CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206317Orig1s000

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 AMENDMENT

Application number:	206317
Supporting document/s:	1
Applicant's letter date:	March 24, 2014
CDER stamp date:	March 24, 2014
Product:	Triferic (ferric pyrophosphate)
Indication:	Treatment of iron deficiency in adult patients with hemodialysis-dependent chronic kidney disease (HDD-CKD)
Applicant:	Rockwell Medical, Inc. (Rockwell Medical) 30142 S. Wixom Rd. Wixom, MI 48393
Review Division:	Division of Hematology Oncology Toxicology (Division of Hematology Products)
Reviewer:	C.J. George Chang, DVM, MS, PhD, DABT
Supervisor/Team Leader:	Todd Palmby, PhD
Division Director:	John K. Leighton, PhD, DABT (Ann Farrell, MD)
Project Manager:	Kimberly Scott, Senior Program Manager

Reason for This Amendment

Section 3, Studies Submitted, of the original Pharmacology and Toxicology review from 12/22/2014 contains errors. The list of studies that were not reviewed, under section 3.2, includes studies with an unrelated drug that were not submitted to NDA 206317.

Review Section Amended

Section 3.2 of the original Pharmacology/Toxicology review is amended as specified below. Section 3.2 that follows should replace section 3.2 in the original review.

3 Studies Submitted

3.2 Studies Not Reviewed

Study#	Title	Module
Toxicology		
(b) (4) 60010 and Amendment 1	Soluble ferric pyrophosphate: A 13-week intravenous infusion toxicity study with a 4-week recovery period in beagle dogs **(previously reviewed)**	4.2.3.2
PHX00001	Intravenous dosage-range developmental study of SFP in female rabbits	4.2.3.2
(b) (4) 70008	Soluble ferric pyrophosphate: A 13-week intravenous toxicity study with a 4-week recovery period in Sprague Dawley rats **(previously reviewed)**	4.2.3.2
(b) (4) 70008 Amendment 1	Soluble ferric pyrophosphate: A 13-week intravenous toxicity study with a 4-week recovery period in Sprague Dawley rats – Adding toxicokinetics report **(previously reviewed)**	4.2.3.2
PHX00003	Intravenous dosage-range developmental toxicity of SFP in female rats	4.2.3.2
Pharmacology		
(b) (4) 1234-004	Evaluation of the effect of SFP on cardiac action potential parameters measured in isolated canine purkinje fibers **(previously reviewed;** no additional review is made and captured within this report**)**	4.2.1.3

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/s/

CHING-JEY G CHANG
02/23/2016

TODD R PALMBY
02/23/2016

MEMORANDUM

Triferic (ferric pyrophosphate)

Date: December 23, 2014
To: File for NDA 206317
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review for Triferic conducted by Dr. Chang, and secondary memorandum and labeling provided by Dr. Palmby. I concur with Dr. Palmby's conclusion that Triferic may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
12/23/2014

MEMORANDUM

Date: December 22, 2014
From: Todd R. Palmby, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
To: File for NDA 206317 Triferic (ferric pyrophosphate)
Re: Approvability for Pharmacology and Toxicology
Indication: Treatment of iron deficiency in adult patients with hemodialysis-dependent chronic kidney disease (HDD-CKD)

Non-clinical pharmacology and toxicology literature and original reports for studies to support Triferic (ferric pyrophosphate) for the treatment of iron deficiency in adult patients with hemodialysis-dependent chronic kidney disease (HDD-CKD) were reviewed by George Ching-Jey Chang, DVM, MS, PhD, DABT. Studies conducted with ferric pyrophosphate and submitted in this NDA include pharmacology, pharmacokinetics and ADME, safety pharmacology, general toxicology, genetic toxicology and reproductive and developmental toxicology.

Ferric pyrophosphate is an iron replacement product intended to deliver iron to the systemic circulation by addition to dialysate. The iron from ferric pyrophosphate can then bind to transferrin and be transported to erythroid precursor cells to be incorporated into hemoglobin. Iron derived from ferric pyrophosphate bound to human apotransferrin in vitro. The crystal structure for the binding of ferric pyrophosphate to human transferrin showed that the pyrophosphate was involved in the binding of iron to transferrin and that iron bound in a conformation similar to that described in the literature. It is unclear whether pyrophosphate coordinates iron binding to transferrin in vivo. Based on nonclinical studies and clinical trials, the primary potential toxicities associated with Triferic administration are related to iron delivery. Therefore, the Established Pharmacologic Class (EPC) of “iron replacement product” that is assigned to other iron products approved for use in patients with chronic kidney disease was assigned to Triferic.

Ferric pyrophosphate was administered intravenously in repeat-dose toxicology studies in rats and dogs. The primary findings in these studies were increases in the amounts of elemental iron in multiple tissues in both rats and dogs, including hepatocytes, Kupffer cells, renal tubular epithelium, and zymogen granules of the pancreas. The exposure (C_{max} and AUC_{0-24}) of iron increased less than proportionally to ferric pyrophosphate dose levels, which suggests that iron binding to transferrin reached saturation, the removal of unbound iron from serum was rapid and iron distribution into tissues was greater.

Ferric pyrophosphate was clastogenic in the chromosomal aberration assay in Chinese hamster ovary (CHO) cells in the presence of metabolic activation, but

was not clastogenic in the absence of metabolic activation. Ferric pyrophosphate was not mutagenic in the bacterial reverse mutation (Ames) test nor was it clastogenic in the in vivo micronucleus assay in mouse bone marrow. Rodent carcinogenicity studies were not required to support an NDA submission for Triferic for the proposed indication. The Applicant submitted non-product specific published reports of lifetime carcinogenicity studies with other iron-containing compounds, which suggest that these types of products do not pose a carcinogenic risk.

In a fertility and early embryonic development study in rats, no adverse effects on fertility, reproduction or embryo toxicity were noted up to the maternally toxic dose of 40 mg/kg. In embryo-fetal developmental toxicity studies in rats and rabbits, ferric pyrophosphate did not cause maternal or developmental toxicity at doses up to 30 mg/kg/day in rats and 20 mg/kg/day in rabbits. Post-implantation loss due to early resorptions, abnormal placentae, decreased fetal body weight and fetal head and vertebral malformations occurred at doses of 90 mg/kg/day in rats and vertebral malformations occurred at 40 mg/kg/day in rabbits. These dose levels were maternally toxic. In a pre- and post-natal development study in rats, reductions in the number of live offspring and lower offspring body weights occurred at the maternally toxic dose of 90 mg/kg/day. There were no adverse effects on survival of offspring at doses up to 30 mg/kg/day, or on behavior, sexual maturation or reproductive parameters of offspring at any dose level. Based on these findings, pregnancy category C was assigned to Triferic. No exposure relationship between animals and humans was included in product labeling for Triferic because there is no defined total amount of iron delivered to a patient treated with Triferic due to administration through the dialysate.

Recommendation: I concur with Dr. Chang's conclusion that submitted pharmacology and toxicology data support the approval of NDA 206317 for Triferic. There are no outstanding non-clinical issues that would preclude the approval of Triferic for the proposed indication.

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/s/

TODD R PALMBY
12/22/2014

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Supervisor/Team Leader:	Todd Palmby, PhD
Division Director:	John K. Leighton, PhD, DABT (acting) (Ann Farrell, MD)
Project Manager:	Amy H. Chi, RN, MSN

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 206317 are owned by Rockwell Medical, Inc. (Rockwell Medical) or are data for which Rockwell Medical has obtained a written right of reference. Any information or data necessary for approval of NDA 206317 that Rockwell Medical does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a

Reference ID: 3676761

previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 206317.

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1 Executive Summary

1.1 Introduction

Rockwell Medical (the Applicant) submitted on March 24, 2014, a new drug application (NDA) under Section 505(b)(1) for the use of Triferic as an iron replacement product for the treatment of iron deficiency in adult patients with hemodialysis-dependent chronic kidney disease (HDD-CKD). Triferic (ferric pyrophosphate) is a mixed ligand iron where Fe^{+3} is covalent bound to pyrophosphate and citrate. Triferic is to be delivered via hemodialysate, serving as an iron therapy to compensate for the increased iron losses in patients with CKD through hemodialysis.

1.2 Brief Discussion of Nonclinical Findings

Ferric pyrophosphate is an iron replacement product intended to deliver iron to the systemic circulation, which can then bind to transferrin and be transported to erythroid precursor cells to be incorporated into hemoglobin. The Applicant conducted an in vitro study which demonstrated that iron derived from ferric pyrophosphate bound to human apotransferrin. A study was conducted in which crystal structure was solved for the binding of iron to human transferrin, which involved pyrophosphate and in which iron bound in a conformation similar to that described in the literature.

Ferric pyrophosphate induced a slight transient increase in blood pressures, increase in heart rate, lengthening of QT and QTc interval and decrease in RR interval duration noted in a safety pharmacology study in Beagle dogs. The magnitude of QT prolongation was <10% as compared to baseline. These nonclinical cardiovascular findings were not noted consistently in trials submitted.

In repeat-dose toxicology studies, rats and dogs were administered ferric pyrophosphate intravenously. Findings in these studies included transient and reversible decreases in body weight and food consumption. Dose-dependent increases in the amounts of element iron were noted in multiple tissues in both rats and dogs, including hepatocytes, Kupffer cells, renal tubular epithelium, and zymogen granules of the pancreas. The exposure (C_{max} and AUC_{0-24}) of iron increased less than proportionally to ferric pyrophosphate dose levels, suggesting saturation of iron binding to transferrin, a rapid removal of unbound iron from serum and an enhanced iron distribution into tissues.

Ferric pyrophosphate was not mutagenic in the bacterial reverse mutation (Ames) test. FP was clastogenic in the chromosomal aberration assay in Chinese hamster ovary (CHO) cells in the presence of metabolic activation, but was not clastogenic in the absence of metabolic activation. FP was not clastogenic in the mouse micronucleus assay.

In a fertility and early embryonic development study in rats at doses up to 40 mg/kg, no adverse effects on fertility or reproduction were noted in males or females. Maternal

toxicity occurred at 40 mg/kg, but no toxicity was noted in the developing embryos. In embryo-fetal development toxicity studies in rats and rabbits, ferric pyrophosphate did not cause maternal or developmental toxicity at doses up to 30 mg/kg/day in rats and 20 mg/kg/day in rabbits. Maternally toxic doses cause developmental toxicity, resulting in post-implantation loss due to early resorptions, abnormal placentae, decreased fetal body weight and fetal head and vertebral malformations at 90 mg/kg/day in rats and vertebral malformations at 40 mg/kg/day in rabbits. In a pre- and post-natal development study in rats, the maternally toxic dose of 90 mg/kg/day resulted in reductions in the number of live offspring and lower offspring body weights. There were no adverse effects on survival of offspring at doses up to 30 mg/kg/day, or on behavior, sexual maturation or reproductive parameters of offspring at any dose level.

1.3 Recommendations

1.3.1 Approvability

There are no pharmacology/toxicology issues that would preclude the approval of Triferic for the proposed indication.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

There is no actual defined dose of iron pyrophosphate delivered to a patient per administration of Triferic due to its delivery through dialysate. Therefore, ratios of systemic exposure (AUC) between animals and humans were not provided in sections of the product label describing the results of animal studies.

2 Drug Information

2.1 Drug: Soluble Ferric Pyrophosphate

CAS Registry Number: 85338-24-5, 1275612-31-1

Generic Name: ferric pyrophosphate

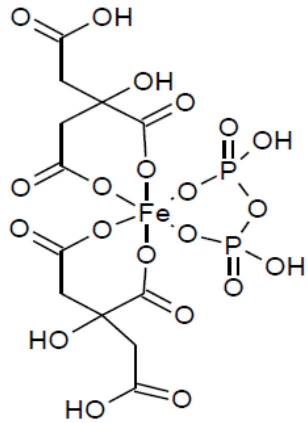
Code Name: soluble ferric pyrophosphate, SFP, H61

Chemical Name:
Iron (3+) cation; 2-oxidopropane-1,2,3-tricarboxylate; diphosphate 1,2,3-propanetricarboxylic acid, 2-hydroxy-, iron (3+), diphosphate

Molecular Formula/Molecular Weight:
$(Fe)_3(C_6H_5O_7)_2(P_2O_7)_2$ / approximately (b) (4) g/mol

Structure or Biochemical Description:

Figure 1 Chemical Structure of ferric pyrophosphate



(Excerpted from the Applicant's submission)

Pharmacologic Class: Iron replacement product

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND-51290 and DMF [redacted] (b) (4)

2.3 Drug Formulation

Triferic solution for addition to bicarbonate concentrate is composed of ferric pyrophosphate, a mixed ligand iron compound in which iron is covalently bound to pyrophosphate and citrate. Triferic is a clear, green or greenish-yellow, sterile solution of ferric pyrophosphate in water (Water for Injection, USP), packaged in 5 mL size, [redacted] (b) (4) low-density, polyethylene [redacted] (b) (4) containers (ampoules). Each 5 mL single use ampoule contains 27.2 mg elemental iron (5.44 mg Fe/mL) in water for injection.

At the time of use, Triferic solution is admixed, [redacted] (b) (4) with liquid bicarbonate concentrate (2.5 gal) [redacted] (b) (4).

2.4 Comments on Novel Excipients

Triferic does not contain novel excipients.

2.5 Comments on Impurities/Degradants of Concern

There are no safety issues regarding impurities or degradants for Triferic.

A discussion on safety of [redacted] (b) (4) On October 21, 2014, CMC reviewer posed a question to the Pharmacology/Toxicology Team regarding whether there was a safety

concern from (b) (4) as the Applicant proposed a specification of (b) (4)% limit of (b) (4) in the ampoule solution.

On September 18, 2014, the following CMC information request was sent to the Applicant:

"Specify whether (b) (4), if present in the complex or drug product, will be transferred across the dialysis membrane and its affect to the safety of the patient."

On October 3, 2014, the Applicant sent a response to this information request, on which the CMC review team based the request for input.

The Applicant stated that with a 2 μM dose, the (b) (4) ((b) (4) in the dialysate is undetectable. (b) (4)

Based on the justification provided by the Applicant, the pharmacology/toxicology team has a low level of concern for the proposed maximum levels of (b) (4) that may be formed from administration of Triferic with a specification of (b) (4)% in the ampoule solution.

2.6 Proposed Clinical Population and Dosing Regimen

Triferic contains iron in the form of ferric pyrophosphate and is administered to patients via transfer from hemodialysis solution, across the dialyzer membrane to the blood. The proposed clinical population includes adult patients with hemodialysis-dependent chronic kidney disease (HDD-CKD).

The proposed dose preparation is as followed:

SFP Concentrate is admixed, (b) (4) with liquid bicarbonate concentrate (2.5 gal) (b) (4)

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2.7 Regulatory Background

This is the original NDA submission for Triferic.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

Study#	Title	Module
(b) (4) 2008-2009	Iron binding of soluble ferric pyrophosphate chelates to human apotransferrin	4.2.1.1
RCK00101AARF	Crystal structure of human transferrin with Rockwell iron pyrophosphate	4.2.1.1

Safety Pharmacology

Study#	Title	Module
(b) (4) 1234-003	Evaluation of the effects of SFP on cloned HERG channels expressed in human embryonic kidney (HEK293) cells **(previously reviewed;** additional review is made and captured within this report**)**	4.2.1.3
(b) (4) 1234-005	A pulmonary safety evaluation of intravenously administered SFP in male rats **(previously reviewed;** additional review is made and captured within this report**)**	4.2.1.3
(b) (4) 1234-001	Neurobehavioral evaluation of intravenously administered SFP in rats **(previously reviewed;** additional review is made and captured within this report**)**	4.2.1.3
(b) (4) 1234-002	A cardiovascular safety evaluation of intravenously administered SFP in the beagle dog **(previously reviewed;** additional review is made and captured within this report**)**	4.2.1.3

Repeat-Dose Toxicology

Study#	Title	Module
(b) (4) 70009	Soluble ferric pyrophosphate: A 26-week intravenous infusion toxicity study with a 4-week recovery period in Sprague Dawley rats **(previously reviewed;** additional review is made and captured within this report**)**	4.2.3.2
(b) (4) 60011	Soluble ferric pyrophosphate: A 39-week intravenous infusion toxicity study with a 4-week recovery period in beagle dogs	4.2.3.2

Genetic Toxicology

Study#	Title	Module
AMS00705	Evaluation of soluble ferric pyrophosphate in the *Salmonella typhimurium-Eschericia coli* reverse mutation (Ames) assay **(previously reviewed;** additional review is made and captured within this report**)**	4.2.3.3.1
CAB00705	In vitro mammalian chromosome aberration assay in Chinese hamster ovary cells challenged with soluble ferric pyrophosphate **(previously reviewed;** additional review is	4.2.3.3.1

Reference ID: 3676761

	made and captured within this report**)**	
MNA00505	Evaluation of soluble ferric pyrophosphate in the mouse bone marrow micronucleus assay **(previously reviewed)**	4.2.3.1

Reproductive and Developmental Toxicology

Study#	Title	Module
PHX00005	Intravenous fertility and general reproduction toxicity study of SFP in rats	4.2.3.5.1
PHX00004	Intravenous developmental toxicity study of SFP in pregnant female rats **(previously reviewed;** additional review is made and captured within this report**)**	4.2.3.5.2
PHX00002	Intravenous developmental toxicity study of SFP in pregnant female rabbits **(previously reviewed;** additional review is made and captured within this report**)**	4.2.3.5.2
PHX00010	Intravenous developmental and perinatal/postnatal reproduction toxicity study of SFP in rats, including a postnatal behavior/functional evaluation (**previously reviewed**; no additional review is made and captured within this report)	4.2.3.5.2

3.2 Studies Not Reviewed

Study#	Title	Module
Toxicology		
(b) (4) 60010 and Amendment 1	Soluble ferric pyrophosphate: A 13-week intravenous infusion toxicity study with a 4-week recovery period in beagle dogs **(previously reviewed)**	4.2.3.2
PHX00001	Intravenous dosage-range developmental study of SFP in female rabbits	4.2.3.2
(b) (4) 70008	Soluble ferric pyrophosphate: A 13-week intravenous toxicity study with a 4-week recovery period in Sprague Dawley rats **(previously reviewed)**	4.2.3.2
(b) (4) 70008 Amendment 1	Soluble ferric pyrophosphate: A 13-week intravenous toxicity study with a 4-week recovery period in Sprague Dawley rats – Adding toxicokinetics report **(previously reviewed)**	4.2.3.2
PHX00003	Intravenous dosage-range developmental toxicity of SFP in female rats	4.2.3.2
Pharmacology		
(b) (4) 1234-004	Evaluation of the effect of SFP on cardiac action potential parameters measured in isolated canine purkinje fibers **(previously reviewed;** no additional review is made and captured within this report**)**	4.2.1.3

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Reference ID: 3676761

3.3 Previous Reviews Referenced

Sally J. Hargus, PhD previously reviewed Study PHX00010 (Serial 051 under IND-051290) in 2009.

4 Pharmacology

4.1 Primary Pharmacology

Study title: Iron binding of soluble ferric pyrophosphate chelates to human apotransferrin

Study no.:	(b) (4) 2008-0009
Study report location:	4.2.1.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not specified
GLP compliance:	No
QA statement:	N/A
Drug, lot #, and % purity:	SFP; see the table below for lot numbers and purity information

Table 1 Information on SFP Samples Tested

Sample Lot	Manufacturer	LOD (%)	Iron Content (%w/w)	Pyrophosphate Content (%w/w)	Citrate (%w/w)	Phosphate (%w/w)	Sulfate (%w/w)
107805	(b) (4)						(b) (4)
112362							
113101							
126412							
0804536							
0804537							
0804538							

(Excerpted from the Applicant's submission)

This study compared between the rate of iron uptake (kinetics of iron absorption) by human apotransferrin for SFP products manufactured by two different suppliers, the (b) (4) .

Key Study Findings

- Rate of iron uptake by apotransferrin is faster with (b) (4) (3 lots; all under (b) (4)) versus (b) (4) (requiring nearly (b) (4)). The difference in uptake rate by transferrin could be related to (b) (4) .

Methods

A solution of human apotrasferrin and an iron source (SFP sample) was mixed and the binding of iron to apotranferrin was monitored at 470 nm (the absorption maximum for ferric transferrin) at several time intervals until a defined point of saturation was reached. The difference between each absorbance reading over the entire scan was calculated to determine the rate of change of absorbance over time. The last time interval in which the absorbance value changed by $1x10^{-4}$ AU or more was designated as the time required for 100% iron uptake ("100% iron loading time"), and the absorbance value at the point was then extrapolated using the average blank absorbance to determine the point at which 50% of the iron had reached ("50% iron loading time").

Validation

- All glassware and disposable cuvettes were acid washed prior to use, to decrease sources of iron contamination.
- Nitrilotriacetic acid (NTA) was utilized as a control chelating agent. The NTA-iron chelate existed as a monomeric molecular species and directly reacting with apotransferrin immediately, and was as a control solution for comparison with the SFP sample preparations.
- The experimental absorbance maxima for ferric transferrin were within expected theoretical values, suggesting that uptake of all available iron in solution.

Results

Results showed that the rate and extent of uptake of iron by apotransferrin is significantly faster with (b) (4) samples (of (b) (4); within (b) (4) than with (b) (4) (approximately (b) (4)) (see tables below).

Table 2 Composition and Content of Lots of SFP Tested

Sample Lot	Manufacturer	LOD (%)	Iron Content (%w/w)	Pyrophosphate Content (%w/w)	Citrate (%w/w)	Phosphate (%w/w)	Sulfate (%w/w)
107805	(b) (4)						(b) (4)
112362							
113101							
126412							
0804536							
0804537							
0804538							

(Excerpted from the Applicant's submission)

Table 3 SFP Iron Uptake Rates (2:1 Iron:Transferrin Molar Ratio)

Sample Lot	100% Iron Loading Time (min)	Absorbance (AU)	50% Iron Loading Time (min)	Absorbance (AU)
112362				(b) (4)
107805				
126412				
113101				
0804536				
0804537				
0804538				
Iron:NTA Control				

(Excerpted from the Applicant's submission)

Note from Reviewer: Drug Product for Nonclinical Profiling and Clinical Product:
The nonclinical studies were conducted using food-grade, (b) (4) ferric pyrophosphate. The clinical SFP product is clinical-grade.

Study title: Crystal structure of human transferrin with Rockwell iron pyrophosphate

Study no.:	RCK0101.AARF
Study report location:	4.2.1.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 19, 2013 (research start date)
GLP compliance:	No
QA statement:	N/A
Drug, lot #, and % purity:	Iron pyrophosphate, lot and purity information not specified

Key Study Findings

- The crystal structure was solved for the binding of iron to human transferrin which involved pyrophosphate.
- The overall structure exhibited an open conformation replicating an apotranferrin structure in the literature.

Methods

The 4 transferrin samples went through (b) (4)

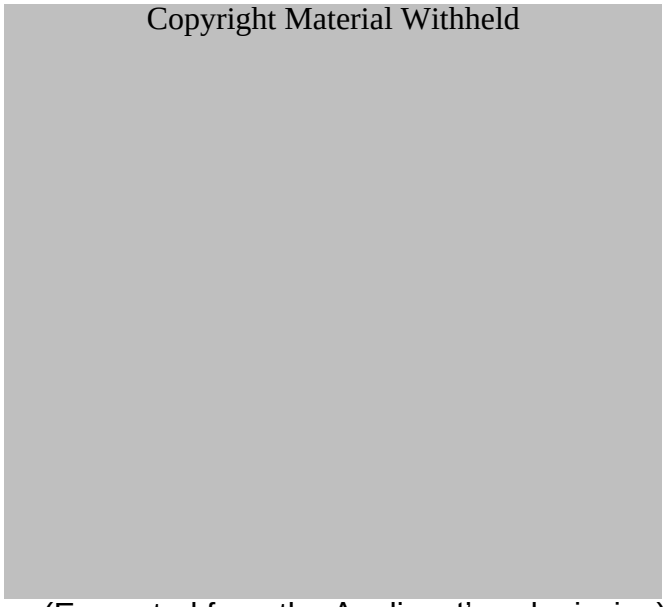
Validation

- The overall structure exhibited an open conformation replicating an apotransferrin structure in the literature.
- No electronic density for iron was noted, indicating that the starting material did not contain iron.

Results

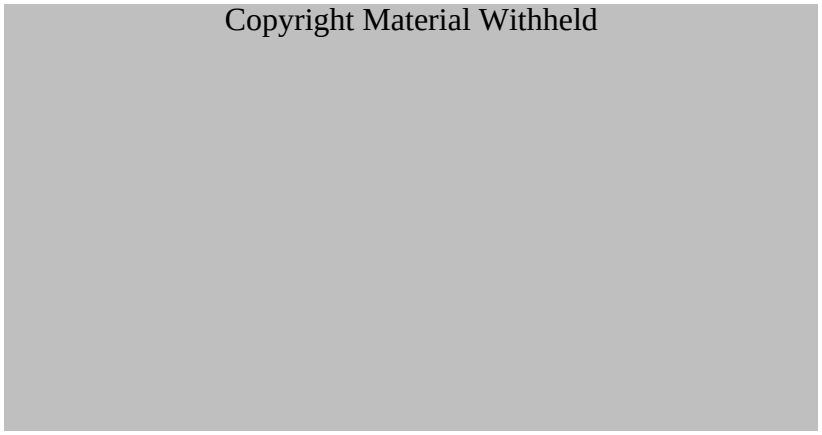
The structure exhibited a full enclosed C-lobe with an encapsulate iron and carbonate molecule, identical to all iron bound human transferrin C-lobe structures reported in the literature. The N-lobe exhibited a more open confirmation, similar to that reported for either bismuth-NTA (nitrilotriacetic acid) or iron-sulfate bound form. The N-lobe contained clear electron density for iron and pyrophosphate molecule. The pyrophosphate binds on the far side of the iron relative to the main protein chelators in a similar location as NTA in the bismuth-NTA complex. See figures below.

