increase (14.5%) in urea nitrogen in 400 mg/kg/day females, as compared to control. No other changes were noted.

Urinalysis

No urinalysis was conducted.

Gross Pathology

Necropsy was performed on all animals, regardless of whether they died while on study or were sacrificed at study termination. Animals were examined for palpable masses, signs of external and internal abnormalities, signs of injury/adverse effects in thoracic, cranial, and abdominal cavities.

Necropsy revealed that the cecum, colon, ileum, and jejunum were distended with gas in high dose males. Similar observations were made in high dose females, along with gas distention in the stomach. Gas distention was also observed in a few mid-dose males in the TK group. There were no other significant treatment-related findings.

Organ Weights

Organ weights were recorded at necropsy for all surviving main study animals and the TK animals treated with 1200 mg/kg/day. Paired organs were weighed together.

There were several changes in organ weights in the crofelemer treatment groups. However, the significance of these changes is not apparent, since they did not correlate to histopathological changes. There was a < 21% decrease in kidney weight in males at both 400 and 1200 mg/kg/day, as compared to control. In both males and females, there was a decrease in salivary, mandibular gland weight (~25-30%) in main study and TK animals at 400 mg/kg/day (females only) and 1200 mg/kg/day (males and females), as compared to control. There was an increase in thyroid/parathyroid weight in males at the high dose; however, the sponsor attributed this increase to the presence of excess non-thyroid/parathyroid tissue, which was identified under microscopic examination. In high dose TK females, there was a decrease in weights of brain (~6%), adrenal (~33%), heart (~17%), kidney (~18%), liver (~12.5%), lungs with bronchi

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(~24%), pituitary (~15.6%), and salivary/mandibular glands (~30%), as compared to controls.

Histopathology

Microscopic examination was conducted on tissues from animals in the control, 400 and 1200 mg/kg/day treatment groups, all animals found dead and those euthanized in extremis.

Adequate Battery: Yes
The following organs/tissues were harvested and processed for histopathological analysis.

- Adrenal (2)*
- Aorta
- Bone with marrow [femur]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*
- Epididymis (2)*
- Eye including optic nerve (2)
- Gallbladder
- GALT [gut associated lymphoid tissue]
- Gastrointestinal tract:
  - esophagus
  - stomach [glandular and nonglandular]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2)* with oviduct (2)
  - testis (2)*
- Gross lesions
- Heart*
- Joint, tibiofemoral
- Kidney (2)*
- Lacrimal gland, exorbital (2)
- Larynx
- Liver [3 sections collected; 2 examined]*
- Lung with bronchi [collected whole; 2 sections examined]*
- Lymph nodes: mandibular and mesenteric
- Mammary gland [process females only]
- Pancreas
- Pituitary*
- Prostate and seminal vesicle (2)
- Salivary gland, mandibular/sublingual [2 collected; 1 examined]*[b]
- Salivary gland, parotid [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen*
- Thymus*
- Thyroid/parathyroid (2)*
- Tongue
- Trachea
- Ureter (2)
- Urinary bladder
- Uterus [both horns]/Cervix
- Vagina

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[a]Bone marrow smears were collected at the scheduled necropsy and held.
[b]The combined weight of the right mandibular/sublingual salivary gland was obtained.

(2) Paired organ
*Weighed organ

Peer Review: no

Histological Findings: Microscopic evaluations revealed lesions in lungs, trachea, and/or larynx in 7 of the animals which died prior to terminal sacrifice. The lesions were likely a result of gavage injury, aspiration of test article, and/or presence of test article. In the remaining 20 animals which died while on study, the cause of death was listed as “undetermined” due to lack of supporting microscopic changes. In males at the high dose, mild inflammation was noted in the esophagus. Lymphoid depletion in the thymus

at 400 and 1200 mg/kg/day and necrosis in the lymphoid cortex of the thymus were observed in high dose females.

Special Evaluation

None

Toxicokinetics

Samples for TK analysis were collected from 3 animals/sex/dose group at predose and at 0.5, 1.5, 4, 6, and 8 h post dose on Days 1, 56 (1200 mg/kg/day group only) and 91.

TK analysis was conducted only on plasma samples from the 1200 mg/kg/day dose group because plasma concentration of crofelemer was below the limit of quantitation at the lower doses. The applicant's table below summarizes the TK results for the 1200 mg/kg/day dose group.

Day	Sex	AUC_{0-8} (hr•µg/mL)	$AUC_{0-tlast}$ (hr•µg/mL)	C_{max} (µg/mL)	T_{max} (hr)	Accumulation ratio (Day 56/Day 1) AUC_{0-8}	$AUC_{0-tlast}$	C_{max}
1	Male	1.37	1.19	0.255	0.500	NA	NA	NA
	Female	2.70	2.70	0.681	8.00	NA	NA	NA
	Combined	2.04	1.95	0.468	4.25	NA	NA	NA
56	Male	16.3	16.3	2.38	1.50	11.9	13.7	9.33
	Female	16.5	16.5	3.88	0	6.12	6.12	5.70
	Combined	16.4	16.4	3.13	0.750	9.03	9.91	7.52

NA = not applicable.

In the 1200 mg/kg/day dose group, females showed slightly higher systemic exposure to crofelemer than males. On Day 1, C_{max} was reached at 0.5 h in males and 8 h in females, whereas it was reached at 1.5 h in males on Day 56. Plasma levels of crofelemer were higher in both sexes on Day 56 than on Day 1 indicating accumulation of drug with increased duration of use.

Dosing Solution Analysis

Dosing formulations were evaluated for stability, concentration and homogeneity at various times during the study period.

Study title: 26-week oral toxicity study of crofelemer administered to rats followed by a 4-week recovery period

Study no.:	(b) (4) 1482-002
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 1, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Crofelemer; Lot #s AA0067003, AA0067029, AA0067007, AA0067002, AA0067004, AA0067005, AA0067006; Purity 90-110%

Key Study Findings

Six males and six females at 600 mg/kg/day (high dose) and 2 females each in the 200 and 60 mg/kg/day dose groups died prior to scheduled study termination. Dosing error and lymphoid tumor were the cause of death in 8 and 1 animal, respectively, while the cause of death was undetermined in the remaining animals. Clinical signs, which were reversed at the end of the recovery period, included audible and/or difficult breathing in 2 and 9 animals treated with 400 and 1200 mg/kg/day crofelemer, respectively, and 2 control animals (one male and one female). Four high dose males were observed as being thin during the dosing period. Slight changes in body weight were reversed at the end of the recovery period in both sexes. Changes in hematology and clinical chemistry parameters at the mid- and high doses during the dosing period normalized after the treatment free period. However, there were several hematology and clinical chemistry changes which were only observed in recovery animals, including an increase in platelet count in all recovery males treated with crofelemer, slight increases chloride and calcium levels in high dose and mid-dose females (calcium only), increase in creatinine in high dose females, increase in albumin at the mid- and high dose, and increase in glucose in all crofelemer treated recovery females. Brown and white discoloration in the lung (w/bronchi) was observed at 600 mg/kg/day in one male and one female. Microscopic examination revealed an accumulation of pigmented macrophages around airways, secondary to aspirated test article. Inflammatory reaction was observed in both sexes and foreign material was noted in airway of the female animal, suggesting that these findings were likely related to dosing procedure. Brown and black discoloration in the duodenum of high dose animals was not observed at recovery necropsy; however, one male and one female had mild and moderate green discoloration of the duodenum, respectively, at recovery. Organ weight changes were observed in high dose animals only, which reversed at the end of recovery period, except for thyroid/parathyroid weights in males. In recovery females, there was a ~34.3% increase in absolute weight of lung with bronchi and a 48% increase relative to brain weight, as compared to control animals. Based on these findings, a NOAEL dose was not established.

Reference ID: 3168746

Methods

Doses:	60, 200, 600 mg/kg/day
Frequency of dosing:	Twice daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg/dose
Formulation/Vehicle:	Aqueous solution/phosphate buffered saline, pH 7.4
Species/Strain:	Rats/CD[Crl:CD(SD)]
Number/Sex/Group:	Main study: 20/sex/group, 5/sex/group (recovery group) TK study: 3/sex (control), 9/sex/group (crofelemer)
Age:	6 weeks of age
Weight:	218-257 g (Males), 171-211 g (Females)
Satellite groups:	Yes
Unique study design:	No
Deviation from study protocol:	GLP deviation: Protocol was unsigned at the time animals were received at testing facility Protocol deviations: No documentation that dosing formulation was returned to refrigerator between doses on Day 26; Dosing formulation not refrigerated after first dose on Day 46; Dosing formulation not stored in refrigerator prior to first daily dose on Day 156

Observations and Results

Mortality

Animals were observed twice daily for signs of morbidity, injury and mortality.

There were unscheduled deaths during the course of the study (shown in the sponsor's table below). There were deaths of 6 high dose males, 5 high dose females, and 2 females each in the 200 and 60 mg/kg/day dose groups. The probable causes of death were dosing error, undetermined, and lymphoid tumor.

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Mortality				
Dose Level (mg/kg/day)	**Animal Number**	**Sex**	**Day of Death**	**Probable Cause of Death**
60	2211	F	183	Undetermined
60 (TK)	2610	F	20	Not examined
200	2312	F	36	Dosing error
200	2313	F	11	Undetermined
600	1414	M	145	Undetermined
600	1415	M	75	Lymphoid tumor
600	1418	M	15	Dosing error
600	1419	M	34	Dosing error
600 (TK)	1805	M	178	Undetermined
600 (TK)	1806	M	90	Dosing error
600	2401	F	23	Dosing error
600	2413	F	175	Dosing error
600	2416	F	210	Dosing error
600 (TK)	2806	F	23	Not examined
600 (TK)	2807	F	31	Dosing error

Clinical Signs

A detailed clinical examination was conducted once a week on all main study animals. Clinical examination included an assessment of the skin, fur, eyes, nose, oral cavity, thorax, abdomen, external genitalia, limbs, feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects (tremors, convulsions, reactivity to handling, bizarre behavior), and presence of palpable masses.

Audible and/or difficult breathing was observed in 2 and 9 animals treated with 400 and 1200 mg/kg/day crofelemer, respectively. One control male and female also had audible breathing. Other clinical signs included discolored hair, red wet/dry material around mouth, forelimbs, or nose in 1-3 animals/sex in all dose groups, including controls. Four high dose males were observed as being thin during the dosing period. At the end of the recovery period, these clinical signs were reversed.

Body Weights

Body weights for all main study animals were measured one day prior to initiation of the treatment, weekly thereafter during the dosing period, and at necropsy. Body weights of TK animals were collected but not reported.

There were no significant decreases in body weight during the course of the study (both dosing and recovery phases). High dose males showed a slight decreasing trend in body weight towards the end of the dosing period, as compared to controls. These data are summarized in the applicant's table below:

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Mean Body Weight Gains, kg
(Day -1 to 175)

	Male		Female	
Dose Level	Body Weight Gain	% of Control	Body Weight Gain	% of Control
0 mg/kg/day	371.1	NA	142.4	NA
60 mg/kg/day	386.4	104	128.9	91
200 mg/kg/day	394.7	106	138.9	98
600 mg/kg/day	329.4	89	132.8	93

NA-Not Applicable

At the end of the recovery period, there were no statistically significant differences in body weight in any of the dose groups.

Feed Consumption

Food consumption was measured weekly during the dosing period for all main study animals.

There were decreases in food consumption in high dose males during Weeks 11 and 21; however, food consumption was similar to control values in the following weeks. At the end of the dosing phase, food consumption in all groups was lower than Week 1 values. There were no significant changes in food consumption in females. Overall, food consumption was similar across all dose groups during the dosing period.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted pretest and prior to terminal necropsy for all main study animals.

No treatment-related effects on ophthalmoscopic were noted.

ECG

Not conducted

Hematology

Blood samples for hematological analysis were collected from all main study animals prior to terminal and recovery necropsies. The following parameters were measured: leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, absolute reticulocytes, percent reticulocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and large unstained cells. The following coagulation parameters were measured as well: activated partial thromboplastin time (APTT) and prothrombin time.

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There were no treatment-related changes in hematology parameters in males during the dosing phase. There was a decrease (~21-23% as compared to control) in absolute reticulocyte count in 200 and 600 mg/kg/day treated females at the end of dosing. There was a ~66% increase in neutrophils at the high dose and a 50% and 35% increase in eosinophils at the mid- and high dose, respectively, in females at the end of the dosing period. The increase in eosinophils at the high dose was not statistically significant. In the recovery group, there was an increase of 30.6%, 31.6%, and 9.3% in platelet counts in the low, mid-, and high dose males, respectively, as compared to controls. In recovery females, there was a ~49% decrease in monocyte count at the low dose. There was a decreasing trend in monocyte count in mid- and high-dose recovery animals; however, these decreases were not statistically significant from controls. There were no treatment-related changes on coagulation parameters during the dosing phase. There was a ~20% increase in APTT in 60 mg/kg/day males at the end of recovery, as compared to control. No other changes were observed.

Clinical Chemistry

Blood samples for clinical chemistry analysis were collected from all main study animals prior to terminal and recovery necropsies. The following clinical chemistry parameters were measured: sodium, potassium, chloride, calcium, phosphorus, alkaline phosphatase, total bilirubin, gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase, urea nitrogen, creatinine, total protein, albumin, globulin, albumin/globulin ratio, cholesterol, and glucose.

There was an increase of ~33% in total bilirubin, a 17% decrease in urea nitrogen, and a 20% decrease in cholesterol at the high dose in terminal males, as compared to controls. There were no significant treatment-related changes in crofelemer treated females at the end if the dosing period. There were slight increases in chloride and calcium levels in high dose and mid-dose females (calcium only) at the end of the recovery period, as compared to controls. There was also a 32% increase in creatinine (high dose only), 13% and 10.3% increase in albumin at the mid- and high dose, respectively, and a 29%, 25.7% and 40.6% increase in glucose at the low, mid-, and high doses, respectively, in recovery females, as compared to controls. These changes were not observed at the end of the dosing phase. Therefore, the significance of these findings is not clear.

Urinalysis

The following parameters were analyzed for urine samples: urine volume, specific gravity, pH.

There were no significant treatment-related effects on urinalysis parameters. The slight decrease in specific gravity observed in low and high dose males normalized to control values at the end of the recovery period.

Gross Pathology

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All main study animals underwent necropsy at terminal sacrifice and end of recovery period. The abdominal, thoracic, and cranial cavities, external surfaces, and organs were examined for abnormalities and/or injuries.

Terminal necropsy revealed black and brown discoloration in the duodenum of high dose males and females. One male and 2 females had mild black discoloration and 2 females had moderate black discoloration, respectively. Mild brown discoloration was observed at the mid-dose (1/sex) and in 2 males and 1 female at the high dose. Moderate brown discoloration was observed in 3 animals/sex at the high dose. These findings normalized at the end of the recovery period. However, one male and one female had mild and moderate green discoloration of the duodenum, respectively.

Brown and white discoloration in the lung (w/bronchi) was observed at 600 mg/kg/day in one male and one female. Microscopic examination revealed an accumulation of pigmented macrophages around airways, secondary to aspirated test article. Inflammatory reaction was observed in both sexes and foreign material was noted in the airway of female animal, suggesting that these findings were likely related to dosing procedure. No other significant treatment-related macroscopic findings were reported.

Organ Weights

The following organs were harvested and weighed at necropsy: brain, adrenal glands, epididymides, heart, kidneys, liver, lung with bronchi, pituitary gland, salivary gland, mandibular/sublingual glands, spleen, testes, thymus, and thyroid/parathyroid glands. Paired organs were weighed together. Absolute organ weights were and weights relative to body and brain were also calculated.

