

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPLICATION NUMBER: 21-135**

**PHARMACOLOGY REVIEW(S)**

Strongin

SEP 28 2000

**PHARMACOLOGIST'S REVIEW OF NDA 21-135  
(Amendment Dated May 3, 2000)**

**Reviewer:** David B. Joseph, Ph.D.,  
Pharmacologist (HFD-180)

**Sponsor and Address:** Luitpold Pharmaceuticals, Inc.  
Shirley, New York

**Drug:** Venofer® (Iron Sucrose Injection), 20 mg iron/ml

**Other Names:** iron sucrose/iron saccharate/Ferrum Hausmann IV®/  
Hippiron® (veterinary product)

**Drug Class:** iron supplement/hematinic

**Date of Submission:** May 3, 2000 (Amendment)

**Date of Receipt by HFD-180:** May 3, 2000 (Amendment)

**Date of Review:** September 7, 2000

**Submission Contents:**

Segment III Study of Effects on Pre- and Post-Natal Development  
in CD Rats by Intravenous Infusion Administration

**Segment III Study of Effects on Pre- and Post-Natal Development  
in CD Rats by Intravenous Infusion Administration**

**Study #** LPL 004/993100

**Testing Laboratory:** \_\_\_\_\_  
\_\_\_\_\_

**Study Dates:** 5/19/99-4/28/00

**GLP Compliance:** A statement of compliance was included.

**QA Report:** Yes (x) No ( )

**Animals:** pregnant Sprague Dawley (Cr1:CD®BR) rats,  
age 8-9 weeks, 178-259 g

**METHODS:** Pregnant rats were treated intravenously with Venofer® (lot # 807119) on days 6 through 19 of gestation, and on days 1, 4, 7, and 10 of lactation. Dose levels of 0 (0.9% NaCl), 1.5, 4.5, and 13 mg Fe/kg/day were used (25 females/group), with corresponding dose volumes of 13, 1.5, 4.5, and 13 ml/kg, respectively. The authors stated that dose selection was based on available toxicity data, although no details were provided. The drug or vehicle was administered by a 4-hr infusion in restrained conscious animals. The dose rates were 6.25, 18.75, and 54.17 µg Fe/kg/min in the low-, middle-, and high-dose groups, respectively. Venofer® (20 mg Fe/ml) was diluted in 0.9% NaCl to a concentration of 1 mg Fe/ml, prior to administration. Two dose levels of Venofer® were proposed in the original protocol, as reviewed in IND (Pharmacologist's review dated June 3, 1999). However, this study was performed using three dose levels, as requested by the Division of Gastrointestinal and Coagulation Drug Products in a teleconference with the Sponsor on December 17, 1998.

The following parameters were recorded for the pregnant females.

**Clinical Signs:** daily (non-dosing and dosing days)

**Bodyweight:** days 1, 2, 3, 6, 8, 10, 12, 14, 16, 18, and 20 of gestation, days 1, 4, 7, 10, 14, 18, and 21 of lactation

**Food Consumption:** days 3-5, 6-7, 8-9, 10-11, 12-13, 14-15, 16-17, and 18-19 of gestation, days 1-3, 4-6, 7-9, 10-13, 14-17, and 18-20 of lactation

**Parturition and Duration of Gestation:** three times daily starting on day 20 of gestation

All females were permitted to deliver and raise their offspring until weaning (day 21 postpartum). Non-pregnant females were sacrificed on day 25 after mating. Females that delivered were sacrificed after weaning, and the number of implantation sites was recorded. On day 4 postpartum, litters that contained more than ten pups were reduced to ten by culling, resulting in five males and five females in each litter, whenever possible. The following parameters were recorded for the offspring.

**Clinical Signs:** daily, starting at 24 hr after birth (day 1)

**Mortality:** daily

**Sex:** days 1, 4, 21

**Bodyweight:** days 1, 4 (before culling), 8, 12, 14, 18, 21, and 28

**Surface Righting Reflex:** day 1 until 100% success

**Startle Reflex:** day 11 until 100% success

**Air Righting Reflex:** day 14 until 100% success

**Pupil Reflex:** day 20

At weaning, 20 male and 20 female offspring were selected from each dose group to form the adult F<sub>1</sub> generation (1 male and 1 female from each of 20 litters). These animals were used for assessment of physical and sexual maturation, and reproductive performance. Pups that were not selected were sacrificed on or shortly after day 21. The following observations were recorded for the F<sub>1</sub> generation.

**Clinical Signs:** daily

**Bodyweight:** weekly for males until termination; weekly for females until mating was detected, days 0, 3, 7, 10, 14, 17, and 20 of gestation, and days 1, 4, 7, and 14 of lactation

**Sexual Maturation:** day 28 until maturation for females; day 35 until maturation for males

**Accelerating Rotarod Test:** 3 trials at 4 weeks of age

**Actimat Test (spontaneous activity):** 5 weeks of age; activity was categorized as low level (head turning and similar small movements) and high level (walking, rearing, and whole body movements)

**Passive Avoidance Test:** 7-8 weeks of age

At approximately 10 weeks of age, males and females from the same treatment group were paired in a 1:1 ratio, without pairing of siblings. Mating was confirmed by the presence of a copulation plug and by the presence of spermatozoa in vaginal smear. The day of mating confirmation was designated as day 0 of gestation. The time elapsed between the initial pairing and detection of mating was recorded. Pregnant females were observed three times daily for evidence of parturition, starting on day 20 of gestation. All F<sub>1</sub> females were allowed to deliver and raise their offspring until day 14 of lactation. F<sub>1</sub> females

were sacrificed on day 14, and the number of implantation sites was recorded. The following observations were recorded for the F<sub>2</sub> pups, which were sacrificed on day 14 postpartum.

**Clinical Signs:** daily

**Mortality:** daily

**Bodyweight:** days 1, 4, 8, 12, and 14

**Sex:** days 1 and 14

All animals (F<sub>0</sub>, F<sub>1</sub>, and F<sub>2</sub>) were examined externally and internally for macroscopic abnormalities.

**RESULTS:**

**Clinical Signs in Dosed Animals:** Evidence of local irritation in the tail, such as scabs, missing tip, dark discoloration, and black or damaged tip, was observed in the 4.5 and 13 mg Fe/kg/day groups (6/25 and 12/25, respectively). The poor tail condition prevented dosing on a few occasions in these groups. Three rats in the 4.5 mg Fe/kg/day group were not dosed on one occasion, and nine rats in the 13 mg Fe/kg/day group were not dosed on up to three occasions. Alopecia was observed in the control and treated groups.