Figure 2 Crystal Structure of Human Transferrin Bound to SFP (Solved at 2.6 Å Resolution)



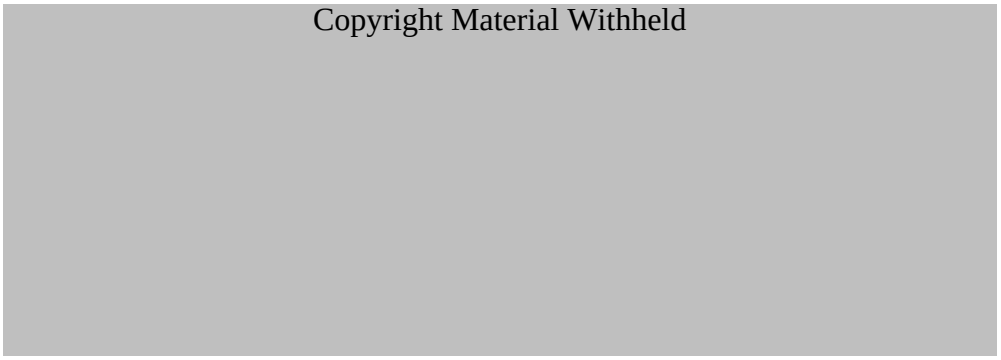
(Excerpted from the Applicant's submission)

Figure 3 Binding of Iron Pyrophosphate to the N-Lobe of Human Transferrin



(Excerpted from the Applicant's submission)

Figure 4 Binding of Iron Carbonate to the C-Lobe of Human Transferrin



(Excerpted from the Applicant's submission)

4.2 Secondary Pharmacology

No secondary pharmacology reports were submitted or summarized.

4.3 Safety Pharmacology

Study title: Evaluation of the effects of SFP on cloned HERG channels expressed in human embryonic kidney (HEK293) cells

Study no.:	1234-003
Study report location:	4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 19, 2007
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	SFP (b) (4), Batch #126412 (non-GMP and (b) (4)), 12% Fe by weight

Key Study Findings

- SFP did not inhibit hERG-mediated potassium current (I_{Kr}) at concentrations up to 1,000 µg/mL (approximately 1 µM).

Methods

Concentrations:	1, 10, 100, 1000 µg/mL
Formulation/Vehicle:	Physiological salt solution (PSS)
Cell Line:	HEK293 cell line stably expressing hERG channels
Positive Control:	Cisapride (0.048 µg/mL; 100 nM)
Negative Control:	PSS
Deviation from study protocol:	Concentration verification and homogeneity analysis of the superfusate formulations tested were not conducted. SFP stock solution (at 10 mg/mL) used to prepare the superfusate formulation was analyzed. *(Reviewer note: The impact of this deviation cannot be assessed.)*

Table 4 Study Design for hERG Test for SFP

Study Design		
Treatment Number	Concentration Level	Number of Cells
1	Vehicle (PSS)	4
2	1 µg/mL SFP	3
3	10 µg/mL SFP	3
4	100 µg/mL SFP	3
5	1000 µg/mL SFP	3
6	0.048 µg/mL Cisapride	4

(Excerpted from the Applicant's submission)

Validation

- HEK293 cells stably expressing the hERG potassium channel was transformed with the adenovirus 5 DNA, and co-transfected with the hERG cDNA and G418-resistant gene incorporated into a modified pCDNA3 plasmid.

- Vehicle perfusion had no effect on hERG-mediated potassium currents, while cisapride (the positive control, at 0.048 µg/mL (100 nM)) produced approximately 80.75% inhibition. See table below.

Results

HEK293 cells were cultured, harvested, and plated onto sterile glass cover slips one day prior to electrophysiological evaluation.

Following whole-cell patch clamp and demonstration of stable hERG current for at least 2 minutes, the test and control articles were applied in PSS via superfusion with flow rate at 3.2 to 3.6 per minute, and currents were recorded from HEK293 cells. Once stably patch clamped in whole cell mode, HEK293 cells were held at a potential of -80 mV. hERG-mediated currents were evoked by application of a depolarizing voltage command step to +40 mV for 2 seconds and followed by a repolarizing command step to -50 mV for 1.5 seconds. Peak tail currents from the last 30 seconds of the baseline period were averaged and compared to 30 seconds of data recorded in the presence of the test solutions. Current inhibition is reported in percent according to inhibition (%) = $100*(1-(I_{test}/I_{baseine}))$ where I test was the peak tail current measured in the presence of the test solution, and $I_{baseline}$ was the peak tail current measured prior to exposure to the test solution.

Perfusion of SEP at concentrations of 1, 10, 100, and 1000 µg/mL produced no meaningful inhibition of hERG-mediated potassium currents (see table below).

Table 5 Effect of SFP on hERG Current Inhibition

Concentration	Inhibition, % Mean	SEM	N
Vehicle (PSS)	0.47	1.122	4
1 µg/mL SFP	1.36	0.465	3
10 µg/mL SFP	2.24	0.497	3
100 µg/mL SFP	1.41	1.692	3
1000 µg/mL SFP	4.28	1.712	3
0.048 µg/mL Cisapride	80.75	1.391	4

(Excerpted from the Applicant's submission)

Dosing Solution Analysis

The stock solution of SFP was within 95-96% of the target concentration and was homogenous, but the SFP testing solutions were not analyzed.

Study title: A pulmonary safety evaluation of intravenously administered SFP in male rats

Study no.: 1234-005
Study report location: 4.2.1.3
Conducting laboratory and location: (b) (4)
Date of study initiation: April 19, 2007
GLP compliance: Yes
QA statement: Provided
Drug, lot #, and % purity: SFP, Batch #126412 (non-GMP and (b) (4) product), 11.5% Fe by weight

Key Study Findings

- No drug-related changes in respiratory rate or minute volume. Drug-related pulmonary effect included a slight increase in tidal volume at 250 mg/kg without impact on the effective minute ventilation.
- The no-observed-adverse-effect-level (NOAEL) of FSP in rats for pulmonary function is 250 mg/kg when administered via 1-hour infusion.

Methods

Doses: 0, 62.5, 125, and 250 mg/kg (0, 7.2, 14.4, and 28.8 Fe/kg)
Frequency of dosing: Once
Dose volume: 5 mL/kg/hour
Route of administration: IV infusion (1 hour) through a surgically implanted jugular catheter
Formulation/Vehicle: Lactated Ringer's Solution (LRS)

Species/Strain:	CD® [Crl:CD® (SD)] rats
Number/Sex/Group:	7-8 males
Satellite groups:	None
Study design:	See table below
Deviation from study protocol:	None

Table 6 Study Design of SFP Pulmonary Safety Pharmacology Study in Male Rats

Experimental Design			
Group Number	Dose Level		Number of Male Animals
	mg SFP/kg	mg Fe/kg	
1	0	0	7[b]
2	62.5	7.2	8
3	125	14.4	8
4	250	28.8	8

[a]Test article or vehicle was administered to up to two animals/group/day over a five day period.
[b]Due to complications with the catheter for one animal at 0 mg SFP/kg (animal number 406), only 7 animals were dosed and monitored.

(Excerpted from the Applicant's submission)

Observations and Results

Table 7 Measurements Performed in the SFP Pulmonary Safety Pharmacology Study in Male Rats

Morbidity:	Twice daily
Mortality:	Twice daily
Clinical Observation (including injury):	Prior to dosing on Day 1, at the end of the infusion, and following completion of the pulmonary monitoring period (approximately 4 hours following the end of the infusion)
Availability of food:	Twice daily
Availability of water:	Twice daily
Body weight:	Prior to dosing
Pulmonary function: Including respiratory rate, tidal volume, minute volume (in plethysmograph tower)	At least 1 hour prior to dosing (baseline recording), for 4 hours following the end of the infusion (Food and water were not available to the animals during pulmonary recording sessions.)

Mortality

No drug-related early mortalities occurred in the study.

Clinical Signs

Drug-related clinical signs of toxicity included red material around the nose or eyes (≥62.5 mg/kg), red urine, red material in the cage pan, red discharge from the penis, and/or red discolored hair in the abdominal and anogenital area (≥125 mg/kg).

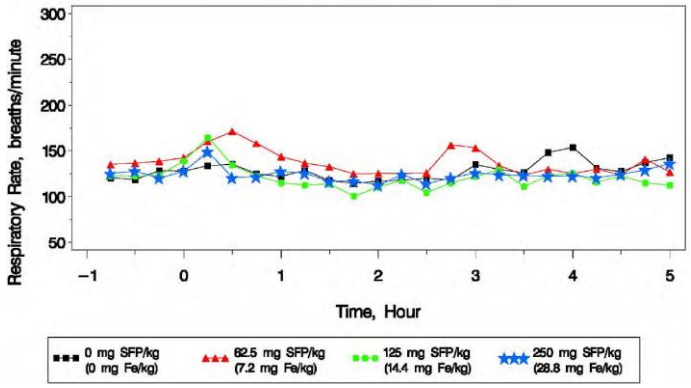
Body Weight

No drug-related changes in body weight were noted.

Pulmonary Function

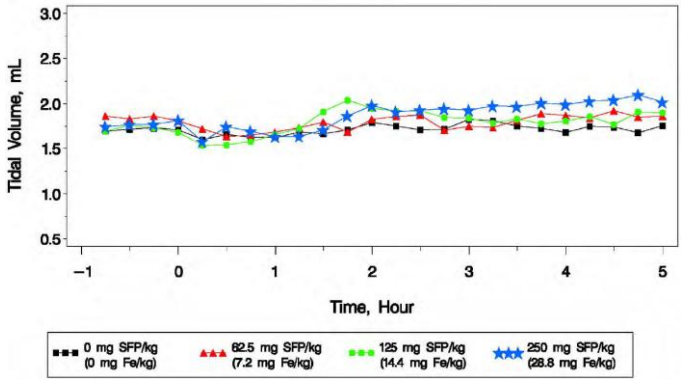
No drug-related changes in respiratory rate or minute volume. Mean tidal volume at 250 mg/kg increased near the end of the infusion and peaked at approximately 0.2 – 0.3 mL above the baseline and above the same period when compared to controls. It remained slightly increased over baseline and control for the duration of the monitoring period. As the effective minute ventilation was unaffected, this slight increase was not considered adverse. See figures below.

Figure 5 Group Mean Respiratory Rates in Male Rats



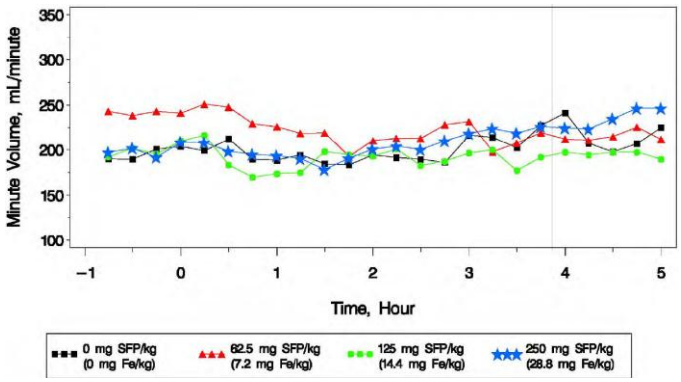
(Excerpted from the Applicant's submission)

Figure 6 Group Mean Tidal Volume in Male Rats



(Excerpted from the Applicant's submission)

Figure 7 Group Mean Minute Volume in Male Rats



(Excerpted from the Applicant's submission)

Dosing Solution Analysis

Mean SFP concentrations, calculated from the measured iron content, were 99-101% of the target concentrations.

Study title: Neurobehavioral evaluation of intravenously administered SFP in rats

Study no.:	1234-001
Study report location:	4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 9, 2006
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	SFP, Batch #125140, 11.7% Fe by weight

Key Study Findings

- Early mortalities occurred in rats at doses >400 mg/kg.
- Drug-related signs of toxicity included decreases in body temperature (at doses ≥62.5 mg/kg) and body weight (>5% at doses ≥125 mg/kg in males; 5% at 500 mg/kg in females), incidence of decrease activity, low posture, altered gait and/or mobility, altered respiratory patterns (at doses ≥125 mg/kg).
- No specific drug-related neurotoxicity findings were noted.
- No adverse CNS effects were observed at dose up to 500 mg/kg (approximately 58 mg Fe/kg).

Methods

Doses: MTD Phase: 200, 400, 700, 1000 mg/kg (approximately 23, 46, 81, and 115 mg Fe/kg, respectively)
FOB Phase: 0, 62.5, 125, 250, 500 mg/kg

	(approximately 0, 7, 14, 29, and 58 mg Fe/kg, respectively)
Frequency of dosing:	Once
Dose volume:	5 mL/kg/hour
Route of administration:	IV infusion (1 hour)
Formulation/Vehicle:	Lactated Ringer's for Injection (LRS)
Species/Strain:	CD [Crl:CD®(SD)] rats
Number/Sex/Group:	MTD Phase: 2 FOB Phase: 10
Satellite groups:	None
Study design:	See table below This study had two phases: 1. Maximum tolerated dose (MTD) phase and 2. Functional observational battery (FOB) phase.
Deviation from study protocol:	The stability study of dosing formulations was not conducted in accordance with GLP regulations, and the homogeneity of test article was not assessed.

Table 8 Study Design of MTD Phase of Neurobehavioral Safety Pharmacology Study in Rats

Group Assignments				
Group Number	Dose Level		Number of Animals	
	(mg SFP/kg)	(mg Fe/kg)[a]	Male	Female
MTD Phase				
1	200	23	2	2
2	400	46	2	2
3	1000	115	2	2
4	700	80.5	2	2
FOB Phase				
5	0	0	10	10
6	62.5	7.2	10	10
7	125	14.4	10	10
8	250	28.8	10	10
9	500	57.5	10	10
[a]SFP is 11.5% Fe by weight				

(Excerpted from the Applicant's submission)

Observations and Results

Table 9 Measurements Performed in the Neurobehavioral Safety Pharmacology Study in Rats

Morbidity and mortality:	Twice daily at least
Clinical observation: including respiratory and circulatory effects, autonomic effects (salivation), nervous system effects (including tremors, convulsions,	MTD Phase: Prior to dosing, 30 minutes after the start of infusion, at the end of infusion, and 2,4, and 24 hours after the end of infusion FOB Phase: Prior to dosing, 30 minutes after the start of infusion, at the end of infusion, and following each FOB examination.

reactivity to handling, and bizarre behavior)	
Functional observational battery: Activity and arousal, posture, rearing, bizarre behavior, clonic and tonic movements, gait, mobility, stereotypy, righting reflex, response to stimulus (approach, clinic, tail pinch, touch), palpebral closure, pupil response, piloerection, exophthalmos, lacrimation, salivation, and respiration, forelimb and hindlimb grip, hindlimb splay, pain perception, body temperature	Prior to dosing, shortly after infusion, and at approximately 24 hours after the end of infusion
Body weight:	Prior to dosing
Gross pathology:	At necropsy – found dead in MTD Phase or at scheduled sacrifice (Found dead in the FOB Phase were not examined.)

Mortality

MTD Phase
The early mortalities that occurred in the MTD Phase are listed in the following tables. The MTD was determined to be 400 mg/kg.

FOB Phase
Two rats died at 500 mg/kg.

Table 10 Early and Scheduled Mortalities in Males

Group, Animal Number	Fate	Day
200 mg SFP/kg (23 mg Fe/kg)		
2001	terminal necropsy	2
2002	terminal necropsy	2
400 mg SFP/kg (46 mg Fe/kg)		
2005	terminal necropsy	2
2006	terminal necropsy	2
1000 mg SFP/kg (115 mg Fe/kg)		
2009	found dead	1
2010	died prior to euthanasia	2
700 mg SFP/kg (80.5 mg Fe/kg)		
2013	found dead	2
2014	terminal necropsy	2

(Excerpted from the Applicant's submission)

Table 11 Early and Scheduled Mortalities in Females

Group, Animal Number	Fate	Day
200 mg SFP/kg (23 mg Fe/kg)		
2003	terminal necropsy	2
2004	terminal necropsy	2
400 mg SFP/kg (46 mg Fe/kg)		
2007	terminal necropsy	2
2008	terminal necropsy	2
1000 mg SFP/kg (115 mg Fe/kg)		
2011	terminal necropsy	2
2012	died prior to euthanasia	2
700 mg SFP/kg (80.5 mg Fe/kg)		
2015	found dead	2
2016	terminal necropsy	2

(Excerpted from the Applicant's submission)

Clinical Signs

MTD Phase: Drug-related clinical signs of toxicity included decreased activity, ataxia, skin cold to touch, slow or shallow breathing (above ≥700 mg/kg), and discolored (red) hair in the anogenital region (1000 mg/kg).

FOB Phase: Procedure-related findings included reduced body temperature at all dose levels, infrequent incidences of decrease activity, low posture, altered gait and/or mobility, and altered respiratory pattern in a few rats at doses ≥125 mg/kg. No specific drug-related neurotoxicity signs were noted. See FOB results table below.

Body Weight

Drug-related decreases in body weight was noted in males (>5% at doses ≥125 mg/kg) and females (5% at 500 mg/kg).

Reference ID: 3676761

Functional Observational Battery (FOB)

No drug-related changes were noted for the following categories of observations at predose, end of dose, and 24-hours post end of dose (see table below). The decreases in body temperature noted at the end of the treatment or 24 hours after the treatment ended were more related to the general condition, instead a specific neurobehavior response.

Table 12 Group Summary of FOB Results in Rats

Dose (mg/kg/day)	0	62.5	125	250	500
Number of rats treated	10	10	10	10	10
Continuous Endpoints - Males					
	End of Dose				
Physiological – Body temperature (ºC)	36.3	35.0**	34.4**	34.8**	34.2**
	24 hours post-end of dose				
Physiological – Body temperature (ºC)	36.3	36.1	35.5	36.2	34.6*
Continuous Endpoints - Females					
	End of Dose				
Physiological – Body temperature (ºC)	37.2	36.1**	34.5**	34.8**	34.6**
	24 hours post-end of dose				
Physiological – Body temperature (ºC)	36.8	36.6	36.2	36.2	34.9

* Significantly different from control ($p<0.05$); ** significantly different from control ($p<0.01$)

Dosing Solution Analysis

For the MTD Phase, the SFP concentrations were 108 to 136% to the targets; for the FOB Phase, the SFP concentrations were 91 to 97% to the targets.

Study title: A cardiovascular safety evaluation of intravenously administered SFP in the beagle dog

Study no.:	1234-002
Study report location:	4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 29, 2005
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	SFP, Batch #125140, 11.5% Fe by weight

Key Study Findings

- Early mortalities occurred at SFP doses ≥250 mg SFP/kg; the maximum tolerated dose (MTD) of SFP is 125 mg/kg. Drug-related signs of toxicity included decrease activity, emesis, and slight body weight decreases.

Reference ID: 3676761

- Drug-related, reversible changes in CV parameters noted included slight transient increase in blood pressures (at all dose levels; <25%), increase in heart rate (at 500 mg/kg; ≤50%), lengthening QT and QTc interval (≥250 mg/kg), and decrease in RR interval duration (at 500 mg/kg). The magnitude of QT prolongation was <10% compared to baseline.

- The no-observed-adverse-effect-level (NOAEL) for CV effects is 125 mg/kg, when C_{max} was 10.1 and 8.5 mg Fe/dL and AUC was 225.1 and 160.2 mg Fe*h/dL in males and females, respectively.

Methods

Doses:	MTD Phase: 60, 90, 150, 300, 500 mg SFP/kg (6.9, 10.4, 17.3, 34.5 and 57.5 mg Fe/kg) CV Phase: 0, 125, 250, 500 mg SFP/kg (0, 14.4, 28.8, 57.5 mg Fe/kg) TK Phase: 0, 125, 250, 500 mg SFP/kg (0, 14.4, 28.8, 57.5 mg Fe/kg)
Frequency of dosing:	MTD Phase: Once at ascending dose levels (every 2-3 days) CV Phase: Once at each ascending dose and with a 7-day washout period between each administration TK Phase: Once at each ascending dose and with a 7-day washout period between each administration
Dose volume:	5 mL/kg/hour
Route of administration:	IV infusion (1 hour) MTD Phase: A temporary catheter CV Phase: A surgically instrumented femoral vascular access port (VAP) TK Phase: A temporary catheter
Formulation/Vehicle:	Lactated Ringer's Injection, USP (LRS)
Species/Strain:	Beagle dogs
Number/Sex/Group:	MTD Phase: 2 CV Phase: 3 TK Phase: 3 (except for 2 females at 500 mg/kg)
Satellite groups:	N/A
Study design:	See tables below. Prior to study initiation, the animals in CV phase were surgically implanted with venous VAP for dosing and radio-transmitters for measuring body temperature, blood pressures, heart rate,

Reference ID: 3676761

	and ECG
Deviation from study protocol:	The homogeneity test for the dosing solutions was not conducted.

Observations and Results

Table 13 Measurements Performed in the Cardiovascular Safety Pharmacology Study in Dogs

Morbidity, mortality, injury, availability of food and water:	Twice daily
Clinical observation: including respiratory and circulatory effects, autonomic effects (salivation), nervous system effects (including tremors, convulsions, reactivity to handling, and bizarre behavior)	MTD Phase: Prior to dosing, 30 minutes after the start of infusion, at the end of infusion, and 2,4, and 24 hours after the end of infusion CV Phase: Prior to dosing, at the end of infusion, and following completion of the 24-hour CV monitoring period. TK Phase: Prior to dosing, at the end of infusion, and following completion of the 24-hour CV monitoring period.
Cardiovascular Endpoints: Body temperature, systolic, diastolic, and mean arterial blood pressures, heart rate, ECG (RR, PR, QRS, QT, and QTc)	Continuously from at least 2 hours predose till at least 24 hours following completion of the 1-hour infusion
Body weight:	1 day prior to dosing
Toxicokinetics:	Blood samples collection: Prior to dose, near the end of infusion, at 0.5, 1, 3, 6, 24, 48, and 72 hours following completion of the infusion

Mortality

Three early mortalities occurred in this study: One male and one female dogs at 500 mg/kg during the CV phase, and one female dog at 250 mg/kg during the TK phase of this study.

CV Phase: One male (Day 2) and one (Day 32) at 500 mg/kg. The male was found dead with a needle found of outside the vascular port during jacket removal, suggesting an undetermined portion of the dose had been administered subcutaneously. For the female found dead on Day 32, red material and red mucoid feces were present in the cage pan the day prior to death.

Reference ID: 3676761

TK Phase: One female at 250 mg/kg was moribund euthanized on Day 5, the animal exhibited signs of decreased activity, prostration, difficult/audible breathing, slow gum capillary refill time, skin cold to touch, and moribundity.

Clinical Signs

Drug-related clinical signs of toxicity included emesis during infusion in dogs in all dose groups, and decrease in activity in one male and two females following infusion at 500 mg/kg. The activity returned to normal within 24-hour monitoring period.

Body Weight

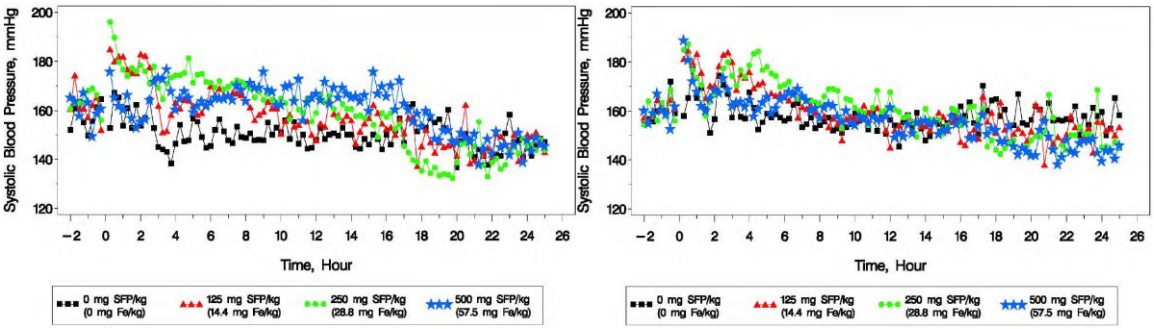
Drug-related changes in body weight included a slight decrease during the week following the dose at 250 mg/kg. (Body weights were not measured in 500 mg/kg group.)

Cardiovascular Evaluation

Drug-related CV effects noted included:

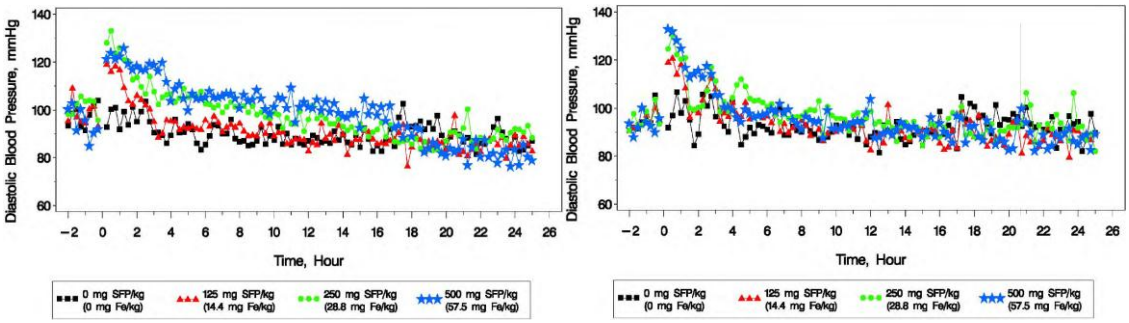
- Slight increase in blood pressures (systolic, diastolic, and mean arterial pressures) at doses ≥125 mg/kg during infusion which peaked at approximately 20 to 30 mmHg (≤25%) above baseline or controls. See figures below.