Statistically significant changes in organ weights were observed in high dose animals. In males, there was ~12%, ~19%, and ~18.1% decrease in absolute kidney, liver and thyroid/parathyroid gland weights, respectively, as compared to controls. These changes normalized at the end of the recovery, except for the thyroid/parathyroid which had slightly higher absolute weight and a statistically significantly higher relative weight (to brain). In high dose females, there was ~23.5% increase in absolute weight of lung with bronchi (27.35% and 24.5% increase relative to body and brain weight, respectively) and ~17.6% increase in absolute spleen weight (with 21.4% and 18.7% increase relative to body and brain weight, respectively), as compared to controls. In recovery females, there was a ~34.3% increase in absolute weight of lung with bronchi and a 48% increase relative to brain weight, as compared to control animals. The increase in lung weight was accompanied by changes in histopathology, likely related to presence of test article or aspiration of test article.

Histopathology

Adequate Battery: Yes

The following organs/tissues were harvested and processed for histopathological analysis.

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- Adrenal (2)*
- Aorta
- Bone with marrow [femur]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*
- Epididymis (2)*
- Eye including optic nerve (2)
- Gastrointestinal tract:
  - esophagus
  - stomach [glandular and nonglandular]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2)* with oviduct (2)
  - testis (2)*
- Gross lesions
- Heart*
- Joint, tibiofemoral
- Kidney (2)*
- Lacrimal gland, exorbital (2)
- Larynx
- Liver [3 sections collected; 2 examined]*
- Lung with bronchi [collected whole; 2 sections examined]*
- Lymph nodes: mandibular, mesenteric, and regional where applicable
- Mammary gland [process females only]
- Pancreas
- Peyer's patch
- Pituitary*
- Prostate and seminal vesicle (2)
- Salivary gland, mandibular/sublingual [2 collected; 1 examined]*[b]
- Salivary gland, parotid [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen*
- Thymus*
- Tissue masses
- Thyroid/parathyroid (2)*
- Tongue
- Trachea
- Ureters (2)
- Urinary bladder
- Uterus [both horns]/Cervix
- Vagina

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[a]Bone marrow smears were collected at the scheduled necropsy and held.
[b]The combined weight of the right mandibular/sublingual salivary gland was obtained.
*Organ weighed
(2) Paired organ

Peer Review: No (slides only reviewed by a veterinary pathologist)

Histological Findings: The applicant's tables below summarize the histopathological findings in terminal and recovery necropsies.

Test Article-Related Microscopic Changes
Terminal Necropsy
Male and Female

Dose level: mg/kg/day	0		60		200		600	
Sex	M	F	M	F	M	F	M	F
Number Examined	15	15	15	15	15	15	15	15
Stomach, glandular								
Macrophages, pigmented	0	0	NA	0	0	0	8	12
-minimal	0	0		0	0	0	8	12
Small intestine, duodenum								
Macrophages, pigmented	0	0	0	0	7	4	13	14
-minimal	0	0	0	0	6	4	2	0
-mild	0	0	0	0	1	0	4	6
-moderate	0	0	0	0	0	0	7	8
Lymph node, mesenteric								
Macrophages, pigmented	13	13	NA	0	12	13	11	13
-minimal	12	12		0	10	12	0	2
-mild	1	1		0	2	1	9	8
-moderate	0	0		0	0	0	2	3
Peyers patch								
Macrophages, pigmented	1	0	NA	0	3	0	9	12
-minimal	0	0		0	3	0	7	10
-mild	1	0		0	0	0	2	2

N/A – Not Applicable/Not Available

Test Article-Related Microscopic Changes
Recovery Necropsy
Male and Female

Dose level: mg/kg/day	0		60		200		600	
Sex	M	F	M	F	M	F	M	F
Number Examined	5	5	5	5	5	5	5	5
Stomach, glandular								
Macrophages, pigmented	0	0	NA	NA	NA	NA	5	4
-minimal	0	0					5	4
Small intestine, duodenum								
Macrophages, pigmented	0	0	0	0	2	1	5	5
-minimal	0	0	0	0	2	1	0	0
-mild	0	0	0	0	0	0	1	3
-moderate	0	0	0	0	0	0	4	2
Lymph node, mesenteric								
Macrophages, pigmented	4	5	NA	NA	NA	NA	5	5
-minimal	4	4					1	0
-mild	0	1					3	5
-moderate	0	0					1	0
Peyers patch								
Macrophages, pigmented	1	0	NA	NA	NA	NA	5	5
-minimal	1	0					3	5
-mild	0	0					2	0

There was an increase in pigmented macrophages in the stomach, small intestine, lymph node, Peyer's patches, which did not normalize after the recovery period. There was a dose-dependent increase in the incidence and severity of pigmented macrophages in the larynx, trachea and lungs. The pigmented macrophages were mixed with cellular and inflammatory exudate, which resolved at the end of recovery. However, the pigmented macrophages persisted at the end of the recovery period.

There was one animal with a lymphoid tumor, which was labeled as the cause of death of this animal.

Special Evaluation

None

Toxicokinetics

Blood samples for TK analysis were collected predose and 0.5, 1.5, 4, 6, and 8 h following the first daily dose on Days 1, 90, and 180. Samples were frozen and shipped to the sponsor for analysis. The CRO did not conduct TK analysis.

Dosing Solution Analysis

Samples were collected for homogeneity, stability, and concentration testing and stored at ~ -20 ^{0}C. However, the samples were not analyzed and were discarded.

Study title: A 9-month oral toxicity study of enteric coated SP303 in dogs

Study no.:	(b) (4) -288022
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 16, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SP303-250 mg delayed release tablets, Lot # 980011, 980191, SP303-500 mg delayed release tablets, Lot # 900010, 980050 Placebo to match enteric coated SP303 500 mg tablets, Lot # R12162, R12477 Purity not specified

Key Study Findings

There were no unscheduled deaths during the course of the study. Crofelemer produced dose-dependent increases in incidence of gastrointestinal (GI)-related adverse effects, including black mucoid feces and/or diarrhea, rust colored diarrhea, black diarrhea, red material in feces, and/or emesis (containing tablets, food and/or rust colored material) at high dose (400 mg/kg/day), as compared to control animals. Statistically significant decreases in body weight and food consumption and hematology changes suggestive of regenerative anemia were observed at the high dose. Changes in clinical chemistry at the high dose were likely related to nutritional deficits experienced by the animals due to decreased food consumption. Macroscopic

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examination revealed discoloration of the GI tract and lymph nodes at doses > 175 mg/kg/day suggestive of test article remnants. Histological findings were related to irritation and macrophage infiltration in the GI tract, as suggested by the increase in GI-related clinical signs in crofelemer treated animals, especially at the high dose. The target organ of toxicity of crofelemer in dogs after 9 months of oral administration was the GI system. The NOAEL dose was 50 mg/kg/day, based on changes in clinical chemistry and histopathology at doses $\geq$ 175 mg/kg/day.

Methods

Doses:	0, 50, 175, 600 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	Not clear from sponsor's protocol
Formulation/Vehicle:	Crofelemer (or placebo equivalent to high dose of test article) tablets were placed into size 11 gelatin capsules
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4/sex/group
Age:	6 months old at dose administration
Weight:	9.3 to 10.4 kg (Males), 7.8 to 9.9 kg (Females)
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Because of low food consumption, some high dose animals were allowed to feed overnight. Others were offered 1 cup of warm beef boullion to stimulate appetite on several occasions. This modification was done only in the high dose group.

Observations and Results

Mortality

Animals were observed twice daily for morbidity and mortality. They were also observed at the time of dosing and 2-3 h post-dose.

There were no unscheduled deaths during the course of the study. All animals survived to study termination.

Clinical Signs

Animals were observed twice daily for morbidity and mortality. They were also observed at the time of dosing and 2-3 h post-dose.

Animals at the mid- and high-dose (175 and 600 mg/kg/day, respectively) experienced increased incidences of gastrointestinal (GI) adverse effects, including black mucoid

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feces and/or diarrhea, rust colored diarrhea, black diarrhea, and/or red material in feces. Much lower incidences of similar GI disturbances were observed at the low dose, as compared to the mid- and high dose groups; however, all crofelemer-treatment groups produced GI effects, unlike placebo control. The incidence of abnormal excreta and related effects observed 3 h post-dose are summarized in the sponsor's table below:

Text Table 1: Incidences (Total Occurrence/No. of Animals) of Selected Excreta-Related Findings - 3 Hours Post-Dosing

	Males				Females			
Parameter Dose (mg/kg/day):	0	50	175	600	0	50	175	600
Soft feces	7/3	3/1	8/4	15/4	0/0	0/0	0/0	0/0
Green mucoid feces	1/1	0/0	8/3	4/3	0/0	0/0	0/0	0/0
Red mucoid feces	1/1	9/3	45/4	40/4	0/0	5/3	40/4	66/4
Mucoid feces	13/4	17/4	11/4	13/4	0/0	0/0	0/0	0/0
Black mucoid feces	0/0	3/2	25/4	28/4	0/0	1/1	27/4	54/4
Black mucoid diarrhea	0/0	1/1	9/3	13/3	0/0	3/1	7/3	16/4
Diarrhea rust in color	0/0	1/1	58/4	95/4	1/1	3/2	23/4	60/4
Red material on cage floor	0/0	0/0	2/2	13/4	0/0	0/0	0/0	0/0
Diarrhea	4/2	2/2	14/3	14/4	0/0	0/0	5/2	12/4
Feces containing tablet(s)	3/3	0/0	2/1	0/0	3/1	0/0	2/1	0/0
Feces containing white material	7/4	0/0	3/2	2/2	4/2	0/0	0/0	1/1
Mucoid feces rust in color	0/0	8/3	69/4	43/4	0/0	2/1	31/4	44/4
Diarrhea black in color	0/0	0/0	5/2	13/4	0/0	0/0	3/2	10/3
Soft feces black in color	0/0	1/1	2/2	3/2	0/0	0/0	1/1	4/3
Feces containing red material	1/1	3/2	27/4	51/4	0/0	2/2	23/4	63/4
Mucoid diarrhea rust in color	0/0	1/1	19/3	17/4	0/0	2/1	11/3	9/4
Soft feces rust in color	0/0	0/0	11/4	7/3	0/0	0/0	4/3	7/3
Yellow mucoid feces	1/1	1/1	2/2	1/1	3/2	0/0	1/1	0/0
Feces containing black material	0/0	0/0	0/0	1/1	0/0	0/0	0/0	1/1
Swollen urogenital area	0/0	0/0	0/0	0/0	7/2	1/1	1/1	1/1
Feces black in color	0/0	0/0	0/0	0/0	0/0	0/0	1/1	1/1
White mucoid feces	0/0	0/0	0/0	0/0	1/1	0/0	0/0	0/0

NA = Not applicable

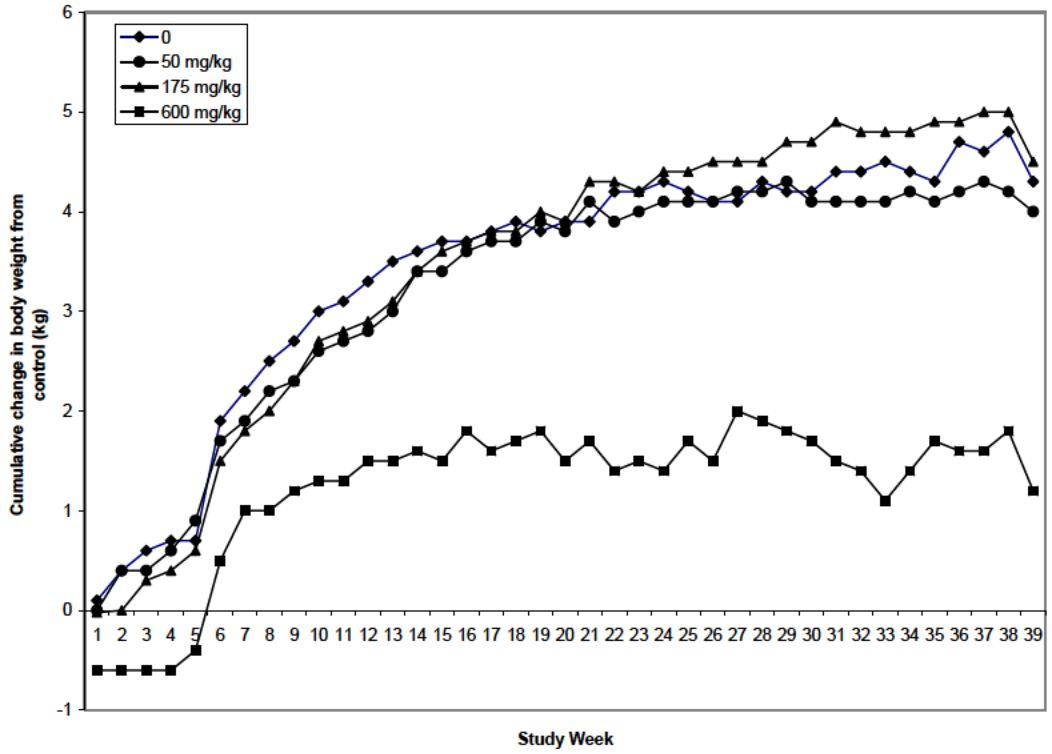
There was also a higher incidence of emesis (containing tablets, food and/or rust colored material) at high dose, as compared to control animals, although emesis was observed sporadically in all treatment groups. All other findings were similar to placebo-treatment and/or did not show a dose-response. Therefore the target organ of toxicity was the GI system in dogs treated daily with crofelemer for 9 months.

Body Weights

Body weights were recorded beginning one week prior to initiation of the treatment period and continued weekly until necropsy.

Lower body weights and body weight gains were consistently observed beginning during Week 2 and Week 9 in males and females, respectively, treated with 600 mg/kg/day, as compared to control. There were occasional minor decreases in body weight at the mid-dose. However, there were no overall treatment-related effects in the

low and mid-dose groups, as compared to controls. The graph below shows the cumulative change in body weight from control, recorded weekly.



Feed Consumption

Individual feed consumption was recorded beginning the week prior to initiation of the treatment period and continued weekly until necropsy.

Males and females treated with 600 mg/kg/day crofelemer showed statistically significant decreases in feed consumption during the course of the study. Decreases ranged from 15-39% in males and 14-43% in females, as compared to controls. Because of low food consumption, some high dose animals were allowed to feed overnight. Others were offered 1 cup of warm beef boullion to stimulate appetite on several occasions. This modification was done only in the high dose group.

Ophthalmoscopy

All study animals were subjected to ophthalmologic examinations, which were conducted prior to initiation of the treatment period and near the end of the treatment period.

There were no treatment-related findings revealed in the ophthalmology examinations in all test animals.

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ECG

Multilead (I, II, and III) ECGs were recorded for all animals one week prior to initiation of the treatment period and during study weeks 19 and 38. ECGs were evaluated by a veterinary cardiologist.

There were no significant treatment-related changes in ECG recordings during the course of the study. One control animal had an intraventricular conduction disturbance that was observed during Study Week 38.