**Mortality in Dosed Animals:** There were no deaths.

**Bodyweight and Food Consumption in Dosed Animals:** Weight gain was significantly reduced in the 4.5 and 13 mg Fe/kg/day groups on days 6-8 of gestation, with loss of weight occurring in the 13 mg Fe/kg/day group. However, weight gain was unaffected from day 8 through 20 of gestation, and there were no changes in postpartum bodyweight. The pregnant control females weighed 256 ± 15 g on day 6 of gestation, and 349 ± 27 g on day 20. The control females weighed 257 ± 20 g on day 1 postpartum, and 327 ± 21 g on day 21 postpartum. Food consumption was reduced by 9-18% in the 4.5 and 13 mg Fe/kg/day groups on days 6-9 of gestation, correlating with the reduction in weight gain in these groups. Food intake was normal during the remainder of the study.

**In-Life Observations:** There were no changes in the duration of pregnancy, litter size, pup mortality at delivery, pup weight, and survival of pups during the first 21 days postpartum. All females with surviving pups reared their offspring until weaning.

on day 21. Pregnancy and litter observations are summarized in the following table.

F <sub>0</sub> Females Parameter	Dose (mg Fe/kg/day)			
	0	1.5	4.5	13
Initial Group Size	25	25	25	25
# Pregnant	23	24	24	23
# With Total Litter Loss Postpartum	0	2	0	1
# Rearing Pups to Weaning	23	22	24	22
Gestation (days)*	21.4	21.6	21.4	21.3
<b>Day 1 Postpartum*</b>				
Litter Size (total)	13.0	11.9	12.8	12.1
Litter Size (living)	12.7	11.6	12.5	12.1
Mortality (%)	2.2	2.4	2.7	0.3
Litter Weight (g)	74.8	74.5	74.6	70.0
Pup Weight (g)	5.9	6.5	6.0	5.9
<b>Day 4 (pre-culling)*</b>				
Litter Size	11.7	11.5	12.1	11.5
Mortality (%)	9.6	3.1	5.4	4.4
Litter Weight (g)	96.2	108.0	101.3	94.2
Pup Weight (g)	8.1	9.5	8.4	8.3
<b>Day 4 (post-culling)*</b>				
Litter Size	9.5	9.7	9.7	9.5
Litter Weight (g)	77.9	92.6	81.5	78.9
Pup Weight (g)	8.1	9.6	8.4	8.3
<b>Day 21*</b>				
Litter Size	9.4	9.7	9.5	9.5
Mortality (%)	0.4	0.0	2.9	0.5
Litter Weight (g)	416.2	466.6	434.2	425.3
Pup Weight (g)	43.8	48.3	45.8	45.0

\*Mean values.

Total litter loss occurred in two females in the 1.5 mg Fe/kg/day group, and in one female in the 13 mg Fe/kg/day group. There was no significant change in the sex ratio. Males comprised 46.4%, 48.3%, 54.1%, and 53.4% of the control, low-, middle-, and high-dose litters, respectively, on day 1, with similar results observed on days 4 and 21.

There were no clinical signs in pups prior to weaning. Hair loss on forelimbs was observed in all treated groups on weeks 12-18. Sexual maturation was unaffected in offspring from Venofer®-treated females. The average age of sexual maturation was 44.8 days in control males (balanopreputial separation) and 34.3 days in control females (vaginal opening).

Weight gain was increased by 31-56% in the offspring of all treatment groups on days 1-10 postpartum. Bodyweight in adult F<sub>1</sub> males was unaffected by Venofer® treatment. Similarly, bodyweight in adult F<sub>1</sub> females before mating and during gestation was unaffected. Control F<sub>1</sub> males weighed 86 ± 7 g on week 4, and 550 ± 43 g on week 16. Control F<sub>1</sub> females weighed 78 ± 6 g on week 4, 250 ± 23 g on week 10 (pre-mating), 261 ± 25 g on day 0 of gestation, 412 ± 31 g on day 20 of gestation, 322 ± 32 g on day 1 postpartum, and 361 ± 23 g on day 14 postpartum.

There were no effects on the performance of pups (F<sub>1</sub> generation) in the pre-weaning developmental tests, which included surface righting, startle response, air righting, and pupil reflex. These results are summarized below.

Dose (mg Fe/kg/day)	Mean Age of Litters at 100% Success			Pupil Reflex (Day 20)
	Surface Righting	Startle Response	Air Righting	% Successful
0	23.9	34.8	37.5	100
1.5	23.7	34.6	37.1	100
4.5	24.1	34.6	37.3	100
13	23.8	34.5	37.5	100

There were no effects on the performance of the F<sub>1</sub> generation in the post-weaning behavioral tests, which included the rotarod test, actimat (spontaneous movement) test, and passive avoidance test.

Venofer® had no effect on the estrous cycle or pre-coital interval in F<sub>1</sub> rats. The mating and fertility parameters of the F<sub>1</sub> generation are summarized in the following table.

F <sub>1</sub> Generation Parameter	Dose (mg Fe/kg/day)			
	0	1.5	4.5	13
Initial Group Size (M/F)	20/20	20/20	20/20	20/20
<b>Males</b>				
Induced Pregnancy	20	19	18	20
Survived to Termination	20	20	20	20
Mating (%)	100	95	100	100
Conception Rate (%)	100	100	90	100
Fertility Index (%)	100	100	90	100
<b>Females</b>				
# Pregnant	20	20	18	20
# With Total Litter Loss	0	1	0	0
Rearing Young to Weaning	20	19	18	20
Mating (%)	100	100	100	100
Conception Rate (%)	100	100	90	100
Fertility Index (%)	100	100	90	100
Gestation Index (%)	100	100	100	100

Conception Rate = (number of pregnant animals/number of mated animals) x 100.  
Fertility Index = (number of pregnant animals/number of paired animals) x 100.

Gestation Index was not defined.

There were no treatment-related effects on the reproductive performance of the F<sub>1</sub> generation. One male in the 1.5 mg Fe/kg/day group failed to mate. Two mated females in the 4.5 mg Fe/kg/day group did not achieve pregnancy. Both of these females exhibited normal estrous cycles. There was no treatment-induced change in gestation length.

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ON ORIGINAL

Litter parameters from the F<sub>1</sub> adults are summarized in the following table.