Figure 8 Group Mean Systolic Pressures in Dogs (Left: Males; Right Females)



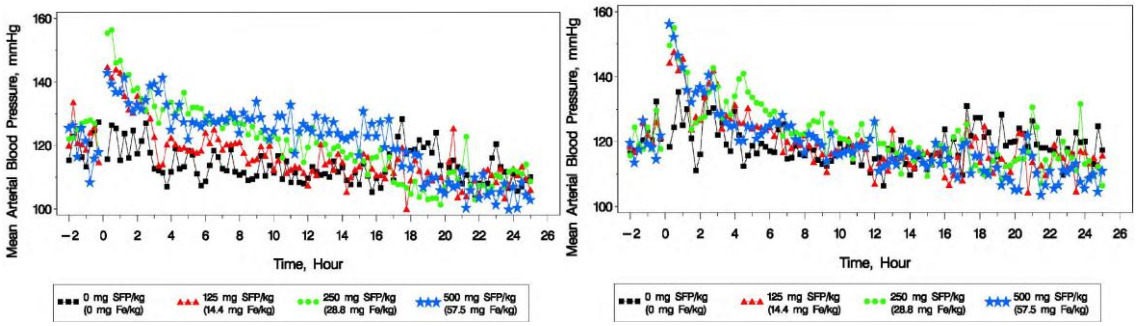
(Excerpted from the Applicant's submission)

Figure 9 Group Mean Diastolic Pressures in Dogs (Left: Males; Right: Females)



(Excerpted from the Applicant's submission)

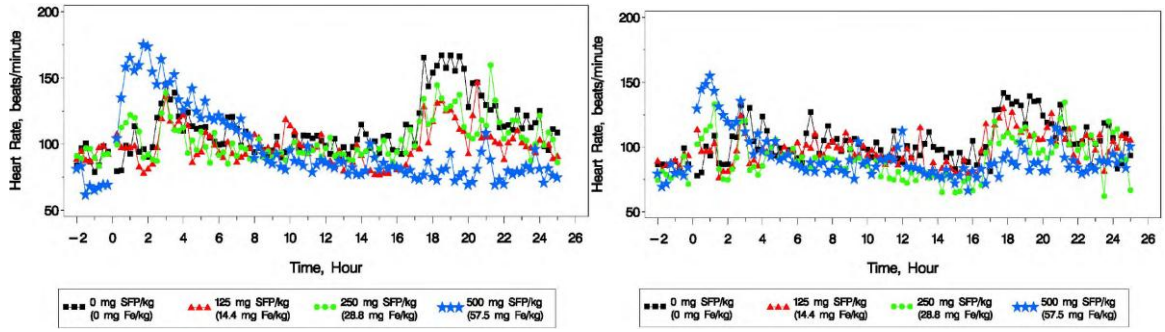
Figure 10 Group Mean Arterial Blood Pressures in Dogs (Left: Males; Right: Females)



(Excerpted from the Applicant's submission)

- Increased (~50%) heart rate at 500 mg/kg which returned to near control/baseline values within 3 hours post the end of infusion for dogs, except for one dog that returned to control values within 8 hours (see figure below).

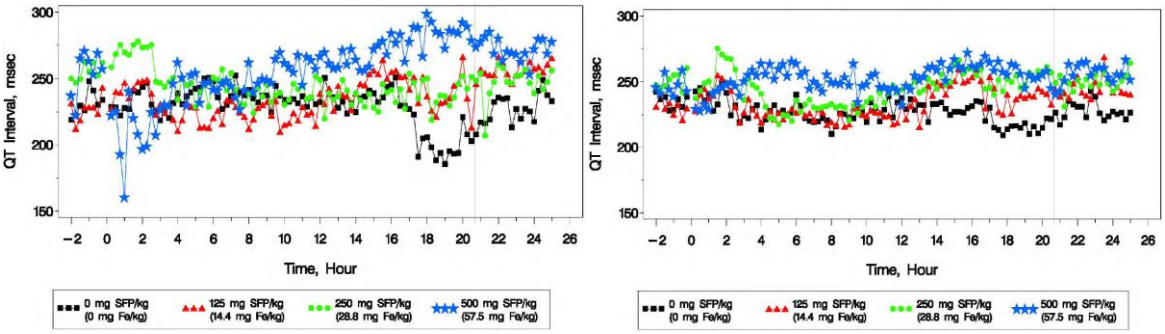
Figure 11 Group Mean Heart Rats in Dogs (Left: Males; Right: Females)



(Excerpted from the Applicant's submission)

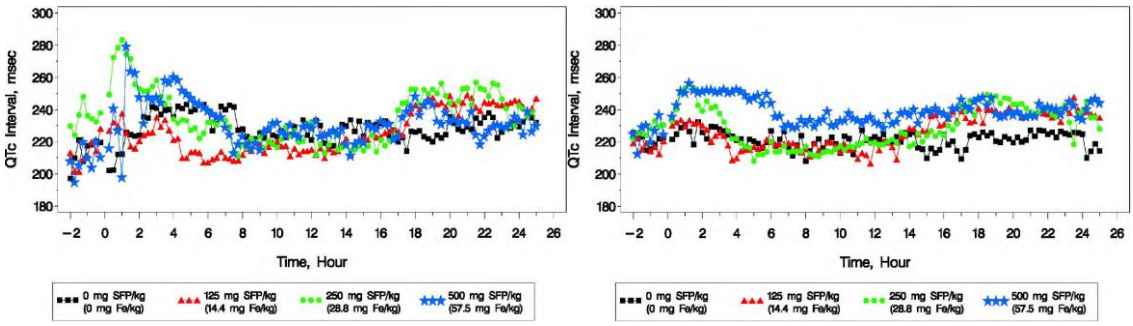
- Slightly lengthened QT and QTc intervals were noted at doses ≥250 mg/kg. These intervals were approximately 30-40 msec longer in males and 25 -30 msec longer in females than baseline /control, and they returned to baseline/control values within 3 hours for dogs at 250 mg/kg and within 2-17 hours for dogs at 500 mg/kg. See figures below.

Figure 12 Group Mean QT Intervals in Dogs (Left: Males; Right: Females)



(Excerpted from the Applicant's submission)

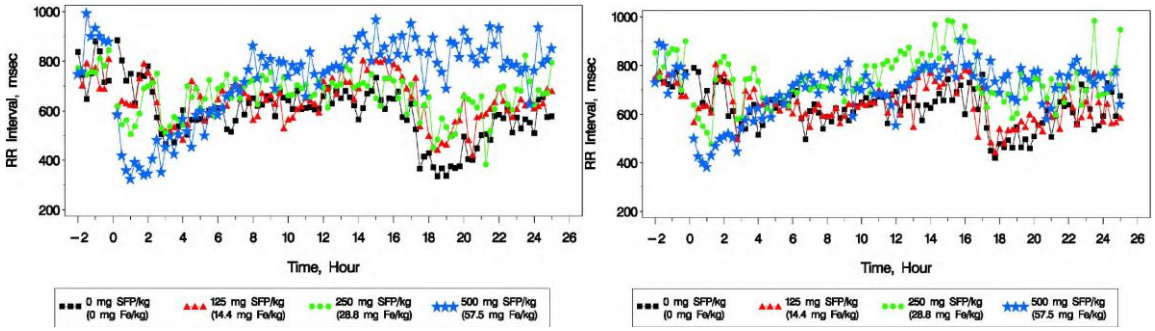
Figure 13 Group Mean QTc Intervals in Dogs (Left: Males; Right: Females)



(Excerpted from the Applicant's submission)

- Slight decrease in RR interval duration at 500 mg/kg were noted but returned to the baseline/control level within 3 hours (see figure below).

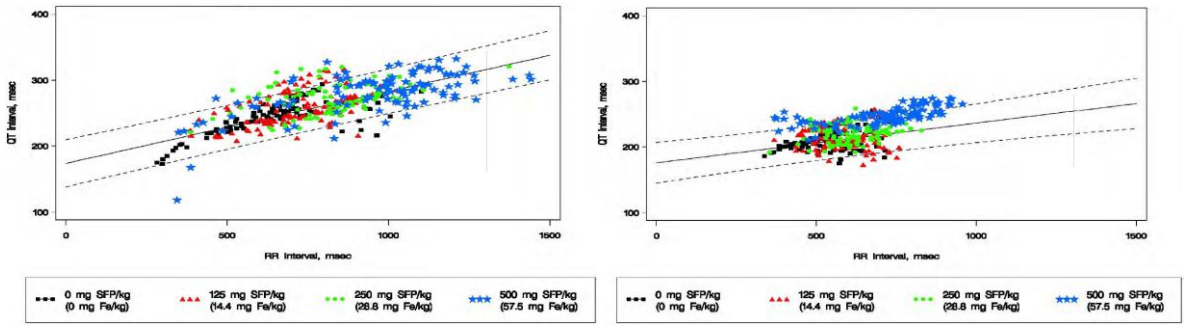
Figure 14 Group Mean of Mean RR Intervals in Dogs (Left: Males; Right: Females)



(Excerpted from the Applicant's submission)

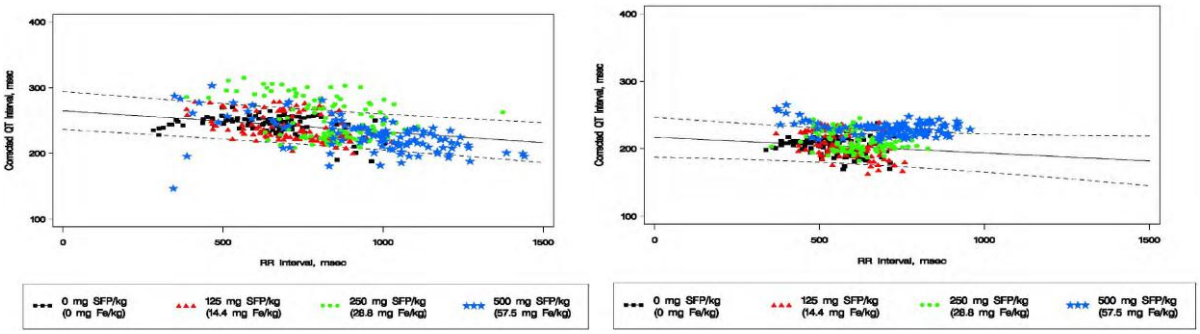
- QT interval vs RR interval and QTc vs RR interval Plots: The magnitude of QT prolongation was <10% (approximately 15 msec) compared to baseline. See two sets of figures as examples.

Figure 15 QT Interval vs. RR Interval in Dogs (Left: Male #6005M; Right: Female #6009F)



(Excerpted from the Applicant's submission)

Figure 16 Corrected QT Interval vs. RR Interval in Dogs (Left: Male #6005M; Right: Female #6009F)



(Excerpted from the Applicant's submission)

Toxicokinetics

The peak serum iron concentration occurred near the end of infusion, with mean $t_{1/2}$ ranging from 18.8 to 34.9 hours resulting in serum iron above baseline levels for at least 72 hours. The peak exposure to iron increased proportionally with dose level, but total exposure reached a plateau between 250 and 500 mg/kg. There was no gender difference in exposure. See table below.

Table 14 Group Mean TK Parameters for Serum iron in Dogs following single IV Dose of SFP

Dose Level (mg/kg)	Sex		C_{max} (µg/dL)	T_{max} (hr)	λ z (1/hr)	t½ (hr)	$AUC_{0\text{-last}}$ (hr*µg/dL)	$AUC_{0\text{-inf}}$ (hr*µg/dL)	% Extrapolation $AUC_{0\text{-inf}}$
0 (Vehicle)	Male	**Mean**	**149**	**9.67**	**ND**	**ND**	**7479**	**ND**	**ND**
		SD	15.5	13.4	-	-	815	-	-
		CV%	10.4	139	-	-	11	-	-
125	Male	**Mean**	**10059**	**1.5**	**0.037**	**18.8**	**225092**	**242638**	**7.2**
		SD	229	0	0.0022	1.1	26345	29656	1.0
		CV%	2.3	0	5.8	5.7	12	12	13.8
250	Male	**Mean**	**19503**	**0.83**	**0.0322**	**21.6**	**367260**	**396884**	**7.4**
		SD	860	0	0.0024	1.6	34310	42677	1.5
		CV%	4.4	0	7.5	7.3	9	11	20.2
500	Male	**Mean**	**28177**	**0.83**	**0.0278**	**25.0**	**360662**	**394679**	**8.6**
		SD	1104	0	0.0009	0.8	44374	47045	0.4
		CV%	3.9	0	3.3	3.3	12	12	4.6
0 (Vehicle)	Female	**Mean**	**179**	**18.7**	**ND**	**ND**	**11086**	**ND**	**ND**
		SD	14.5	26.5	-	-	1655	-	-
		CV%	8.1	142	-	-	15	-	-
125	Female	**Mean**	**8498**	**1.28**	**0.0210**	**33.2**	**160232**	**194703**	**17.4**
		SD	119	0.387	0.0014	2.13	24223	36404	2.9
		CV%	1.4	30.3	6.7	6.5	15	19	16.7
250	Female	**Mean**	**15465**	**0.83**	**0.0204**	**34.9**	**239567**	**291632**	**16.9**
		SD	1227	0	0.0041	6.3	52222	82128	5.7
		CV%	7.9	0	20.0	18.2	22	28	34.0
500	Female	**Mean**	**25298**	**0.83**	**0.0229**	**30.4**	**210410**	**237200**	**11.0**
		SD	928	0	0.0020	2.7	28787	41888	3.6
		CV%	3.7	0	8.9	8.9	14	18	32.7

ND = Not determined since estimation of a valid half-life is not possible without SFP administration. SD = Standard Deviation CV = Coefficient of Variation

(Excerpted from the Applicant's submission)

Dosing Solution Analysis

Results from dosing solution analysis indicated that the formulations were 77 to 97% of targets (81-95% for MTD Phase; 77-97% for CV Phase; 92-95% for TK Phase).

6 General Toxicology

6.1 Single-Dose Toxicity

Not reviewed

6.2 Repeat-Dose Toxicity

Study title: Soluble ferric pyrophosphate: A 26-week intravenous infusion toxicity study with a 4-week recovery period in Sprague Dawley rats

Study no.:	70009 ((b) (4)
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 13, 2006
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	Soluble ferric pyrophosphate (SFP); Batch #126412 (non-GMP and (b) (4) product); 12.0% iron by weight

Key Study Findings

- At the doses ≥30 mg/kg, early mortalities occurred within the 1st week; the doses for those study groups were reduced to ≤20 mg/kg initially on Day 9 and then to ≤10 mg/kg on Day 30.
- Dose-dependent increases in the amounts of element iron were noted in multiple tissues in all study groups, including mainly the cytoplasm of histiocytes, hepatocytes, Kupffer cells, renal tubular epithelium, and zymogen granules of the pancreas. Those increases of tissue storage remained at the end of the recovery phase.
- Dose-dependent increase in incidence and severity of Infusion-site inflammation was noted. These inflammatory findings remained at the end of the recovery phase.
- Group mean T_{max} values for iron were similar in all SFP-treated groups, and exposure to iron was similar in both genders. The AUC_{0-24} increased less than proportionally with dose levels, suggesting saturation of iron binding to transferrin and a rapid removal of unbound iron from serum with an enhanced iron distribution into tissues.
- The NOAEL of SFP in rats was 2 (20/10/2) mg/kg in this study.

Methods

Doses:	0, 10/5/0.5, 20/10/2, 30/15/5, and 40/20/10 mg/kg/day
Frequency of dosing:	3 times/week for 26 consecutive weeks (see deviations below for specific duration of revised dosages)
Route of administration:	Intravenous infusion (1-hour) at 5 mL/kg/hour
Dose volume:	Varied among dose groups
Formulation/Vehicle:	Lactated Ringer's Solution, USP (LRS)
Species/Strain:	Sprague-Dawley rats (Crl:CD (SDS))
Number/Sex/Group:	Main study: 10-11 Recovery Phase: 5-6 Toxicokinetic phase: 3-7
Age:	8 to 10 weeks (at treatment start)
Weight:	Males: 228 – 298 g Females: 171 – 234 g
Satellite groups:	See table below.
Unique study design:	See table below.
Deviation from study protocol:	Changes of doses: 1. Doses were reduced started on Day 9 to 0, 5, 10, 15 and 20 mg/kg, for Groups 1-5, due to toxicity noticed. 2. Doses were reduced started on Day 30 to 0, 0.5, 2, 5 and 10 mg/kg, for Groups 1-5, due to further toxicity noted. Replacement animals: Multiple spare rats were added to the study groups, as mortalities occurred within the first 5 days of dosing.

Reference ID: 3676761

Table 15 Study Design for the Rat Toxicity Study

Group No.	Group Name	Dose Level[#]		Dose Conc. (mg/mL)	Number of Animals**					
					Main Study		Recovery Phase		Toxicokinetic Phase	
		mg SFP/kg	mg Fe/kg*		M	F	M	F	M	F
1	Control @	0	0	0	10	10	5	5	3	3
2	Low Dose	10/5/0.5	1.17/0.59/0.06	2/1/0.1	10	10	5	5	6	6
3	Mid Dose	20/10/2	2.34/1.17/0.23	4/2/0.4	10	10	5	5	6	6
4	Mid High Dose	30/15/5	3.51/1.76/0.59	6/3/1	10	11	6	5	6	6
5	High Dose	40/20/10	4.68/2.34/1.17	8/4/2	10	10	5	5	7	6

@ Control animals received only Lactated Ringer's Solution.

As indicated above, because of the development of adverse clinical signs and mortality early in the study, the range of SFP dose levels initially administered (i.e., 10 through 40 mg SFP/kg) was reduced to 5 through 20 mg SFP/kg beginning on May 9, 2006 (Day 9 for Replicate A) and was further reduced to 0.5 through 10 mg SFP/kg beginning on May 30, 2006 (Day 30 for Replicate A). In order to keep the other dosing parameters (i.e., infusion rate and volume) unchanged, the SFP concentration in each dosing formulation was reduced appropriately with each reduction in the range of dose levels administered.

* SFP was 12.0% Fe by weight. The tabulated dose levels (expressed as iron) corresponding to the SFP dose levels were calculated on the basis of the iron concentration value (i.e., 11.7%) originally provided by the Sponsor.

** Because of deaths that occurred during the first 6 days of dosing, one spare male was added each to the Toxicokinetic Phase (5022F) and to the Recovery Phase (4022D) and one spare female was added to the Main Study (4522A). Each of these additional animals commenced dosing on the study day achieved by the corresponding decedent but was not categorized as a replacement animal. Accordingly, in each case, data generated for the decedent as well as its "additional" counterpart have been incorporated into this report.

Abbreviations: M = male; F = female

(Excerpted from the Applicant's submission)

Observations and Results

Table 16 Measurements Performed in Rat Toxicology Study

Mortality:	Once daily
Cage side clinical signs:	Once daily
Body weights:	Once weekly (from Week -1) and prior to scheduled sacrifice
Food consumption:	Qualitatively measured daily (from Week -1)
Electrocardiography (ECG waveforms - Leads I, II, III, aVR, aVL, and aVF): heart rate, PR interval, QRS duration, QT and QTc intervals	Pretest (Week -1), near the end of Week 26 (after the 3 infusions for that week), and near the end of the recovery period (Week 30)
Ophthalmoscopy:	Pretest, near the end of the treatment period (Week 26), and near the end of the recovery period (Week 30)
Hematology:	Pretest, Weeks 13, 26, and 30
Clinical chemistry:	Pretest, Weeks 13, 26, and 30
Coagulation:	Pretest, Weeks 13, 26, and 30
Urinalysis:	Pretest, Weeks 13, 26, and 30
Gross pathology:	At necropsy
Organ weights:	At necropsy
Histopathology:	At necropsy; on all tissue sections from main-study animals, and potential target organs (jejunum and

Reference ID: 3676761

	ileum with GALT, mesenteric and retropharyngeal lymph nodes, spleen, and thymus) and any gross lesions from recovery animals
Toxicokinetics: serum iron	For the first dose in Weeks 1, 9, 18, and 26 (Days 1, 57, 120, and 180): Before infusion began, at the end of infusion, and at 0.5, 3 and 24 hours after completion of the infusion (for Group 1) and at 0.5, 1, 3, 6, 26, and 47 hours after completion of the infusion (for Groups 2-5)

Mortality

Sixty-four (64) rats died or were euthanized early across all main-study and TK animals of all study groups (including controls). Some of the early mortalities were due to failing catheter or the catheter could not be surgically repaired. The gender-combined mortality rate for deaths from other causes (including bacteremia and/or organ failure following systemic spread of infection from the infusion sites) was 17%, 10%, 17%, 30%, and 26% at doses of 0, 10, 20, 30, and 40 mg/kg, respectively. These mortalities were predose, surgical procedure-related and possibly SFP dose-impacted (i.e., SFP affected on the recovery of surgical lesions). See table below.

Table 17 Early Mortality Tables in Rat Repeat-Dose Toxicity Study

	Males					Females				
Group Number =	1	2	3	4	5	1	2	3	4	5
Rats that died or were euthanized early (Main-study, space, then TK)*	1001A 1004A 1008B 1018F	2006B 2009B 2012C 2017E	3010B 3016E 3017E	4002A 4005A 4007B 4008B 4012C 4013D 4015D 4118E	5001A 5002A 5009B 5012C 5014D 5015D 5019F 5020F	1501A 1503A 1511C 1515D 1518F	2503A 2505A 2513C 2517E	3504A 3505A 3509B 3510B 3513D 3514D 3516E 3517E 3518E 3520F	4503A 4505A 4506B 4507B 4508B 4510B 4512C 4513C 4516E 4517E 4518E 4521F	5502A 5507B 5508B 5515D 5519F 5520F
Total rats on study	18	21	21	22	22	18	21	21	22	21
Number of deaths	4	4	3	8	8	5	4	10	12	6
Total										
Procedure-related**	1	3	1	0	2	2	2	5	7	1
Other causes	3	1	2	8	6	3	2	5	5	5
Mortality rate (%)										
Overall	22%	19%	14%	36%	36%	28%	19%	48%	55%	29%
Other causes***	17%	10%	10%	36%	27%	17%	10%	24%	23%	24%

* Numbers are the individual animal numbers of affected rats.

** Rats that were euthanized because the catheter became non-patent and catheter replacement surgery was no longer possible.

*** Excludes procedure-related deaths

(Excerpted from the Applicant's submission)

Clinical Signs

No drug-related signs of clinical toxicity were noted.

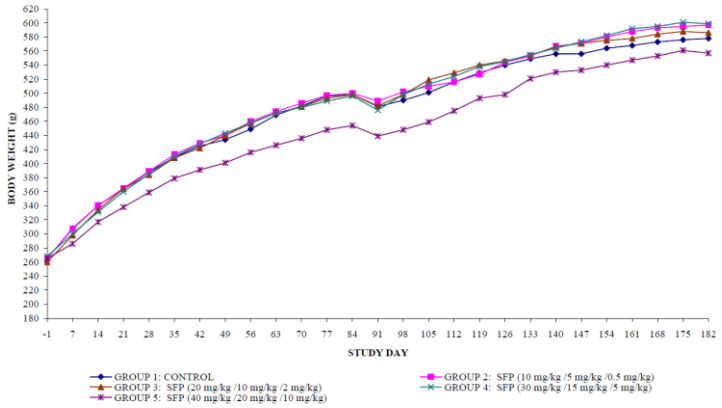
Pre-dose, surgical procedure-related and possibly SFP dose-impacted signs of toxicity noted included limited use of appendages, the presence of dry brown material around

the muzzle, dull/ungroomed/matted fur, fur thinning, partial ptosis, and various changes (including crusts, redness, swelling, scabs, lumps, wounds, and thickening) of the skin.

Body Weights

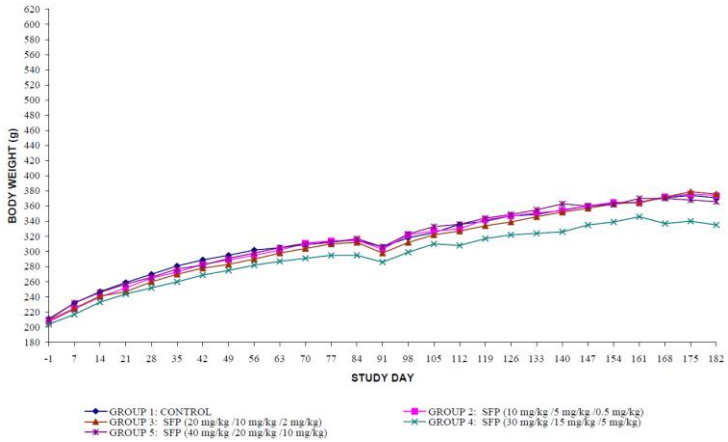
Decreases in body weight compared to controls were noted at week 13 (9%) in males at 40/20/10 mg/kg, which remained at the end of the recovery period (15%). The females were not affected.

Figure 17 Group Mean Body Weights in Male Rats



(Excerpted from the Applicant's submission)

Figure 18 Group Mean Body Weights in Female Rats



(Excerpted from the Applicant's submission)

Feed Consumption

Transient decrease in food consumption was noted in all SFP-treated groups with resolution within a few weeks within the treatment period, except for the females at dose ≥30/15/5 mg/kg for which the decrease persisted until the end of the treatment (≤ 17%). Food consumption did not change in the recovery period for other groups.

Ophthalmoscopy

unremarkable

Hematology

Slight increase in lymphocyte and monocyte and slightly decrease in erythron mass in males at doses ≥30 mg/kg. These findings were most likely secondary to the inflammation at the IV infusion sites and other tissues and impacted by SFP dose. The increased lymphocytes and monocytes remained at the end of the recovery phase. See table below.

Table 18 Group Mean Hematological Changes in Rats

Index	Mean Values			Percent deviation from Concurrent Control											
	Control 0 mg/kg			10/5/0.5 mg/kg			20/10/2 mg/kg			30/15/5 mg/kg			40/20/10 mg/kg		
	W13	W26	W30	W13	W26	W30	W13	W26	W30	W13	W26	W30	W13	W26	W30
Males															
RBC (10^6/µL)	9.45	9.28	9.72	-	-	-	-	-	-	-	11↓*	-	-	-	-
Hemoglobin (g/L)	161	156	173	-	-	-	-	-	-	-	15↓*	-	-	-	-
Hematocrit (L/L)	0.49	0.45	0.49	-	-	-	-	-	-	-	13↓*	-	-	-	-
Total WBC (10^9/L)	13.90	11.19	8.13	-	-	73↓	-	-	-	-	42↑	134↑	-	-	10↑
Neutrophil (10^9/L)	5.22	4.78	2.23	-	58↓	-	-	48↓	-	12↑	67↑	227↑	19↓	29↓	61↑
Monocyte (10^9/L)	0.65	0.41	0.33	-	-	12↓	-	-	19↓	-	98↑*	327↑	14↓	49↑	42↑
Females															
Neutrophil (10^9/L)	2.58	2.67	1.91	-	-	30↑	45↓	39↑	84↑	15↑	41↓	13↑	11↑	18↑	24↑
Monocyte (10^9/L)	0.60	0.36	0.27	-	11↓	19↑	18	31↑	130↑	-	33↓	-	23↓	14↑	26↑

W: Week; *: p<0.05; **: p<0.01

Clinical Chemistry

Slight increase in alkaline phosphatase and globulin and slight decrease of albumin were noted at doses ≥30 mg/kg. These changes were most likely secondary to inflammation at the infusion site and other tissues and SFP dose-impacted. See table below.