Hematology

Blood samples from fasted study animals were collected prior to initiation of treatment, during study week 19, and at necropsy. The following parameters were analyzed:

Total Leukocyte Count (White Cells)
Erythrocyte Count (Red Cells)
Hemoglobin
Hematocrit
Mean Corpuscular Volume (MCV)
Mean Corpuscular Hemoglobin (MCH)
Mean Corpuscular Hemoglobin Concentration (MCHC)
Platelet Count (Platelet)
Prothrombin Time (Pro Time)
Activated Partial Thromboplastin Time (APTT)
Reticulocyte Count[a]
Percent (Reticulocyte)
Absolute (Retic Absolute)
Differential Leukocyte Count - Percent and Absolute
-Neutrophil
-Lymphocyte
-Monocyte
-Eosinophil
-Basophil
Platelet Estimate[b]
Red Cell Morphology (RBC Morphology)[b]

Reticulocyte count was evaluated on Weeks 19 and 38 only.

There were several changes in hematology parameters in the high dose group (males and females) which were suggestive of regenerative anemia, including increased reticulocyte counts, increased platelet counts, decreased hemoglobin and hematocrit, decreased MCV, MCH, and MCHC. There was also an increase (16.5% as compared to control) in red blood cell count in high dose males at Week 38 only. In crofelemer-treated females, there was 30% increase (during Week 19) and a 23% and 20% increase at the mid- and high dose, respectively, (during Week 38) in the percentage of neutrophils, as compared to controls. There was a 38% decrease in the percentage of lymphocytes at the high dose during Week 19 and a 19% and 40% decrease during Week 38 at the mid- and high-dose groups, respectively, as compared to controls. The changes in the high dose group are summarized in the table below.

	Males		Females	
	Week 19	Week 38	Week 19	Week 38
Parameter	% Change from control		% Change from control	
Hemoglobin	-28.7%	-24.3%	-37.7%	-36.8%
Hematocrit	No change	-25.8%	-32.5%	-34.2%
MCV	-24.1%	-29.8%	-27.4%	-22.8%
MCH	-27.4%	-35.1%	-33.8%	-25.8%
MCHC	-4.8%	-7.8%	-9.5%	-4.3%

Platelet	35.3%	60%	71.9%	111.2%
Retic %	116.7%	275%	500%	240%
Retic count	130.5%	300%	566.7%	196.9%

No other hematology changes related to treatment with crofelemer were noted.

Clinical Chemistry

Blood samples from fasted study animals were collected prior to initiation of treatment, during study week 19, and at necropsy. The following parameters were analyzed:

Albumin
Total Protein
Globulin [by calculation]
Albumin/Globulin Ratio (A/G Ratio) [by calculation]
Total Bilirubin (Total Bili)
Urea Nitrogen
Creatinine
Alkaline Phosphatase (Alkaline Phos'tse)
Alanine Aminotransferase (Alanine Transfer)
Aspartate Aminotransferase (Aspartat Transfer)
Gamma Glutamyltransferase (Glutamyl Transfer)
Glucose
Total Cholesterol (Cholesterol)
Calcium
Chloride
Phosphorus
Potassium
Sodium

There were slight decreases (< 14% as compared to placebo treatment) in albumin at both Weeks 19 and 38 in high dose males and in total protein levels mid- and high dose males at Week 19 only. There was a statistically significant decrease (~32%, as compared to control) in alkaline phosphatase activity at the high dose at Week 19 only; there was a decreasing trend in alkaline phosphatase activity in all dose groups at Week 38. At Week 38, there was a statistically significant decrease in alanine aminotransferase and aspartate aminotransferase activity of ~42% and 47%, respectively, as compared to controls. There was a decrease ($\leq$ 20%, compared to controls) in cholesterol at Weeks 19 and 38 in high dose males. Decreases in serum calcium, potassium and sodium which ranged from < 5% to < 18% of controls were also noted at the high dose males during weeks 19 and 38.

In females, there were slight decreases in albumin and total protein levels during Week 19, although a dose response was not evident. There was also a 50% decrease in bilirubin level at Week 38 in high dose females, as compared to controls. There was a ~68% and ~42% increase in aspartate aminotransferase activity at Week 19 and 38, respectively, and a 50% decrease in glutamyltransferase activity at Week 19 only in high dose females, as compared to controls. There was a ~36.5% and < 20% decrease in serum cholesterol level at the high dose females only at week 19 and 38, respectively, as compare to control. There were minor (~5-7%) decreases in calcium at the high dose at both time points.

Overall, the changes in clinical chemistry parameters were likely related to the nutritional deficit experienced most severely by animals at the high dose.

Urinalysis

Reference ID: 3168746

The following urinalysis parameters were evaluated:

Specific Gravity (SG)	Ketones (KET)
pH	Bilirubin (BIL)
Urobilinogen (URO)	Occult Blood (BLD)
Total Volume (TVOL)	Leukocytes (LEU)
Color (CLOR)	Nitrites (NIT)
Appearance (APP)	Microscopy of Sediment
Protein (PRO)	[Tabular abbreviations appear
Glucose (GLU)	on individual tables]

There were no significant treatment-related changes in urinalysis parameters in either sex.

Gross Pathology

Study animals were euthanized with sodium pentobarbital and subjected to necropsy. External surfaces, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities and their contents were examined.

Discoloration (red, black, red streaks, gray or green) of portions of the gastrointestinal tract was observed in males and females at the mid and high dose. Discoloration of lymph nodes was also observed in most crofelemer-treated animals. These findings are suggestive of test article remaining in the gastrointestinal tract. No other significant findings indicative of systemic toxicity related to crofelemer treatment were noted.

Organ Weights

The following organs were weighed from all animals at necropsy: adrenal glands, brain, heart, kidneys, liver, ovaries (without oviducts), spleen, testes, thymus, and thyroid with parathyroid.

At the high dose, thyroid/parathyroid weight decreased by ~47% and ~33% in males and females, respectively, as compared to control animals. There was also a decrease (~12-19%) in brain weight in males at all doses of crofelemer, although a dose response was not evident. In females, a 27% decrease in brain weight was only noted at the high dose.

Histopathology

Adequate Battery: Yes
The following tissues/organs were harvested and prepared for histopathological analysis:

Adrenal glands (2)
Aorta
Bone with marrow
  Femur
  Rib - costochondral junction
Bone marrow smear[a]
Brain (forebrain, midbrain, hindbrain)
Eyes with optic nerve (2)[b]
Gallbladder
Gastrointestinal tract
  Esophagus
  Stomach (cardiac, fundic, pyloric)
  Duodenum
  Jejunum
  Ileum
  Cecum
  Colon
  Rectum
Heart
Kidneys (2)
Liver (sections of two lobes)
Lungs (including bronchi, fixed by inflation with fixative)
Lymph nodes
  Mesenteric
  Suprapharyngeal
Ovaries (without oviducts)
Pancreas
Peripheral nerve (sciatic)
Pituitary
Prostate
Salivary glands
  [submaxillary (2)]
Skeletal muscle (vastus medialis)
Skin (with mammary gland)
Spinal cord (cervical, midthoracic, lumbar)
Spleen
Testes with epididymides(2)[c]
Thymus
Thyroid [with parathyroids (2)]
Trachea
Urinary bladder
Uterus with vagina
Gross lesions

Peer Review: The slides were reviewed by a study pathologist only. There was no secondary review.

Histological Findings: Histological findings were related to irritation and macrophage infiltration in the GI tract, as suggested by the increase in GI-related clinical signs in crofelemer treated animals, especially at the high dose. These findings are summarized in the sponsor's table below:

Text Table 2: Gastrointestinal Pathology Findings

	Males				Females			
Parameter Dose (mg/kg/day):	0	50	175	600	0	50	175	600
Cecum								
Congestion, lamina propria	1	1	3	0	0	0	3	0
Infiltrate, macrophage	0	0	4	4	0	0	2	4
Pigment - macrophage	0	0	3	4	0	0	2	4
Inflammation, acute	0	0	0	1	0	0	0	4
Crypt abscesses	0	0	0	0	0	0	0	3
Colon								
Congestion, lamina propria	2	0	1	0	0	0	0	3
Infiltrate, macrophage	0	0	2	2	0	0	0	4
Pigment - macrophage	0	0	1	2	0	0	0	4
Inflammation, acute	0	0	0	2	0	0	0	2
Crypt abscesses	0	0	0	0	0	0	0	3
Duodenum								
Dilatation, crypt	1	0	0	0	1	0	0	0
Congestion	0	1	0	1	0	0	0	2
Ileum								
Congestion - ileo-colic junction	0	1	0	1	0	0	0	2
Jejunum								
No findings								
Rectum								
Congestion, lamina propria	1	1	1	1	0	0	2	1
Erythrocytosis, lymphoid tissue	0	1	0	0	0	1	0	0
Infiltrate, macrophage	0	0	3	4	0	0	2	4
Pigment - macrophage	0	0	2	4	0	0	2	4
Inflammation, acute	0	0	1	1	0	0	0	3

Macrophage infiltration (histiocytosis) in the lymph nodes was also observed in animals at all crofelemer doses, as shown in the sponsor's table below.

Text Table 3: Lymph Node Pathology Findings

	Males				Females			
Parameter Dose (mg/kg/day):	0	50	175	600	0	50	175	600
Mesenteric								
Hyperplasia, lymphoid	4	3	1	1	4	3	3	1
Erythrocytosis, sinus	0	4	3	1	0	1	4	2
Histiocytosis, sinus	0	4	4	4	0	2	0	3
Basophilic bodies-macrophage	0	0	2	4	0	0	1	1
Histiocytosis, medullary cords	0	1	4	4	0	1	4	4
Pigment - macrophages	0	0	3	4	0	1	2	3
Suprapharyngeal								
Hyperplasia, lymphoid	3	4	4	3	4	3	4	4
Erythrocytosis, sinus	2	0	0	0	1	0	0	0
Pigment, green	1	0	0	0	0	0	0	1
Histiocytosis, medullary cords	0	0	4	4	0	0	2	3
Mediastinal								
Histiocytosis, sinus	NA	1	NA	0	NA	NA	NA	NA
Erythrocytosis, sinus	NA	1	NA	0	NA	NA	1	NA
Hyperplasia, lymphoid	NA	NA	NA	NA	NA	NA	1	NA
Iliac								
Erythrocytosis, sinus	NA	1	NA	1	NA	NA	NA	NA
Pigment - macrophages	NA	0	NA	1	NA	NA	NA	NA

NA = Not Applicable

Macrophage infiltration (Kupffer cells) was also observed in the liver in both sexes at mid and high dose of crofelemer. These findings are shown in the sponsor's table below:

Text Table 4: Liver Pathology Findings

	Males				Females			
Parameter Dose (mg/kg/day):	0	50	175	600	0	50	175	600
Liver								
Inflammation, nonsuppurative	2	4	4	2	4	3	2	1
Vacuolation, cytoplasmic	2	3	0	0	3	1	1	0
Pigment - Kupffer cells	0	0	3	4	1	1	3	4
Basophilic bodies - Kupffer cells	0	0	4	4	0	1	4	4

There were no other significant treatment-related findings.

Toxicokinetics

Samples for TK analysis were collected from all dogs prior to dosing and at 2, 6, 12, 18, and 24 h post-dose on Days 0, 29, 135, and 270. Prepared plasma samples were frozen and shipped to the sponsor for analysis. The CRO did not perform the TK analysis[3].

Dosing Solution Analysis

Not specified

7 Genetic Toxicology

Crofelemer was tested in an Ames assay (Study # SP303-F-021) and the data were previously reviewed under IND 51,818 by Dr. Tanveer Ahmad. His review is included verbatim below.

[3] The Division agreed at an End of Phase 2 Meeting on May 5, 2004 that the ADME studies conducted to date were adequate and additional studies were not needed for registration of crofelemer.

Reference ID: 3168746

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

Ames Test
(Report #SP303-F-021)

Testing Laboratories: [redacted] (b) (4)

Dates Studies Started and Completed: January 21, 1991 and March 25, 1991.

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538.

Concentration Employed: 1-10000 mcg/plate

Solvent Control: Water and dimethyl sulfoxide (DMSO)

Source of Metabolic Activation: Aroclor 1254 induced rate liver microsomal enzymes (S-9 mix).

Drug Batch No.: T9636

Criteria of Positivity: A two fold increase in the number of revertant colonies above the solvent control value in strains TA 98 and TA 100, or threefold increase in the number of revertant colonies above the solvent control value in strains TA 1535, TA 1537 and TA 1538 are considered positive provided if the effect is seen in at least two consecutive dose levels.

Methods: Ames test was conducted to assess the mutagenic potential of the drug by measuring its ability to induce reverse mutations at selected loci of several strains of Salmonella typhimurium [TA 98, TA 1537 and TA 1538 (frame shift); TA 100 and TA 1535 (baser pair substitution)] in the presence and absence of S-9 activation (Arocol 1254-induced rate liver microsomal enzyme mixture). The method used is plate incorporation method. Vehicle (deionized and distilled water), drug (1-10,000 mcg/plate) and positive controls [NaN_3 (1.0 mcg/plate), 2-aminoanthracene (0.5 mcg/plate), 2-nitrofluorene (1.0 mcg/ml) and ICR-191 (2 mcg/plate)] were plated in triplicate with tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 in the presence and absence of S-9 mix and incubated for 48-72 hours. Revertant colonies were counted.

Results: The drug inhibited the growth of most of the strains at 10,000 mcg/plate. Drug was not mutagenic in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix). Increase in mutant colonies was noted in all microbial strains employed in the presence of positive control (with or without S-9 Mix).

Reference ID: 3168746

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells

Study no.:	SP-303-AT-001
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 9, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SP303E-2, Lot # PL-401R, Purity not specified

Key Study Findings

Under the conditions tested, SP-303 did not produce chromosomal aberrations at concentrations of up to 600 μg/mL, independent of metabolic activation.

Methods

Cell line:	Chinese Hamster Ovary (CHO) cells
Concentrations in definitive study:	-S9 mix: 2.5, 5.0, 10.0, 20.0, 35.0, 50.0, 75.0, 100, 150, and 200 μg/mL for 17.8 h +S9 mix: 50, 100, 200, 300, 350, 400, 500, 600 μg/mL for 3 h
Basis of concentration selection:	Solubility of the test article was the basis of concentration selection. In solubility studies, a concentration of 156 mg/mL was prepared without any precipitates. Concentrations of 21.2, 30.2, 43.0, 61.4, 87.8, 125, 179, 256, 366, 524, 748, 1070, 1530, 2180, and 3120 μg/mL were tested in the initial trial (±S9).
Negative control:	Cell culture medium (McCoy's 5a)
Positive control:	Mitomycin C (-S9), Cyclophosphamide (+S9)
Formulation/Vehicle:	Solution in dimethylsulfoxide (DMSO)/ Solvent control: DMSO
Incubation & sampling time:	Initial trial: 3 h treatment, harvest at 20 h Confirmatory trial: 3 h and 17.8 h (+S9 and –S9 mix, respectively), harvest at 20 h

Study Validity

A study was considered valid if all of the following criteria were satisfied: (1) negative and vehicle control cultures contain <5% cells with chromosomal aberrations, (2) positive control result is higher than vehicle/negative control ($p<0.01$), (3) assay

includes the highest applicable dose (target dose 10 mM or 5 mg/mL) or dose exceeding the solubility limit in culture medium if test system is negative for chromosomal aberrations or there is no significant decrease (≥50%) in confluence or mitotic index, (4) assay includes at least 3 analyzable doses, (5) clear evidence of dose response.