F <sub>1</sub> Generation Parameter	Dose (mg Fe/kg/day)			
	0	1.5	4.5	13
<b>Day 1 Postpartum</b>				
Litter Size (total)	13.9	15.9	15.7	14.4
Litter Size (living)	13.3	15.7	15.1	14.3
Mortality (%)	3.6	1.7	3.9	0.7
Litter Weight* (g)	78.9	96.2*	96.2*	88.3*
Pup Weight (g)	6.0	6.2	6.2	6.2
<b>Day 4 Postpartum</b>				
Litter Size	13.0	15.0	14.8	14.1
Mortality (%)	6.3	6.4	5.1	2.1
Litter Weight (g)	107.0	126.9*	127.3*	124.2*
Pup Weight (g)	8.4	8.4	8.7	8.9
<b>Day 14 Postpartum</b>				
Litter Size	12.6	14.5	14.8	13.7
Mortality (%)	8.5	9.2	5.4	4.4
Litter Weight (g)	323.7	360.6	372.9	364.8
Pup Weight (g)	26.1	24.9	25.6	26.8

Mean values are listed.

\*  $p \leq 0.05$

There were no effects on pup weight and mortality. There was a slight increase in litter weight in all Venofer®-treatment groups, due to a smaller number of pups in the control litters. There was no significant change in the sex ratio. Males comprised 49.6%, 51.4%, 54.9%, and 49.1% of the control, low-, middle-, and high-dose litters, respectively, on day 1, with similar results observed on day 14. The live birth index and viability index (day 4) was unchanged in the treatment groups, as summarized in the following table.

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F <sub>1</sub> Generation Dose (mg Fe/kg/day)	Number of Pregnant Animals	Live Birth Index (%)	Day 4 Viability Index (%)
0	20	96.4	97.3
1.5	19*	98.3	95.3
4.5	18	96.2	98.7
13	20	99.3	98.6

Live birth index = (number of live offspring on day 1/total number of offspring born) x 100.

Day 4 viability index = (number of live offspring on day 4 (before culling)/number of live offspring on day 1) x 100.

\*Excluding one female with total litter loss.

There was a single incidence of total litter loss (13 alive on day 1) and one litter with a 71% mortality rate (14 alive on day 1) in the 1.5 mg Fe/kg/day group.

**Terminal Evaluations:** Signs of local irritation in the tail, as described previously, were observed in the macroscopic examination of F<sub>0</sub> females treated with 4.5 or 13 mg Fe/kg/day. Aside from this change, there were no treatment-related effects in the F<sub>0</sub> females. There were no treatment-related changes in the F<sub>1</sub> pups that were sacrificed on day 21 postpartum. Small testes and epididymides was observed in 3/20 adult F<sub>1</sub> males in the 4.5 mg Fe/kg/day group. This change was probably unrelated to treatment, since there was no incidence in the 13 mg Fe/kg/day group. No macroscopic changes were observed in the F<sub>2</sub> pups. Venofer® treatment had no effect on post-implantation loss in the F<sub>0</sub> and F<sub>1</sub> litters, as summarized in the following table.

Parameter	Dose (mg Fe/kg/day)			
	0	1.5	4.5	13
<b>F<sub>0</sub> Generation</b>				
Implantations	13.7	12.5	13.6	13.4
Post-Implantation Loss (%)	5.5	4.5	6.9	8.5
<b>F<sub>1</sub> Generation</b>				
Implantations	14.2	16.2	16.2	14.6
Post-Implantation Loss (%)	5.6	1.7	4.0	2.6

Post-Implantation Loss = [(number of implantations - number of live pups)/number of implantations] x 100.

Mean values are listed.

**Conclusions:** Intravenous administration of Venofer® to pregnant rats produced impaired weight gain during the first two days of treatment (days 6-8 of gestation) at dose levels of 4.5 and 13 mg Fe/kg/day, but had no effects on other pregnancy-related parameters. There were no effects on the development of the F<sub>1</sub> generation and F<sub>2</sub> pups.

**SUMMARY AND EVALUATION:**

This study was performed using three dose levels, as requested by the Division of Gastrointestinal and Coagulation Drug Products in a teleconference with the Sponsor on December 17, 1998.

Treatment of female rats with Venofer® during pregnancy and lactation, using doses of 0, 1.5, 4.5, or 13 mg Fe/kg/day i.v., produced no effects on the viability at birth, long-term survival, growth, development, and reproductive performance of the offspring. Venofer® was well tolerated in the pregnant females, with no effects on gestation. Local irritation and reduced weight gain on days 6-8 of gestation were the only adverse reactions observed in the females. The use of dosing on every third day during lactation was appropriate, since Venofer® is intended to be administered not more than three times per week in humans.

The basis of dose selection was not described in detail. However, the use of the selected dose levels appears to be justified based on results from a 13-week intravenous study in rats using three doses per week (Pharmacologist's Review of NDA 21-135 Dated April 13, 2000). The tolerated dose in this study was 3 mg Fe/kg/dose. Heavy accumulation of iron in liver, kidneys, and spleen, and a reduction in weight gain were observed in the 10 and 30 mg Fe/kg/dose groups. Elevation of serum levels of ALT, AST, and bilirubin occurred in the 30 mg Fe/kg/dose group.

**APPEARS THIS WAY  
ON ORIGINAL**

**RECOMMENDATIONS:**

None.

*/S/*

David B. Joseph Ph.D.  
Pharmacologist, HFD-180

*9/7/00*  
Date

Comments:

*/S/*

Jasti B. Choudary, B.V.Sc., Ph.D.  
Supervisory Pharmacologist, HFD-180

*9/28/2000*  
Date

cc:

Orig NDA 21-135

HFD-180

HFD-181/CSO

HFD-180/Dr. Choudary

HFD-180/Dr. Joseph

IND 57,103

HFD-180

R/D Init.: J. Choudary 8/2/00

DJ/deg: 9/7/00

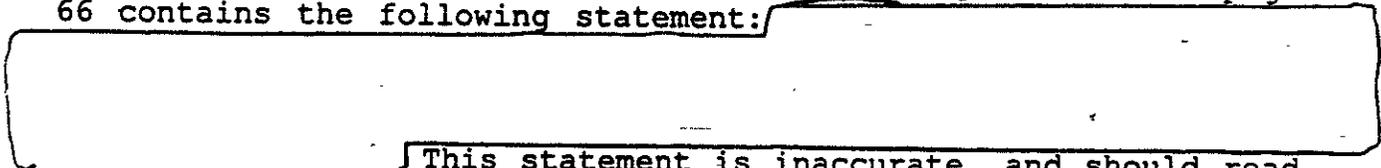
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ADDENDUM TO PHARMACOLOGY REVIEW OF NDA 21-135  
DATED APRIL 18, 2000

The proposed version of the pregnancy labeling section on page 66 contains the following statement:



This statement is inaccurate, and should read as follows: "Reproduction studies have been performed in rats and rabbits at doses up to 0.5 times and 1 times the human dose (based on a body surface area comparison), respectively, and have revealed no evidence of impaired fertility or harm to the fetus due to Venofer®."