Table 19 Group Mean Clinical Chemistry Changes in Rats

Index	Mean Values			Percentage deviation from Concurrent Control											
	Control 0 mg/kg			10/5/0.5 mg/kg			20/10/2 mg/kg			30/15/5 mg/kg			40/20/10 mg/kg		
	W13	W26	W30	W13	W26	W30	W13	W26	W30	W13	W26	W30	W13	W26	W30
Males															
ALP (U/L)	98	69	77	-	23↑	26↓	-	13↑	-	-	194↑*	-	-	67↑	-
Globulin (g/L)	34	32	33	12↓	-	-	-	-	-	12↓	19↑	-	-	-	-
Albumin (g/L)	42	38	44	-	-	-	-	-	-	-	24↓*	-	-	-	-
Females															
ALP (U/L)	51	32	32	16↑	56↑	13↑	24↑	163↑	59↑	20↑	53↑	12↓	12↑	156↑	47↑

Index	Mean Values			Percentage deviation from Concurrent Control											
	Control 0 mg/kg			10/5/0.5 mg/kg			20/10/2 mg/kg			30/15/5 mg/kg			40/20/10 mg/kg		
	W13	W26	W30	W13	W26	W30	W13	W26	W30	W13	W26	W30	W13	W26	W30
Globulin (g/L)	28	29	31	-	14↑	-	25↑*	24↑*	19↑	14↑	24↑*	10↓	11↑	28↑*	-
Albumin (g/L)	49	44	50	-	-	-	14↓	14↓	14↓	14↓	11↓	12↑	-	11↓	-

W: Week; *: p<0.05; **: p<0.01

No changes in coagulation parameters were noted.

Urinalysis

unremarkable

Gross Pathology

Infusion-site inflammation was accompanied by secondary or reactive changes in tissue in rats that survived to the end of the treatment period and in early decedents. The incidence and severity of infusion site lesions remained elevated among drug-treated animals at the end of the recovery phase. See tables below.

Table 20 Gross Pathology Findings in Rats – At the End of Treatment Phase

Dose Level: mg/kg	**0**		**10/5/0.5**		**20/10/2**		**30/15/5**		**40/20/10**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	**7**	**8**	**8**	**8**	**9**	**6**	**6**	**5**	**7**	**7**
Lymph node										
Cyst	0	0	1	0	0	0	0	0	0	0
Dark area	0	0	0	0	1	0	0	0	0	0
Dark discoloration	0	0	1	0	0	0	0	0	1	0
Enlargement	0	0	3	1	1	0	3	1	1	0
Infusion site										
Dark material	0	0	0	0	0	0	0	1	0	1
Mass	1	1	3	2	6	5	5	4	4	5
Mass 1	0	0	0	0	0	0	0	0	1	0
Mass 2	0	0	0	0	0	0	0	0	1	0
Mottled	0	0	0	0	1	0	0	0	0	0
Pale material	3	4	4	4	2	1	1	0	1	0
Thickening	2	3	2	3	1	2	0	2	1	2

M – Male
F - Female

Table 21 Gross Pathology Findings in Rats - At the End of Recovery Phase

Dose Level: mg/kg	**0**		**10/5/0.5**		**20/10/2**		**30/15/5**		**40/20/10**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	**5**	**3**	**4**	**4**	**5**	**3**	**3**	**3**	**2**	**4**
Lymph node										

Enlargement	0	0	2	0	0	0	2	0	1	0
Skin and subcutis										
Dark discoloration	0	0	0	0	0	0	1	0	0	0
Mass	0	0	0	0	0	0	1	0	0	1
Thickening	1	0	0	0	0	1	0	0	0	0
M – Male F - Female										

Organ Weights

Moderate increases in spleen and slight increase in liver weights were noted. The group-mean spleen weights approximated to that of controls at the end of the recovery period. These findings of increased weight correlated with the accumulation of iron in spleen and liver were noted microscopically.

Table 22 Group Mean Organ Weight Changes in Rats

Index	Mean Values		Percentage deviation from Concurrent Control					
	Control 0 mg/kg		10/5/0.5 mg/kg	20/10/2 mg/kg	30/15/5 mg/kg		40/20/10 mg/kg	
	W26	W30	W26	W26	W26	W30	W26	W30
Males								
Spleen/Body Weight ratio	0.19	0.17	**26↑**	**26↑**	**74↑***	**71↑**	**42↑**	**29↑**
Females								
Spleen/Body Weight ratio	0.21	0.23	38↑	38↑	43↑*	13↑	43↑*	-

W: Week; recovery period data for 10/5/0.5 and 20/10/2 mg/kg groups are not listed due to table space limitation.
*: $p<0.05$; **: $p<0.01$

Histopathology

Adequate Battery: Yes

Peer Review: Not conducted

Histological Findings

Increases in incidence and amount of iron accumulation were noted in the cytoplasm of multiple tissues including mainly the hepatocytes, Kupffer cells, renal tubular epithelium, the zymogen granules of the pancreas, and spleen (see tables below). Occasionally, the same material was also noted in adrenal, brain, epididymis, bone marrow, heart, kidney, liver, pituitary, skin/subcutis, thymus and uterus in all study groups (including controls) with higher incidences and severities in SFP-treated groups. Brown pigment/material was stained positive with Perls' Prussian Blue stain method for iron. Iron accumulation remained at the end of the recovery period.

Table 23 Major Histopathology Findings in Rats - At the End of Treatment Phase

Dose Level: mg/kg	**0**	**10/5/0.5**	**20/10/2**	**30/15/5**	**40/20/10**

Sex	M	F	M	F	M	F	M	F	M	F
Number Examined	**7**	**8**	**8**	**8**	**9**	**6**	**6**	**5**	**7**	**7**
Liver										
Pigmentation	1	2	0	0	0	0	3	5	7	6
Kidney										
Pigmentation	0	0	0	0	0	0	0	3	0	4
Pigmentation, histiocytic	0	0	0	1	0	0	2	1	3	3
Spleen										
Pigmentation, histiocytic	7	8	2	1	2	1	6	5	7	7
Extramedullary hematopoiesis	0	0	1	0	1	1	2	1	1	0
M – Male F - Female										

Table 24 Major Histopathology Findings in Rats - At the End of the Recovery Period

Dose Level: mg/kg	0		10/5/0.5		20/10/2		30/15/5		40/20/10	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	**5**	**3**	**4**	**4**	**5**	**3**	**3**	**3**	**2**	**4**
Liver										
Pigmentation	1	0	0	0	0	0	1	0	1	4
Kidney										
Pigmentation	1	0	0	0	0	0	0	0	0	4
Pigmentation, histiocytic	0	1	0	0	1	0	2	0	0	1
Spleen										
Pigmentation, histiocytic	5	3	1	0	0	0	3	3	2	4
Extramedullary hematopoiesis	0	0	0	0	0	0	1	0	0	0
M – Male F - Female										

Special Evaluation

Toxicokinetics

Reviewer Comments: Though serum samples generated were measured for total iron, unbound iron binding capacity (UIBC), ferritin, transferrin concentration and transferrin saturation (TSat; %) for calculating the total iron binding capacity (TIBC), only the serum iron data were presented in the toxicokinetics report.

For Day 1 TK data, T_{max} occurred at the end of the infusion (data not shown). At doses ≥30 mg/kg, serum iron concentrations tended to be higher in males than females. Iron C_{max} and AUC_{0-25} values were higher in females than males for controls. The increase in iron C_{max} and AUC_{0-25} values in SFP-treated groups were less than proportional with dose and the increases from control values were greater for males than females. See table below.

Table 25 Exposure to Iron on Day 1 (10 to 40 mg/kg) for Rats

	Males					Females				
SFP dose (mg/kg) =	0	10	20	30	40	0	10	20	30	40
Fe dose (mg/kg) =	0	1.17	2.34	3.51	4.68	0	1.17	2.34	3.51	4.68
*HED (mg SFP/patient) =	0	97	195	292	389	0	97	195	292	389
C_{max} (mg/dL)	0.23	0.59	0.88	1.22	1.99	0.39	0.70	0.79	0.97	1.42
AUC_{0-25} (hr*mg/dL)	4.3	6.2	7.8	10.0	13.8	7.7	9.8	8.8	9.8	11.1

*HED = human equivalent dose calculated as per FDA guidance on selecting a safe starting dose level for the first clinical trial, assuming that rats = 6 kg/m^2, humans = 37 kg/ m^2, and average patient weight = 60 kg.

(Excerpted from the Applicant's submission)

After repeated dosing of SFP at 0.5, 2, 5, and 10 mg/kg three times per week up to 180 days in the study, iron C_{max} increased less than proportionally with SFP dose, and increase from control was greater for males than females but exposure to females was greater than males. T_{max} occurred within 5-60 minutes after infusion completion.

On Day 57, serum iron AUC_{0-25} increased less than proportionally with SFP dose, while on Days 120 and 180 the iron AUC_{0-25} were similar in all study groups. Iron C_{max} and AUC_{0-25} values generally decreased with repeated dosing. See table below.

Table 26 Exposure to Iron on Days 57, 120, and 180 (0.5 to 10 mg/kg) for Rats

	Males					Females				
SFP dose (mg/kg) =	0	0.5	2	5	10	0	0.5	2	5	10
Fe dose (mg/kg) =	0	0.06	0.23	0.59	1.17	0	0.06	0.23	0.59	1.17
*HED (mg SFP/patient) =	0	5	19	49	97	0	5	19	49	97
C_{max} (mg/dL)										
Day 57	0.27	0.31	0.49	0.63	1.00	0.39	0.43	0.72	0.81	0.73
Day 120	0.22	0.28	0.33	0.68	0.70	0.35	0.42	0.51	0.68	0.80
Day 180	0.22	0.22	0.29	0.65	0.67	0.33	0.32	0.29	0.40	0.76
AUC_{0-25} (hr*mg/dL)										
Day 57	4.9	3.8	4.2	5.3	5.9	8.7	7.6	9.1	8.0	9.6
Day 120	4.2	3.9	3.4	4.8	4.5	8.4	7.4	6.6	6.7	8.2
Day 180	4.4	3.4	3.6	3.9	4.3	7.2	6.0	6.0	6.5	6.8

*HED = human equivalent dose calculated as per FDA guidance on selecting a safe starting dose level for the first clinical trial, assuming that rats = 6 kg/m^2, humans = 37 kg/ m^2, and average patient weight = 60 kg.

(Excerpted from the Applicant's submission)

Dosing Solution Analysis

Dose analyses were conducted for samples collected in Weeks 1, 2, 5, 13, and 26. No iron was detected in the vehicle samples, and all low dose samples were below the limit of quantitation (LOQ). All Week 1, 2, 5, 13 and 26 samples were within 100±10% (90% - 99%) of the target concentrations or were below limit of quantitation (LOQ) with the progressive lowering of the dose levels.

Study title: Soluble ferric pyrophosphate: a 39-week intravenous infusion toxicity study with a 4-week recovery period in beagle dogs

Study no.:	60011 ((b) (4)
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 19, 2006
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	Soluble ferric pyrophosphate (SFP), Batch #126412 (non-GMP and (b) (4) product), 12.0% of iron by weight

Key Study Findings

- Early mortalities (2) occurred in the study with septicemia or chronic inflammation that involved multiple organs.
- Transient and reversible decreases in in body weight and body weight gain were noted.
- Tissue accumulation of iron was noted in hepatocytes, gallbladder, kidneys, heart, adrenals, Peyer's patches, ovaries, and infusion sites. Other drug-related findings included hepatic microgranulomas, minimal lymphoid hyperplasia of gallbladder mucosa, and minimal renal tubular basophilia. At the end of the recovery phase, iron accumulation, hepatic microgranulomas, and lymphoid hyperplasia in gallbladder remained.
- T_{max} for iron occurred near completion of the infusion. C_{max} and AUC_{0-25} increased less than proportionally with increasing dose during Weeks 1, 13, 26, and 39 and without gender difference. C_{max} and AUC_{0-25} values were similar at same dose levels in Weeks 1, 26, and 39; the Week 13 C_{max} and AUC_{0-25} values were slightly lower than other weeks.
- The lowest observed adverse effect level (LOAEL) for SFP is at 5 mg/kg.

Methods

Doses:	5, 10, 20, and 30 mg SFP/kg (0, 0.6, 1.2, 2.3, and 3.5 mg Fe/kg)
Frequency of dosing:	3 times per week for 39 consecutive weeks
Route of administration:	IV infusion in 1 hour
Dose volume:	5 mL/kg/hour (actual volume adjusted based on the most recent practical body weight of each dog)
Formulation/Vehicle:	Lactated Ringer's Solution, USP (LRS)
Species/Strain:	Beagle dogs
Number/Sex/Group:	Main Study: 4 Recovery Phase: 2 for Groups 1, 4, and 5 0 for Groups 2 and 3
Age:	6 – 8 months
Weight:	Males: 7.4 – 9.6 kg Females: 7.0 – 9.1 kg
Satellite groups:	None
Unique study design:	See table below
Deviation from study protocol:	1. For multiple animals, the dosing in the sixth month was administered through temporarily-placed catheter (instead of a centrally-placed catheter) due to catheter blockage at the infusion site (around the femoral veins). 2. Under-dosing occurred in multiple dogs at the first dosing in Week 26; they received doses at -10% to -33% of the target doses.

Table 27 Study Design for Dog Toxicology Study

Group Number	Group Designation	Dose Level		Dose Conc. (mg/mL)	Main Study		Recovery Phase	
		mg SFP/kg	mg Fe/kg*		Males	Females	Males	Females
1	Control	0	0	0	4	4	2	2
2	Low Dose	5	0.6	1	4	4	---	---
3	Mid Dose	10	1.2	2	4	4	---	---
4	Mid-High Dose	20	2.3	4	4	4	2	2
5	High Dose	30	3.5	6	4	4	2	2

*SFP was 12.0% Fe by weight.

(Excerpted from the Applicant's submission)

Observations and Results

Table 28 Measurement Performed in Dog Toxicology Study

Mortality:	At least once daily
Cage side clinical signs:	Once daily
Body weights:	Once weekly (from Week -1) and prior to scheduled sacrifice
Food consumption:	Qualitatively; at least once daily (from Week -1 and on)
Electrocardiography (ECG waveforms -	Pretest (Week -1), near the end of Week 39 (after the 3 infusions for that week), and near the end of the

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Leads I, II, III, aVR, aVL, and aVF): heart rate, PR interval, QRS duration, QT and QTc intervals	recovery period (Week 43)
Ophthalmoscopy:	Pretest, near the end of the treatment period (Week 39), and near the end of the recovery period (Week 43)
Hematology:	Pretest, Weeks 13, 39, and 43
Clinical chemistry:	Pretest, Weeks 13, 39, and 43
Coagulation:	Pretest, Weeks 13, 39, and 43
Urinalysis:	Pretest, Weeks 13, 39, and 43
Gross pathology:	At necropsy
Organ weights:	At necropsy
Histopathology:	At necropsy; on all tissue sections from main-study animals, and potential target organs (jejunum and ileum with GALT, mesenteric and retropharyngeal lymph nodes, spleen, and thymus) and any gross lesions from recovery animals
Toxicokinetics: serum iron	For the first dose in Weeks 1, 13, 26, and 39: Before infusion began, at the end of infusion, and at 0.5, 1, 3, 6, 24, and 48 hours after completion of the infusion
Synovial fluid:	At necropsy

Mortality

Two early mortalities occurred in the study; the causes of death were pre-dose, surgical procedure-related. One (5 mg/kg group male; 2002B) was found with septicemia and other (20 mg/kg group male; 4005E) with multiple chronic inflammatory processes, including chronic pleuritis, chronic interstitial pneumonia and chronic pericarditis.

Clinical Signs

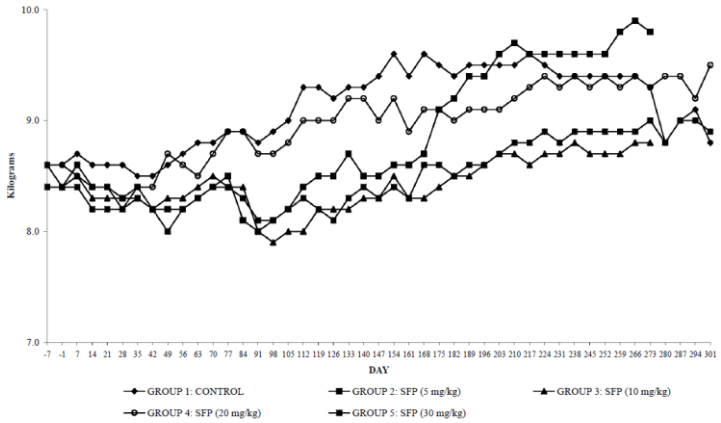
Signs of toxicity included thin body condition and variation in feces (color, consistency, composition, and amount), which correlated with the findings of body weight reduction.

Body Weights

No drug-related changes in body weight were noted in females. Drug-related, mild decreases of body weight were noted in males at doses ≥5 mg/kg (≤9%) between Weeks 12 and 15 (Days 84 and 105).

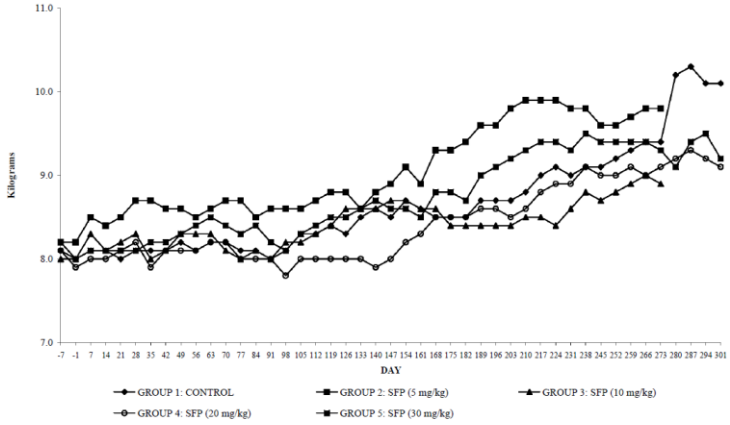
Reference ID: 3676761

Figure 19 Group Mean Body Weights of Male Dogs



(Excerpted from the Applicant's submission)

Figure 20 Group Mean Body Weights of Female Dogs



(Excerpted from the Applicant's submission)

Table 29 Group Mean Body Weight Changes in Dogs

Index	Mean			Percentage deviation from Concurrent Control									
	Control 0 mg/kg			5 mg/kg		10 mg/kg		30 mg/kg			20 mg/kg		
	W15	W39	W43	W15	W39	W15	W39	W15	W39	W43	W15	W39	W43
Males													
Body Weight (kg)	9.0	9.3	8.8	9↓	-	11↓	-	3↓	-	-	9↓	-	-
Females													
Body Weight (kg)	8.3	9.4	10.1	-	-	-	-	-	-	-	-	-	-

W: Week

Feed Consumption

Drug-related decreases in food consumption were noted in both genders at doses ≥5 mg/kg (≤15%). At the end of the recovery group, the food consumption for 30 mg/kg females remained lower than controls.

Table 30 Group Mean Food Consumption Changes in Dogs

	Mean Values	Percentage deviation from Concurrent Control

Index	Control 0 mg/kg			5 mg/kg		10 mg/kg		30 mg/kg			20 mg/kg		
	W13	W39	W43	W13	W39	W13	W39	W13	W39	W43	W13	W39	W43
Males													
Food Consumption (g)	335	310	354	15↓	-	-	-	306	-	-	11↓	-	-
Females													
Food Consumption (kg)	328	308	347	11↓	-	-	-	23↓	20↓	26↓	-	15↓	-

W: Week

Ophthalmoscopy

unremarkable

ECG

unremarkable

Hematology

Increases in neutrophil and monocyte and slight and transient decreases in erythron mass (RBC, Hemoglobin, and hematocrit) were noted in all SFP-treated groups of animals. The neutrophil and monocyte changes last to the end of treatment phase, suggested a mainly subchronic inflammation in the infusion sites. Those changes were mostly comparable to controls at the end of the recovery phase.

Table 31 Procedure-Related Changes in Hematology in Dog Study

Index	Mean Values			Percentage deviation from Concurrent Control									
	Control 0 mg/kg			5 mg/kg		10 mg/kg		30 mg/kg			20 mg/kg		
	W13	W39	W43	W13	W39	W13	W39	W13	W39	W43	W13	W39	W43
Males													
RBC (10^6/µL)	5.99	6.61	6.72	-	-	15↓	-	-	-	-	-	-	-
Hemoglobin (g/L)	134	151	148	-	-	17↓	-	-	-		-	-	-
Hematocrit (L/L)	0.40	0.43	0.43	-	-	17↓	-	-	-	-	-	-	-
Total WBC (10^9/L)	12.07	10.51	10.85	77↑	-	-	18↓	-	-	23↓	66↑	-	-
Neutrophil (10^9/L)	8.37	6.81	7.44	94↑	15↓	128↑	24↓	-	-	35↓	91↑	-	-
Monocyte (10^9/L)	0.71	0.61	0.58	110↑	25↓	113↑	10↑	24↑	20↑	43↓	69↑	13↑	-
Females													
RBC (10^6/µL)	6.38	6.73	5.99	-	-	17↓	-	18↓	-	15↑	13↓	-	-
Hemoglobin (g/L)	141	153	136	-	-	21↓	-	19↓	-	15↑	13↓	-	-
Hematocrit (L/L)	0.42	0.44	0.39	-	-	19↓	-	19↓	-	15↑	14↓	-	-
Total WBC (10^9/L)	14.89	10.93	11.79	-	23↓	31↑	-	36↑	-	-	27↑	-	11↓
Neutrophil (10^9/L)	10.51	6.73	7.30	-	20↓	38↑	-	45↑	-	-	37↑	-	-
Monocyte (10^9/L)	0.92	0.43	0.63	28↑	-	64↑	42↑	73↑	44↑	32↓	21↑	-	38↓

W: Week

Clinical Chemistry

unremarkable

Urinalysis

unremarkable

Gross Pathology

Dose-dependent dark/discoloration/mottling of lymph nodes were noted in animals in all study groups (including controls). For the two early-mortality male dogs, the findings suggested inflammation involved multiple organs. See tables below.

No calcium pyrophosphate crystals were noted from the synovial fluid smears collected at necropsy from each of the relevant dogs (data not presented).

Table 32 Gross Pathology at the End of Dosing Phase in Dogs

Dose Level: mg/kg	0		5		10		20		30	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Lymph node										
Dark area	0	0	0	0	0	0	0	0	0	1
Dark discoloration	1	0	1	0	2	3	4	3	4	2
Enlargement	0	1	0	1	0	0	1	0	1	0
Mottled	0	0	1	1	1	1	0	1	0	1
Infusion site										
Constriction	1	1	0	1	1	0	1	2	1	2
Dark area	3	2	1	2	2	2	3	1	1	2
Dark material	1	0	0	1	0	1	0	0	1	1
Mass	0	0	0	2	0	0	0	0	1	1
Mottled	0	0	1	0	1	1	1	0	0	0
Pale material	0	0	1	0	1	1	1	1	2	1
Thickening	2	1	3	1	2	4	3	1	3	1
M – Male F - Female										

Table 33 Gross Pathology at the End of Recovery Phase in Dogs

Dose Level: mg/kg	0		20		30	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Number Examine	**2**	**2**	**1**	**2**	**2**	**2**
Lymph node						
Dark area	0	0	0	2	2	2
Enlargement	1	1	0	0	0	0
Mottled	0	0	1	0	1	0
Infusion site						
Constriction	0	1	0	0	1	1
Dark discoloration	0	0	0	0	1	1
Dark material	0	0	0	1	0	0
Mottled	0	0	0	0	0	1
Pale discoloration	0	0	0	0	1	0
Thickening	1	1	0	1	2	2

Dose Level: mg/kg	0		20		30	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Number Examine	**2**	**2**	**1**	**2**	**2**	**2**
M – Male F - Female						

Table 34 Gross Pathology in Early Mortality Dogs

Tissue / Observation	Group: / Sex: / Number:	2 M 1	4 M 1
AORTA			
DARK MATERIAL		0	1
COLON			
DARK AREA		0	1
ESOPHAGUS			
DARK MATERIAL		0	1
HEART			
DARK AREA		0	1
PALE DISCOLORATION		0	1
THICKENING		0	1
INFUSION SITE			
THICKENING		1	1
LUNG			
ADHESION		1	1
DARK AREA		0	1
DARK DISCOLORATION		1	0
MASS		0	1
SOFT		1	0
LYMPH NODE			
DARK DISCOLORATION		1	1
ENLARGEMENT		1	1
PERICARDIUM			
DARK DISCOLORATION		0	1
DARK MATERIAL		0	1
THICKENING		0	1
PEYER'S PATCH			
DARK DISCOLORATION		0	1
RECTUM			
DARK AREA		0	1
SPLEEN			
PALE DISCOLORATION		0	1
SMALL		1	1
SKIN & SUBCUTIS			
SCAB		0	1
WOUND		0	1

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THORACIC CAVITY		
DARK MATERIAL	0	1
DARK FLUID	1	0
OPAQUE FLUID	0	1
PALE MATERIAL	1	1
THICKENING	1	0
THYMUS		
NOT FOUND	1	0
DARK DISCOLORATION	0	1
DARK MATERIAL	0	1
URINARY BLADDER		
DARK AREA	0	1

(Excerpted from the Applicant's submission)

Organ Weights

Dose-dependent increases in liver weight were noted in animals of both genders at doses ≥10 mg/kg; the increase remained at the end of the recovery period. This increase in liver weights was most probably related to hepatocellular iron accumulation and the incidence of hepatic microgranulomas.

Table 35 Group Mean Relative Organ Weight to Body Weight Ratios in Dogs

Index	Mean Values		Percentage deviation from Concurrent Control					
	Control 0 mg/kg		5 mg/kg	10 mg/kg	20 mg/kg		30 mg/kg	
	W40	W43	W40	W40	W40	W43	W40	W43
Males								
Liver/Body Wt. Ratio (100%)	3.09	3.07	-	16↑	17↑	19↑	24↑	33↑
Females								
Liver/Body Wt. Ratio (100%)	3.83	3.10	27↓	14↓	-	10↑	-	20↑

W: Week; *: $p<0.05$

Histopathology
Adequate Battery: Yes; see table below.