Summary of Chromosomal Aberrations Assay Treatment Schedule in Hours

Test	Test Article	Wash	Colcemid®	Fixation
Initial Trial				
- S9	0	3.0	18.0	20.0
+ S9	0	3.0	18.0	20.0
Confirmatory Trial				
- S9	0	17.8	18.0	20.0
+ S9	0	3.0	18.0	20.0

Results

All cultures were run in duplicate. The data table below summarizes results from each confirmatory assay carried out in the absence of S9. Each data point represents the mean of 2 sets of evaluations (100 cells scored per evaluation). At 50 μg/mL, SP-303 induced an increase in the % pf cells with aberrations, as compared to solvent and negative controls. Since there was excessive toxicity observed at concentrations ≥ 75 μg/mL, a dose response was not apparent. The assay was therefore repeated. In the repeat assay, no aberrations were observed in cells treated with SP-303 at concentrations up to 51.5 μg/mL. Concentrations ≥ 67 μg/mL produced excessive toxicity and were therefore not evaluated. In the repeat assay, 36.1 μg/mL produced a statistically significant increase in % polyploid cells, a finding that was not observed in the first assay or at concentrations > 36.1 μg/mL in the repeat assay.

Group	Conc (μg/mL)	Time* (h)	Mitotic index(%)	% cells with aberrations	% PP**	% E***
Culture Medium	--	17.8-20	6.4	0.5	1.5	0
DMSO (μl/mL)	10		6.9	1.5	2.0	0
MMC	0.08			28****	1.5	0
SP-303	10		4.6	1.5	1.0	0
	20		4.6	1.5	2.5	0
	35		6.1	0.5	3.5	0.5
	50		3.2	7.5****	1.5	0
	75		1.9	Excessive toxicity		
	100		1.3			
	150		0.1			
	200		0.0			
Culture Medium	--	17.8-20	10.7	0	2.0	0.0
DMSO	10 μl/mL		12.0	0	2.5	0.0
MMC	0.100			30.0****	2.5	0.5
SP-303	20.6		6.6	0.5	7.5	0.0
	36.1		8.1	2.0	11.5****	0.0
	51.5		4.5	1.5	6.5	0.0
	67		2.5	Excessive toxicity		

	82.4		1.7	Excessive toxicity
	103		0.9	
	129		0.1	
	155		0.5	

*Treatment time to harvest time
** % polyploidy cells
*** % endoreduplications
**** p≤0.01, compared to solvent control

The table below summarizes the results from the confirmatory assay conducted in the presence of S9 mix. Each data point represents the mean of 2 sets of evaluations (100 cells scored per evaluation). Under the conditions of this study, SP-303 did not produce an increase in chromosomal aberrations, as compared to negative and solvent controls, in the presence of metabolic activation. However, there were increases in % PP and % E. The assay was repeated and these results were observed again.

Group	Conc (µg/mL)	Time* (h)	Mitotic index(%)	% cells with aberrations	% PP**	% E***
Culture Medium	--	3-20	8.7	0.5	0.0	0.5
DMSO (µl/mL)	10		6.9	2.0	0.5	0.0
CP	5.0			48.0****	3.0	0.0
SP-303	50.0		8.7	2.0	10.5****	0.0
	100		6.7+	2.0	40.0****	2.5
	200		0.3	Excessive toxicity		
	300		2.4	5.0	1.0	11.5****
	350		0.5	Excessive toxicity		
	400		0.6			
	500		0.3			
	600		0.1			
Culture Medium	--	3-20	12.2	1.0	3.5	0.5
DMSO	10 µl/mL		11.6	0.5	2.5	1.0
CP	5.0			43.4****	6.0	0.0
SP-303	36.1		7.9	1.5	5.0	2.0
	51.5		13.2	3.5	5.0	4.5
	103		13.7	Excessive toxicity		
	155		5.5	1.0	21.0****	11.0****
	225		6.6	Excessive toxicity		
	310		4.8	1.5	7.0	13.0****
	375		2.9	Excessive toxicity		
	450		2.2			

*Treatment time to harvest time
** % polyploidy cells
*** % endoreduplications
**** p≤0.01, compared to solvent control

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

Study title: Mutagenicity test on SP-303 in the *in vivo* rat micronucleus assay

Study no:	SP-303-AT-002
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 9, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SP303E-2 (Lot No. PL-4-1R), Purity not specified

Key Study Findings

SP-303 was not clastogenic under the conditions of this assay at doses of up to 50 mg/kg IP in the rat.

Methods

Doses in definitive study:	25, 50 mg/kg
Frequency of dosing:	Single
Route of administration:	Intraperitoneal
Dose volume:	20 mL/kg
Formulation/Vehicle:	Solution/Corn Oil
Species/Strain:	Rat/Crl:CD(SD)IGS BR
Number/Sex/Group:	6 males/group
Satellite groups:	6 males each in control and High dose (used to replace main study animals which died on study)
Basis of dose selection:	Dose ranging finding study 1 (200, 500, 800, 1500, 2000 mg/kg) Dose range finding study 2 (50, 100, 200 mg/kg)
Negative control:	Corn Oil
Positive control:	Cyclophosphamide (60 mg/kg)

Study Validity

In order to be considered clastogenic, the following criteria were applied: a statistically significant positive response for at least one dose and a dose response. The result was considered to be negative unless both criteria were met.

Results

Two dose-ranging studies were conducted before the doses of 25 and 50 mg/kg were chosen for the definitive study. Doses greater than 50 mg/kg produced significant mortality in a majority of the animals. The maximum tolerated dose was 200 mg/kg, based on the observations from the dose range finding studies. Clinical signs of toxicity

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observed in the definitive study included soft feces, fecal stains, chromodacryorrhea, red crust around nose, and/or hypoactivity. Clinical signs were observed at 50 mg/kg and increased at 2 days post-dose. There were no signs of bone marrow toxicity in any of the SP-303 treatment groups. There was no increase in micronucleated polychromatic erythrocytes (PCE) at both doses of SP-303, as compared to vehicle. The positive control produced a statistically significant increase in % micronucleated PCEs. Therefore, under the conditions tested, SP-303 was not clastogenic. The results of the definitive micronucleus assay are summarized in the sponsor's table below:

TEST ARTICLE: SP303

ASSAY: 19288

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCEs MEAN OF 2000 PER ANIMAL ± S.E. MALES	RATIO PCE:NCE MEAN ± S.E. MALES
CONTROLS				
VEHICLE	Corn Oil	24 hr	0.10 ± 0 04	0.73 ± 0.13
		48 hr	0 04 ± 0 02	0 75 ± 0.05
POSITIVE	CP 60.0 mg/kg	24 hr	3.19 ± 0.33*	0.80 ± 0.12
TEST ARTICLE	25.0 mg/kg	24 hr	0.12 ± 0 03	0.83 ± 0.07
	50.0 mg/kg	48 hr	0.07 ± 0 04	0.62 ± 0.08

* Significantly greater than the corresponding vehicle control, $p<0.01$.
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

The sponsor did not conduct carcinogenicity studies with SP-303. Carcinogenicity studies will be conducted post-marketing, as agreed upon at the pre-NDA meeting on January 19, 2011 with the Agency.

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9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: A study of the effects of microencapsulated Provir on fertility and early embryonic development to implantation in rats

Study no.:	(b) (4) -288017
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 20, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SP303 Delayed Release Beads, Lot# CB8MN01A04 Placebo for SP303 Delayed release beads, Lot CC8MN01P04 Purity not specified

Key Study Findings

SP-303 did not affect fertility or reproductive performance in male and female rats when administered orally at doses of up to 738 mg/kg/day.

Methods

Doses:	0, 123, 369, 738 mg/kg/day
Frequency of dosing:	Once a day
Dose volume:	N/A
Route of administration:	Oral gavage with flexible rubber catheter (followed by 2-3 mL water flush)
Formulation/Vehicle:	Gelatin capsule (appropriate amount of test article weighted into gelatin capsules)/Placebo in gelatin capsule
Species/Strain:	Rats/Crl:CD(SD)IGS BR
Number/Sex/Group:	25/sex/group
Satellite groups:	None
Study design:	Males: Dosed with placebo or test article for 28 days prior to mating and continued through mating until one day prior to scheduled sacrifice Females: Dosed with placebo or test article for 14 days prior to mating and continued through mating until gestation day (GD) 7
Deviation from study protocol:	Not specified

Observations and Results

Mortality

Animals were checked twice daily for signs of morbidity and mortality.

Two males at the high dose died soon after initiation of treatment (Day 2) and one mid-dose male died during week 3 of treatment. One of the high dose males likely died of intubation injury, as indicated by a torn esophagus. The other high dose male and the mid-dose male had red contents in the thoracic cavity, although the exact cause of death could not be identified. The sponsor proposed that it was related to dosing procedure. The mid-dose male showed signs of hypoactivity and labored respiration while the high dose male was gasping on the day of death.

In females, there were several animals which died while on study or were euthanized. Two control animals, 1 mid-dose, and 2 high dose animals were found dead between Weeks 2-5. Both control group females and 1 high dose female had a perforated esophagus, which was indicative of dosing-related injury. The other females (1 high dose and 1 mid-dose) had red and clear fluid contents, respectively, in the thoracic cavity. The sponsor concluded that these animals may have died due to dosing-error; however, there were no other findings to support this conclusion. One high dose female was euthanized on study day 45 (3 days after completion of mating period) because this animal gained weight consistent with pregnancy, although no signs of mating were observed.

Clinical Signs

Clinical signs were assessed everyday throughout the study period, specifically at time of dosing and one hour post-dose, and as needed.

Clinical signs in males observed during the dosing period included dried red matting on forelimbs in a few mid- and high dose animals, rales, gasping and/or labored respirations in a few animals in all SP-303 dose groups, and decreased defecation in 3-4 animals in the mid and high dose groups. Clinical signs in females during the dosing period included hair loss on ventral thoracic or abdominal area, unkempt appearance, decreased urination in 1-2 animals in the mid and high dose groups. Wet, yellow staining of the ventral abdominal or urogenital area, tan and clear matting around the mouth were observed in mid- and high-dose animals as well. Overall, the incidence and types of clinical signs observed did not correlate with significant adverse effects related to treatment.

Body Weight

Body weights were measured twice a week for males throughout the dosing period until study day 59. For females, body weights were recorded twice weekly throughout the dosing period, beginning with initiation of vaginal smearing and continuing until evidence of mating (day 24). In females where no evidence of mating was observed, twice weekly body weight measurements were recorded until necropsy. After confirmed mating, pregnant females were weighed on gestation days (GD) 0, 3, 7, 10, and 15.

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In males, body weight gain increased slightly during the course of the study. Although these changes were statistically significant during certain study intervals, as compared to control males, body weights in SP-303-treated males were similar to control animals over the course of the entire dosing period. In females, there were no differences in pre-mating or gestation body weights related to SP-303 treatment.

Feed Consumption

Food consumption was recorded on the corresponding body weight days for each sex.

There were statistically significant increases in food consumption in males during Study Days 0-7, and 10-14 in mid and/or high dose animals (~8-13%, as compared to controls). There were minor increases in weekly food consumption during specific study interval in females treated with SP-303. From Study Day 17-21, there was a 7.9% and 15.9% increase in food consumption in the mid and high dose females, respectively, as compared to controls. During Study Days 21 to 24, there was an 11.1% and 14.3% increase in food consumption at the low and high doses, respectively, as compared to controls. During gestation, ~12%, 10.7%, and 9.7% increases in food consumption were observed in high dose females during Study intervals 0-3, 3-7, and 7-10 days, respectively.

Toxicokinetics

Not conducted

Dosing Solution Analysis

Not provided

Necropsy

All animals were subjected to full necropsy at scheduled sacrifice. Animals which died on study or were sacrificed prior to study completion were necropsied if possible. Necropsy included an examination of the external surface, all orifices, the thoracic, abdominal and pelvic cavities, viscera, and external surfaces of brain and spinal cord. The following tissues and organs were harvested and preserved for histopathological examination, if needed: cervix, coagulating gland, ovaries and oviduct (2), pituitary, prostate, seminal vesicles, right testes with epididymis and vas deferens, uterus with vagina, any and all gross lesions. One high dose female which died on Study Day 33 did not undergo organ/tissue harvest. The following organ weights were determined: testes, epididymides (total and cauda), ovaries, oviducts, brain and pituitary. Absolute organ weights and relative organ weights (relative to body weight) are reported.

Dark red lungs were observed in the mid and high dose males that died on study. Both high dose males that died on study had adhesions and thickening in the pericardium and test article was observed in the stomach. In the males which survived to the end of

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the study, small coagulating glands (1 male each at low dose and high dose), dilated pelvis (1 high dose male), thickened stomach and/or distended ureter (1 high dose male), and small seminal vesicles (1 low dose male) were observed. There were no significant treatment-related changes in absolute or relative organ weights in males.

In females which survived until the end of the study, there were no treatment-related changes noted at necropsy. There were no changes in absolute organ weights associated with treatment. There was a slight statistically significant decrease (~14.9%, as compared to control) in the relative pituitary weight at the low dose and high dose only. This was not observed in the mid-dose-treatment groups.

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

1. Estrous cycle: Vaginal smears were evaluated daily beginning 10 days prior to initiation of the treatment period and continuing through the 14-day pre-pairing period. After pairing, vaginal smears were evaluated until evidence of mating (presence of sperm in vaginal smear or vaginal copulatory plug) or until termination of mating.

2. Breeding: Males, which received treatment for 28 days prior to pairing, were mated with females at the same dose level on a 1:1 basis. If evidence of mating was not present, then a proven male was mated with the female in the same dose level for an additional 5 days. Mating and fertility indices were calculated as follows:

$$\text{Male (Female) Mating Index (\%)} = \frac{\text{No. of Males (Females) with Evidence of Mating or Confirmed Pregnancy}}{\text{Total No. of Males (Females) Used for Mating}} \times 100$$

$$\text{Male Fertility Index (\%)} = \frac{\text{No. of Males Siring at Least 1 Litter}}{\text{Total No. of Males Used for Mating}} \times 100$$

$$\text{Female Fertility Index (\%)} = \frac{\text{No. of Females with Confirmed Pregnancy}}{\text{Total No. of Females Used for Mating}} \times 100$$

Mating and fertility indices in males and females were not affected by SP-303 treatment. The mean number of days between pairing and conception were similar between SP-303-treated animals (males and females) and controls. The table below summarizes the mating and fertility indices for males and females after SP-303 treatment.

	Males		Females	
Dose (mg/kg/day)	Mating Index (%)	Fertility Index (%)	Mating Index (%)	Fertility Index (%)
0	100	95.2	100	100
123	100	100	100	100
369	100	100	100	95.8
738	95.8	91.3	100	95.7

3. Uterine examination (GD 15): Pregnant females were euthanized on GD15 by carbon dioxide inhalation and contents of the thoracic and abdominal cavities

were examined. The following endpoints were assessed: examination of uterus and ovaries, number and location of all embryos, early resorptions and total implantation sites, embryo viability, and staining of uteri with no macroscopic evidence of implantation to identify early implantation loss. The group mean litter basis and proportional litter basis were calculated using the methods below:

1. Group Mean Litter Basis:

$$\text{Postimplantation Loss/Litter} = \frac{\text{No. Dead Embryos, Resorptions (Early/Late)/Group}}{\text{No. Gravid Females/Group}}$$

2. Proportional Litter Basis:

$$\text{Summation per Group (\%)} = \frac{\text{Total Postimplantation Loss/Litter/Group (\%)}^{a}}{\text{No. of Litters/Group}}$$

$$a = \frac{\text{No. Dead Embryos, Resorptions (Early/Late)/Litter}}{\text{No. Implantation Sites/Litter}} \times 100$$

There were no treatment-related changes in embryonic data, as compared to controls.