/S/

David B. Joseph, Ph.D.  
Pharmacologist, HFD-180

9/25/00  
Date

Noted

/S/

9/25/00

- cc:
- Orig NDA 21-135
- HFD-180
- HFD-181/CSO
- HFD-180/Dr. Choudary
- HFD-180/Dr. Joseph
- HFD-345 Dr. Viswanathan
- IND \_\_\_\_\_
- HFD-180

R/D Init.: J. Choudary 9/25/00  
DBJ/9/22/00

APPEARS THIS WAY  
ON ORIGINAL

*Spencer*

APR 18 2000

NDA 21-135

REVIEW # 1

Sponsor and Address: Luitpold Pharmaceuticals, Inc.  
Shirley, New York

Reviewer: David B. Joseph, Ph.D.,  
Pharmacologist (HFD-180)

Date of Submission: Initial - August 6, 1999  
Amendment - September 22, 1999  
Amendment - October 19, 1999

Date of HFD-180 Receipt: Initial - August 9, 1999  
Amendment - September 22, 1999  
Amendment - October 19, 1999

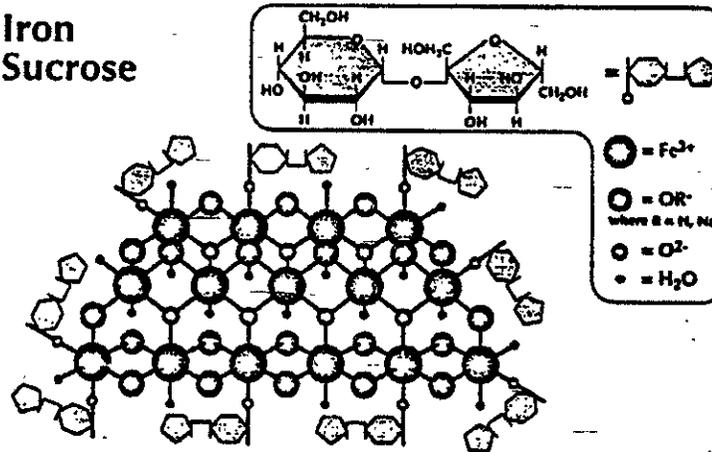
Date of Review: April 13, 2000

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original Summary

Drug: Venofer® (Iron Sucrose Injection), 20 mg iron/ml

Iron  
Sucrose



MW≈43,200 Daltons

Molecular Formula:  $[Na_2Fe_5O_8(OH) \cdot 3(H_2O)]_n \cdot m(C_{12}H_{22}O_{11})$

n = degree of iron polymerization

m = number of sucrose molecules in complex with  
iron(III)-hydroxide

Chemical Name: Iron(III)-hydroxide sucrose complex

Formulation: Venofer® Iron Sucrose Injection. Each vial contains 100 mg of iron in the form of iron(III) hydroxide-sucrose complex, in 5 ml of water for injection with pH adjusted to 10.5-11.1 with NaOH.

Other Names: Iron sucrose/iron saccharate/Ferrum Hausmann IV®/Hippiron® (veterinary product).

Category: Iron supplement/hematinic

Related Drugs/INDs/NDAs: IND \_\_\_\_\_ Venofer® (Iron Sucrose Injection), Luitpold Pharmaceuticals, Inc.

Proposed Marketing Indications: Venofer® is indicated for treatment of the following: dialysis-associated anemia

Dose: The recommended dose for adults is 100 — mg iron (2 — mg iron/kg/dose) administered one to three times per week by intravenous drip infusion (100 mg iron/15 min, with dilution of Venofer® in 0.9% NaCl), or slow injection into dialysis line (100 mg iron/5 min, with undiluted Venofer®). The total recommended dose is 1000 mg iron given in 10 doses, with repetition if needed.

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13-Week I.V. Infusion Toxicity in Dogs with 3 Doses Per Week	LPL 002/992102	807119	32
<b>Reproductive Toxicology:</b>			
Segment I Fertility and Early Embryonic Study in Rats	LPL 003/992500	807119	38
Segment II Intravenous Teratogenicity Study in Rats <sup>1</sup>	VFR 16-974254	676109 692109A1	41
Segment II Intravenous Teratogenicity Study in Rabbits	VFR026/983656	711119	46
<b>Genetic Toxicology:</b>			
Bacterial Mutation Assay <sup>1</sup>	96/VFR012/1211	572109	57
Bacterial Mutation Assay <sup>1</sup>	265	---	58
Mammalian Cell Mutation Assay <sup>1</sup>	VFR 014/971264	572109	59
Mouse Micronucleus Test <sup>1</sup>	96/VFR013/1243	572109	60
Chromosome Aberration Test in Human Lymphocytes <sup>1</sup>	VFR 4/950317	330109A2	61
<b>Special Toxicology Studies:</b>			
Perivenous Tolerance in Rabbits <sup>1</sup>	VFR 2/951737	330109A2	63
Intra-arterial Tolerance in Rabbits <sup>1</sup>	VFR 1/951736	330109A2	63

<sup>1</sup> Study was reviewed in IND \_\_\_\_\_

Several studies that were included in this application were previously reviewed in IND \_\_\_\_\_. These studies include the following: distribution in rats; distribution in minipigs; excretion and transfer to offspring in rats; acute toxicity in rats and mice with intravenous, subcutaneous, and oral administration; acute toxicity in rats and mice with intravenous and subcutaneous administration; 7-day intravenous toxicity in rats; 13-week IV infusion study in rats with weekly dosing; 13-week IV infusion study in dogs with weekly dosing; Segment II intravenous teratogenicity study in rats; two bacterial mutation studies; mammalian cell mutation study; mouse micronucleus test;

chromosome aberration test in cultured human lymphocytes; perivenous tolerance study in rabbits; intra-arterial tolerance study in rabbits. The reviews of these studies were incorporated into the present review.

#### PHARMACOLOGY:

Preclinical pharmacology studies were not included in this application. The pharmacological activity of Venofer® (iron sucrose) was characterized in clinical studies only. Venofer® was shown to augment the hematopoietic response to recombinant human erythropoietin (r-HuEPO) in patients with anemia of renal failure. - Treatment of anemic patients with Venofer® produced increases in hemoglobin, serum ferritin, and transferrin saturation.

#### ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION (ADME):

##### 1. Absorption

##### Absorption of $^{59}\text{Fe}$ in Rats Treated with [ $^{59}\text{Fe}$ ]Iron Sucrose, [ $^{59}\text{Fe}$ ]Iron Polymaltose, and [ $^{59}\text{Fe}$ ]Iron Dextran.