Table 36 List of Tissues Weighed and/or Examined in the Dog Toxicology Study

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

ORGANS/TISSUES	Retain (•)	Weigh (√)	Examine (€)
Small intestine, duodenum	•		€
Small intestine, jejunum	•		€
Small intestine, ileum	•		€
Spinal cord (cervical)	•		€
Spleen	•	√	€
Sternum + marrow	•		€
Stomach	•		€
Testes	•d	√	€
Thymus	•	√	€
Thyroid lobes + parathyroids	•	√	€
Tongue	•		€
Trachea	•c		€
Urinary bladder	•		€
Uterus	•	√	€
Vagina	•		€
Abnormal findings	•		€
Additional Tissues presented below			
Infusion site	•		€
Tarsal/metatarsal joint (left)	•		€
Stifle and coxofemoral joints (both sides)	•		

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

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

(Excerpted from the Applicant's submission)

Peer Review: Not conducted

Histological Findings

Microscopic findings included iron accumulation in multiple organs (≥5 mg/kg), dose-dependent increase in incidence and severity of hepatic microgranuloma, minimal lymphoid hyperplasia of the gallbladder mucosa (≥20 mg/kg), and minimal renal tubular basophilia (at 30 mg/kg). All findings remained at the end of the recovery period, except for renal tubular basophilia.

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The organs with iron accumulation included adrenal, bone marrow, spleen, lungs, lymph nodes, pancreas, mammary gland, liver (Kupffer cells), and ovary in animals of all study groups (including controls) by Perls' Prussian Blue stain method. Drug-related iron accumulation was noted in hepatocytes, gallbladder, kidneys, heart, adrenals, Peyer's patches (data not presented), ovaries, and infusion sites. The iron pigment accumulation remained at the end of the recovery period.

Other findings included minimal lymphoid hyperplasia in gallbladder, renal tubular basophila, and inflammation at injection sites. At the end of recovery phase, most findings remained except for renal tubular basophilia.

Table 37 Histopathology Findings in Dogs - At the End of Treatment

Dose Level: mg/kg	**0**		**5**		**10**		**20**		**30**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Liver										
Microgranuloma	1	0	1	1	0	2	0	0	0	0
Pigment, brown	0	0	3	1	4	4	4	4	4	4
Gall bladder										
Lymphoid hyperplasia, minimal	0	0	0	0	0	0	1	0	0	1
Kidney										
Tubular basophilia, minimal	0	0	0	0	0	0	0	0	0	2
Pigment, brown	0	0	0	0	1	2	0	2	1	2
Bone marrow (femur)										
Pigment, brown	4	3	3	4	4	4	4	4	4	4
Spleen										
Pigment, brown	1	2	1	4	4	3	4	4	4	4
Lung										
Pigment, brown	0	0	1	0	3	3	3	2	2	1
Lymph nodes										
Pigment, brown	1	0	2	1	3	4	4	4	4	4
Pancreas										
Pigment, brown	0	0	0	0	0	0	0	0	2	0
Mammary gland										
Pigment, brown	0	1	0	1	0	3	0	2	0	4
Adrenal										
Pigment, brown	0	0	0	0	0	0	0	0	1	0
Ovary										
Pigment, brown	-	0	-	0	-	0	-	0	-	2
Infusion site										
Constriction	1	1	0	1	1	0	1	2	1	2
Dark area	3	2	1	2	2	2	3	1	1	2
Dark material	1	0	0	1	0	1	0	0	1	1
Mass	0	0	0	2	0	0	0	0	1	1
Mottled	0	0	1	0	1	1	1	0	0	0

Dose Level: mg/kg	0		5		10		20		30	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number Examined	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Pale material	0	0	1	0	1	1	1	1	2	1
Thickening	2	1	3	1	2	4	3	1	3	1
M – Male F - Female										

Table 38 Histopathology Findings in Dogs - At the End of the Recovery Phase

Dose Level: mg/kg	0		20		30	
Sex	**M**	**F**	**M**	**F**	**M**	**F**
Number Examine	**2**	**2**	**1**	**2**	**2**	**2**
Liver						
Microgranuloma	0	0	1	2	2	2
Pigment, brown	0	0	1	2	2	2
Gall bladder						
Lymphoid hyperplasia, minimal	0	0	1	0	0	1
Kidney						
Tubular basophilia, minimal	0	0	0	0	0	0
Pigment, brown	0	0	1	0	1	1
Bone marrow (femur)						
Pigment, brown	2	2	1	2	2	2
Spleen						
Pigment, brown	1	0	1	2	2	2
Lung						
Pigment, brown	0	0	1	2	1	1
Lymph nodes						
Pigment, brown	0	0	1	2	2	2
Pancreas						
Pigment, brown	0	0	0	0	0	0
Mammary gland						
Pigment, brown	0	0	0	1	0	2
Adrenal						
Pigment, brown	0	0	0	0	0	0
Ovary						
Pigment, brown	-	0	-	1	-	2
Infusion site						
Phlebitis, fibrosis	0	2	1	2	2	2
Microgranuloma	1	0	1	1	0	0
Pigment, brown	0	0	0	0	0	2
Cellular infiltrate, mononuclear	0	0	0	1	1	0
M – Male F - Female						

Toxicokinetics

Reviewer's Note: Though serum samples were measured for total iron, unbound iron binding capacity (UIBC), ferritin, transferrin concentration and transferrin saturation (TSat; %) for calculating the total iron binding capacity (TIBC), only the serum iron concentration results were presented in the toxicokinetics report.

Serum iron concentrations tended to increase with SFP dose level, and there were no gender differences. Group mean T_{max} for serum iron wee similar among all SFP dose groups and occurred near completion of the 1-hour infusion. Serum concentrations of iron were slightly higher during Week 1 compared to later time points at doses of 20 and 30 mg/kg SFP. C_{max} and AUC_{0-25} values were similar at similar dose levels in Weeks 1, 26, and 39; those values were lower in Week 13. The apparent elimination half-life ($t_{½}$) was not estimated due to insufficient data in the terminal elimination phase.

Serum exposure to iron increased less than proportionally to the increase in dose level, which suggested saturation of iron binding to transferrin and a rapid removal of unbound iron from the serum and movement into cells.

Table 39 Toxicokinetics of SFP in Dogs

	Males				Females			
Group Number =	2	3	4	5	2	3	4	5
Dose Level (mg SFP/kg) =	5	10	20	30	5	10	20	30
*HED (mg SFP/patient) =	162	324	649	973	162	324	649	973
C_{max} (µg/dL)**								
Week 1	420	550	880	1140	420	530	800	1210
Week 13	340	460	690	850	390	470	570	1020
Week 26	450	530	730	920	470	530	710	960
Week 39	490	550	820	1150	470	600	850	1110
$AUC_{(0-25)}$ (h*µg/dL)***								
Week 1	5500	6500	8800	11000	5300	6300	7500	11000
Week 13	3000	3900	6500	7900	4200	4400	5300	9300
Week 26	5000	5900	6500	8800	6100	5200	7400	8600
Week 39	6100	6000	7800	9600	5000	6400	8600	10200

*HED = human equivalent dose calculated as per FDA guidance on selecting a safe starting dose level for the first clinical trial, assuming that dogs = 20 kg/m^2, humans = 37 kg/ m^2, and average patient weight = 60 kg.

**Rounded to the nearest 10 µg/dL.

***Rounded to the nearest 100 h*µg/dL.

(Excerpted from the Applicant's submission)

Dosing Solution Analysis

Dose analyses were conducted for samples collected in Weeks 1, 13, 26, and 39. No iron was detected in the vehicle samples, and all low dose samples were below the limit of quantitation (LOQ). All other SFP samples were within 100±10% (88%-102%) of the target concentrations, except for Week 1 mid dose formulation which was 88% of the target concentration.

Reference ID: 3676761

7 Genetic Toxicology

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

Study title: Evaluation of soluble ferric pyrophosphate in the *Salmonella typhimurium-Escherichia coli* reverse mutation (Ames) assay

Study no.:	AMS00705
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 22, 2005
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	SFP, Lot # 125140, ≥98%

Key Study Findings

SFP was negative for mutagenicity in the *Salmonella typhimurium-Escherichia coli* reverse mutation assay in the presence and absence of mammalian metabolic activation system (S9) under the conditions tested.

Reference ID: 3676761

Methods

Strains:	*Salmonella typhimurium* Strains TA98, TA100, TA1535, TA1537 *Escherichia coli Strain* WP2*uvr*A
Concentrations in definitive study:	0, 0.5, 5, 50, 500, 5000 µg SFP/plate (0, 0.06, 0.6, 5.9, 58.5, 585 µg Fe/plate)
Basis of concentration selection:	Chosen according to cytotoxicity results (under maximum tolerated concentration (MTC))
Negative control:	Saline (0.9%)
Positive control:	See table below for list of positive controls used
Formulation/Vehicle:	Dimethyl sufoxide (DMSO) for all positive controls
Incubation & sampling time:	48 to 72 hours; sampling time not specified

Table 40 Test Strain Genotypes and Mutations

TESTER STRAIN GENOTYPES and MUTATIONS						
Tester Strain	*his*[a] Mutation	Additional Mutations		R-factor Plasmid		Type of Mutation
		Repair	LPS[b]			
TA1537	*hisC3076*	*uvr*B[c]	rfa	–	–	Frame shift (GC)
TA98	*his*D3052	*uvr*B[c]	rfa	pKM101	–	Frame shift (GC)
TA100	*his*G46	*uvr*B[c]	rfa	pKM101	–	Base pair substitution (GC)
WP2uvrA	*TrpE65*	*uvr*A[c]	–	–	–	Base pair substitution (AT)
TA1535	*his*G46	*uvr*B[c]	rfa	–	–	Base pair substitution (GC)

Abbreviations: *his* = Histidine; LPS = lipopolysaccharide

[a] = Histidine mutation incorporated
[b] = LPS mutation at the lac locus for deficiency in bacterial cell wall LPS barrier
[c] = Ultraviolet light damage repair gene B or A (*uvr*B or *uvr*A) deletion

(Excerpted from the Applicant's submission)

Table 41 Positive Controls Used in the Ames Assays

Positive Controls		
Tester Strain	With S9 mix	Without S9 mix
TA1537	2.5 µg 2-AMN in DMSO	50 µg 9-AA in DMSO
TA98	2.5 µg 2-AMN in DMSO	20 µg 2-NF in DMSO
TA100	2.5 µg 2-AMN in DMSO	5 µg SA in DMSO
WP2*uvr*A	25 µg 2-AMN in DMSO	1 µg 4-NQO in DMSO
TA1535	2.5 µg 2-AMN in DMSO	5 µg SA in DMSO

Abbreviations:
9-AA = 9-Aminoacridine; SA = Sodium azide; 2-NF = 2-Nitrofluorene; 2-AMN = 2-Aminoanthracene; 4-NQO = 4-Nitroquinoline 1-oxide

(Excerpted from the Applicant's submission)

Study Validity

- Cytotoxicity was defined as a decrease in the number of colonies and/or background lawn density compared to vehicle control.

- A cytotoxicity test was conducted to determine the maximum-tolerated concentration (MTC) of SFP. MTC was defined as the highest concentration that resulted in a ≤50% decrease in the number of colonies with a normal or slight decrease in the background lawn.
- Concentrations that decreased the number of colonies by more than 50% were considered the MTC.
- In the absence of toxicity or solubility limits, the MTC did not exceed 5000 µg/plate.
- The colony numbers in the vehicle control were within the following range (±10%)
- Positive controls showed a significant increase (>2-fold) compared to the vehicle control in the same test strain in the presence and the absence of S9.
- Positive Response Determination:
  a. When a concentration-related increase in revertants was observed in which the number of revertants exceed the control value by at least 2-fold (in Strains TA98, TA100, or WP2uvrA) or at least 3-fold (in Strains TA1535 or TA1537) for at least two successive concentrations.
  b. When the above criteria were met for only one concentration, the determination of a positive response was made on the basis of scientific judgment relative to the quality of the concentration response finding.
- Negative Response Determination: When the criteria for a positive response were not met and the conditions for a valid assay were satisfied.
- The cultures of all 5 tester strains used in the assays had an optical density value from 1.007 to 1.333, which indicated that the bacteria used were in the log growth phase.
- The sterility test conducted with the vehicle, top agar, DMSO and S9 mixture showed no contamination.
- The number of revertants observed for the vehicle control was in the acceptable range for each tester strain and in the historical range in each tester strain.
- The positive controls produced the anticipated response relative to the historical range in each tester strain.

Results

Cytotoxicity Tests: No cytotoxicity was observed at the concentration of 0, 5, 50, 500, and 5000 µg SFP/plate (0, 0.06, 0.6, 5.9, 58.5, and 585 µg Fe/plate), based on the findings of normal colony numbers (revertants) and a normal background lawn in all bacteria strains tested.

Definitive Tests: At concentrations of 312.5 to 5,000 µg SFP/plate (37 to 585 µg Fe/plate) in the presence and absence of S9, SFP did not induce an increase in the mean number of revertants per plate in any of the test strains, when compared with the respective positive controls.

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Table 42 Acceptable ranges of the colony number in the vehicle control for tests to be validated

Tester Strains	With or Without S9
TA98	20 - 50
TA100	75 - 200
TA1535	5 - 20
TA1537	2 - 25
WP2*uvr*A	5 - 40

(Excerpted from the Applicant's submission)

Table 43 Summary of Definitive Ames Assay Results

	Mean Revertants Per Plate with Standard Deviation									
	TA1537		TA98		TA100		WP2*uvr*A		TA1535	
Dose/Plate	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Revertant Colony Growth With Metabolic Activation [a]										
Vehicle Control	5.3	0.38	43.3	5.60	161.0	1.53	31.3	5.54	13.3	1.64
Test Article 312.5 µg	7.3	1.64	33.3	6.05	152.7	7.24	33.7	2.71	16.0	8.50
625 µg	8.0	0.58	36.3	1.26	131.3	20.62	26.0	4.04	15.7	0.77
1250 µg	5.7	0.19	37.3	8.66	115.0	20.00	30.5	1.06	10.3	4.53
2500 µg	6.7	1.64	38.0	8.89	133.0	5.00	36.3	2.22	9.7	4.02
5000 µg	4.7	0.69	32.3	2.50	153.0	6.81	35.3	2.99	12.3	5.50
Positive Control [b]	56.3	15.75	302.7	90.51	660.0	219.61	270.7	65.46	36.7	9.10
Revertant Colony Growth Without Metabolic Activation [a]										
Vehicle Control	5.7	0.51	27.7	2.50	123.0	6.56	30.3	2.01	9.7	0.69
Test Article312.5 µg	7.0	1.15	22.3	0.69	119.0	4.36	31.0	3.61	7.3	1.50
625 µg	7.7	1.26	19.0	5.00	114.7	11.28	30.7	2.99	8.0	2.08
1250 µg	8.7	0.19	21.7	3.53	108.7	4.07	31.7	0.69	9.3	1.50
2500 µg	6.7	0.38	23.3	1.26	105.0	8.62	29.7	1.17	8.3	0.51
5000 µg	7.0	0.58	19.0	6.11	109.0	12.06	33.0	4.51	7.3	1.50
Positive Control [c]	87.7	8.78	773.3	71.02	613.3	56.02	474.7	36.40	397.3	44.17

[a] Background Lawn Normal unless otherwise denoted, P=Precipitate, T=Thinning, A=Absent

[b]

TA1537	2-Aminoanthracene	2.5 µg/plate	TA1537	9-Aminoacridine	50 µg/plate
TA98	2-Aminoanthracene	2.5 µg/plate	TA98	2-Nitrofluorene	20 µg/plate
TA100	2-Aminoanthracene	2.5 µg/plate	TA100	Sodium azide	5 µg/plate
WP2*uvr*A	2-Aminoanthracene	25 µg/plate	WP2*uvr*A	4-Nitroquinoline 1-oxide	1 µg/plate
TA1535	2-Aminoanthracene	2.5 µg/plate	TA1535	Sodium azide	5 µg/plate

(Excepted from the Applicant's submission)

Concentration Verification

Results from the concentration formulation analyses indicated that the SFP solutions were all within acceptable range (±15%), except for the 3.15 mg/mL sample (171% higher than the target concentrations (in terms of Fe).

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In vitro mammalian chromosome aberration assay in Chinese hamster ovary cells challenged with soluble ferric pyrophosphate

Study no.:	CAB00705
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 8, 2005
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	SFP, Lot # 125140, ≥98%

Key Study Findings

SFP was negative for chromosome aberration in Chinese hamster ovary (CHO) cells exposed to up to or close to the maximum-tolerated concentration (MTC) without metabolic activation, and was positive with metabolic activation.

Methods

Cell line:	CHO cells
Concentrations in definitive study:	1. Without S9 a. 4 hours: 312.5, 625, 1250, 2500, 5000 µg/mL b. 19 hours: 3.12, 6.25, 12.5, 25, 50 µg/mL 2. With S9 4 hours with S9: 18.75, 37.5, 75, 150, 300 µg/mL
Basis of concentration selection:	Chosen according to cytotoxicity results (concentrations under MTC)
Negative control:	Saline (0.9% sodium chloride)
Positive control:	Without S9: Mitomycin C (MMC; 0.3 µg/mL) With S9: Cyclophosphamide monohydrate (CP; 15 µg/mL)
Formulation/Vehicle:	Saline for SFP Sterile purified water for positive controls
Incubation & sampling time:	Without S9: 4 and 19 hours; With S9: 4 hours

Study Validity

The sterility test, cell growth status, results of vehicle and positive controls, and concurrent cytotoxicity test were used to evaluate the study validity and the results are summarized below:

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- The concentration verification results of the dosing solutions were within acceptable ranges for the target concentrations, except for the 4 hr-S9 500, 250, and 31.25 mg/mL (which were 87, 83, and 84% to the target concentrations, respectively).
- The CHO cells used were from Passage Numbers 11 to 20 and had a normal growth rate and without contamination in the cell cultures.
- Chromosome aberrations in the CHO cells from the vehicle control group were in the acceptable low ranges in both the presence and absence of S9.
- There was statistically significant increase in aberrant cells in positive controls compared to vehicle controls in both the presence and absence of S9, which indicated a normal S9 activity.
- The concurrent cytotoxicity assay results indicated that the highest suppression of cell number still approximated a 50% decrease, supporting the acceptability of both the 4- and 19-hour assay.

Results

Cytotoxicity assays (initial and repeated) were first performed to determine the MTC. CHO cells were exposed up to 5000 µg/mL for 4 hours with and without S9 and for 19 hours without S9, and CHO cells then evaluated for growth and survival. Without S9, the MTC of SFP for CHO cells was 5000 µg/mL for the 4-hour assays (63% of relative growth) and was 50 µg/mL for the 19-hour assays (37.8% of relative growth). With S9, the MTC of SFP was 100 µg/mL (39% of relative growth) for the 4-hour assay. See tables below.

Table 44 Cytotoxicity Results in Chromosome Aberration Test – Initial Testing

SFP (µg/mL)	% Relative Growth		
	4 Hour With S9	4 Hour Without S9	19 Hour Without S9
0	100	100	100
9.8	NA	NA	83.7
19.5	NA	NA	57.7
39.1	NA	NA	51.0
78.1	NA	NA	47.1
156.25	54.9	103	46.2
312.5	40	96.4	50
625	40	90.5	30.8
1250	19.2	74.1	26
2500	22.7	75.9	26
5000	14.4	63.2	4.8

NA = not applicable

(Excerpted from the Applicant's submission)

Table 45 Cytotoxicity Results in Chromosome Aberration Test – Repeated Testing

SFP (µg/mL)	% Relative Growth	
	With S9	Without S9
	4-Hour Exposure	
0	100	100
18.75	80.0	NA
37.5	78.5	NA
75	68.9	NA
150	45.2	NA
300	23.7	NA
312.5	NA	120.6
625	NA	92.4
1250	NA	78.2
2500	NA	72.3
5000	NA	63.0
	19-Hour Exposure	
0	NA	100
3.125	NA	66.7
6.25	NA	61.3
12.5	NA	57.7
25	NA	48.6
50	NA	37.8

NA = not applicable

(Excerpted from the Applicant's submission)

Table 46 Cytotoxicity Results in Chromosome Aberration Test - Refined Testing

SFP (µg/mL)	% Relative Growth
	4 Hour With S9
0	100
6.25	143
12.5	76.8
25	66.1
50	53.5
100	39.5
200	31
400	10.8

(Excerpted from the Applicant's submission)

The definitive assay was performed to assess clastogenicity. In this assay, CHO cells were exposed up to 150 µg/mL for 4 hours with S9, up to 5000 µg/mL for 4 hours without S9, and up to 25 µg/mL for 19 hours without S9. SFP was negative for chromosome aberration in CHO cells exposed at up to 5000 µg/mL for 4 hours without metabolic activation and up to 25 µg/mL for 19 hours, and was positive at

concentrations as low as 37.5 µg/mL with the presence of metabolic activation (see tables below).

Table 47 Summary of Chromosome Aberration Assay Results

SFP	Cells Scored	Total Aberrant Cells		Total Aberrations	
(µg/mL)	(n)	(n)	(%)[a]	(n)	(%)[b]
4-Hour Exposure With S9					
0	200	1	0.5	2	1
37.5	200	14*	7	25	14.5
75	200	31*	15.5	79	39.5
150	200	34*	17	76	38
CP (15)	125	86*	68.8	202	161.6
4-Hour Exposure Without S9					
0	200	0	0	0	0
1250	200	3	1.5	3	1.5
2500	200	0	0	0	0
5000	200	2	1	2	1
MMC (0.3)	100	63*	63	137	137
19-Hour Exposure Without S9					
0	200	5	2.5	6	3
6.25	200	3	1.5	3	1.5
12.5	200	7	3.5	8	4
25	200	4	2	4	2
MMC (0.3)	100	83*	83	243	243

Abbreviations: CP = cyclophosphamide monohydrate, MMC = Mitomycin C.
[a] Total cells with aberrations/total number of cells scored times 100.
[b] Total number aberrations/total number of cells scored times 100.
* Statistically significant at level of 0.05; p = 0.000

(Excerpted from the Applicant's submission)

Table 48 Results of Statistical Analysis (Cochran-Armitage Test) for Chromosome Aberration Assay

	Probability of Trend Test	
	Treatment vs. Control	Positive Control vs. Negative Control
4 hour with S9	0.000*	0.000*
4 hour without S9	0.274	0.000*
19 hour without S9	0.459	0.000*

* Statistically significant at level of 0.05

(Excerpted from the Applicant's submission)

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

Study title: Evaluation of soluble ferric pyrophosphate in the mouse bone marrow micronucleus assay

Study no:	MNA00505
Study report location:	4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 21, 2005
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	SFP, Lot # 125140, 11.7% iron (≥98%)

Key Study Findings

SFP was negative for clastogenicity when given intravenously to ICR mice of both genders at up to the maximum tolerated dose level of 200 mg/kg (approximately 23 Fe/kg) twice.

Methods

Doses in definitive study:	0, 100, 200, and 400 mg/kg (0, 12, 23, 47 mg Fe/kg, respectively)
Frequency of dosing:	2 consecutive days
Route of administration:	Intravenous administration
Dose volume:	10 mL/kg
Formulation/Vehicle:	Saline (0.9% NaCl)
Species/Strain:	ICR mice
Number/Sex/Group:	5
Satellite groups:	None
Basis of dose selection:	Based on the results of a dose-range-finding assay conducted earlier. The highest dose was approximately 80% of the MLD, and the lower doses were ½ and ¼ of the MLD.
Negative control:	Saline (0.9% NaCl)
Positive control:	Cyclophosphamide monohydrate (CP)

Study Validity

- A treatment group (400 mg/kg) was excluded from final data analysis when more than 40% of animals died or displayed bone marrow toxicity with a normochromic to polychromic erythrocyte (PCE/NCE) ratio of <0.3.
- At least two treatment groups (100 and 200 mg/kg) per gender did not show excessive toxicity (PCE/NCE ratios ≥0.3).
- For a Positive Response:

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a. The level of micronucleated PCE (MPCE) frequency in the treatment group is significantly greater than the concurrent vehicle control group, and
b. An increase in MPCE frequency occurred in a dose-dependent manner in the treated animals compared to controls.

- For a Negative Response: When the criteria for a positive response had not been met and the conditions for a valid study have been satisfied.
- The mice displayed a stable and sensitive response to the vehicle and positive controls that was consistent with historical control data at the testing facility.
- The final interpretations of equivocal findings would be made on the basis of scientific judgment.
- Dose verification results were within an acceptable range (±10% to targets) for all dosing formulations used on Days 1 and 2, with the exception of Day 2 dosing solutions for 100 mg/kg and 200 mg/kg groups (81% and 60% to targets).

Results

Dose-ranging study:
Mortalities occurred at 500 mg/kg/day. SFP did not produce bone marrow toxicity at either 250 or 500 mg/kg (see table below).

Table 49 PCE/NCE Ratios in the Dose Range-Finding Assay

Dose (mg/kg)	Animal Number	Males			Females		
		PCE	NCE	PCE/NCE	PCE	NCE	PCE/NCE
Vehicle Control 0	1	426	578	0.74	519	501	1.04
	2	506	496	1.02	526	478	1.10
	3	605	406	1.49	569	452	1.26
	4	526	413	1.27	560	552	1.01
	5	600	517	1.16	598	441	1.36
Mean ± Standard Deviation		1.14 ± 0.28			1.15 ± 0.15		
Soluble Ferric Pyrophosphate 250	1	579	657	0.88	NA	NA	NA
	2	389	613	0.63	NA	NA	NA
	3	535	585	0.91	NA	NA	NA
	4	477	524	0.91	NA	NA	NA
	5	549	472	1.16	NA	NA	NA
Mean ± Standard Deviation		0.90 ± 0.19					
Soluble Ferric Pyrophosphate 500	1	(a)	(a)	(a)	(a)	(a)	(a)
	2	(a)	(a)	(a)	625	464	1.35
	3	(a)	(a)	(a)	555	466	1.19
	4	417	658	0.63	682	503	1.36
	5	336	698	0.48	644	515	1.25
Mean ± Standard Deviation		0.56 ± 0.11[b]			1.29 ± 0.08		

NCE = normochromatic erythrocytes
PCE = polychromatic erythrocytes
(a) = animal died before bone marrow harvest so PCE and NCE not evaluated
NA= Not Applicable
[b] = Standard Deviation and mean based on two animals; SOP requires a minimum of three animals to use the value

(Excerpted from the Applicant's submission)

Definitive study:
Mortalities that occurred on Day 1 (within 1 hours postdose) and Day 2 (within 1 hours postdose) led to insufficient sampling from the 400 mg/kg/day group; samples from this group were not assessed for micronucleated PCE (MPCE) frequency (see table below).