4. Spermatogenic endpoint evaluations: Sperm count, sperm motility, sperm production rate, and sperm morphology were evaluated for each study male. For each treated male, the right epididymis was weighed and sperm count was described as count in millions/g tissue). Sperm samples were collected, counted and evaluated for changes in motility. The left testis and epididymis were collected and homogenized and evaluated for homogenization resistant spermatid count and sperm production rate.

There was no treatment-related effect on sperm count (reported as millions of sperm/g of tissue) in the testis or epididymis in study males. There was no significant treatment-related effect on weight of testis (left/right weighed individually) or left epididymis. The sperm production rate (defined as (#sperm/g tissue)/6.1 days) in the left testis did not vary between control and SP-303-treated males. The rate of turnover of germinal epithelium is 6.1 days. An assessment of sperm motility also showed that SP-303 did not affect this parameter either. There was a slight statistically significant decrease in the percentage of morphologically normal sperm in 369 mg/kg/day-treated males, as compared to controls; however, this effect was not observed at either the low or high doses. There were no differences any of the other parameters evaluated in the morphological examination (normally shaped head separated from flagellum, head absent with normal flagellum, head absent with abnormal flagellum, misshapen head with normal flagellum, misshapen head with abnormal flagellum, degenerative flagellar defect with normal head, other flagellar defects with normal head).

9.2 Embryonic Fetal Development

Study title: A dose range-finding study of the effects of microencapsulated Provir on embryo/fetal development in rats

Study no.:	(b) (4)-288015
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 20, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SP303 delayed release beads, Lot # CB8MN01A04 Placebo for SP-303 delayed release beads, Lot # MN01P Purity not specified

Methods

Doses:	0, 123, 307, 615, 922, 1229 mg/kg/day (active crofelemer)
Frequency of dosing:	Once a day
Dose volume:	N/A
Route of administration:	Oral gavage with flexible rubber catheter (followed by 2-3 mL water flush)
Formulation/Vehicle:	Microencapsulated enteric coated beads/1229 mg/kg/day placebo
Species/Strain:	Rats/Crl:CD(SD)IGS BR
Number/Sex/Group:	8 females/group
Satellite groups:	None
Study design:	Pregnant females were dosed once daily from GD 6-GD 17. Laparohysterectomies were performed on GD 20 and maternal and fetal parameters were evaluated.
Deviation from study protocol:	Not specified

Key Study Findings

In this dose range-finding study, pregnant rats (8/group) were administered 123, 307, 615, 922, 1229 mg/kg/day microencapsulated enteric coated SP-303 (or placebo) orally via gastric tube from GD6-GD17. Several animals, including controls, died on study or had to be euthanized in extremis prior to scheduled sacrifice. Clinical signs included rales and labored respirations, unkempt appearance, and staining on various body surfaces. There was a decreasing trend in maternal body weight during the dosing period, with statistically significant decreases in mean body weight on GD 17, 18 and 20 (14.6%, 13.5%, and 10.6%, respectively, as compared to controls). It was concluded that the dosing procedure was likely to blame for the decrease in body weight gain observed in control animals, as compared to their historical control data. SP-303 treatment was maternally toxic at 1229 mg/kg/day producing a ~37% decrease in

maternal body weight gain, as compared to control, over the entire gestation period. There was also a decrease in gravid uterine weight at the highest dose of SP-303 (18.1%, as compared to control). There were no treatment-related effects on fetal weight, litter sizes, post implantation losses, fetal sex ratios, and mean number of corpora lutea and implantation sites. SP-303 treatment did not produce any external malformations or variations, similar to placebo. In conclusion, there was significant maternal toxicity at the highest dose of SP-303. The sponsor chose to pursue 123, 369, and 738 mg/kg/day for the definitive segment II study in rats. This reviewer agrees with the sponsor's rationale.

Study title: A study of the effects of microencapsulated provir on embryo/fetal development in rats

Study no.:	(b) (4)-288019
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 20, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SP303 beads, Lot # CB8MN01A04 Placebo for SP-303 beads, Lot # CC8MN01P04 Purity not specified

Key Study Findings

SP-303 was not teratogenic at doses of up to 738 mg/kg/day in rats when administered orally from GD 6-17 as microencapsulated enteric coated beads. The NOAEL dose for maternal and developmental toxicity was 738 mg/kg/day.

Methods

Doses:	0, 123, 369, 738 mg/kg/day active crofelemer
Frequency of dosing:	Once daily
Dose volume:	Capsules were opened and beads were poured into syringe bore connected to rubber catheter
Route of administration:	Oral gavage via a flexible rubber catheter (followed by a 2-3 mL water flush)
Formulation/Vehicle:	Microencapsulated enteric coated beads/placebo microencapsulated enteric coated beads
Species/Strain:	Rat/Crl:CD(SG)IGSBR
Number/Sex/Group:	25 females/group
Satellite groups:	6 females/group for TK samples
Study design:	Sexually mature females (~70 days old) were mated with males and confirmed pregnant (GD 0) before being moved to separate cages.

	Animals were dosed from GD6-GD17. Laparohysterectomy was performed on GD20. Animals which died prior to terminal sacrifice underwent necropsy and implantation sites and corpora lutea were recorded. Recognizable fetuses were examined.
Deviation from study protocol:	None which affected the outcome of the study

Observations and Results

Mortality

All study animals were observed twice daily for signs of mortality and morbidity.

Four animals (one from control, two from 369 mg/kg/day, and one from 738 mg/kg/day dose groups) died prior to scheduled termination. Three were found dead (control, high dose, and one mid-dose animal) and one was euthanized (second mid-dose animal) in extremis. The mid-dose females died on GD 6 and 18 due to injuries related to intubation errors. The high dose animal died on GD 18 also due to intubation errors. The cause of death of the control animal on GD 13 was not apparent.

Clinical Signs

Clinical signs were assessed twice daily for all animals, with observations one hour post-dose as well.

Clinical signs such as hair loss on forelimbs, rales, dried red material around nose, red or brown matting around mouth or on forelimbs were observed in all animals, including controls. In SP-303-treated animals, clinical signs were observed sporadically and did not show a dose response.

Body Weight

Body weights were measured on GD 0, daily from GD 6-18, and on GD 20. Gravid uterine weights were determined at necropsy on GD 20 (day of scheduled laparohysterectomy).

Body weights were not affected by SP-303 treatment, as compared to placebo, over the entire study period (GD 0 – GD 20). There were minor increases in body weight in the low dose groups, as compared to control, during specific study intervals. However, there were no dose dependent changes in body weight over the entire duration of the study. Gravid uterine weights in SP-303-treated animals were similar to controls.

Feed Consumption

Food consumption was measured on GD 0, 6-18 (daily), and on GD 20.

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Increases in food consumption were observed in mid- and high dose animals at specific study intervals (GD 12-18, GD 17-18, GD 18-20). These increases ranged between 10-14%, as compared to controls. A dose-dependent relationship was not apparent. The CRO did note that food consumption in the control animals in this study was slightly lower than previously observed in historical controls.

Toxicokinetics

Samples were collected and shipped to the sponsor for further analysis. The CRO did not analyze the samples.

Dosing Solution Analysis

Not provided

Necropsy

All animals surviving until scheduled sacrifice underwent a full necropsy. Animals which died while on study were also necropsied to determine cause of death (if possible) and record all internal and external abnormalities. The thoracic, abdominal and pelvic cavities were examined and abnormalities were recorded.

The cause of death of the mid- and high dose animals was determined to be dosing related injury, as indicated by lodging of dosing canula in the esophagus, perforated esophagus, and/or red fluid contents in thoracic cavity. One animal each from the mid- and high-dose groups died on GD 18. One mid-dose animal was euthanized on GD 6. The control animal which died on GD 13 had scabbing on buccal and nasal surfaces, dark red lobes on the lungs, red fluid contents in the thoracic cavity, and brown matting on the ano-genital region. The cause of death on the control animal was not apparent.

In animals which survived until termination, no other significant differences between SP-303 and control treatment groups were identified.

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

The following parameters were assessed after laparohysterectomy on GD 20. The location and number of all early and late resorptions, the total number of resorptions, fetuses and corpora lutea were recorded. The group mean litter basis and proportional litter basis were calculated using the formulas below:

Reference ID: 3168746

1. Group Mean Litter Basis:

$$\text{Postimplantation Loss/Litter} = \frac{\text{No. Dead Fetuses, Resorptions (Early/Late)/Group}}{\text{No. Gravid Females/Group}}$$

2. Proportional Litter Basis:

$$\text{Summation per Group (\%)} = \frac{\Sigma \text{ Postimplantation Loss/Litter (\%)}^{a}}{\text{No. of Litters/Group}}$$

$$a = \frac{\text{No. Dead Fetuses, Resorptions (Early/Late)/Litter}}{\text{No. Implantation Sites/Litter}} \times 100$$

The cesarean section data is summarized in the sponsor's table below.

TABLE 9

PROJECT NO. (b) (4) 288019 A STUDY OF MICROENCAPSULATED PROVIR ON EMBRYO/FETAL DEV. IN RATS PAGE 1
SPONSOR:SHAMAN PHARMACEUTICALS SUMMARY OF MEAN FETAL DATA AT THE SCHEDULED NECROPSY

GROUP	SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1 TOTAL	135	132	267	0	11	0	11	278	313	35	NA	19
MEAN	7.1	6.9	14.1	0.0	0.6	0.0	0.6	14.6	16.5	1.8	3.7	
S.D.	1.97	1.72	1.90	0.00	0.61	0.00	0.61	1.80	2.34	1.77	0.19	
2 TOTAL	111	119	230	0	12	0	12	242	271	29	NA	17
MEAN	6.5	7.0	13.5	0.0	0.7	0.0	0.7	14.2	15.9	1.7	3.7	
S.D.	2.24	1.94	2.37	0.00	0.92	0.00	0.92	2.41	1.89	1.26	0.22	
3 TOTAL	118	127	245	0	18	0	18	263	306	43	NA	18
MEAN	6.6	7.1	13.6	0.0	1.0	0.0	1.0	14.6	17.0	2.4	3.7	
S.D.	2.06	2.21	2.62	0.00	1.28	0.00	1.28	2.57	1.91	2.70	0.18	
4 TOTAL	136	142	278	0	9	0	9	287	329	42	NA	21
MEAN	6.5	6.8	13.2	0.0	0.4	0.0	0.4	13.7	15.7	2.0	3.8	
S.D.	1.81	2.30	2.90	0.00	0.75	0.00	0.75	2.96	1.80	1.95	0.25	

None significantly different from control group
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 123 MG/KG/DAY 3- 369 MG/KG/DAY 4- 738 MG/KG/DAY

These data are further clarified (% per litter) in the table below:

Dose (mg/kg/day) Parameter	0 (Control)	123	369	738
Corpora Lutea	16.5	15.9	17.0	15.7
Implantation Sites	14.6	14.2	14.6	13.7
Viable fetuses (%)	95.9	95.2	93.4	97.0
Dead Fetuses (%)	0	0	0	0
Early resorptions (%)	4.1	4.8	6.6	3.0
Late resorptions (%)	0	0	0	0
Total resorptions (%)	4.1	4.8	6.6	3.0

Pre-implantation loss (%)	10.7	11.0	13.5	13.7
Post-implantation loss (%)	4.1	4.8	6.6	3.0
Males (%)	50.3	47.9	48.2	49.4
Females (%)	49.7	52.1	51.8	50.6
Male fetal weights (g)	3.8	3.8	3.8	3.9
Female fetal weights (g)	3.6	3.6	3.7	3.7
Combined fetal weights (g)	3.7	3.7	3.7	3.8

Overall, there were no statistically significant differences in laparohysterectomy findings between controls and SP-303 treated animals. SP-303 did not affect litter size, sex ratio, the number of early or late resorptions, pre- or post-implantation losses, and fetal weights.

Offspring (Malformations, Variations, etc.)

Each fetus was weighed, sexed, and underwent a detailed examination of external surfaces and internal cavities to identify all malformations and variations. Alizharin Red S staining was used to examine the skeleton.

There were no fetal effects (malformations or variations) at SP-303 doses of up to 738 mg/kg/day. No fetal effects were observed in control animals either. No visceral findings were reported in any dose groups. Skeletal observations included unossified sternbrae #5 and/or #6, ossified cervical centrum #1, presence of 14th rudimentary ribs and 7th cervical ribs, and unossified hyoid in all dose groups. Reduced ossification of the 13th rib(s), bent rib(s), 27 presacral vertebrae, malaligned and/or unossified sternebrae were also observed, but not in all dose groups. The observations are summarized in the sponsor's table below.

TABLE 13

PROJECT NO. (b) (4) 288019 A STUDY OF MICROENCAPSULATED PROVIR ON EMBRYO/FETAL DEV. IN RATS
SPONSOR:SHAMAN PHARMACEUTICALS NUMBER OF FETUSES AND LITTERS WITH VARIATIONS - SUMMARY

	FETUSES			
DOSE GROUP:	1	2	3	4
NUMBER EXAMINED EXTERNALLY	267	230	245	278
NUMBER WITH FINDINGS	0	0	0	0
NUMBER EXAMINED VISCERALLY	267	230	245	278
NUMBER WITH FINDINGS	0	0	0	0
NUMBER EXAMINED SKELETALLY	267	230	245	278
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	29	23	20	6
CERVICAL CENTRUM #1 OSSIFIED	33	33	45	53
14TH RUDIMENTARY RIB(S)	32	35	30	28
HYOID UNOSSIFIED	6	9	10	3
REDUCED OSSIFICATION OF THE 13TH RIB(S)	0	1	0	1
7TH CERVICAL RIB(S)	1	4	1	1
BENT RIB(S)	0	1	1	0
14TH FULL RIB(S)	1	0	1	0
27 PRESACRAL VERTEBRAE	1	2	2	0
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	2	0	0	1
STERNEBRA(E) #1,#2,#3 AND/OR #4 UNOSSIFIED	1	1	0	0
ISCHIUM UNOSSIFIED	0	1	0	0

1- 0 MG/KG/DAY 2- 123 MG/KG/DAY 3- 369 MG/KG/DAY 4- 738 MG/KG/DAY

Study title: A dose-ranging finding study (DRF) of effects of enteric coated SP303 on embryo/fetal development in rabbits

Study no.:	(b) (4)-288016
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 20, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SP303 50 mg enteric coated tablets, Lot # J00154.02D R11195 SP303 125 mg enteric coated tablets, Lot # J00172.01A R11618 Placebo for SP303 50 mg enteric coated tablets, Lot # J00172.01A R11196 Placebo for SP303 125 mg enteric coated tablets, Lot # J00154.05C R11616 Purity not specified

Methods

Doses:	0, 100, 250, 500, 750, 1000 mg/kg/day
Frequency of dosing:	Twice daily
Dose volume:	# capsules/animal/day: 6-8, 2, 2-4, 4-6, 4-8, 6-8 for 0, 100, 250, 500, 750, and 1000 mg/kg/day, respectively
Route of administration:	Oral
Formulation/Vehicle:	Size 0 gelatin capsules/SP-303 placebo
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	6/group
Satellite groups:	None
Study design:	The doses for this DRF study were based on a 5-day repeat dosing study in non-pregnant animals. Sexually mature rabbits (~ 6months old, 2767-4207 g body weight) were inseminated and administered human chorionic gonadotropin (IV) to induce ovulation. Day of insemination was designated GD 0. Treatment with SP303 was initiated on GD 7 and continued through GD 20.
Deviation from study protocol:	Not specified

Key Study Findings

In this DRF study, rabbits were administered SP303 at doses of up to 1000 mg/kg/day in twice daily doses through the period of gestation to determine the adverse effects, if any, on the developing fetus and pregnant mothers. There were numerous unscheduled deaths. All animals in the 1000 mg/kg/day group died, one on GD 5 and

the remaining five between GD 18-21. There were 3 deaths in the 750 mg/kg/day group and 1 death each in the 250 mg/kg/day and control groups. Cause of death of the control, 750 mg/kg/day and high dose animals was likely related to dosing (asphyxiation due to presence of tablet/capsules in esophagus or oral cavity). Cause of death of the 250 mg/kg/day animal was not identified. Of the animals which died, only the 250 mg/kg/day female, two 750 mg/kg/day females, and 4 1000 mg/kg/day females were gravid at time of death. One female each at 500 and 750 mg/kg/day aborted on GD 26 and 25, respectively. Maternal and developmental parameters were assessed in all dosing groups, except for the high dose group. Under the conditions of this study, no fetal variations or malformations were observed in any of the treatment groups, including controls. Maternal toxicity included body weight loss at doses greater than 500 mg/kg/day. Body weight loss and decreased body weight gain were observed in control animals during GD 7-21. However, these data do not agree with the sponsor's historical controls, leading to the conclusion that the dosing regimen was affecting maternal health. Based on these observations, the sponsor concluded that doses for the definitive study would be 50, 200, and 400 mg/kg/day.