Methods: Male Sprague-Dawley rats weighing an average of 259 g were given an iron-deficient diet for 10 days to achieve an iron depleted state. However, the parameters related to iron status (i.e. hemoglobin, ferritin, and transferrin saturation) were not measured. Rats were administered a single intravenous injection of 10 mg  $^{59}\text{Fe}$  (average of 39 mg Fe/kg) using one of the following iron preparations: iron sucrose (Venofer®, lot # 559209A2), iron polymaltose (Amylofer®), iron dextran VIT, or iron dextran BP/USP (4 males/group). Iron polymaltose, iron dextran VIT, and iron dextran BP/USP were used for the purpose of comparison. Serum content of radioactivity was measured during a 28-day period following injection, and the total amount of radioactive iron in serum was estimated. The elimination of  $^{59}\text{Fe}$  from serum was characterized for all iron preparations tested.

Results: The serum levels of  $^{59}\text{Fe}$  and the kinetic parameters are summarized in the following tables.

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% Radioactive Dose in Serum (Mean $\pm$ SD)				
Day	Iron Sucrose	Iron Polymaltose	Iron Dextran VIT	Iron Dextran BP/USP
0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0
0 (6 hr)	2.7 $\pm$ 0.7	2.9 $\pm$ 0.4	38.6 $\pm$ 9.0	26.2 $\pm$ 5.5
1	0.7 $\pm$ 0.2	0.8 $\pm$ 0.1	3.4 $\pm$ 0.8	3.7 $\pm$ 0.7
2	0.5 $\pm$ 0.4	0.5 $\pm$ 0.2	0.3 $\pm$ 0.2	0.5 $\pm$ 0.3
3	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1
4	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1	0.4 $\pm$ 0.2	0.4 $\pm$ 0.2
7	0.5 $\pm$ 0.3	0.3 $\pm$ 0.1	0.4 $\pm$ 0.2	0.2 $\pm$ 0.0
14	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0
21	0.1	0.1	0.2	0
28	0	0	0	0.2

n = 4 on days 0-14

n = 2 on days 21 and 28

Compound	$K_e$ (hr <sup>-1</sup> )	MRT (hr)	$t_{1/2}$ (hr)
Iron Sucrose	0.60	1.66	1.15
Iron Polymaltose	0.59	1.69	1.17
Iron Dextran (VIT)	0.14	7.17	4.97
Iron Dextran (BP/USP)	0.13	7.65	5.30

$K_e$  - elimination rate constant

MRT - mean residence time

Iron was rapidly cleared from serum in rats injected with [<sup>59</sup>Fe]iron sucrose, with only 2.7% of the total dose of <sup>59</sup>Fe remaining after 6 hr. The  $t_{1/2}$  value measured with [<sup>59</sup>Fe]iron sucrose was 1.15 hr. Similar results were observed with [<sup>59</sup>Fe]iron polymaltose, whereas removal of iron was slower with both of the iron dextran preparations. Low levels (<1% of total dose) of <sup>59</sup>Fe were detected for up to 21 days following administration of [<sup>59</sup>Fe]iron sucrose.

In summary, <sup>59</sup>Fe was rapidly cleared from serum in rats following intravenous injection of [<sup>59</sup>Fe]iron sucrose or [<sup>59</sup>Fe]iron polymaltose, whereas the removal of <sup>59</sup>Fe was slower following administration of [<sup>59</sup>Fe]iron dextran.

## 2. Distribution

### Distribution of <sup>59</sup>Fe in Rats Treated with [<sup>59</sup>Fe]Iron Sucrose.

Note: This section contains a review of two distribution studies in rats.

**Methods:** In the first study, Male Sprague Dawley rats weighing an average of 259 g were given an iron-deficient diet for 10 days to achieve an iron depleted state. However, the parameters related to iron status (i.e. hemoglobin, ferritin, and transferrin saturation) were not measured. Rats were given a

single intravenous injection of 10 mg  $^{59}\text{Fe}$  (average of 39 mg Fe/kg) using one of the following iron preparations: iron sucrose (Venofer<sup>®</sup>, lot # 559209A2), iron polymaltose (Amylofer<sup>®</sup>), iron dextran VIT, or iron dextran BP/USP (4 males/group). Iron polymaltose, iron dextran VIT, and iron dextran BP/USP were used for the purpose of comparison. Distribution in red blood cells was measured on days 0-28. Distribution was measured in kidneys, spleen, and liver on days 14 and 28.

In the second study, the cellular distribution of iron was characterized in rats (Gamerding and Pietzonka, Zeitschrift für die Gesamte Experimentelle Medizin Bd, 128s: 148-157, 1956). Rats weighing 150 g were injected with iron saccharate (iron sucrose), -2 mg/day ( $\approx$  13 mg/kg/day), for a total of 120 mg over 2 months. It was not stated whether the dose level referred to iron or iron sucrose. Liver, spleen, kidney, lung, and heart were stained with hematoxylin-eosin, iron-hematoxylin, picrofuchsin, scarlet stain, and Turnbull's blue.

Results: Distribution of  $^{59}\text{Fe}$  in RBCs (red blood cells) is summarized in the following table.

% Radioactive Dose in RBCs				
Day(s)	Iron Sucrose Mean $\pm$ SD	Iron Polymaltose Mean $\pm$ SD	Iron Dextran VIT Mean $\pm$ SD	Iron Dextran BP/USP Mean $\pm$ SD
0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
0.25	3.6 $\pm$ 0.10	2.6 $\pm$ 0.7	4.6 $\pm$ 1.3	13.2 $\pm$ 2.4
1	16.4 $\pm$ 3.9	16.0 $\pm$ 1.7	9.6 $\pm$ 0.9	13.4 $\pm$ 2.5
2	33.3 $\pm$ 7.4	28.6 $\pm$ 4.2	21.9 $\pm$ 2.2	26.9 $\pm$ 3.0
3	36.1 $\pm$ 3.8	42.4 $\pm$ 2.6	35.9 $\pm$ 6.3	32.3 $\pm$ 4.4
4	38.4 $\pm$ 6.2	49.2 $\pm$ 6.5	34.8 $\pm$ 5.6	33.7 $\pm$ 3.5
7	60.7 $\pm$ 7.7	59.5 $\pm$ 12.2	49.7 $\pm$ 8.4	42.5 $\pm$ 7.7
14	80.0 $\pm$ 17.3	8.45 $\pm$ 0.73	64.9 $\pm$ 10.6	63.5 $\pm$ 5.4
21	90.8	93.4	70.3	71.2
28	89.4	88.8	67.2	70.9

N = 4 on days 0-14

N = 2 on days 21, 28

In rats injected with [ $^{59}\text{Fe}$ ]iron sucrose,  $^{59}\text{Fe}$  accumulated gradually in RBCs. A plateau was achieved at 21 days, when radioactive iron measured in RBCs was equal to 90% of the total radioactive dose. Similar results were observed with [ $^{59}\text{Fe}$ ]iron polymaltose, whereas accumulation was lower in rats injected with either form of [ $^{59}\text{Fe}$ ]iron dextran.