Table 50 Mortalities in the 400 SFP mg/kg/day Group But Not in Positive Control Group - Definitive Study

			Day 1 (Post Dose)			Day 2 (Post Dose)		
Dose (mg/kg)	Sex	Animal Number	0-60 Minutes	1 Hour	4 Hour	0-60 Minutes	1 Hour	4 Hour
Soluble Ferric Pyrophosphate 400	M	1	Dead	NA	NA	NA	NA	NA
	M	2*	H	H	H	NA	NA	NA
	M	3	Dead	NA	NA	NA	NA	NA
	M	4	Dead	NA	NA	NA	NA	NA
	M	5	Dead	NA	NA	NA	NA	NA
	F	1	H	H	A	H	H	H
	F	2	Dead	NA	NA	NA	NA	NA
	F	3	A	A	A	Dead	NA	NA
	F	4	Dead	NA	NA	NA	NA	NA
	F	5	A	A	A	H	H	A
Cyclophosphamide 50	M	1	NA	NA	NA	A	A	A
	M	2	NA	NA	NA	A	A	A
	M	3	NA	NA	NA	A	A	A
	M	4	NA	NA	NA	A	A	A
	M	5	NA	NA	NA	A	A	A
	F	1	NA	NA	NA	A	A	A
	F	2	NA	NA	NA	A	A	A
	F	3	NA	NA	NA	A	A	A
	F	4	NA	NA	NA	A	A	A
	F	5	NA	NA	NA	A	A	A

A = No observable abnormalities
H = Lethargic
* This mouse died after the 4-hour observation period on day 1 of dosing, but prior to the day 2 dose
NA = Not Applicable

(Excepted from the Applicant's submission)

SFP did not significantly increase MPCE frequency at 0, 100 and 200 mg/kg/day (0, 12 and 23 mg/kg/day, respectively). CP significantly increased MPCE frequency in both sexes, and the frequencies of vehicle and CP groups were within the historical range at (b) (4). See table below.

Table 51 Micronucleus Frequency and PCE/NCE Ratios - Definitive Study

		Mean ± Standard Deviation	
Dose (mg/kg)	Sex	% MPCE[a]	PCE/NCE Ratio
Vehicle Control 0	M	0.60 ± 0.55	0.83 ± 0.22
	F	0.40 ± 0.55	1.26 ± 0.38
Soluble Ferric Pyrophosphate 100	M	0.60 ± 0.55	0.93 ± 0.16
	F	0.60 ± 0.55	1.05 ± 0.25
Soluble Ferric Pyrophosphate 200	M	1.33 ± 1.53 (b)	0.76 ± 0.13
	F	0.20± 0.45	1.26 ± 0.10
Soluble Ferric Pyrophosphate 400	M	(c)	(c)
	F	(d)	(d)
Cyclophosphamide 50	M	16.40± 5.03	1.09± 0.11
	F	9.40 ± 4.45	1.18± 0.30

MPCE = micronucleated polychromatic erythrocytes
NCE = normochromatic erythrocytes
PCE = polychromatic erythrocytes
[a] = Micronucleus frequency was represented as MPCE per 2000 PCE.
b = values based on three mice
c = 4 mice died shortly after first dose, mouse 2 died before second dose
d = mice 2 and 4 died shortly after first dose, mouse 3 died shortly after second dose

(Excepted from the Applicant's submission)

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Intravenous fertility and general reproduction toxicity study of SFP in rats

Study no.:	PHX00005
Study report location:	4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 4, 2006
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	Soluble ferric pyrophosphate (SFP), Batch #126412 (non-GMP and (b) (4) product), 11.5% iron by weight

Key Study Findings

- SFP caused decreases in body weight and food consumption, but did not affect reproductive organ weight, sperm count, sperm motility, mating or fertility outcomes for males or estrous cycle, pregnancy rate, implantation, or embryonic development in females.

- The NOAELs for SFP for reproductive toxicity were 10 and 40 mg/kg/dose in males and females, respectively.

Methods

Doses:	Males: 1st and 2nd weeks: 0, 10, 20, and 40 mg/kg (0, 1.15, 2.3, and 4.6 mg Fe/kg) 3rd and 4th weeks: 0, 2, 5, and 10 mg/kg (0, 0.23, 0.575, and 1.15 mg Fe/kg) Females: 0, 10, 20, 40 mg/kg (0, 1.15, 2.3, and 4.6 mg Fe/kg)
Frequency of dosing:	Males: Three times each week for 7 weeks (45 days) Females: Three time each week for 6 weeks (38 days)
Dose volume:	Not specified (assumed at 5 mL/kg/hour)
Route of administration:	IV infusion (1 hour; through lateral tail vein)
Formulation/Vehicle:	Lactated Ringer's Injection, USP
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	25
Satellite groups:	None
Study design:	See table below. After 29 days of dosing, male rats were caged with virgin females for up to a 16-day cohabitation period, during which time dosing continued. The day when female rats with spermatozoa observed in vaginal smear contents and/or a copulatory plug *in situ* were considered DG 0. All surviving females were euthanized on DG 13. After mating was confirmed, male rats were removed and dosing continued in females on Days 0, 4, and 7 of presumed gestation (DGs 0, 4, 7). The females were maintained an additional six days (to DG 13) and euthanized on DG 13.
Deviation from study protocol:	The stability data for SFP dosage formulations provided by the Applicant was not conducted in compliance with GLP regulations.

Observations and Results

Table 52 Study Design for Male Rat Fertility

Reference ID: 3676761

Dosage Group	Number of Male Rats	SFP Dosage (mg/kg)[a]	Fe Dosage (mg/kg)[a,b]	SFP Concentration (mg/mL)[a]	Volume (mL/kg)	Infusion Duration (hours)	Assigned Male Rat Numbers
I	25	0 (Vehicle)	0	0	5	1	16701 - 16725
II	25	10 / 2	1.15 / 0.23	2 / 0.4	5	1	16726 - 16750
III	25	20 / 5	2.3 / 0.575	4 / 1	5	1	16751 - 16775
IV	25	40 / 10	4.6 / 1.15	8 / 2	5	1	16776 - 16800

a. Male rats were administered dosage levels of 0 (Vehicle), 10, 20 or 40 mg/kg (approximately 0, 1.15, 2.3 or 4.6 mg Fe/kg) for the first two weeks of the dosage period; the dosages levels were lowered to 0 (Vehicle), 2, 5 and 10 mg/kg (approximately 0, 0.23, 0.575 and 1.15 mg Fe/kg) after the first two weeks of the dosage period.
b. The test article is 11.5% iron (Fe) by weight.

(Excerpted from the Applicant's submission)

Table 53 Study Design for Female Rat Fertility

Dosage Group	Number of Female Rats	SFP Dosage (mg/kg)	Fe Dosage (mg/kg)[a]	SFP Concentration (mg/mL)	Volume (mL/kg)	Infusion Duration (hours)	Assigned Female Rat Numbers
I	25	0 (Vehicle)	0	0	5	1	16801 - 16825
II	25	10	1.15	2	5	1	16826 - 16850
III	25	20	2.3	4	5	1	16851 - 16875
IV	25	40	4.6	8	5	1	16876 - 16879, 5470[b], 16881 - 16886, 5478[c], 16888 - 16900

a. The test article is 11.5% iron (Fe) by weight.
b. Rats 16880 and 16887 were found dead during dosage administration on day 1 of study (DS 1) and were replaced with rats 5470 and 5478, respectively.

(Excerpted from the Applicant's submission)

Observations and Results

Table 54 Measurements Performed in Rat Fertility and Early Embryo Development Study

Mortality:	Acclimation period: Twice Post-acclimation: At least twice daily
Cage side clinical signs: Including abortions, premature deliveries, and death:	For female rats: Weekly during the pre-treatment period. During treatment period: Before each infusion and within 2 hours of completion of each infusion. Non-treatment days: Once daily
Body weights:	Males: Acclimation period: At least weekly Dosage period: Daily Females: Acclimation & pre-treatment periods: At least weekly Treatment and post-treatment periods: Daily

Food consumption:	Males: Dosage period: weekly (except during cohabitation) Females: Weekly to cohabitation and on GDs 0, 4, 7, 8, 10, and 13.
Estrous cycling and mating	Vaginal cytology: 14 days before initiation of the dosage period, and 14 days before the initiation of cohabitation, and until spermatozoa were observed in the vaginal smear content and/or a copulatory plug was observed during the cohabitation period
Gross pathology, sperm analysis, and male organ weights (testes, epididymides, seminar vesicles, prostate), and female pregnancy data (placenta, number and distribution of corporal lutea, implantation sites, embryos):	Early mortalities Scheduled euthanasia: Males: After completion of cohabitation period Females: GD 13

Mortality

One non-drug-related early mortality of male rat in vehicle control group occurred on Day 52. One non-drug-related, early female mortality (20 mg/kg) occurred on Day 10.

Clinical Signs

Dose-dependent increases in alopecia and sparse hair coat were noted in both genders.

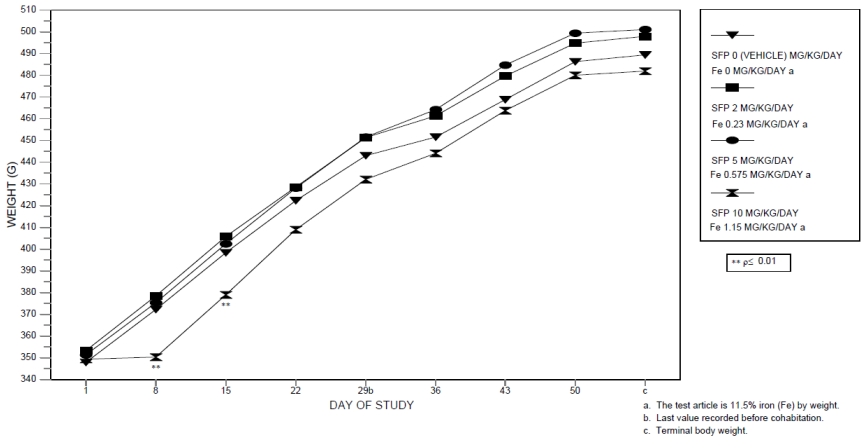
Other drug-relate findings included scab on right site of back or tail, discoloration of injection site, and tip of tail missing were noted mainly in females at 40 mg/kg.

Body Weight

Males:
Minimal decreases in body weight and body-weight gain were noted during the dosing period prior to the initiation of cohabitation in males at 10 mg/kg (see figures and tables below) and in females at 40 mg/kg for the precohabitation and gestation period.

Reference ID: 3676761

Figure 21 Group Mean Body Weight in Male Rats - Fertility Study



(Excerpted from the Applicant's submission)

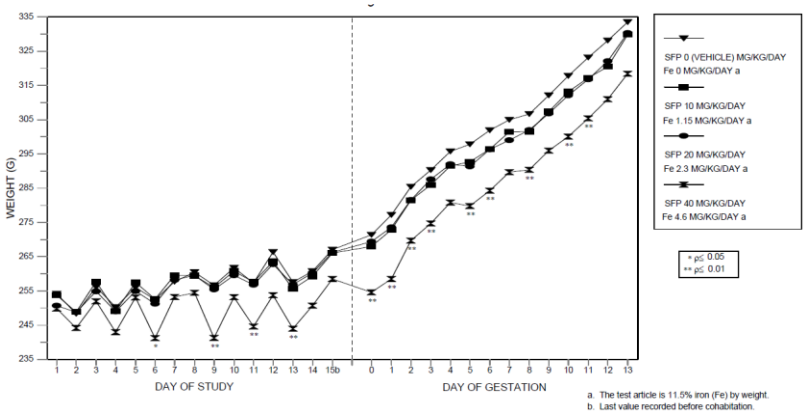
Table 55 Group Mean Body Weights (g) of Males - Fertility Study

Parameter	Dose (mg/kg/day)			
	0	10/2	20/5	40/10
Number of rats tested	25	25	25	25
Day 8	372.3	378.5 (102)	375.4 (101)	**350.3** (94)**
Day 15	398.4	405.9 (102)	402.5 (101)	**379.0** (95)**
Day 29[$]	443.1	451.4 (102)	451.6 (102)	432.1 (98)
Day 50	486.3	494.8 (102)	499.4 (103)	480.0 (99)

[$] The last body weight measurement before cohabitation.
* Significantly different from control ($p<0.05$); ** significantly different from control ($p<0.01$)
Values in parentheses are percent to respective controls.

Females:
Minimal decreases in body weight were noted during the dosing period prior to the initiation of cohabitation in females at 40 mg/kg and for the precohabitation and gestation period. No changes in body weight at the gestational period. See figure and tables below.

Figure 22 Group Mean Body Weights of Female Rats - Fertility Study



(Excerpted from the Applicant's submission)

Precohabitation Phase

Table 56 Group Mean Body Weights (g) for Female Rats – Precohabitation Infertility Study

Parameter	Dose (mg/kg/day)			
	0	10	20	40
Number of rats tested	25	25	24[!]	25
Day 8	260.5	259.5 (100)	259.6 (100)	254.5 (98)
Day 9	256.7	256.0 (100)	255.5 (100)	**241.3** ** **(94)**
Day 13	257.6	255.6 (99)	256.9 (99)	**244.0** ** **(95)**
Day 15	267.1	266.1 (100)	266.3 (100)	258.5 (97)

[!] Excluded values for rats that were found dead or aborted.
[$] The last body weight measurement before cohabitation.
* Significantly different from control ($p<0.05$); ** significantly different from control ($p<0.01$)
Values in parentheses are percent to respective controls.

Gestation Phase

Table 57 Group Mean Maternal Body Weights (Gestation Phase)

Parameter	Dose (mg/kg/day)			
	0	10	20	40
Number of rats tested	25	25	24[!]	25
Number of rats pregnant	25	25	23	23
Day 0	271.4	268.0	269.4	**254.6** ** **(94)**
Day 3	290.3	286.0	287.6	**274.7** ** **(95)**
Day 6	301.9	296.4	296.3	**284.3** ** **(94)**

Parameter	Dose (mg/kg/day)			
	0	10	20	40
Day 10	317.8	313.2	312.1	**300.1** (94)**
Day 13	333.5	329.8	330.3	318.4 (96)

[!] Excluded values were from rats that were found dead or aborted.
[$] The last body weight measurement before cohabitation.
* Significantly different from control ($p<0.05$); ** significantly different from control ($p<0.01$)
Values in parentheses are percent to respective controls.

Feed Consumption

Males:
Decreases in food consumption were noted during the dosing period prior to the initiation of cohabitation in males at 40/10 mg/kg.

Table 58 Group Mean Food Consumption (g/kg/day) in Male Rats - Fertility Study

Parameter	Dose (mg/kg/day)			
	0	10/2	20/5	40/10
Number of rats tested	25	25	25	25
Days 1 - 8	68.2	69.1	68.2	**55.0** (81)**
Day 1 - 29[$]	65.4	65.9	66.1	**63.4** (97)*
Day 1 - 50	60.7	60.8	60.7	**58.9** (97)*

[$] The last body weight measurement before cohabitation.
* Significantly different from control ($p<0.05$); ** significantly different from control ($p<0.01$)
Values in parentheses are percent to respective controls.

Females:
Minimal decreases in food consumption was noted during the dosing period prior to the initiation of cohabitation in females at 40 mg/kg for the precohabitation and early gestation period (GDs 4-7).

Table 59 Group Mean Relative Feed Consumption - Precohabilitation Period

Parameter	Dose (mg/kg/day)			
	0	10	20	40
Number of rats tested	25	25	24[!]	25
Days 1 - 8	68.9	69.6	70.0	**65.8** (96)*
Days 8 – 15[$]	69.6	68.8	69.1	67.0
Days 1 – 15[$]	69.4	69.2	69.6	**66.5** (96)**

[!] Excluded values were from rats that were found dead.
[$] The last body weight measurement before cohabitation.
* Significantly different from control ($p<0.05$); ** significantly different from control ($p<0.01$)

Table 60 Group Mean Relative Feed Consumption (g/kg/day) - Gestational Period (Rats)

Parameter	Dose (mg/kg/day)			
	0	10	20	40
Number of rats tested	25	25	24[!]	25
Number of rats pregnant	25	25	23	23
Days 0 - 4	22.6	22.0	21.8	21.9
Days 4 – 7	23.5	**22.1* (94)**	**21.7** (92)**	**22.0* (94)**
Days 7 - 10	24.1	22.9	23.1	22.4
Days 10 – 13	24.9	23.8	4.1	24.6
Days 0 - 13	23.7	22.7	22.6	22.7

[!] Excluded values were for rats that were found dead or aborted.
* Significantly different from control (p<0.05); ** significantly different from control (p<0.01)

Necropsy

Organ Weights: unremarkable

Gross Pathology

Dose-dependent increases in tan pancreas were noted for both genders (see tables below).

Table 61 Gross Pathology in Male Rats

Parameter	Dose (mg/kg/day)			
	0	10/2	20/5	40/10
Number of male rats tested	25	25	25	25
Pancreas: tan	1	6	**14***	**24****

* Significantly different from control (p<0.05); ** significantly different from control (p<0.01)

Table 62 Gross Pathology in Female Rats

Parameter	Dose (mg/kg/day)			
	0	10	20	40
Number of male rats tested	25	25	25	25
Pancreas: tan	2	9	**22****	**23****

* Significantly different from control (p<0.05); ** significantly different from control (p<0.01)

Mating and Fertility Parameters

- No drug-related changes in mating and fertility parameters, organ weights and sperm parameters evaluated for males were noted.

- No drug-related changes in estrous cyclicity, mating and fertility and Caesarean-sectioning or litter parameters evaluated for females were noted. Pregnancy occurred in 23 to 25 rats per group.

Reference ID: 3676761

Detail results on mating and fertility are summarized below:

Males: unremarkable

Females:

Estrous Cycle: unremarkable

Reproductive and Fertility Indices: unremarkable

Uterine and Ovarian Examination: unremarkable

9.2 Embryonic Fetal Development

Study title: Intravenous developmental toxicity study of SFP in female rats

Study no.:	PHX0004
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 24, 2006
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	SFP, Lot #125140, 11.5% of iron by weight

Key Study Findings

- Early mortalities at 90 mg/kg/day occurred on GDs 11 and 17.
- SFP adversely affected embryo-fetal development mainly at maternally toxic dose level of 90 mg/kg/day. Maternal toxicity included decreases in body weight gain and food consumption.
- Drug-related developmental toxicity included increase in post-implantation loss; resorption and placental abnormalities; decreases in litter size and, live fetuses, and fetal body weight; delayed fetal ossification; and increases in fetal head, ribs, and/or vertebral malformations at 90 mg/kg/day. Slight decrease in delayed fetal ossification (phalanges) was noted at 30 mg/kg/day.
- The NOAEL for both maternal and fetal developmental toxicity is 30 mg/kg/day for 11 days (GDs 7 – 17).

Methods

Doses:	0, 10, 30, and 90 mg/kg/day (0, 1.15, 3.45, and 10.35 mg Fe/kg/day)
Frequency of dosing:	Presumed gestation days (GDs) 7 through 17
Dose volume:	5 mL/kg/hour

Route of administration:	IV infusion (1 hour)
Formulation/Vehicle:	Lactated Ringer's Injection, USP
Species/Strain:	Rats Crl:CD(SD)
Number/Sex/Group:	25 females (timed mated)
Satellite groups:	TK animals; see table below
Study design:	See table below
Deviation from study protocol:	SFP, Lot #125140, 11.5% iron by weight

Table 63 Study Design of Rat Developmental Toxicology Study

Dosage Group	Number of Rats	SFP Dosage (mg/kg/day)	Fe Dosage (mg/kg/day)[a]	SFP Concentration (mg/mL)	Volume (mL/kg)	Infusion Duration (hours)	Assigned Rat Numbers	
							Main	Satellite
I	25	0 (Vehicle)	0	0	5	1	17901 - 17925	NA
II	25 + 6[b]	10	1.15	2	5	1	17926 - 17950	15976 - 15981
III	25 + 6[b]	30	3.45	6	5	1	17951 - 17975	15982 - 15987
IV	25 + 6[b]	90	10.35	18	5	1	17976 - 18000	15988 - 15993

a. The test article is 11.5% iron (Fe) by weight.
b. Eighteen additional rats assigned for blood sample collection.
NA – Not applicable

(Excerpted from the Applicant's submission)

Observations and Results

Table 64 Assessments in Embryo-Fetal Toxicity Study in Rats

Mortality:	Acclimation period: At least weekly and on GD 0 Treatment period and on: At least twice daily
Cage side clinical signs:	Before each infusion and within 2 hours of completion of each infusion Post-treatment period: Once daily
Body weights:	Acclimation period: At least weekly and on GD 0 Study period: Once daily
Food consumption:	Main study rats: GDs 0, 7, 10, 12, 15, 18, and 21. Toxicokinetic rats: GDs 0, 7, 10, 12, 15, and 20.
Gross pathology - Maternal and fetal examination:	GD 21
Toxicokinetics - serum iron:	On GD 7: Shortly before the beginning of the infusion, after completion of the infusion, and at 0.5, 1, 3, 6, and 24 hours after completion of infusion. On GD 17: Shortly before the beginning of the infusion, after completion of the infusion, and at 0.5, 1, 3, 6, 24, 48, and 72 hours after completion of infusion.

Mortality

Drug-related early mortalities or moribund euthanasia (a total of 3) occurred at 90 mg/kg/day on GDs 11 and 17.

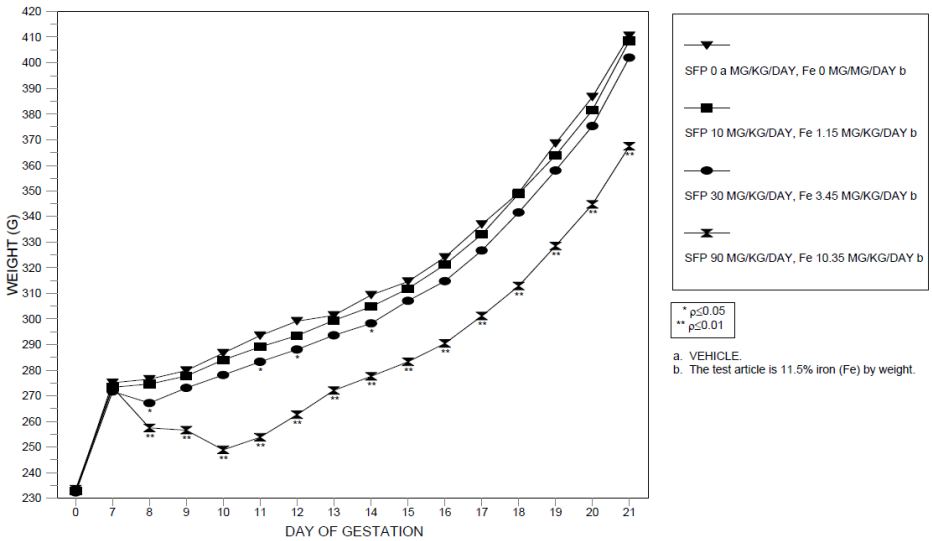
Clinical Signs

Clinical signs of toxicity included discoloration (purple, black, green, and/or white) at tail vein injection sites at ≥30 mg/kg/day, sparse hair coat at 90 mg/kg/day, and scabbing on tail and absence of tail tip at ≥10 mg/kg/day.

Body Weight

Moderate decreases body weight and body weight changes were noted on GDs 7 to 21 at 90 mg/kg (see figure and tables below).

Figure 23 Maternal Body Weights – F0 Rats



(Excerpted from the Applicant's submission)

Table 65 Group Mean of Maternal Body Weights – F0 Rats

Parameter	Dose (mg/kg/day)			
	0	10	30	90
Number of Rats Tested	25	25	25	25[!]
Number of Rats Pregnant	23	24	23	25
GD 7	275.1	273.3 (99)	271.7 (99)	273.3 (99)
GD 8	276.4	274.6 (99)	267.2* (97)	**257.5** (93↓)**
GD 14	309.3	304.8 (99)	298.3* (96)	**277.6** (90↓)**
GD 21	410.6	408.5 (100)	402.0 (98)	**367.4** (90↓)**

[!] Excluded values for rats that were found dead or aborted (weight of does averaged at 90 mg/kg/day: 24 for GD 12 and 23 for GDs 13 - 21).
* Significantly different from control (p<0.05); ** significantly different from control (p<0.01)
Values in parentheses are percent to respective controls.

Table 66 Group Mean Maternal Body Weight Changes – F0 Rats

Parameter	Dose (mg/kg/day)

	0	10	30	90
Number of rats tested	25	25	25	25
Number of rats pregnant	25	24	23	25
Number of Rats averaged	23	24	23	21-24[!]
Weight changes (GD 0-21)	178.2	175.7 (99)	169.7 (95)	**132.3** (74↓)**
Weight gain (GD 0-7)	42.6	40.4 (95)	39.5 (93)	39.8 (93)
Treatment phase weight gain (GD 7-18)	74.2	75.5 (102)	69.8 (94)	**37.7** (51↓)**
Post-treatment weight gain (GD 18-21)	61.3	59.7 (97)	60.5 (94)	**52.9* (86↓)**

[!] Excluded values for rats that were found dead or aborted.
* Significantly different from control (p<0.05); ** significantly different from control (p<0.01)
Values in parentheses are percent to respective controls.

Feed Consumption

Significant decreases in the absolute and relative food consumption were noted at 90 mg/kg during the treatment phase (see tables below).