Study title: A study of the effects of enteric coated SP303 on embryo/fetal development in rabbits

Study no.:	(b) (4)-288018
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 20, 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SP303 50 mg enteric coated tablets, Lot # C-90714B27-A SP303 125 mg enteric coated tablets, Lot# 970139 Placebo for SP-303 50 mg enteric coated tablets, Lot # C-97014B26B Placebo for SP-303 125 mg enteric coated tablets, Lot # 970138 Purity not specified[4]

Key Study Findings

Crofelemer did not produce teratogenic effects when administered to rabbits. However, an increase in fetal resorptions and abortions (8 total) was observed in the 400 mg/kg/day crofelemer-treated dams, as compared to control treatment (3 abortions). There were deaths in the dams in all dose groups (3 controls, 1 mid-dose, 2 high dose

[4] Purity was not provided for each lot # of drug used. However, the sponsor stated that the potency of the 50 mg and 125 mg enteric coated tablets was 101.1% and 102.8%, respectively.

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animals). The sponsor stated that the number of deaths in the control group did not match historical data and suggested that these deaths may have resulted from dose administration (Control and high dose animals received 5-10 capsules by mouth daily.)

Methods

Doses:	0, 50, 200, 400 mg/kg/day
Frequency of dosing:	Twice daily
Dose volume:	# of capsules/animal/dose: 5-10, 1-2, 3-6, 5-10 for 0, 50, 200, 400 mg/kg/day, respectively
Route of administration:	Oral
Formulation/Vehicle:	Gelatin capsules/Placebo for SP-303
Species/Strain:	Rabbit/White New Zealand Rabbits
Number/Sex/Group:	22 females/group
Satellite groups:	Yes (6 females for the 50, 200 and 400 mg/kg/day SP-303 dose groups only) for TK
Study design:	Sexually mature white rabbits (~5.5 months of age, 2523-3757 g) were inseminated (recorded as GD 0) and administered human chorionic gonadotropin to induce ovulation. SP-303 (or placebo) treatment began on GD 7 and continued through GD 20.
Deviation from study protocol:	None which affected the outcome of the study

Observations and Results

Mortality

Animals were observed twice daily for morbidity and mortality from GD 0 through GD 29.

Three control, 1 mid-dose, and 2 high-dose animals, which were all gravid, died prior to scheduled sacrifice. The control animals died on GD 16, 22, and 18 and showed clinical signs such as rapid/labored respiration, wet and/or dry material on bedding, around mouth, or forelimbs, and hunched posture. One of these control females had one tablet lodged in the esophagus at the level of the larynx. At necropsy, these animals presented with abscesses in the larynx and/or perforated esophagus. The sponsor proposed that the cause of death of control animals is likely related to dosing procedure since these findings do not match their historical data for segment II studies in untreated rabbits (placebo/vehicle control). The high dose animals died on GD 14 and 16 and showed clinical signs such as labored respirations, red material around mouth 1 h post dose, suggesting that these deaths may have been related to dose administration as well. The mid-dose animal which died did not present any significant clinical signs after dosing or prior to death and was unremarkable at necropsy. Therefore the cause of death of the mid-dose animal was not clear.

Clinical Signs

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Animals were observed one hour post-dose and twice daily from GD 0 through GD 29.

Eight animals in the high dose group aborted between GD 17-27. Decreased defecations, wet/dry red material in cage, on forelimbs, or around mouth, rapid/labored respirations, nasal congestion, and swollen neck were observed in these females. The mid-dose female which aborted experienced similar clinical signs, in addition to swollen urogenital area, soft stool and diarrhea followed by decreased defecation.

In animals which survived to laparohysterectomy, clinical signs such as decreased defecation occurred at a higher incidence in high dose animals, while the mid- and low-dose animals had incidences similar to controls. The sponsor attributed incidences of swollen neck, labored respirations, red/dry matting around mouth or on other body surfaces to dosing procedure. Hair loss on various surfaces and nasal discharge were observed sporadically and at incidences similar to controls.

Body Weight

Maternal body weight measurements were recorded on GD 0, GD 7-21 (daily), GD 24 and 29. Gravid uterine weights were calculated for each animal at necropsy.

There were increases and decreases in mean body weight change throughout gestation. There was no obvious dose relationship. The body weight changes are shown in the sponsor's table below.

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TABLE 6
A STUDY OF SP303 ON EMBRYO/FETAL DEVELOPMENT IN RABBITS
MEAN BODY WEIGHT CHANGES (GRAMS) DURING GESTATION

GROUP :		1	2	3	4
DAY 0- 7	MEAN	303.	298.	278.	334.
	S.D./N	146.7/18	89.6/22	110.4/21	126.7/20
DAY 7- 8	MEAN	-7.	-4.	-8.	-51.
	S.D./N	77.5/18	38.6/22	58.4/21	71.3/20
DAY 8- 9	MEAN	10.	4.	-1.	-20.
	S.D./N	51.7/18	44.5/22	39.8/21	41.4/20
DAY 9- 10	MEAN	-28.	0.	2.	-6.
	S.D./N	90.6/18	36.2/22	24.6/21	39.8/20
DAY 10- 11	MEAN	-33.	-3.	-3.	-45.
	S.D./N	64.2/18	28.8/22	51.5/21	49.3/20
DAY 11- 12	MEAN	-28.	-6.	22.*	-53.
	S.D./N	39.7/18	42.6/22	77.0/21	49.7/20
DAY 12- 13	MEAN	-24.	-11.	-8.	-19.
	S.D./N	71.7/18	71.1/22	49.7/21	59.2/20
DAY 13- 14	MEAN	-42.	22.**	2.	-17.
	S.D./N	71.3/18	59.9/22	48.4/21	59.7/19
DAY 14- 15	MEAN	-32.	25.	8.	-7.
	S.D./N	61.1/18	65.6/22	58.4/21	93.4/19
DAY 15- 16	MEAN	1.	-14.	-13.	-6.
	S.D./N	58.1/18	79.7/22	66.8/20	101.1/19

DAY 16- 17 MEAN	-38.	-20.	-11.	-66.
S.D./N	64.2/17	71.9/22	183.4/20	107.0/18
DAY 17- 18 MEAN	9.	-6.	-2.	-52.
S.D./N	47.6/16	57.4/22	190.3/20	73.4/17
DAY 18- 19 MEAN	-7.	4.	-14.	-8.
S.D./N	74.6/16	48.3/22	54.5/20	89.5/17
DAY 19- 20 MEAN	-15.	5.	11.	-28.
S.D./N	56.7/16	43.9/22	54.3/20	78.6/17
DAY 20- 21 MEAN	24.	-9.	-6.	-19.
S.D./N	120.1/16	49.3/22	43.8/19	72.5/17
DAY 21- 24 MEAN	159.	84.	120.	155.
S.D./N	67.7/15	94.7/21	77.8/19	129.7/12
DAY 24- 29 MEAN	178.	151.	70.**	195.
S.D./N	129.5/15	80.6/21	115.9/19	70.5/10
DAY 7- 10 MEAN	-26.	-1.	-7.	-77.
S.D./N	70.9/18	61.3/22	53.0/21	88.7/20
DAY 10- 13 MEAN	-85.	-19.	10.**	-117.
S.D./N	119.5/18	76.2/22	84.1/21	89.2/20
DAY 13- 21 MEAN	-98.	7.	-8.	-182.
S.D./N	206.2/16	282.6/22	160.0/19	269.1/17

1- 0 MG/KG/DAY 2- 50 MG/KG/DAY 3- 200 MG/KG/DAY 4- 400 MG/KG/DAY

** = Significantly different from the control group at 0.01 using Dunnett's test
MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES
NONGRAVID WEIGHT(S) NOT INCLUDED IN CALCULATION OF MEAN

There were no treatment-related effects on gravid uterine weights in any dosing groups.

Feed Consumption

Food consumption was recorded daily through the gestation period.

There were statistically significant increases in food consumption in the low- and/or mid-dose groups only, as compared to controls, although these changes were not dose dependent. Overall, mean food consumption during gestation was similar between control and high dose groups.

Toxicokinetics

Samples for TK were collected at 0 (pre-dose), 1, 4 (prior to second daily dose), 5, 8, and 24 h after the first daily dose on GD 7 and GD 20 and shipped to the sponsor. The CRO did not conduct TK analysis and no TK data from these animals were submitted in this study report.[5]

Dosing Solution Analysis

[5] The Division agreed at an End of Phase 2 Meeting on May 5, 2004 that the ADME studies conducted to date were adequate and additional studies were not needed for registration of crofelemer.

Reference ID: 3168746

Not specified

Necropsy

All surviving animals underwent a full necropsy. Laparohysterectomy was conducted on GD 29. All animals which died prior to scheduled termination also underwent necropsy.

Necropsy findings of the animals which died prior to scheduled termination are summarized in the table below.

Table: Necropsy findings of dams which received crofelemer and died prior to scheduled study termination

Group	DOD*	Finding
Control	16	• Abscess in larynx, perforated esophagus • Placebo tablets in stomach
	22	• Abscess in larynx, dark red, firm lungs • Placebo in stomach
	18	• Placebo in stomach
200 mg/kg/day	16	• No abnormal findings
400 mg/kg/day	14	• Thick brown material in trachea, dark red area on larynx, test article in mouth, dark red apical lobe of left lung
	16	• Test article in trachea at level of larynx and in stomach, died of asphyxiation

* DOD: Day of death during gestation

Eight high dose animals aborted between GD 17-27. None of the aborted fetuses showed any external malformations. At necropsy, test article was present in the stomach of 2 of these females, while thick brown material in the oral cavity and around larynx, along with white purulent material near the thyroid and nodules on lungs were noted in another female. The remaining females which aborted did not have remarkable findings at necropsy.

Of the females which survived to scheduled termination, necrotic lobes or lobules of the liver were observed in one control and one high dose animal, where the control animal also had liver adhesions. One female each in the 200 and 400 mg/kg/day groups had abscesses in the oral cavity, and another in the mid-dose group had a reddened cervical lymph node. Two 200 mg/kg/day females also had white precipitate in the amniotic fluid.

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

Laparohysterectomy included an examination of uterus (implantation sites, early/late resorptions, pre-/post-implantation loss) ovaries, corpora lutea, fetal number and location, sex ratio, and fetal weights.

One mid-dose and 8 high-dose females aborted prior to scheduled laparohysterectomy. Of these, the mid-dose animal and 2 of the high dose animals were gravid. The uterine

Reference ID: 3168746

findings of animals which died or aborted prior to terminal sacrifice are summarized in the table below.

Table: Uterine findings of dams treated with crofelemer which died or aborted prior to scheduled necropsy

Group	DOS*/Aborted	Uterine Findings
Control	GD 16	8 normally developing implantations
	GD 22	Entirely resorbed litter
	GD 18	7 normally developing implantations, 2 early resorptions
200 mg/kg/day	GD 16	9 normally developing implantations, 2 early resorptions
400 mg/kg/day	GD 14	Entirely resorbed litter (early resorptions)
	GD 16	7 normally developing implantations
	Abortions/Uterine Findings	
400 mg/kg/day (8 animals)	Aborted GD 17-27	Two late, two early resorptions
		Aborted one fetus, one mummified late resorption, five normal fetuses and one former implantation *in utero*
		5 normally developing implantations, one former implantation
		One early resorption
		One late resorption, 3 late resorptions, 3 normal fetuses in utero
		One fetus and 2 late resorptions, 7 fetuses and 3 late resorptions in utero
		One fetus and two late resorptions, 5 fetuses in utero
		8 late resorptions

*DOS: Died on Study

The findings from the animals which survived until scheduled laparohysterectomy are summarized in the sponsor's table below.

TABLE 10

PROJECT NO. (b) (4)288018 A STUDY OF SP303 ON EMBRYO/FETAL DEVELOPMENT IN RABBITS PAGE 1
SPONSOR:SHAMAN PHARMACEUTICAL SUMMARY OF MEAN FETAL DATA AT THE SCHEDULED NECROPSY

GROUP	SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1 TOTAL	58	48	106	0	6	0	6	112	161	49	NA	15
MEAN	3.9	3.2	7.1	0.0	0.4	0.0	0.4	7.5	10.7	3.3	46.3	
S.D.	2.23	2.18	2.15	0.00	0.63	0.00	0.63	2.26	3.39	2.66	7.05	
2 TOTAL	81	65	146	0	21	0	21	167	247	80	NA	22
MEAN	3.7	3.0	6.6	0.0	1.0	0.0	1.0	7.6	11.2	3.6	47.4	
S.D.	1.73	1.73	2.85	0.00	2.28	0.00	2.28	2.17	2.35	2.90	6.41	
3 TOTAL	55	65	120	0	2	0	2	122	213	91	NA	19
MEAN	2.9	3.4	6.3	0.0	0.1	0.0	0.1	6.4	11.2	4.8	47.5	
S.D.	1.76	2.36	2.79	0.00	0.32	0.00	0.32	2.81	2.49	2.95	7.83	
4 TOTAL	28	40	68	0	7	2	9	77	114	37	NA	10
MEAN	2.8	4.0	6.8	0.0	0.7	0.2	0.9	7.7	11.4	3.7	43.7	
S.D.	1.75	1.63	2.62	0.00	1.25	0.63	1.29	2.31	2.17	2.54	7.47	

None significantly different from control group
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 50 MG/KG/DAY 3- 200 MG/KG/DAY 4- 400 MG/KG/DAY

Overall, there were no treatment-related effects on number of fetuses, sex ratio, early/late resorptions, pre-/post-implantation loss, implantation sites, corpora lutea, or fetal weight, when results from individual animals are normalized by litter size.

Offspring (Malformations, Variations, etc.)

Excised fetuses were examined for evidence of external and internal malformations and variations.

Of the fetuses which were aborted, no external malformations were identified. In fetuses examined at laparohysterectomy on GD 29, there were a total of 6, 7, 3, and 3 (combined external, soft tissue, and skeletal) malformations in the control, 50, 200 and 400 mg/kg/day dose groups, respectively. When normalized by litter size, the number of malformations per groups was not statistically significant. Although a correlation between maternal toxicity in the high dose animals and the number of abortions was not apparent, it cannot be overlooked that there was a higher number of abortions at the high dose, as compared to all other dose groups.

There was a statistically significant increase in the skeletal variations (% per litter) at the low dose only, as compared to control. The variations observed in mid- and high-dose SP-303 groups were similar to those observed in the control groups. The sponsor's table below summarizes the external, visceral and skeletal variations observed in study animals.