Distribution of  $^{59}\text{Fe}$  in kidneys, liver, and spleen is summarized in the following tables.

% Radioactive Dose At 14 Days				
Tissue	Iron Sucrose Mean	Iron Polymaltose Mean	Iron Dextran VIT Mean	Iron Dextran BP/USP Mean
Kidneys	0.8	0.9	0.7	0.4
Spleen	1.2	1.2	3.5	3.9
Liver	5.1	8.5	4.8	2.7
Feces	2.2	3.0	3.1	3.4
Urine	1.5	0.0	0.0	0.2
RBC + Serum	80.2	84.6	65.1	63.6
Sum	100.7	103.5	85.2	71.6

% Radioactive Dose At 28 Days				
Tissue	Iron Sucrose Mean	Iron Polymaltose Mean	Iron Dextran VIT Mean	Iron Dextran BP/USP Mean
Kidneys	0.8	0.6	0.7	0.7
Spleen	0.3	0.9	3.3	3.3
Liver	3.2	4.6	9.5	3.4
Feces	2.8	3.6	4.3	4.7
Urine	3.1	0.0	0.0	0.1
RBC + Serum	89.4	88.8	67.2	71.1
Sum	99.5	98.5	84.9	83.1

At 14 days after injection with [<sup>59</sup>Fe]iron sucrose, 5% of the total dose of <sup>59</sup>Fe was present in liver, with 1% in the kidneys and spleen. Distribution in RBCs and serum accounted for 80% of the radioactive dose. On day 28, the levels of <sup>59</sup>Fe were slightly reduced in liver and spleen. The pattern of distribution was similar following injection with [<sup>59</sup>Fe]iron polymaltose, whereas slight differences were observed with the [<sup>59</sup>Fe]iron dextran preparations.

The cellular distribution of iron in rats treated with iron sucrose (second study) is described below.

**Liver:** Abundant coarsely granular iron deposits were observed in Kupffer cells. Some cells were enlarged enough to produce obstruction of the capillary lumen. Much less iron was observed in parenchymal cells, vascular endothelial cells, and adventitial cells. There was no evidence of cell death, chronic inflammation, fibrosis, or cirrhosis.

Spleen: Reticular and epithelial cells were swollen from heavy deposits of iron (course granular). Fine granular iron deposits were observed in the vascular endothelial and adventitial cells.

Kidney: Fine granular deposits of iron were observed in glomeruli, epithelial cells of proximal tubule, and vascular endothelial cells.

Lung: Moderate to heavy iron deposits were found in connective tissue cells. Fine granular deposits were observed in endothelial and adventitial cells.

Heart: Slight deposits were observed in some connective tissue cells.

In summary, the results indicate that blood, liver, kidneys, and spleen are the major sites of distribution of  $^{59}\text{Fe}$  following intravenous administration of [ $^{59}\text{Fe}$ ]iron sucrose in rats. This study yielded a complete distribution profile for days 14 and 28 post-injection, since the total  $^{59}\text{Fe}$  measured accounted for 100% of the radioactive dose. However, distribution was measured on days 14 and 28 only. Therefore, no information was revealed regarding the distribution at early time points. It is clear that there are specific patterns of iron distribution among different cell types in liver, spleen, kidney, lung, and heart.

Distribution of [ $^{59}\text{Fe}$ ]Iron Sucrose Following Intravenous Injection in Rats.

Methods: Four OFA Icolbm:SD female rats were injected with [ $^{59}\text{Fe}$ ]iron sucrose (Venofer<sup>®</sup>), 32.5 mg Fe/kg i.v., on the morning after giving birth. Radioactivity was measured in tissue at 28 days after injection. Average weight of rats was 307 g.

Results: Tissue distribution of  $^{59}\text{Fe}$  is summarized in the table below.

<u>Tissue</u>	<u>% Radioactive Dose At 28 Days <math>\pm</math> SD</u>
Blood	34.7 $\pm$ 7.9
Liver	34.1 $\pm$ 7.5
Spleen	1.1 $\pm$ 0.7
Kidneys	1.7 $\pm$ 0.4
Stomach	0.1 $\pm$ 0.0
Intestines	0.6 $\pm$ 0.2
Brain	0.1 $\pm$ 0.0

$^{59}\text{Fe}$  content in bone marrow was not determined. Distribution within blood components was characterized over the course of 28 days, as described below.

Day After Injection	% Radioactive Dose	
	RBC	Serum
0 (6 hours)	1.34	1.29
7	31.3	0.67
14	29.7	0.26
21	29.5	0.10
28	34.5	0.23

**Conclusion:** Blood and liver are the major sites of iron distribution in rats at 28 days following a single high-dose injection of iron sucrose. Blood and liver each contained 34-35% of the total radioactive dose. At least 98% of the  $^{59}\text{Fe}$  measured in blood was present in red blood cells beginning at 7 days. Slight accumulation (1-2% of dose) was detected in spleen and kidneys.

**Kinetic Analysis of [ $^{52}\text{Fe}$ ]Iron Sucrose in Minipigs Using Positron Emission Tomography.**

**Methods:** Four female SPF Göttingen minipigs weighing  $21 \pm 2.8$  kg were fasted overnight. Minipigs were anesthetized by injection with azaperone, zoletel, and atropine sulfate (0.04 mg/kg i.m.) and ventilated with a 35%/65% mixture of  $\text{O}_2$  and  $\text{N}_2\text{O}$ . Anesthesia was maintained by continuous intravenous infusion of pentobarbital (8 mg/kg/hr) and pancuron bromide (0.25 mg/kg/hr). Catheters were placed in the carotid artery and jugular vein, for iron sucrose injection and blood sampling, respectively. [ $^{52}\text{Fe}$ ]Iron sucrose complex was synthesized using a method developed by Vifor International AG, Switzerland. [ $^{52}\text{Fe}$ ]Iron sucrose was combined with 5 ml of Venofer<sup>®</sup>. 100 mg  $\text{Fe}^{3+}$  was administered as an i.v. bolus over 10 minutes.  $^{52}\text{Fe}$  levels were measured by positron emission tomography (PET) for up to 6 hr post-dose in blood, liver, bone marrow, kidney and brain. Proportions of  $^{52}\text{Fe}$  and its daughter radionuclide  $^{52}\text{Mn}$  were estimated by calculation ( $^{52}\text{Fe}$   $t_{1/2} = 8.3$  hr).