Table 67 Group Mean Absolute Food Consumption (g/day) – F0 Female Rats

Parameter	Dose (mg/kg/day)			
	0	10	30	90
Number of Rats Tested	25	25	25	25
Number of Rats Pregnant	23	24	23	25
Number of Rats averaged	22-23	23-24	22-23	22-24[!]
GDs 0 - 21	25.0	24.2 (97)	23.8* (95)	**21.0** (84↓)**
GDs 0 - 7	23.4	22.4 (96)	22.0 (94)	22.5 (96)
Early phase of Treatment (GD 7-10)	22.8	22.3 (98)	**20.5** (90↓)**	**10.7** (47↓)**
Treatment phase (GD 7-18)	24.9	24.6 (99)	23.7 (95)	**18.8** (76↓)**
Post-treatment phase (GD 18-21)	28.5	27.8 (98)	28.2 (99)	**25.4** (89↓)**

[!] Excluded values for rats that were found dead or aborted.
* Significantly different from control (p<0.05); ** significantly different from control (p<0.01)
Values in parentheses are percent to respective controls.

Table 68 Group Mean Relative Food Consumption (g/kg/day) – F0 Female Rats

Parameter	Dose (mg/kg/day)			
	0	10	30	90
Number of Rats Tested	25	25	25	25
Number of Rats Pregnant	23	24	23	25
Number of Rats averaged	22-23	23-24	22-23	22-24[!]
GDs 0 - 21	79.0	77.7 (98)	77.4 (98)	**73.4** (93)**
GDs 0 - 7	92.1	88.6 (96)	**87.2** (95)**	88.7 (96)
Early phase of Treatment (GD 7-10)	81.2	80.6	**75.1**	**41.0**

		(99)	**(92)**	**(51↓)**
Treatment phase (GD 7-18)	81.8	81.6 (100)	80.1 (98)	**67.8**** **(83↓)**
Post-treatment phase (GD 18-21)	74.9	74.0 (100)	76.5 (102)	75.6 (101)

! Excluded values for rats that were found dead or aborted.
* Significantly different from control (p<0.05); ** significantly different from control (p<0.01)
Values in parentheses are percent to respective controls.

Necropsy

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

Uterine-examination toxicity included increase in post-implantation loss, resorption, placental abnormalities, and decrease in litter size and the number of live fetuses at 90 mg/kg (see table below).

Table 69 Cesarean Sectioning Examination (Uterine Examination) in F0 Rats

Dose (mg/kg/day)	0	10	30	90
Number of females tested	25	25	25	25
Number of females pregnant	23	24	23	25
Number found dead or moribund sacrificed	0	0	0	**3**** **(12)**
Number of females aborted and sacrificed	0	0	0	**1**
Number of females pregnant at necropsy*	23	24	23	**21**
Corpora lutea Mean number per female	16.8	16.8	16.3	16.8
Implantation sites Mean number per litter	15.3	15.1	15.2	15.1
Litter sizes	14.4	14.6	14.6	**12.4**
Live fetuses	331	350	335	**261**
Live fetuses - average	14.4	14.6	14.6	**12.4**
Dead fetuses	0	0	0	0
Resorptions	1.0	0.5	0.6	**2.7**
Early resorptions	22	13	14	**55**
Mean	1.0	0.5	0.6	**2.6**
Late resorptions	0	0.	**1**	**1**
Mean	0.0	0.0	0.0	0.0
Dams with any resorption	11 (47.8)	8 (33.3)	13 (56.5)	**13** **(61.9)**
Does with all conceptuses resorbed	0	0	0	**2** **(9.5)**
Does with variable fetuses	23 (100)	24 (100)	23 (100)	19 (91)
Placentae appeared normal	23 (100)	24 (100)	23 (100)	**18**** **(95)**

Cesarean section was conducted on GD 29
* Significantly different from control (p<0.05); ** significantly different from control (p<0.01)
Values in parentheses are percent to respective controls.
The number of females pregnant at necropsy does not include the number of females aborted.

Offspring Data (Malformations, Variations, etc.):

Offspring developmental toxicity included decrease in fetal body weight and litter size, delayed fetal ossification, and increase in fetal head, ribs, and/or vertebral malformations at 90 mg/kg; slight delayed fetal ossification (phalanges) at 30 mg/kg (possibly secondary to the transient reduced maternal weight gain early in gestation).

Table 70 Litter Observation (Cesarean-Delivered Fetuses) Summary - Rats

Dose (mg/kg/day)	0	10	30	90
Number of females treated	25	25	25	25
Number of females pregnant	23	24	23	25
Number found dead	0	0	0	**3** **(12)**
Number of females aborted and sacrificed	0	0	0	1
Number of females pregnant at necropsy*	23	24	23	**21**
Litter with one or more live fetuses	23	24	23	19
Implantations	15.3	15.1	15.2	15.0
Litter sizes				
Live fetuses	331	350	335	261
Live fetuses - average	14.4	14.6	14.6	**13.7**
Live male fetuses	171	168	167	133
% Live male fetuses/litter	51.1	48.2	49.7	51.1
Live fetal body weights (g/litter)	5.48	5.41	5.35	**5.03****
Male fetuses	5.62	5.56	5.52	**5.14****
Female fetuses	5.35	5.28	5.18	**4.92****
% Resorbed conceptuses/litter	5.9	3.5	4.2	**8.8**
Fetal Alteration				
Litters with fetuses with any alteration observed	4 (17.4)	6 (25.0)	5 (21.7)	**8** **(42.1)**
Fetuses with any alteration observed	4 (1.2)	8 (2.3)	**11*** **(3.3)**	**15**** **(5.7)**
% fetuses with any alteration/litter	1.2	2.4	3.1	**6.1**

Cesarean section was conducted on GD 21
* Significantly different from control ($p<0.05$); ** significantly different from control ($p<0.01$)
Values in parentheses are percent to respective controls.
The number of females pregnant at necropsy does not include the number of females aborted.

Table 71 Malformation in F1 Rats

Dose (mg/kg/day)		0	10	30	90
Number of litters evaluated		23	24	23	19
Number of fetuses evaluated		331	350	335	261
Live		331	350	335	261
Malformations					
Soft tissue alteration					
Cleft palate	Litter incidence (%)	0 (0)	1 (4.2)	0 (0)	1 (5.3)
	Fetal incidence (%)	0 (0)	1(0.3)	0 (0)	1 (0.4)

Dose (mg/kg/day)		0	10	30	90
Number of litters evaluated		23	24	23	19
Number of fetuses evaluated		331	350	335	261
Live		331	350	335	261
Fetal skeletal alteration					
Ribs: wavy	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**2 (10.5)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**3 (2.2)****
Skull: Eye socket, Small	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**
Skull: Premaxilla, Incompletely ossified	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**
Skull: Maxilla, Incompletely ossified	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**
Skull: Palate, Incompletely ossified	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**
Skull: Nasal misaligned	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**
Cervical Vertebrae: Cervical rib present at 7th cervical vertebrae	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**2 (10.5)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**4 (3.0)**
Cervical vertebrae: Arch, has appearance like 7th	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**
Cervical	Litter	0	0	0	**1**

Dose (mg/kg/day)		0	10	30	90
Number of litters evaluated		23	24	23	19
Number of fetuses evaluated		331	350	335	261
Live		331	350	335	261
vertebrae: Centrum, bifid	incidence (%)	(0)	(0)	(0)	**(5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**
Thoracic vertebrae: Centrum, bifid	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**2 (1.5)**
Sacral vertebrae: Hemivertebrae	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**2 (1.5)**
Sacral vertebrae: Arch, small	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**2 (1.5)**
Sacral vertebrae: Centrum, bifid	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**
Caudal vertebrae: Hemivertebrae	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**
Caudal vertebrae: Arch, incompletely ossified	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**
Caudal vertebrae: Centra, fused	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**2 (10.5)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**2 (1.5)**
Caudal vertebrae: Centrum, bifid	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal	0	0	0	**1**

Dose (mg/kg/day)		0	10	30	90
Number of litters evaluated		23	24	23	19
Number of fetuses evaluated		331	350	335	261
Live		331	350	335	261
	incidence (%)	(0)	(0)	(0)	**(0.7)**
Ribs: Short	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**
Ribs: Thickened	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**1 (5.3)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**1 (0.7)**

Only the drug-related findings are included in the table.
* Significantly different from control ($p<0.05$); ** significantly different from control ($p<0.01$)
Values in parentheses are percent to respective controls.

Table 72 Fetal Ossification Sites - Caesarean-Delivered Live Fetuses (GD 21) - Rats

Dose (mg/kg/day)	0	10	30	90
Number of litters evaluated	23	24	23	19
Number of fetuses evaluated	171	180	173	13
Ossification site per fetus per litter				
Forelimb				
Phalanges	8.23	7.96 (97)	**7.76* (94)**	**7.50** (91↓)**
Hind Limb				
Metatarsals	4.88	4.80 (98)	4.72 (97)	**4.47** (92↓)**
Phalanges	6.22	6.10 (98)	5.80 (93)	**5.16** (83↓)**

Only the drug-related findings are included in the table.
* Significantly different from control ($p<0.05$); ** significantly different from control ($p<0.01$)
Values in parentheses are percent to respective controls.

Toxicokinetics

Iron exposure (C_{max} and AUC_{0-25}) increased less than proportionally with SFP doses, suggesting saturation of iron binding to transferrin and potential rapid removal of unbound iron form the serum into cells. The apparent elimination half-life ($t_{1/2}$) was not estimated due to insufficient data.

Table 73 Toxicokinetic Parameters in Pregnant Rats - Developmental Study

Text Table 3. Summary of Toxicokinetic Parameters							
SFP Dosage Level (mg/kg)	Parameter	Day 7 of Gestation			Day 17 of Gestation		
		C_{max} (µg/dL)	T_{max} (h)	$AUC_{(0-25)}$ (h*µg/dL)	C_{max} (µg/dL)	T_{max} (h)	$AUC_{(0-25)}$ (h*µg/dL)
10	Mean	667	1.1	8744	684	1.1	6442
30	Mean	940	1.1	9957	1002	1.1	7488
90	Mean	4771	1.1	21994	3267	1.1	18957

(Excerpted from the Applicant's submission)

Dosing Solution Analysis

Dose verification results for all dosing solutions were within ±10% (94% to 102%) of the target concentrations.

Study title: Intravenous developmental toxicity study of SFP in female rabbits

Study no.:	PHX0002
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 16, 2006
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	FSP, Lot #125140, 11.5% iron by weight

Key Study Findings

- Mortalities in pregnant rabbits occurred at 40 mg/kg/day.
- Maternal toxicities included reduced body weight gain, increase incidences of ungroomed coat, white periocular substance, red perinasal substance, cold to touch, decreased motor activity, enlarged kidneys, abortions, and injection-site discoloration at 40 mg/kg/day.
- Fetal skeletal alterations were noted at 40 mg/kg, and included low incidences of irregular thoracic vertebral arch and short ribs. No Caesarean-sectioning or littler parameters, gross external, soft tissue, or other skeletal alterations were noted up to 20 mg/kg/day. All ossification site averages were comparable among the dosage groups.
- The no observed adverse effect level (NOAEL) of SFP for maternal and fetal toxicity in rabbits is at 20 mg/kg/day.

Methods

Doses:	0, 10, 20, and 40 mg/kg/day (0, 1.15, 2.3, and 4.6 mg Fe/kg/day)
Frequency of dosing:	GDs 7 through 19 (Days 7 to 19 of presumed gestation)
Dose volume:	5 mL/kg/hour
Route of administration:	IV infusion (1 hour)
Formulation/Vehicle:	Lactated Ringer's Injection, USP
Species/Strain:	New Zealand White [Hra:NZW)SPF]
Number/Sex/Group:	20 Females (timed mated): 2.8 – 4.3 kg on GD 0
Satellite groups:	TK groups (see table below)
Study design:	See table below
Deviation from study protocol:	The stability data for SFP dosing formulations provided by the Applicant was not conducted in compliance with GLP.

Table 74 Study Design for Developmental Toxicology Study in Rabbits

Dosage Group	SFP Dosage[a,b] (mg/kg/day)	Fe Dosage[a,b] (mg/kg/day)	SFP Concentration (mg/mL)	Volume (mL/kg)[c]	Number of Rabbits	Assigned Rabbit Numbers	
						Main	Satellite
I	0 (Vehicle)	0	0	5	20	6201 - 6220	NA
II	10	1.15	2	5	20 + 3[d]	6221 - 6223, 3799[e], 6225 - 6240	6281 - 6283
III	20	2.3	4	5	20 + 3[d]	6241 - 6260	6284 - 6286
IV	40	4.6	8	5	20 + 3[d]	6261 - 6280	3793[f], 6288, 6289

a. The test article was considered 100% active/pure for the purpose of dosage calculations.
b. The test article was 11.5% iron (Fe) by weight.
c. Prepared formulations were administered via intravenous infusion (approximately 1 hour in duration).
d. Nine additional timed-mated rabbits assigned for blood sample collections.
e. Rabbit 6224 was excluded from study and euthanized due to body weight loss and low feed consumption on DG 8 and was replaced with rabbit 3799.
f. Rabbit 6287 was excluded from study and euthanized due to body weight loss and low feed consumption on DG 7 and was replaced with rabbit 3793.
NA - Not applicable

(Excerpted from the Applicant's submission)

Observations and Results

Table 75 Measurements Made in the Embryo-Fetal Toxicology Study in Rabbits

Mortality:	Twice daily
Cage side clinical signs:	Twice on infusion day: Before each daily infusion and within 2 hours of completion of each daily infusion; Once daily during the post-treatment period.
Body weights:	Once weekly
Food consumption:	Once weekly
Gross pathology: Maternal examination, fetal examination	GD 29
Organ weights:	GD 29
Toxicokinetics (TK):	Samples were collected on GDs 7 and 19:

serum iron from satellite rabbits	On GD 7: Shortly before the beginning of the infusion, earl the end of the infusion, and at 0.5, 1, 3, 6, and 24 hours after completion of the infusion. On GD19: Shortly before the beginning of the infusion, earl the end of the infusion, and at 0.5, 1, 3, 6, 24, 48, and 72 hours after completion of the infusion.

Mortality

Drug-related mortalities occurred at 40 mg/kg. Five (5) does at 40 mg/kg/day were found dead after the second dose. Abortion occurred in one doe at 40 mg/kg/day after the 13th dose (GD19); this doe was sacrificed subsequently.

Clinical Signs

Clinical signs related to the administration procedure included increased incidence of injection-site discoloration, ungroomed coat, white periocular substance, red perinasal substance, cold to touch, and decreased motor activity at 40 mg/kg/day.

Table 76 Clinical Signs of Toxicity and Gross Necropsy Findings in Early Mortalities - Pregnant Rabbits

Text Table 2. Clinical and Necropsy Findings for Rabbits that Died					
Dosage (mg SFP/kg/day)	40				
Dosage (mg Fe/kg/day)	4.6				
Rabbit Number	6264	6266	6278	6279	6280
Doses Administered	2	2	2	2	2
Mode of Death	FD	FD	FD	FD	FD
Clinical Observations					
Injection site discoloration	DG 8				
Ungroomed coat		DG 8	DG 8	DG 8	
White periocular substance			DG 7	DG 7	
Red perinasal substance			DG 9	DG 9	
White perinasal substance			DG 9	DG 9	
Decreased motor activity				DG 8	
Lacrimation				DG 8	
Cold to touch				DG 8	
Necropsy Observations					
Kidney(s)					
Large	X	X	X	X	X
Thymus					
Numerous black areas			X		
Numerous red areas					X
Lungs					
Mottled red and dark red			X	X	
FD = Found dead	DG = Day of Gestation				

(Excerpted from the Applicant's submission)

Body Weight

A trend of decreased body weights and decreased body weight gains were noted in rabbits at 40 mg/kg/day during the dosing (GDs 7-20) and post-dosing (GDs 20 – 29) periods when compared to controls (see tables below).

Table 77 Group Means of Maternal Body Weights - Rabbits

Parameter	Dose (mg/kg/day)			
	0	10	20	40
Number of Rabbits Tested	20	20	20	20
Number of Rabbits Pregnant	18	19	19	19
GD 7	3.59	3.57 (99)	3.62 (101)	3.58 (100)
GD 19	3.82	3.78 (99)	3.84 (101)	**3.65 (96)**
GD 29	4.02	4.00 (100)	4.02 (100)	**3.84 (96)**

! Excluded values were from does that were found dead or aborted (weight of does averaged at 40 mg/kg/day: 14 for GD 19 and 13 for GD 29).
* Significantly different from control ($p<0.05$)

Table 78 Maternal Body Weight Changes (kg) in Rabbits

Parameter	Dose (mg/kg/day)			
	0	10	20	40
Number of Rabbits averaged	20	20	20	13!
Weight gain (GDs 0-29)	0.61	0.58 (95)	0.58 (95)	**0.48 (79↓)**
Treatment weight gain (GDs 7-20)	0.24	0.23 (96)	0.24 (100)	**0.20 (83↓)**
Post-treatment weight gain (GDs 20-29)	0.18	0.21 (117)	0.16 (89)	**0.14 (78↓)**

! Excluded values were from does that were found dead or aborted.
* Significantly different from control ($p<0.05$)

Feed Consumption

Decreases in food consumption were noted in rabbits at 40 mg/kg during the dosing (GDs 7-10 (relative values) and GDs 7-20 (absolute values)) and post-dosing (GDs 20 – 29) periods when compared to controls.

Table 79 Maternal Absolute Food Consumption (g/day) - Rabbits

Parameter	Dose (mg/kg/day)			
	0	10	20	40
Number of Rabbits Tested	20	20	20	20
Number of Rabbits Pregnant	18	19	19	19
Number of Rabbits averaged	28	19	19	14!
Early phase of Treatment (GDs 7-10)	173.0	162.7 (94)	175.8 (102)	**137.4 (79↓)**
Treatment phase (GDs 7-20)	174.3	161.2 (92)	172.2 (99)	**151.8** ** **(87↓)**
Post-treatment phase (GDs 20-29)	142.4	139.0 (98)	138.2 (97)	**128.8 (90)**

! Excluded values were from does that were found dead or that aborted.
** Significantly different from control ($p<0.01$)
Values in parentheses are percent of respective controls.

Table 80 Maternal Relative Food Consumption (g/kg/day) - Rabbits

Parameter	Dose (mg/kg/day)			
	0	10	20	40
Number of Rabbits Tested	20	20	20	20
Number of Rabbits Pregnant	18	19	19	19
Number of Rabbits averaged	28	19	19	14[!]
Early phase of Treatment (GDs 7-10)	48.2	45.6 (95)	48.7 (101)	**39.4** ** **(82↓)**
Treatment phase (GDs 7-20)	47.2	44.0 (93)	46.4 (98)	**42.6 (90)**
Post-treatment phase (GDs 20-29)	36.0	35.7 (99)	35.2 (98)	34.0 (94)

[!] Excluded values were from does that were found dead or that aborted.
** Significantly different from control ($p<0.01$)
Values in parentheses are percent of respective controls.

Necropsy

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

No effects for Caesarean-sectioning or litter parameters, including litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percentage of resorbed conceptuses, and percentage of live male fetus. No doe had a litter consisting of only resorbed conceptuses, and there were no dead fetuses; all placentae appeared normal (see table below).

Table 81 Cesarean-Sectioning Observations (Uterine Examination) in Rabbits

Dose (mg/kg/day)	0	10	20	40
Number of females treated	20	20	20	20
Number of females pregnant	18	19	19	19
Number found dead	0	0	0	**5** ** **(26.3)**
Number of females aborted and sacrificed	0	0	0	1
Number of females pregnant at necropsy*	18	19	19	**13**
Corpora lutea Mean number per female	8.9	9.0	8.4	8.9
Implantation sites Mean number per litter	8.6	8.6	8.2	8.5
Litter sizes	8.3	8.5	7.9	8.0
Live fetuses	150	161	151	104
Live fetuses - average	8.3	8.5	7.9	8.0
Dead fetuses	0	0	0	0
Resorptions	0.3	0.2	0.3	0.5
Early resorptions	4	3	3	6
Mean	0.2	0.2	0.3	0.5
Late resorptions	1	0	2	1
Mean	0.0	0.0	0.1	0.1
Doe with any resorption	2 (11.1)	3 (15.8)	5 (26.3)	**7** ** **(53.8)**
Does with all conceptuses resorbed	0	0	0	0
Does with variable fetuses	18	19	19	13

Dose (mg/kg/day)	0	10	20	40
Number of females treated	20	20	20	20
	(100)	(100)	(100)	(100)
Placentae appeared normal	18 (100)	19 (100)	19 (100)	13 (100)

Cesarean section was conducted on GD 29
** Significantly different from the vehicle control group value (p≤0.01).
The number of females pregnant at necropsy does not include the number of females that aborted.

Table 82 Litter Observations (Caesarean-Delivered Fetuses) - Rabbits

Dose (mg/kg/day)	0	10	20	40
Number of females treated	20	20	20	20
Number of females pregnant	18	19	19	19
Number dead due to dosing error	0	0	3	0
Number found dead	0	0	0	**5** (26.3)
Number of females aborted and sacrificed	0	0	0	1
Number of females pregnant at necropsy*	18	19	19	**13**
Litter with one or more live fetuses	18	19	19	13
Implantations	8.6	8.6	8.2	8.5
Litter sizes	8.3	8.5	7.9	8.0
Live fetuses	150	161	151	104
Live fetuses - average	8.3	8.5	7.9	8.0
Live male fetuses	73	88	71	48
% Live male fetuses/litter	47.2	52.6	45.9	46.7
Live fetal body weights (g/litter)	43.95	44.80	43.60	41.95
Male fetuses	45.67	45.44	43.02	42.40
Female fetuses	42.63	44.17	43.44	42.06
% Resorbed conceptuses/litter	4.0	2.3	3.4	**7.1**
Fetal Alteration				
Litters with fetuses with any alteration observed	8	7	9	6
Fetuses with any alteration observed	11 (7.3)	11 (6.8)	15 (9.9)	9 (8.6)
% fetuses with any alteration/litter	10.0	6.5	9.2	10.1

Cesarean section was conducted on GD 29
** Significantly different from the vehicle control group value (p≤0.01).
The number of females pregnant at necropsy does not include the number of females that aborted.

Offspring (Malformations)

Skeletal alterations were noted at 40 mg/kg, and included incidences of irregular thoracic vertebral arch and short ribs. No effects on gross external or soft tissues were noted. All ossification site averages were comparable among the dosage groups. See table below.

Table 83 Malformations in Rabbits

Dose (mg/kg/day)	0	10	20	40

Number of litters evaluated		18	19	19	13
Number of fetuses evaluated		150	161	151	104
Live		150	161	151	104
Total malformations	Litter incidence (%)	8 (44)	7 (37)	9 (47)	6 (46)
	Fetal incidence (%)	11 (7.3)	11 (6.8)	15 (9.9)	9 (8.6)
Malformations					
Fetal gross external alterations	Litter incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)
Fetal Soft tissue alteration	Litter incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	0 (0)
Fetal skeletal alteration					
Thoracic vertebrae, arch, irregular shaped	Litter incidence (%)	0 (0)	0 (0)	0 (0)	**2** (15.4)**
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	**2** (1.9)**
Rib: Short	Litter incidence (%)	0 (0)	0 (0)	0 (0)	2** (15.4)
	Fetal incidence (%)	0 (0)	0 (0)	0 (0)	2** (1.9)

Table 84 Summary of Findings in Early Decedents in Embryo-fetal Toxicity Study in Rabbits

Dosage Group	Rabbit Number	Day of Gestation/ Mode of Death/No. of Doses	Clinical Observations, Body Weights, Feed Consumption, Necropsy Observations and Uterine Contents
40 MKD (4.6 Fe MKD)	6264	DG 9 FD 2	**Clinical Observations**: Injection site discoloration (DG 8). **Body Weights**: Body weight loss of 0.11 kg from DG 7 to DG 8. **Feed Consumption**: Reduced from DG 7 to DG 8 (1 g consumed). **Necropsy Observations**: Both kidneys appeared large; all other tissues appeared normal for slight degree of autolysis. **Uterine Contents**: There were 11 embryos *in utero*. Early developmental age precluded determination of viability and any further evaluation.
	6266	DG 9 FD 2	**Clinical Observations**: Ungroomed coat (DG 8). **Body Weights**: Body weight loss of 0.09 kg from DG 7 to DG 8. **Feed Consumption**: Reduced from DG 7 to DG 8 (23 g consumed). **Necropsy Observations**: Both kidneys appeared large; all other tissues appeared normal. **Uterine Contents**: There were 9 embryos *in utero*. Early developmental age precluded determination of viability and any further evaluation.
	6270	DG 21 A/S 13	**Clinical Observations**: White periocular substance (DG 7). **Body Weights**: Body weight gains fluctuated during the observation period. **Feed Consumption**: Values fluctuated during the dosage period. **Necropsy Observations**: All tissues appeared normal. **Uterine Contents**: There was a single aborted fetus in the litter that appeared normal for developmental age at external and soft tissue evaluation. At skeletal examination, it had not ossified ischia and pubes (which was normal for its developmental age)
	6278	DG 9 FD 2	**Clinical Observations**: White periocular substance (DG 7), white perinasal substance (DG 9), red perinasal substance (DG 9) and ungroomed coat (DG 8). **Body Weights**: Body weight loss of 0.13 kg from DG 7 to DG 8. **Feed Consumption**: Reduced from DG 7 to DG 8 (29 g consumed). **Necropsy Observations**: Both kidneys appeared large; all lobes of the lungs were mottled red and dark red; the thymus contained black areas; all other tissues appeared normal for slight degree of autolysis. **Uterine Contents**: There were 9 embryos *in utero*. Early developmental age precluded determination of viability and any further evaluation.

40 MKD (4.6 Fe MKD)	6279	DG 9 FD 2	**Clinical Observations**: White periocular substance (DG 7); cold to touch, lacrimation, and decreased motor activity (DG 8). **Body Weights**: Body weight loss of 0.16 kg from DG 7 to DG 8. **Feed Consumption**: Reduced from DG 7 to DG 8 (9 g consumed). **Necropsy Observations**: Both kidneys appeared large; all lobes of the lungs were mottled red and dark red; all other tissues appeared normal for slight degree of autolysis. **Uterine Contents**: There were 12 embryos *in utero*. Early developmental age precluded determination of viability and any further evaluation.
	6280	DG 9 FD 2	**Clinical Observations**: Appeared normal. **Body Weights**: Body weight loss of 0.16 kg from DG 7 to DG 8. **Feed Consumption**: Reduced from DG 7 to DG 8 (22 g consumed). **Necropsy Observations**: Both kidneys appeared large; the thymus contained red areas; all other tissues appeared normal for slight degree of autolysis. **Uterine Contents**: There were 12 embryos *in utero*. Early developmental age precluded determination of viability and any further evaluation.