TABLE 14

PROJECT NO.: (b)(4)288018 A STUDY OF SP303 ON EMBRYO/FETAL DEVELOPMENT IN RABBITS PAGE 1
SPONSOR:SHAMAN PHARMACEUTICAL NUMBER OF FETUSES AND LITTERS WITH VARIATIONS - SUMMARY DAY 29

	FETUSES				LITTERS			
DOSE GROUP:	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	106	146	120	68	15	21	19	10
NUMBER WITH FINDINGS	0	0	0	0	0	0	0	0
NUMBER EXAMINED VISCERALLY	106	146	120	68	15	21	19	10
RETROCAVAL URETER	1	3	0	0	1	2	0	0
ACCESSORY SPLEEN	7	6	6	5	6	5	4	3
MAJOR BLOOD VESSEL VARIATION	3	2	5	4	1	2	3	1
GALLBLADDER ABSENT OR SMALL	4	4	6	3	2	2	4	1
NUMBER EXAMINED SKELETALLY	106	146	120	68	15	21	19	10
13TH FULL RIB(S)	43	74	58	27	13	19	15	6
27 PRESACRAL VERTEBRAE	19	42	28	19	11	16	13	7
13TH RUDIMENTARY RIB(S)	15	46	23	17	9	15	14	8
HYOID ARCH(ES) BENT	6	6	4	0	5	6	4	0
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	1	8	7	4	1	6	4	1
ACCESSORY SKULL BONE(S)	0	2	2	0	0	1	2	0
STERNEBRAE WITH THREAD-LIKE ATTACHMENT	3	6	2	1	3	4	2	1
HYOID BODY AND/OR ARCH(ES) UNOSSIFIED	1	3	0	0	1	2	0	0
25 PRESACRAL VERTEBRAE	1	1	0	0	1	1	0	0
7TH STERNEBRA	0	1	1	0	0	1	1	0
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	0	3	3	0	0	2	3	0
REDUCED OSSIFICATION OF THE 12TH RIB(S)	0	1	0	0	0	1	0	0
7TH CERVICAL RIB(S)	0	3	1	0	0	3	1	0
EXTRA SITE OF OSSIFICATION ANTERIOR TO STERNEBRA #1	0	2	0	0	0	2	0	0
ACCESSORY SKULL BONE(S)	1	0	0	1	1	0	0	1

1- 0 MG/KG/DAY 2- 50 MG/KG/DAY 3- 200 MG/KG/DAY 4- 400 MG/KG/DAY

9.3 Prenatal and Postnatal Development

Study title: A study of the effects of microencapsulated Provir on pre- and post-natal development, including maternal function in the rat

Study no.: (b) (4)-288023
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: March 20, 1998
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: SP303 delayed release beads, Lot # CB8MN01A04
Placebo for SP-303 delayed release beads, Lot # CC8MN01P04
Purity not specified[6]

Key Study Findings

Microencapsulated SP-303 was tested in a segment III pre- and post- natal developmental study in rats. Treatment with SP-303 did not affect F_0 pregnancy and lactation, and survival, sex ratio, physical and neurobehavioral development, or reproductive performance of F_1 animals. Maternal (F0) exposure to SP-303 did not

[6] The CRO provided the following statement: "The test article was considered to be (b) (4) active for purposes of dose calculations."

affect fertility parameters of F_1 animals or embryonic development of F_2 generation. Based on these observations, the NOAEL dose for maternal and developmental toxicity was 738 mg/kg/day, the highest dose tested.

Methods

Doses:	0, 123, 369, 738 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	Gelatin capsule followed by water flush of ~2 mL
Route of administration:	Oral gavage via flexible rubber catheter
Formulation/Vehicle:	Appropriate amount of test article (or placebo) was weighed and placed into gelatin capsules
Species/Strain:	Rat/Crl:CD(SD)IGS BR
Number/Sex/Group:	25 females/group
Satellite groups:	None
Study design:	Pregnant females were dosed from gestation day (GD) 6 through lactation day (LD) 20.
Deviation from study protocol:	None which affected the outcome of the study

Observations and Results

F_0 Dams	
Parameters Evaluated	Observations and Results
Survival: Animals were observed twice daily.	• Two high dose females died on GD 14 and 16, 2 mid-dose females died on LD 11 and 12 and 1 control female died on LD 14.The cause of death was intubation error in 3 animals (1 control and 2 high dose) and likely related to test article administration in the mid-dose animals. The control, mid-dose, and one high-dose animal was found dead, while the other high dose animal was moribund and was euthanized. • Macroscopic findings of perforated esophagus (in control and both high dose animals), red fluid contents in thoracic cavity (mid- and high dose animals) and red and yellow matting (mid-dose animals) were observed.
Clinical signs: Animals were observed twice daily.	• Over the course of the entire study, SP-303 produced rales in 4, 7, and 7 animals in the low, mid and high dose groups, as compared to 2 in the control groups.

Reference ID: 3168746

	• At the time of dosing (GD 6-LD 20), the incidence of rales was higher in across all dosing groups and decreased slightly one hour post-dose. There was also an increased incidence of wet/clear matting and/or wet/red material around the mouth in SP-303 treated animals, as compared to controls. No other clear treatment-related clinical signs were observed in F_0 animals. • There were 23, 19, 19, and 21 gravid females in the control, low, mid, and high dose groups at the end of mating. • Of these, 22 control, 19, 19, and 21 SP-303 low, mid-, and high dose females had viable pups. • There were no litter losses. Gestation length was similar between SP-303 and placebo treatment groups.
Body weight: Individual body weights were measured on GD 0, 6, 9, 12, 15, 18, and 20, and LD 1, 4, 7, 14, and 21.	• Mean body weight was not affected by SP-303 treatment during the gestation period. • There was a statistically significant increase in body weight gain in the mid dose group during GD 9-12 only. This finding was likely incidental, since there was no obvious dose response relationship. • There were no statistically significant treatment-related changes in body weight in SP-303-treated animals during lactation, as compared to control.

Feed consumption: Individual food consumption was recorded on GD 0, 6, 9, 12, 15, 18, and 20, and LD 1, 4, 7, 14, and 21.	• There were slight increases (4-9%) in food consumption in SP-303 treatment groups between GD 15 and GD 20, as compared to control. • There was a slight increase (~19%) in food consumption in high dose animals between LD 1–4 only, as compared to control.
Uterine content:	There was no difference in the number of implantation sites, number of pups born, and the number of unaccounted sites between SP-303 and placebo treatment groups.
Necropsy observation: Gross necropsies were performed on each female which died while on study to determine a cause of death, if possible. Animals which failed to deliver also underwent necropsy. Pregnancy status was determined and the sponsor attempted to identify anatomic/pathologic causes for lack of pregnancy. The number of implantation sites, corpora lutea, and early implantation losses were determined, if possible, by using staining techniques.	There were no significant treatment-related changes in gross necropsies across all dose groups.

Toxicokinetics:	• TK sampling was collected on LD 20 from 3 animals/group at pre-dose and 1, 2, 4, 6, and 24 h post-dose on LD 20. These samples were frozen and shipped to the sponsor. Quality control samples of Provir were prepared each day. A stock solution of Provir (200 μg/mL) in USP grade water was prepared. Quality control samples were prepared by spiking 9.5 mL blank plasma with 0.5 mL of the Provir stock solution (final concentration 10 μg/mL Provir in plasma), frozen, and shipped to the sponsor. • The CRO ((b) (4) did not conduct TK analysis) .
Dosing Solution Analysis	Not specified
Other: All animals were allowed to deliver and wean their pups to post natal day (PND) 21 and the day of parturition was noted as PND 0. The number, sex, stillborn, and live pups in each litter were recorded, and each pup was examined for gross malformations. On LD 21, all surviving F_0 females were euthanized by CO_2 inhalation and necropsied. The number of former implantations was recorded, and representative samples of organs were preserved for microscopic analysis.	• There were 3, 6, 6, and 4, F_0 females in the placebo, 123, 369, and 738 mg/kg/day dose groups which did not deliver pups and were euthanized on day 25 post-mating. • One of the control females had one fetus with sirenomelia and anury retained in the uterus. • All remaining animals were non gravid and uteruses were normal.

F_1 Generation	
Parameters Evaluated	Observations and Results
Survival: Individual litters were examined daily for survival and all deaths were recorded. Litter size was recorded from PND 0 to PND 4, and any pups which	• There was no treatment-related effect on litter size, sex ratio, or number of live pups at birth (PND 0) between dosing groups. Pup survival data was similar between all dosing groups at the following intervals: birth to PND 4, PND 4-21.

[7] The Division agreed at an End of Phase 2 Meeting on May 5, 2004 that the ADME studies conducted to date were adequate and additional studies were not needed for registration of crofelemer.

died were necropsied. Litters were randomly culled to 4 pups/sex/group on PND 4.	• During the preweaning period, there were 2, 5, 7, and 3 pups which were found dead from the control, 123, 369, 738 mg/kg/day dose groups, respectively, and one mid dose pup was euthanized in extremis. There were 4, 1, 3, and 4 pups in the control, 123, 369, 738 mg/kg/day dose groups, respectively, missing. • After weaning, all F_1 animals survived to scheduled necropsies.
Clinical signs: Clinical signs were assessed daily and changes in behavior or appearance were recorded. Detailed physical examination was performed on PND 1, 4, 7, 14, and 21 and weekly thereafter until necropsy.	• There were no significant treatment-related clinical signs in F_1 pups during the preweaning period. • In F_1 pups surviving to scheduled necropsy, clinical signs were similar across all dose groups including controls and low in incidence.
Body weight: Body weights were recorded on PND 1, 4, 7, 14, and 21 and weekly thereafter until necropsy. F1 females selected for breeding were weighed on GD 0, 6, 9, 12, 16, and 20. Gravid uterine weight was calculated on GD 20.	• F1 male and female pups in all dose groups were similar in body weight during the preweaning period. • There was a decrease in body weight in high dose males and females during study weeks 7-16 and 7-14, as compared to controls. During Weeks (postnatal study weeks) 7, 8, 9, 10, and 11, there was ~8.2%, 7.5%, 6.3%, 7%, and 12.7% decrease in body weight in high dose males, as compared to corresponding control males. Lower body weights were again observed starting in Week 14, with ~9.2%, 6.6%, and 5.9% decreases in body weight in Weeks 14, 15, and 16, as compared to controls. There were no other changes in body weights in males during the study period. • In high dose females, there was ~11%, 8.75%, and 7% decrease in body weight during postnatal Weeks 7, 8, and 9, as compared to corresponding controls. There were no other changes in body weights in females during the study period. • There were no statistically significant differences in body weight during gestation in any of the dosing groups including controls. • There were no statistically significant differences in initial body weight, terminal body weight and gravid uterine weight between any of the dosing groups, including controls.
Feed consumption:	Not specified
Physical development: Pups	

Reference ID: 3168746

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<td>were sexed individually on PND 0, 4, and 21. Acquisition of balanopreputial separation and vaginal patency were recorded.</td>
<td>

Treatment with SP-303 did not affect acquisition of balanopreputial separation in F_1 pups. The mean ages when balanopreputial separation was observed in females were 41.8, 42.1, 43.0, and 42.7 days in control, 123, 369, and 738 mg/kg/day, respectively. In male pups, balanopreputial separation was observed by PND 49.
Vaginal patency was acquired by F_1 females at the mean ages of 32.3, 33.6, 33.0, and 32.8 days in control, 123, 369, and 738 mg/kg/day, respectively, with all pups having acquired vaginal patency by PND 42. Therefore, SP-303 treatment did not affect this developmental parameter.

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<td>Neurological assessment: Ten/sex/group were randomly selected for neurological assessment (sensory and behavioral testing), which concluded when the oldest pups were 98 days of age. The following investigations were conducted to assess the effect of test article treatment on neurological development/behavior: auditory startle test, motor activity (total and ambulatory activity), and Biel maze swimming trials.</td>
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Overall, responses to the auditory startle test were similar across dose groups. There was a slight increase in the mean average response in mid dose males on PND 60 only. A similar increase was not observed at the high dose.
SP-303 treatment did not affect the motor activity of F_1 pups under the conditions of the assay.
SP-303 treatment did not affect swimming ability, learning, and memory as assessed by the Biel maze swimming trials.

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<td>Reproduction: F_1 pups (25/sex/group) between 6 to 12 days of age were randomly selected, allowed to attain sexual maturity and assessed for reproductive performance. Regularity and duration of estrous cycles were assessed by examining vaginal smears for 10 consecutive days before pairing and continuing until evidence of mating or to end of breeding. F_1 animals were ~86 days old at the time of pairing. Females with signs of mating were then placed in individual</td>
<td>

The mean estrous cycle length was similar across all dose groups (4.2, 4.3, 4.7, and 4.5 days in the control, 123, 369, and 738 mg/kg/day dose groups, respectively).
There were no statistical differences in male and female mating and fertility indices between dosing groups. These data are shown in the table below:

<table>
<tr><td></td><td></td><td colspan="4">Dose (mg/kg/day)</td></tr>
<tr><td>Parameter</td><td></td><td>Control</td><td>123</td><td>369</td><td>738</td></tr>
<tr><td>Mating Index</td><td rowspan="2">Male</td><td>88%</td><td>100%</td><td>96%</td><td>88%</td></tr>
<tr><td>Fertility Index</td><td>88%</td><td>88%</td><td>92%</td><td>84%</td></tr>
<tr><td>Mating Index</td><td rowspan="2">Female</td><td>96%</td><td>100%</td><td>96%</td><td>96%</td></tr>
<tr><td>Fertility Index</td><td>96%</td><td>88%</td><td>92%</td><td>92%</td></tr>
</table>

GD 20 Laparohysterectomy revealed that SP-303 did not affect the number of viable fetuses, implantation sites, corpora lutea, or pre-implantation losses. There were no dead fetuses.

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<td>cages. After 10 days of mating, females without evidence of a vaginal plug were mated a proven male for an additional 5 days. If no sign of mating was observed after a total of 15 days, females were placed in individual cages until necropsy. Laparohysterectomy was performed on GD 20 and fetuses were examined. Female mating index, male mating index, and female fertility index were calculated. The uteri and ovaries were examined. The number of corpora lutea, number and locations of all fetuses, early and late resorptions, and total number or implantation sites were recorded. Each fetus was examined in detail (external surface, palate, external orifices, crown-rump measurements, degrees of autolysis for late resorptions, and skeletal examination (Alizarin Red S staining)). Developmental malformations and variations were recorded.</td>
<td>The number of early resorptions and post implantation losses were higher in SP-303 treated animals, as compared to controls (shown in the table below):
<table>
<tr><td></td><td colspan="4">Dose (mg/kg/day)</td></tr>
<tr><td>Parameter (% per litter)</td><td>Control</td><td>123</td><td>369</td><td>738</td></tr>
<tr><td>Early resorptions</td><td>1.8</td><td>5.6*</td><td>7.2</td><td>7.6**</td></tr>
<tr><td>Post-implantation loss</td><td>2.1</td><td>5.9</td><td>7.2</td><td>7.6</td></tr>
</table>
*Stat sig from control at 0.05
**Stat sig from control at 0.01

Male and female fetal weights in SP-303 treatment groups were similar to controls.
A total of 3 external malformations were observed in control fetuses from 2 litters (umbilical herniation of intestine, omphalocele, and maxillary micrognathia) and in 1 fetus from a single litter (body shorter than normal, filamentous tail). There were no variations recorded in any of the fetuses across all dose groups.