**Results:** Blood iron concentration was measured in the left ventricle of the heart. A rapid increase in blood iron levels was followed by a fast clearance phase at 10-20 min, after which a slow clearance phase appeared. After 4 hr,  $^{52}\text{Fe}$  concentration in blood was reduced to 20-25% of peak value and showed no further decline. Uptake was greatest in liver, while accumulation in bone marrow was about 50% of the amount in liver

after 6 hr.  $^{52}\text{Fe}$  accumulation in liver was fast during the first 30 min, followed by a slower uptake phase. The uptake pattern in kidney was similar to that of blood. No radioactivity was detected in brain.

**Conclusion:** Liver is a major site of iron deposition in minipigs. Rapid clearance from blood after intravenous injection correlates with rapid appearance in liver. Iron accumulation in liver and bone marrow in minipigs is consistent with the known pathways of iron transfer in man (Finch et al., *Medicine*, 49, 1970). Transient renal uptake was probably due to blood flow rather than active uptake.

**Distribution in Pregnant Rabbits and Fetuses.** Pribilla, *Acta Haematologica*, 12(6), 372, 1954.

**Methods:** Pregnant rabbits weighing 3.5-6 kg were given intravenous injection of iron sucrose at doses of 0, 50, or 200 mg Fe/kg (3 rabbits/group) on days 14-27 of gestation. Although not stated, it is assumed that the doses represent the cumulative dose of iron. The rabbits were sacrificed on day 29. Iron content was determined in liver, spleen, uterus, and placenta by an unspecified method. Tissue sections were stained with cadmium sulfate for the detection of ferritin, which appeared as crystals, and hemosiderin, which appeared as granules.

**Results:** The maternal distribution of iron in the liver, spleen, and uterus is summarized in the following table.

Maternal Distribution	Iron (mg%)		
	Dose (mg Fe/kg)		
Organ	0	50	200
Liver	13.5	154.4	254.4
Spleen	54.8	310.7	1088.1
Uterus	1.8	2.3	6.1

Iron concentration in liver and spleen was increased by up to 20-fold in the treated groups, whereas the iron increase in uterus was only 3-fold in the high-dose group.

The fetal distribution of iron in liver and spleen is summarized in the following table.

Fetal Distribution	Iron (mg%)		
	Dose (mg Fe/kg)		
Organ	0	50	200
Liver	80.7	114.6	166.7
Spleen	7.5	88.6	185.0
Placenta	5.8	8.2	159.1

Accumulation of iron was observed in the liver and spleen, as well as the placenta. This suggests that the administered iron crossed the placental barrier.

#### Histological Observations:

Maternal Controls - Large deposits of intracellular and extracellular hemosiderin in spleen; few hemosiderin granules in Kupffer cells; no ferritin was detected.

Fetal Controls - Hemosiderin observed in liver capillary endothelium and hepatocytes; high levels of ferritin in liver were observed, and no hemosiderin or ferritin was found in the spleen or placenta, in contrast to the maternal observations.

50 mg Fe/kg Adults - Large deposits of intracellular (Kupffer cells) and extracellular hemosiderin in spleen and liver; iron deposits in hepatic stellate cells, and small amounts in hepatocytes; large deposits in reticular bone marrow cells.

50 mg Fe/kg Fetuses - Increased hemosiderin and ferritin levels in liver; hemosiderin observed in hepatocytes; no hemosiderin or ferritin was detected in spleen

200 mg Fe/kg Adults - Extremely large accumulation of hemosiderin in spleen, liver, and bone marrow; many deposits of hemosiderin in the uterine wall; ferritin observed in liver, spleen, and bone marrow.

200 mg Fe/kg Fetuses - Increased hemosiderin and ferritin levels in liver; hemosiderin observed in placenta.

Conclusions: Treatment of pregnant rabbits with repeated injection of iron sucrose produced accumulation of iron in the liver and spleen in both mother and fetus, suggesting that the administered iron permeated the placenta. There were differences in the storage form of iron observed in the adults and fetuses. Whereas hemosiderin was the predominant storage form in adults, both ferritin and hemosiderin were observed in fetuses.

**Other Studies on Iron Distribution:** An early study characterized the tissue distribution of iron following the administration of iron saccharate (iron sucrose) in mice and rats (10 mg Fe i.v.). Since iron was detected by histological staining, this study yielded minimal quantitative information about iron deposition. Liver, spleen, and lymph nodes appeared to be the major sites of iron distribution, with lower levels found in bone marrow (Cappell, J Path Bact 33:175, 1930). Another study on mice showed that iron was deposited in hepatic reticuloendothelial cells, spleen, and bone marrow following injection with iron sucrose, 200 mg Fe/kg (Geisser et al., 42:1439, 1992). Serum iron was bound to ferritin and transferrin.

### 3. Excretion

#### Excretion of $^{59}\text{Fe}$ in Rats Treated with [ $^{59}\text{Fe}$ ]Iron Sucrose, [ $^{59}\text{Fe}$ ]Iron Polymaltose, and [ $^{59}\text{Fe}$ ]Iron Dextran.

**Methods:** Male Sprague-Dawley rats weighing an average of 259 g were given an iron-deficient diet for 10 days to achieve an iron depleted state. However, the parameters related to iron status (i.e. hemoglobin, ferritin, and transferrin saturation) were not measured. Rats were injected intravenously with 10 mg  $^{59}\text{Fe}$  (average of 39 mg Fe/kg) using one of the following iron preparations: iron sucrose (Venofer<sup>®</sup>, lot # 559209A2), iron polymaltose (Amylofer<sup>®</sup>), iron dextran VIT, or iron dextran BP/USP (4 males/group). Iron polymaltose, iron dextran VIT, and iron dextran BP/USP were used for the purpose of comparison.

**Results:** Excretion in urine and feces is summarized in the following table. After 28 days, only 5.9% of the radioactive dose was excreted in rats injected with [ $^{59}\text{Fe}$ ]iron sucrose. Urinary  $^{59}\text{Fe}$  comprised about 50% of the total amount excreted following injection with [ $^{59}\text{Fe}$ ]iron sucrose, whereas little or no urinary excretion occurred with the other iron preparations.