DG = day of gestation MKD = mg/kg/day Fe MKD = iron mg/kg/day
FD = Found Dead

(Excerpted from the Applicant's submission)

Toxicokinetics

Serum iron concentration increased with SEP dosage level, but the increases were less than proportional with the dose levels. Total serum iron exposures was similar at doses of 10 and 20 mg/kg/day for AUC_{0-25}, but there was much less of an increase in AUC_{0-25} between 20 and 40 mg/kg/day, suggesting potential saturation of iron binding to transferrin and rapid removal of unbound iron from the serum into cells. There were no significant differences between GDs 7 and 19 TK profiles. See table below.

Table 85 Toxicokinetics of SFP in Pregnant Rabbits

Text Table 3. Summary of Toxicokinetic Parameters

SFP Dosage Level (mg/kg)	Parameter	Day 7 of Gestation			Day 19 of Gestation		
		C_{max} (µg/dL)	T_{max} (h)	$AUC_{(0-25)}$ (h*µg/dL)	C_{max} (µg/dL)	T_{max} (h)	$AUC_{(0-25)}$ (h*µg/dL)
10	**Mean**	**380.3**	**0.83**	**6653.6**	**435.7**	**0.83**	**6103.0**
	S.D.	61.8	0.0	1036.9	27.4	0.0	651.9
	CV%	16.2	0.0	15.6	6.3	0.0	10.7
20	**Mean**	**585.7**	**0.83**	**7009.7**	**621.7**	**0.83**	**6335.1**
	S.D.	151.5	0.0	419.3	85.8	0.0	499.5
	CV%	25.9	0.0	6.0	13.8	0.0	7.9
40	**Mean**	**1429.0**	**0.83**	**9668.9**	**1374.0**	**0.83**	**8092.2**
	S.D.	323.6	0.0	1200.6	296.8	0.0	151.3
	CV%	22.6	0.0	12.4	21.6	0.0	18.9

(Excerpted from the Applicant's submission)

Dosing Solution Analysis

All dosing solutions were within 11% (89% to 99%) of the target concentrations.

9.3 Prenatal and Postnatal Development

Study title: Intravenous development and perinatal/postnatal reproduction toxicity study of SFP in rats, including a postnatal behavior/functional evaluation

Study no.:	PHX00010
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 7, 2006
GLP compliance:	Yes
QA statement:	Provided
Drug, lot #, and % purity:	SFP, Lot #125140, 11.5% iron by weight

Key Study Findings

- Three early mortalities occurred in 90 mg/kg/day dams.
- Maternal and pup effects noted at 90 mg/kg/day included dehydration, soft or liquid feces during lactation, decreases in body weight gain and feed consumption, decreases in gestation and lactation periods, number of delivered pups, number of live born pups, survival pups per litter and mean live litter sizes, and pup weight gain.
- Toxicity noted in F1 generation rats included decreases in mean body weight, body weight gain, and feed consumption values during the postweaning period. SFP adversely affected development of F1 generation pups only at maternal toxic dosage levels.
- No effects in F2 generation fetuses were noted.
- NOAEL for SFP was 30 mg/kg/day for maternal toxicity and for offspring viability and growth.

Methods

Doses:	0, 10, 30, and 90 mg/kg/day (0, 1.15, 3.45, and 10.35 mg Fe/kg/day)
Frequency of dosing:	GDs 7 to 24 (for rats not delivered a litter) or from GD 7 to Lactation Day (LD 20)
Dose volume:	5 mL/kg/hour
Route of administration:	IV infusion (in 1 hour)
Formulation/Vehicle:	Lactated Ringer's Injection, USP
Species/Strain:	Crl:CD(SD) Rats
Number/Sex/Group:	25 females for F0 generation 25/sex for F1 generation
Satellite groups:	None
Study design:	See tables below
Deviation from study protocol:	The stability data for SFP dosing formulations provided by the Applicant was not conducted in compliance with GLP regulations.

Table 86 Study Design for Treatment of F0 Generation Rats in Prenatal/Postnatal Developmental Study

Dosage Group	Number of Rats	SFP Dosage (mg/kg/day)[a,b]	Fe Dosage (mg/kg/day)[a,b]	SFP Concentration (mg/mL)	Volume (mL/kg)	Infusion Duration (hours)	Assigned F0 Generation Rat Numbers
I	25	0 (Vehicle)	0	0	5	1	1501 - 1525
II	25	10	1 15	2	5	1	1526 - 1550
III	25	30	3.45	6	5	1	1551 - 1575
IV	25	90	10.35	18	5	1	1576 - 1600

a. The test article was considered 100% active/pure for the purpose of dosage calculations.
b. The test article is 11.5% iron (Fe) by weight.

(Excerpted from the Applicant's submission)

Table 87 Study Design for F1 Generation Rats in Prenatal/Postnatal Developmental Study

Dosage Group	Maternal Dosage (mg/kg/day)	Number of Rats Per Sex	Assigned F1 Generation Rat Numbers	
			Male	Female
I	0 (Vehicle)	25	3301 - 3325	3201 - 3225
II	10	25	3326 - 3350	3226 - 3250
III	30	25	3351 - 3375	3251 - 3275
IV	90	25	3376 - 3400	3276 - 3300

(Excerpted from the Applicant's submission)

Reviewer's Note: For detailed review of this study, see the review completed by Sally J. Hargus, PhD in 2009 in the Appendix I; the study was originally submitted under IND 51290.

11 Integrated Summary and Safety Evaluation

Mechanism of Action

Triferic contains iron in the form of ferric pyrophosphate and is an iron replacement product to be administered to patients via transfer from hemodialysis solution across the dialyzer membrane to the patients' circulation. The iron can then bind to transferrin and be transported to erythroid precursor cells to be incorporated into hemoglobin.

Safety Pharmacology

Ferric pyrophosphate did not produce meaningful inhibition of hERG-mediated potassium current (I_{Kr}) at concentrations up to 1,000 µg/mL (approximately 1 µM). Ferric pyrophosphate did not induce changes in respiratory rate or minute volume, but induced a slight increase in tidal volume at 250 mg/kg without impact on the effective minute ventilation in rats. Ferric pyrophosphate did not induce specific neurotoxicities in rats. Ferric pyrophosphate induced a slight transient increase in blood pressures, increase in heart rate, lengthening QT and QTc interval, and decrease in RR interval duration. The magnitude of QT prolongation was <10% longer than baseline.

Genetic Toxicology

Ferric pyrophosphate was clastogenic in the in vitro chromosomal aberration assay in CHO cells in the presence of metabolic activation, but was negative in the absence of metabolic activation. Ferric pyrophosphate was not mutagenic in the in vitro bacterial reverse mutation (Ames) test or clastogenic in the in vivo mouse micronucleus assay.

General Repeat-Dose Toxicology – Rats and Dogs

Early mortalities in both rat and dog studies were due to moribundity related to the administration procedure, which involved septicemia or chronic inflammation in multiple organs or infusion sites. Infusion-site inflammation was noted in surviving ferric pyrophosphate-treated animals, and those inflammatory findings remained at the end of the recovery phases in those studies.

Ferric pyrophosphate induced transient and reversible decreases in body weight, body weight gain, and food consumption. Dose-dependent increases in the amount of element iron tissue deposition were noted in multiple tissues (including hepatocytes, Kupffer cells, renal tubular epithelium, and zymogen granules of the pancreas). The increases of tissue iron deposition remained at the end of the recovery phase (Perls' Prussian Blue stain method was applied histologically to examine tissue deposit of iron in tissues).

Group mean T_{max} values for iron were similar in all ferric pyrophosphate-treated groups, and exposure to iron was largely similar in both genders with minor variations. The exposure to iron (C_{max} and AUC_{0-24} values) increased less than proportionally with dose

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levels, suggesting potential saturation of iron binding to transferrin and rapid removal of unbound iron from serum with an enhanced iron distribution into tissues.

Developmental and Reproduction Toxicology

At doses up to 40 mg/kg, no adverse effects on fertility or reproduction were noted in male or female rats. At the maternally toxic dose of 40 mg/kg in rats, early embryonic development was not affected.

Ferric pyrophosphate caused embryo-fetal developmental toxicity only at maternally toxic doses, resulting in post-implantation loss due to early resorptions, abnormal placentae, decreased fetal body weight, reductions in the number of live offspring and body weights, fetal head and vertebral malformations at 90 mg/kg/day in rats and vertebral malformations at 40 mg/kg/day in rabbits.

12 Appendix/Attachments

Appendix I – Development and Reproductive Studies

This appended review was completed by Sally J. Hargus, PhD in 2009 on the prenatal and postnatal development toxicity study submitted under IND 51290 and within this NDA:

2.6.6.6 **Reproductive and developmental toxicology:Prenatal and postnatal development**

Study title: Intravenous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of SFP in Rats, Including a Postnatal Behavioral/Functional Evaluation

Key study findings: Pregnant and lactating rats tolerated daily intravenous administration of SFP at up to 30 mg/kg/day, but three rats died or were euthanized early at 90 mg/kg/day. The only effects at 30 mg/kg/day were local irritation at injection sites, apparent as injection-site discoloration and/or missing or necrotic portions of the tail, sparse hair coat on the underside, and tan pancreas, none of which were considered to reflect systemic toxicity. Other effects at 90 mg/kg/day included apparent dehydration; soft or liquid feces during the lactation period; reduced body weight gain and feed consumption; failure to deliver a litter (four dams) with corresponding reductions in the gestation and lactation indices, the number of delivered pups and the number of liveborn pups; fewer surviving pups per litter and lower mean live litter sizes; and reduced pup weight gain. F1 generation rats in the 90 mg/kg/day maternal dosage group had lower mean body weights, body weight gains and absolute and relative feed consumption values during the post-weaning period. No effects were observed in F1 generation rats with regard to sexual maturation, learning and memory or fertility and reproductive capacity as a result of treatment of the F0 generation dams with SFP, nor were there any SFP-related abnormalities in F2-generation fetuses.

Based on these results, the maternal no-observed-adverse-effect-level (NOAEL) for systemic toxicity of SFP was 30 mg/kg/day, and this was also considered to be the NOAEL for viability and growth in the offspring. Consequently, SFP adversely affected development of the F1 generation pups only at maternally toxic dosage levels.

Study no.: PHX00010
Volume # 1 of 1, and page # 1-425
Study report location: Test Facility (Conducting Laboratory)
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: 07 September 2006
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: SFP, Lot 125140, food-grade test article from (b) (4) (b) (4) (b) (4) conducted analyses; certificate of analysis was provided; 11.5% iron by weight (Appendix E) and no known contaminants.

Reviewer comment: The sponsor has been using the food-grade SFP in studies conducted thus far, including the current study reviewed herein. Testing was conducted for heavy metal contaminants, as well as iron content, citrate, phosphate, and pyrophosphate amounts. It appeared that no endotoxin or sterility testing was done. The sponsor plans to transition to GMP (pharmaceutical grade) product in late 2009, which

will include a specification for endotoxin levels. Vehicle for this study was lactated ringers solution for Injection, USP, which is similar to dialysis buffer.

Methods

Doses:

5.8.1. F0 Generation Rats

Dosage Group	Number of Rats	SFP Dosage (mg/kg/day)[a,b]	Fe Dosage (mg/kg/day)[a,b]	SFP Concentration (mg/mL)	Volume (mL/kg)	Infusion Duration (hours)	Assigned F0 Generation Rat Numbers
I	25	0 (Vehicle)	0	0	5	1	1501 - 1525
II	25	10	1.15	2	5	1	1526 - 1550
III	25	30	3.45	6	5	1	1551 - 1575
IV	25	90	10.35	18	5	1	1576 - 1600

a. The test article was considered 100% active/pure for the purpose of dosage calculations.
b. The test article is 11.5% iron (Fe) by weight.

5.8.2. F1 Generation Rats

Dosage Group	Maternal Dosage (mg/kg/day)	Number of Rats Per Sex	Assigned F1 Generation Rat Numbers	
			Male	Female
I	0 (Vehicle)	25	3301 - 3325	3201 - 3225
II	10	25	3326 - 3350	3226 - 3250
III	30	25	3351 - 3375	3251 - 3275
IV	90	25	3376 - 3400	3276 - 3300

Species/strain: Rat/Crl:CD(SD)
Number/sex/group: 25
Route, formulation, volume, and infusion rate: see table above
Satellite groups used for toxicokinetics: NA
Study design, parameters and endpoints evaluated:

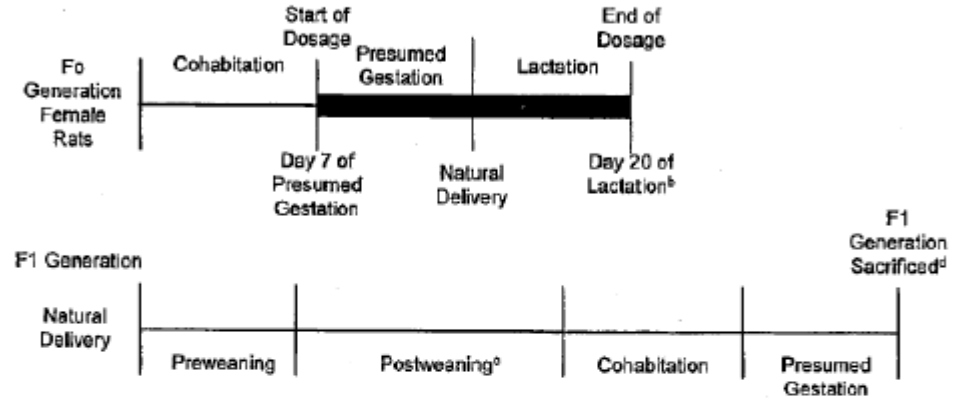
F0 generation female rats were naturally bred, assigned to Groups I through IV (25 per group) upon confirmation of mating, and given SFP or the vehicle once daily via 1-hour intravenous infusion beginning on gestation day (DG) 7 and continuing through either DG 24 (rats that did not deliver a litter) or lactation day (DL) 20. Dosage levels were 0 (Vehicle), 10, 30 and 90 mg/kg/day (0, 1.15,3.45 and 10.35 mg Fe/kg/day, respectively). The F0 generation female rats were allowed to deliver naturally and remained on study until DL 21, at which time their respective litters were weaned. The F0 generation female rats were then euthanized and necropsied on DL 21. In life, F0 generation female rats were observed for clinical signs, maternal behavior, and changes in body weight and feed consumption.

At necropsy, gross pathologic findings in the thoracic, abdominal, and pelvic cavities and the number and distribution of implantation sites were recorded.

F1 generation rats were not directly given SFP, but may have been exposed to it systemically during maternal gestation (in utero exposure) or orally via maternal milk while nursing. F1 generation rats were observed for clinical signs, changes in body weight and feed consumption, sexual maturation, behavioral changes and reproductive

capacity. Fl generation female rats were euthanized and Caesarean-sectioned on DG 21, and gross pathologic findings in the thoracic, abdominal, and pelvic cavities and the number and distribution of corpora lutea were recorded. The reproductive tract was examined, and the number and distribution of implantation sites, early and late resorptions, and live and dead fetuses were recorded. F2 fetuses were weighed, sexed and examined for gross external abnormalities. Fl generation male rats were euthanized after completion of the 21-day cohabitation period, and gross pathologic findings in the thoracic, abdominal and pelvic viscera were recorded. Testes and epididymides were excised, weighed as a pair and retained for possible future histopathological evaluations.

The sponsor provided a schematic representation of Study Design:



Dosage Period

a. For additional details see "Tests, Analyses and Measurements" section of the protocol.
b. F0 generation rats sacrificed day 21 of lactation.
c. Behavioral and functional assessments.
d. Fetal evaluations (all fetuses - external examinations).

Dose levels selected for the current study were based on the results of a previously-conducted developmental toxicity study of SFP in rats (Protocol PHX00004), which was reviewed by Dr. David E. Bailey, 2007, STN 020 and 021. In that study, pregnant rats were given SFP by 1-hour intravenous infusion at a rate of 5 mL/kg/h once daily on days 7 through 17 of presumed gestation at dosages of 0 (Vehicle), 10, 30 and 90 mg/kg/day (0, 1.15, 3.45 and 10.35 mg Fe/kg/day, respectively). The sponsor stated that SFP produced no toxic effects at 10 mg/kg/day. At 30 mg/kg/day, maternal toxicity was limited to reduced weight gain the first day and discolored injection sites in a few rats, and developmental toxicity was limited to a slight increase in delayed fetal ossification. At 90 mg/kg/day, maternal toxicity included reduced weight gain for the first 3 days, discolored injection site, sparse hair coat, various gross lesions, and early death or euthanasia. Developmental toxicity at 90 mg/kg/day included greater post-implantation loss, fewer normal placentae, lower fetal body weight, and a significant increase in fetal head and/or vertebral malformations.

Reviewer comment: The statements by the sponsor are in agreement with Dr. Bailey's review regarding the study outcome.

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RESULTS

RESULTS FOR F_0 IN-LIFE:
Mortality: Three SFP-related deaths occurred at 90 mg/kg/day: two rats (#1577 and #1580) were found dead during dosage administration on the third and second day of treatment, respectively, and another rat (# 1596) was euthanized due to adverse clinical condition and the lack of appropriate maternal behavior on DL 9 after 24 doses of SFP were administered. The cause of death or morbidity for these rats was not apparent from the in-life and postmortem observations, which are summarized in Text Table 1 and detailed in Text Table 2; however, the deaths were considered related to treatment with SFP because the mortalities occurred at the highest dosage level. Common findings in the two rats that died were weight loss, red submandibular lymph nodes, red areas in the thymus and red renal pelvis. Findings in the dam that was euthanized included injection site discoloration (purple) and swelling, vocalization, circling, apparent dehydration, sparse hair coat on the underside, and a tan pancreas.

Two other deaths occurred but were considered by the sponsor to be unrelated to SFP. One rat at 30 mg/kg/day was found dead on DG 19 after 12 doses were administered, and one rat at 10 mg/kg/day was found dead on DL 10 after 25 doses were administered. The cause of death or morbidity for these rats was not apparent from the in-life and postmortem observations, which are summarized in Text Table 1 (page 40) and detailed in Text Table 2 (page 41). These mortalities were not attributed to treatment with SFP due to the lack of common adverse in-life findings and the lack of common gross lesions.

All other rats survived until scheduled sacrifice.

Clinical signs: The following clinical observations occurred during the gestation and/or lactation period in a pattern that suggested a relationship to SFP:
-Injection-site discoloration (purple and/or black) and missing or necrotic portions of the tail (where the injection site was located) in a few rats at all dose levels.
-Sparse hair coat on the underside in a few rats at 30 mg/kg/day and many rats at 90 mg/kg/day.
-Apparent dehydration and failure to deliver a litter at 90 mg/kg/day.
-A significantly increased incidence of soft or liquid feces during the lactation period at 90 mg/kg/day.

Body weight: Daily administration of SFP reduced body weight gain at 90 mg/kg/day during gestation and lactation phases. Body weight gains in the lower dose-level groups were similar to vehicle controls.

Food consumption: Daily administration of SFP reduced feed consumption at 90 mg/kg/day but not at lower dosages.

Toxicokinetics: not measured

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Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): Pregnancy occurred in 24, 24, 24, and 24 of the 25 mated female rats in all groups. However, deaths prior to delivery day resulted in 24, 24, 23, and 23 rats (for 0, 10, 30, and 90 mg/kg/d groups, respectively) that could deliver pups at the end of the gestation period.

Natural delivery observations: The sponsor stated that the following SFP-related natural delivery observations occurred at 90 mg/kg/d:
-Failure to deliver a litter, with total litter losses (100% resorptions) in four dams.
-Cannibalization of the entire litter in one female rat. The dam had 15 empty implantation sites in utero at examination DG 25, but was not observed to have delivered a litter.
-Fewer surviving pups per litter and lower mean live litter sizes
-Reduced pup weight gain at each tabulated interval

Natural delivery and litter observations were similar to controls in the 10 and 30 mg/kg/d groups. However, the number of pups found dead or presumed cannibalized on DL 1 was significantly increased in the 30 mg/kg/d group.

F_0 NECROPSY: The high-dose group was observed to have SFP-related gross lesions at necropsy as described above under mortality results.

RESULTS FOR F1 GENERATION PUPS:
Clinical and necropsy observations: daily administration of sfp was not consistently associated with clinical or necropsy observations in f1 generation pups at any dosage, according to the sponsor.
-No incidences of clinical signs were dosage-dependent
-Clinical signs were observed in limited numbers of litters within the dose group
-Some signs were observed only in the vehicle control group.

RESULTS FOR F1 GENERATION ADULT RATS:
Mortality: There appeared to be no SFP-dependent effects on survival of F1 generation rats at any dose level.

Clinical observations: The only clinical observations considered related to SFP was a greater incidence ($p \leq 0.01$) of swelling of one or both ears in F 1 generation female rats from F0 generation dams that were treated with SFP at 90 mg/kg/day. Ear swelling was noted in two female rats that had both ears swollen during the pre-cohabitation period (week 1 post-weaning to cohabitation), and five female rats that had the right and/or the left ear swollen during the gestation period.

Ear swelling also was noted during the gestation period in one female rat in the 30 mg/kg/day maternal dosage group and during weeks 13 through 17 post-weaning in one male rat in the maternal dosage group. Although ear swelling in female rats from dams treated with SFP at 90 mg/kg/day was considered to be SFP-related, it was not

considered adverse because the growth and development of these F 1 generation rats was not affected. The toxicologic and clinical significance of the ear swelling is unknown.

All other clinical observations in the F1 generation male and female rats were considered unrelated to treatment of the F0 generation dams with SFP because: 1) the incidences were not dosage dependent; 2) the observation occurred in a relatively small number of rats; 3) the observation is common in this species and strain; and/or 4) the observation occurred in only the maternal control group. These clinical observations included bent tail, sparse hair coat (limbs, neck and/or underside), missing/broken and/or misaligned incisors, tip of tail missing and/or swollen, swollen snout, scab on the neck or back, and localized alopecia (limbs).

Body Weights and Body Weight Changes:
Maternal dosages of SFP as high as 30 mg/kg/day in F0 generation dams did not affect body weights or body weight gains for the F1 generation rats, but a maternal dosage of 90 mg/kg/day in the F0 generation dams reduced weight gain in F1 generation rats of both sexes.

Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values:
Maternal dosages of SFP as high as 30 mg/kg/day in F0 generation dam did not affect absolute (g/day) and relative (g/kg/day) feed consumption values in the F1 generation, but a maternal dosage of 90 mg/kg/day in F0 generation dams did reduce feed consumption in F1 generation rats of both sexes.

Absolute feed consumption was significantly reduced in the F1 generation male rats in the 90 mg/kg/day maternal dosage group during the first two weeks of the post-weaning period (8% and 6% reduction from control), during the overall pre-cohabitation period (7% reduction from control) and during the entire post-weaning period (6% reduction from control). Relative feed consumption values were generally comparable among the F1 generation male rats in each group.

Relative feed consumption values were significantly increased in the F1 generation female rats in the 30 and 90 mg/kg/day maternal dosage groups during the first week of the post-weaning period; absolute feed consumption was significantly reduced ($p \leq 0.05$) in the F1 generation female rats in the 90 mg/kg/day maternal dosage group during the second week of the post-weaning period. The increase in relative feed consumption values in the 30 mg/kg/day maternal dosage group was considered transient and not adverse.

Absolute and relative feed consumption values were generally comparable among the F1 generation female rats during the overall pre-cohabitation period and during the entire gestation period.

F_1 behavioral evaluation:
Passive Avoidance: The only difference among groups considered related to SFP was a significant increase ($p \leq 0.05$) in the latency to the second trial in F1 generation female rats from F1 generation dams that were treated with SFP at 90 mg/kg/day.

There were no other statistically significant or biologically important differences in the values for learning, short-term retention, long-term retention, or response inhibition in the F1 generation rats, as evaluated by performance in a passive avoidance paradigm.

Water Maze Performance: NNF

Caesarean-Sectioning and Litter Observations: NNF

F_1 reproduction: NNF

F1 NECROPSY: Maternal dosages of SFP as high as 90 mg/kg/day in F0 generation dams did not produce findings at necropsy in F 1 generation rats.

Terminal Body Weights, Epididymides and Testes Weights and Ratios (%) of Epididymides and Testes Weight to Terminal Body Weight: Maternal dosages of SFP as high as 90 mg/kg/day in F0 generation dams did not affect the absolute weights of testes or epididymides or the ratios of these weights to the
terminal body weight in F 1 generation male rats.

F_2 RESULTS: NNF

shown are an estimate of worst-case. The amount of iron from SFP absorbed by the dialysis patient likely would be dependent on many factors.

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/s/

CHING-JEY G CHANG
12/22/2014

TODD R PALMBY
12/22/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

NDA Number: 206317 **Applicant: Rockwell Medical, Inc. Stamp Date: March 24, 2014**

Drug Name: Triferic (Soluble Ferric Pyrophosphate, SFP) **NDA Type: 505(b)(1) Standard**

On **initial** overview of the NDA 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		Numerous spaces among words are present in many pages of report text of the submitted 39-week, repeat-dose toxicology study in dogs testing SFP.
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		The food-grade ferric pyrophosphate was used in the nonclinical toxicology studies. The pharmaceutical-grade ferric pyrophosphate was used in the clinical trials and will be used clinically. Comparability between the two will be determined during the review of the NDA.
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		Note: The nonclinical in vivo studies were conducted through an IV infusion, while clinical trials were conducted with addition of SFP to dialysis solution.
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		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		No impurity issues were raised prior to the NDA submission. Evaluation of impurities will be conducted during the NDA review.
11	Has the applicant addressed any abuse potential issues in the submission?	N/A	N/A	
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	N/A	N/A	

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

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.

None

C.J. George Chang 5/16/14

Reviewing Pharmacologist Date

Todd Palmby 5/16/14

Team Leader/Supervisor Date

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

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05/16/2014

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