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<td>Necropsy: All animals underwent necropsy, including pups which were found dead or were euthanized in extremis. F_1 females with no evidence of mating also underwent necropsy. Abdominal and thoracic cavities were opened and examined and the numbers of corpora lutea and implantation sites were recorded (if present). Uteri were examined for presence of early implantation losses. F_1 males were euthanized and</td>
<td>

Necropsy of pups (F_1) which were found dead or euthanized in extremis (preweaning) did not reveal any remarkable findings.
Necropsies were also conducted on surplus F_1 pups (scheduled on PND 21) and on pups at the end of behavioral testing. Necropsies revealed dilation of one renal pelvis in one pup each in control and low dose groups and diverticulum in the jejunum in one low dose pup.
Necropsies of F_1 dams did not reveal any abnormal findings in any of the dosing groups.

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necropsied. Organs were preserved for histopathological analysis only if abnormal macroscopic findings were recorded.	

10 Special Toxicology Studies

Study Title: Rabbit Pyrogen Test
Study #: ZB410.007
In this study, adult female white New Zealand rabbits (3/group) were given an intravenous injection of 3 mg/kg SP-303 (vehicle control: 5% dextrose) and rectal temperatures were recorded at 1, 2, and 3 h after dosing. After inoculation, the mean rise in rectal temperature over the course of 3 h was 0.2, 0.2, and 0.4 ^{0}C in each of the SP-303 rabbits. In each of the controls, the mean rise in rectal temperature was 0.0, 0.3, and 0.3 ^{0}C. Therefore, SP-303 was not pyrogenic in rabbits under the conditions tested. These data are summarized in the sponsor's table below.

TABLE 1

Pyrogen Test Data for SP-303

Rabbit No.	Weight (kg)	Dose (ml)	Temperature (°C)				
			Control	1 HR	2 HR	3 HR	Rise
1265	2.5015	25.0	39.3	39.4	39.5	39.3	0.2
1266	2.6001	26.0	39.3	39.2	39.5	39.5	0.2
1267	2.4400	24.4	39.3	39.7	39.3	39.5	0.4
						Sum of Maximum Rise	0.8

Pryogen Test Data for
5% Dextrose Control

Rabbit No.	Weight (kg)	Dose (ml)	Temperature (°C)				
			Control	1 HR	2 HR	3 HR	Rise
1268	2.6723	26.7	39.4	39.3	39.2	39.4	0.0
1269	2.5916	25.9	39.3	39.4	39.6	39.5	0.3
1270	2.8225	28.2	39.2	39.5	39.3	39.5	0.3
						Sum of Maximum Rise	0.6

11 Integrated Summary and Safety Evaluation

Human immunodeficiency virus (HIV) affects the immune system and leads to acquired immunodeficiency syndrome (AIDS). HIV depletes CD4 cells, and patients with low CD4 cells are more susceptible to opportunistic infections. Since the emergence of combination retroviral therapy (highly active antiretroviral therapy (HAART)), HIV/AIDS has now become a chronic, potentially life threatening disease. Although it has significantly decreased mortality, HAART in HIV/AIDS patients leads to progressive immunosupression and increases the propensity for opportunistic infections, such as infectious diarrhea. HIV/AIDS patients suffer from a myriad of GI illnesses anorexia, nausea, vomiting and abdominal pain, in addition to secretory diarrhea, which affect their quality of life. GI intolerance (diarrhea, nausea, vomiting) contributed to poor patient compliance to HAART. At this time, there are no FDA-approved therapies for secretory diarrhea in HIV/AIDS patients. Current treatments for diarrhea include GI motility agents such as loperamide, difenoxin, tincture of opium, and octreotide, which are not specifically indicated for patients with HIV/AIDS. Although effective, these drugs have several shortcomings, such as limited duration of use, potential for abuse and undesirable side effects (constipation, nausea, vomiting, nutritional absorption, thyroid and heart function, etc).

In this NDA, the sponsor is seeking approval to market Crofelemer (NP303, SP-303) for the control and symptomatic relief of diarrhea in patients with HIV/AIDS on anti-retroviral therapy. The proposed oral dose is 125 mg twice daily with or without food. Crofelemer is extracted from the red latex sap of the plant *Croton lechleri* of the family *Euphorbiaceae*, which are found in the western Amazon regions of South America. Crofelemer is a proanthocyanidin oligomer (molecular weight ~170—2500 Dalton) which is composed of 5 to 11 linearly linked monomers. The predominant monomers are (+)-gallocatechin and (-)-epigallocatechin, while (+) catechin and (-)-epicatechin are present in lower amounts.

In support of the NDA, the sponsor conducted pharmacology, pharmacokinetics, general toxicology, genetic toxicology, and reproductive toxicology studies in rodents (mice, rats) and/or non rodents (dogs, monkeys, rabbits).

The mechanism of action of crofelemer was demonstrated *in vitro* and *in vivo*. In human intestinal epithelial cell lines (Caco-2 and T84 monolayers, crofelemer (also referred to as SP-303) was shown to inhibit *V. cholerae* toxin- and *E. coli* heat labile (HL) toxin-induced chloride ion secretion, which is dependent on cAMP/cGMP-mediated pathways. SP-303-mediated inhibition does not affect the function of the chloride ion transporter on the basolateral side of the intestinal cell wall, as demonstrated by a further decrease in chloride current induced by butamide, a specific inhibitor of the basolateral chloride transporter. In an adult mouse model of secretory diarrhea, SP-303 significantly reduced cholera-toxin (CT) induced fluid accumulation (FA) in the small intestine, as compared to positive control (vehicle followed by single dose of cholera toxin). An enteric coated formulation of SP-303 was also tested in the adult mouse model of secretory diarrhea and was equally effective. Crofelemer was shown to inhibit the cystic fibrosis transmembrane regulator (CFTR) chloride channel with an IC_{50} of ~7

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μM and the intestinal calcium-activated TMEM16A chloride channel by a voltage independent mechanism with an IC_{50} of ~6.5 μM. SP-303 did not affect GI transit in mice, as compared to positive control atropine which produced a statistically significant decrease in GI transit.

Crofelemer was shown to bind various proteins *in vitro*, including bovine and serum albumin, collagen and immunoglobulins in human whole plasma. The sponsor suggested that enteric coating may be necessary to deliver the drug to the site of action (small intestine) based on the observation that crofelemer was shown to bind pepsin in simulated gastric fluid.

The safety of orally administered crofelemer (gelatin encapsulated tablets) was tested in rats and dogs at doses of up to 600 mg/kg/day. The NOAEL dose for cardiovascular safety of SP-303 in conscious, freely moving dogs was 600 mg/kg. There were no treatment-related changes in blood pressure (mean systolic, diastolic and mean arterial pressure), heart rate, RR interval, PR interval, QRS interval, QRS duration, QT and corrected QT interval, as compared to placebo treatment. Male rats treated with doses of up to 600 mg/kg of crofelemer did not show significant treatment-related effects on neurobehavioral functions, as measured in the functional observational battery (FOB). In a respiratory safety pharmacology study, 600 mg/kg crofelemer produced some sporadic decreases in respiratory rate and/or minute volume, as compared to control. However, clinical signs, such as difficulty breathing, brown material around mouth, and red material around nose, were observed in 1-3 animals treated with crofelemer at all doses tested.

In human embryonic kidney cells stably expressing the hERG (human ether-a-go-go) K^+ channel, crofelemer was shown to inhibit I_{Kr} in a dose-dependent manner with IC_{50} values of 1.79 μM and 1.75 μM in the first and second set of experiments, respectively, as compared to 10 μM cisapride (positive control) which inhibited I_{Kr} by 99.67% and 100.47% in the first and second sets of experiments, respectively. Crofelemer is poorly absorbed (~1%) after oral administration and is highly bound (~98%) to human plasma protein, suggesting that crofelemer is likely to pose minimal or no cardiovascular risk because of its low bioavailability after oral administration.

In vitro, crofelemer metabolism was enzymatic and dependent on NADPH. Although it inhibited transport of substrates specific for MRP2 and MRP4 and potently inhibited OATP2B1, PEPT1, ASBT, and OATP1A2 transporters, crofelemer failed to induce CYP enzymes CYP1A2, 2B6, and 3A4/5 in human liver microsomes. Crofelemer had an *in vitro* half life and a clearance of 15.3 min and 90.4 μL/min/mg microsomal protein, respectively. When administered to rats, crofelemer had very low oral bioavailability (~1%). The majority of oral administered radiolabeled crofelemer was unabsorbed and excreted in the feces (88.2% of dosed radioactivity), with minimal urinary excretion. At 24 h post-dose, the majority of crofelemer remained in the GI tract, with the majority of radioactivity recovered by 72 h post-dose. The predominant metabolite identified in rat urine after oral administration of crofelemer was 4'-O-methyepigallocatechin, along with several minor metabolites. One of these fractions demonstrated dose-dependent

antiviral activity against (b) (4) A similar metabolic profile was identified in human urine samples as well, suggesting that there are no unique human metabolites.

In acute toxicity studies, the LD_{50} of SP-303 in mice and rats was > 50 mg/kg and >100 mg/kg when administered IV and IP, respectively. When administered orally to rats, crofelemer was well tolerated at doses up to 600 mg/kg and was lethal at ~ 1200 mg/kg. Rales was the predominant treatment-related clinical sign. In dogs, SP-303 was tolerated at up to 1200 mg/kg. The GI system was the target organ of toxicity after oral administration. Treatment related clinical signs included abnormal excreta, decreased food consumption, emesis and weight loss. Adverse GI effects were also observed in the 30-day repeat-dose toxicity study in rhesus monkeys where dose-dependent histopathological effects (increased presence of pigmented macrophages) in the small intestine and cecum were observed when crofelemer was administered orally at up to 100 mg/kg/day.

When administered to mice for 13 weeks at oral doses of up to 1200 mg/kg/day, crofelemer produced numerous dose-dependent deaths (20, 5, and 2 at 1200, 400 and 40 mg/kg/day, respectively), and several of these deaths were attributed to gavage-related injuries; however, the cause of the majority of deaths was unknown. TK analysis was only conducted at the high dose due to poor oral bioavailability and showed that females had slightly higher systemic exposure to crofelemer than males and slight drug accumulation on Day 56 as compared to Day 1. In rats, repeated administration of crofelemer for 26 weeks at doses of up to 600 mg/kg/day produced several unscheduled deaths (11, 2, and 2 at 600, 200, and 60 mg/kg/day, respectively), and dosing error was cited as the cause of death of only 9 of these animals. Changes in body weight, hematology and clinical chemistry which were observed during treatment were generally reversed during the recovery period (4 weeks); however, there are some changes which were observed only in recovery animals, including an increase in platelet count in all recovery males treated with crofelemer, slight increases chloride and calcium levels in high dose and mid-dose females (calcium only), increase in creatinine in high dose females, increase in albumin at the mid- and high dose, and increase in glucose in all crofelemer treated recovery females. There were also several changes in histology and organ weights, likely related to aspiration of test article. In conclusion, a NOAEL dose was not established from either the 13-week mouse or the 26-week rat study.

In the 9-month dog study, there were no unscheduled deaths when crofelemer was administered at oral doses of up to 400 mg/kg/day. The target organ of toxicity at the highest dose was the GI tract. Crofelemer produced dose-dependent increases in incidence of gastrointestinal (GI)-related adverse effects, including black mucoid feces and/or diarrhea, rust colored diarrhea, black diarrhea, red material in feces, and/or emesis (containing tablets, food and/or rust colored material) at high dose (400 mg/kg/day), as compared to control animals. Statistically significant decreases in body weight and food consumption and hematology changes suggestive of regenerative anemia were observed at the high dose. Changes in clinical chemistry at the high dose

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were likely related to nutritional deficits experienced by the animals due to decreased food consumption. Macroscopic examination revealed discoloration of the GI tract and lymph nodes at doses > 175 mg/kg/day suggestive of test article remnants. Histological findings were related to irritation and macrophage infiltration in the GI tract. The NOAEL dose was 50 mg/kg/day, based on changes in clinical chemistry and histopathology at doses $\geq$ 175 mg/kg/day.

Crofelemer was negative in the bacterial reverse mutation assay, chromosomal aberration assay, and rat bone marrow micronucleus assay. Carcinogenicity testing with crofelemer will be conducted as a Phase 4 commitment.

Crofelemer, at oral doses of up to 738 mg/kg/day had no effects on fertility or reproductive performance of male and female rats. Crofelemer was not teratogenic in rats and did not produce maternal toxicity when administered at doses of up to 738 mg/kg/day. In the segment II rabbit study, there were several deaths of dams in all dose groups, including controls, and the increased number of fetal resorptions and abortions in the 400 mg/kg/day dose group. Although crofelemer was not teratogenic, it is not clear that the effects on litters in all dose groups, including controls, are not secondary effects of maternal toxicity, which may have resulted from dose administration. In the segment III study, crofelemer did not produce adverse effects on pre- and postnatal development when administered at doses of up to 738 mg/kg/day in rats.

Crofelemer was also tested in juvenile animals. When administered daily by oral gavage to rats for 14 days (postnatal days 5 to 18) at doses of 50 and 100 mg/kg/day, there were 4 deaths (2 at high dose and 2 at low dose; none in control). There were also decreases in body weight at both doses tested and clinical chemistry changes, as compared to control. In juvenile monkeys (age 6-8 weeks), crofelemer administered by oral gavage at 10, 200 and 500 mg/kg/day for 2 weeks. Lymphoid depletion from the thymus was observed at 200 and 500 mg/kg/day and the no effect dose was 10 mg/kg/day. Crofelemer was also not pyrogenic when administered intravenously (3 mg/kg) to New Zealand White rabbits.

The NOAEL dose of 50 mg/kg/day in dogs in the 9-month oral toxicity study provides ~12-fold margin of safety for the proposed to-be marketed dose of 250 mg/day (4.2 mg/kg/day, based on 60 kg body weight).

In conclusion, from a nonclinical standpoint, there are no significant safety concerns for the proposed dose of crofelemer (125 mg tablet for oral use, twice daily) for the proposed indication, i.e., for the control and symptomatic relief of diarrhea in adult patients with HIV/AIDS on anti-retroviral therapy.

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12 Appendix/Attachments

None

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/s/

SRUTHI T KING
08/02/2012

SUSHANTA K CHAKDER
08/02/2012

Comments on N202292 crofelemer tablets
From: Abigail Jacobs, AD
Date: 8/1/12

1. I agree that there are no outstanding pharm/tox issues and that the pregnancy category should be C.

2. I have discussed other comments with the reviewer and supervisor and they will be addressed as appropriate.

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

/s/

ABIGAIL C JACOBS
08/30/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 202292 **Applicant: Salix Pharmaceuticals** **Stamp Date: December 5, 2011**

Drug Name: Crofelemer **NDA/BLA Type: 505(b)(1)**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		• Carcinogenicity study in mice and rats to be conducted post-approval/Phase 4 commitment (FDA agreed to this during Pre-NDA meeting) • Sponsor conducted the recommended CV and respiratory safety pharmacology studies
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	--	--	Not applicable
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		Sections 8.1, 8.3, 13.1, 13.2 may need to be modified
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		
11	Has the applicant addressed any abuse potential issues in the submission?	--	--	Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	--	--	Not applicable

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

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

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

Sruthi Tallapragada King, Ph.D. — January 18, 2012
Reviewing Pharmacologist — Date

Sushanta Chakder, Ph.D. — January 18, 2012
Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

SRUTHI T KING
01/23/2012

SUSHANTA K CHAKDER
01/23/2012