	% of RADIOACTIVE DOSE			
	Iron Sucrose	Iron Polymaltose	Iron Dextran VIT	Iron Dextran BP/USP
<b>Day 14</b>				
Urine	1.5	0	0	0.1
Feces	2.2	3.0	3.1	2.4
<b>Day 28</b>				
Urine	3.1	0	0	0.2
Feces	2.8	3.6	4.3	4.7

**Conclusion:** Excretion of  $^{59}\text{Fe}$  following intravenous injection of [ $^{59}\text{Fe}$ ]iron sucrose in rats occurred through urinary and fecal routes in approximately equal proportion. Excretion of iron proceeded at a very slow rate, with only 6% of the dose eliminated at 28 days after injection.

**Excretion and Transfer of [ $^{59}\text{Fe}$ ]Iron Sucrose from Female Rats to Offspring.**

**Methods:** Four OFA Icolbm:SD female rats were injected with [ $^{59}\text{Fe}$ ]iron sucrose, 32.5 mg Fe/kg i.v., on the morning after parturition. Radioactivity was measured in milk, urine, feces, and sacrificed pups on days 2-28. Average weight of rats was 307 g.

**Results:**  $^{59}\text{Fe}$  excretion in milk collected for 24 hr is indicated in the following table.

Days Post Partum	$^{59}\text{Fe}$ EXCRETION IN MILK	
	% Dose $\pm$ SD	
8	0.97 $\pm$ 0.24	
12	0.51 $\pm$ 0.11	
16	0.40 $\pm$ 0.08	
20	0.49 $\pm$ 0.18	

No more than 1% of the radioactive dose was found in individual pups sacrificed on days 1 through 28 postpartum (11-17 pups/litter). However, the mean accumulation per litter after 28 days was  $7.17 \pm 1.87\%$  of injected  $^{59}\text{Fe}$ .

Urinary and fecal excretion over 28 days was  $5.07 \pm 0.45\%$  and  $6.09 \pm 1.25\%$ , respectively (mean % dose  $\pm$  SD).

**Conclusion:** The results indicate that iron transfer from female rats to their offspring occurs at low levels. The rate of excretion in milk appears to be greater than the daily combined urinary and fecal excretion. After 28 days, 11% of the radioactive dose was excreted in urine and feces (about 0.39% excreted/day), whereas the mean daily rate of excretion in milk (from table above) was  $0.59 \pm 0.13\%$ . The form of the excreted iron (e.g. ferritin, transferrin) was not characterized.

In summary, iron was rapidly cleared from serum following intravenous administration of Venofer® (iron sucrose) in rats and minipigs. The major sites of iron distribution were liver, bone marrow, and red blood cells, with low levels present in spleen and kidneys. Treatment of pregnant rabbits with repeated injection of iron sucrose produced accumulation of iron in the liver and spleen in both mother and fetus, suggesting that the administered iron permeated the placenta. At 28 days after treatment of rats with Venofer®, only 11% of the administered iron was excreted (5% in urine, 6% in feces). Small quantities of iron were excreted in milk as well.

**TOXICOLOGY:****ACUTE TOXICITY:****Acute Toxicity Studies in Rats and Mice with Intravenous, Subcutaneous and Oral Administration.****Testing Laboratory:** \_\_\_\_\_**Report #:** 223**Study Dates:** March - April, 1982

GLP compliance is not indicated.

**Methods:** Acute toxicity of iron sucrose (Hippiron® 400, containing 2% w/v Fe<sup>3+</sup>) was assessed in Sprague-Dawley rats age 5 weeks, and ICR mice age 4 weeks. Intravenous injection was in caudal vein. Dose rate was 1 ml/min (20 mg Fe/min) in rats and 0.5 ml/min (10 mg Fe/min) in mice.

**Results:** The following table summarizes results from intravenous toxicity studies (10 animals/dose group).

IV Injection Species	Dose	Minimum		Highest	
	Range (mg Fe/kg)	Lethal Dose (mg Fe/kg)	LD <sub>50</sub> (mg Fe/kg)	Nonlethal Dose (mg Fe/kg)	Time of Death
Rat (males)	100-280	140	140	100	10-48 hr
Rat (females)	140-400	200	236	140	10-24 hr
Mice (males)	200-800	280	424	200	1 min-2 days
Mice (females)	280-800	400	438	280	1 min-3 days

Symptoms were similar in both species. Pale eyes, respiratory disturbances, sedation, dark urine and reduction in spontaneous activity was observed. Onset of symptoms was immediate in both species. Dark reddening of liver, kidneys and spleen, and hemorrhagic lesions in stomach, intestines and lungs were found in dead rats and mice. In surviving animals, no abnormalities were found except for yellowish-brown liver and lungs. Histopathological examination was not performed.

Rats were treated with 1000 mg Fe/kg either by subcutaneous injection or oral intubation. No deaths occurred with either dose route. Reduced spontaneous activity was observed with both routes of administration. Following subcutaneous injection, lumps appeared at the injection site, which spread from the back to the front of the body. In several rats, ulceration appeared on the body surface. Enlarged spleens were found during necropsy.

Mice were dosed with 1000 mg iron/kg by subcutaneous and oral routes. Again, no deaths were observed and symptoms were similar to those found in rats. In both rats and mice, the subcutaneous and oral LD<sub>50</sub> values for iron sucrose are greater than 1000 mg Fe/kg.

Conclusion: The acute toxicity of iron sucrose is highly dependent on route of administration. Administration by oral or subcutaneous routes is substantially less toxic than intravenous injection. This was likely due to poor absorption, since iron is known to be poorly absorbed when administered orally. The appearance of lumps on the body surface and the absence of dark red discoloration of viscera after subcutaneous injection are suggestive of poor absorption with this route of administration.

Acute Toxicity Studies in Rats and Mice with Intravenous and Subcutaneous Administration.

Testing Laboratory: \_\_\_\_\_

Study Date: 1975

Methods: No information about strain, age, or weight of animals is stated. Animals were observed for 7 days following administration of Hippiron<sup>®</sup> 400 (iron-sucrose).

Results: Lethality data from intravenous injection of Hippiron<sup>®</sup> 400 is summarized below.

Species	IV Injection		LD <sub>50</sub> (mg Fe/kg)	Highest Nonlethal	
	Dose Range (mg Fe/kg)	Minimum Lethal Dose (mg Fe/kg)		Dose (mg Fe/kg)	Time of Death
Rats (males)	75-200	100	149	75	1-2 days
Rats (females)	150-275	200	235	175	1 day
Mice (males)	100-400	150	236	100	1-4 days
Mice (females)	150-400	250	340	200	1-6 days

At higher doses, a slight sedative reaction was noticed. Iron deposition was observed in liver and kidney.

Lethality data from subcutaneous injection in mice is summarized below.

Species	Subcutaneous Injection		LD <sub>50</sub> (mg Fe/kg)	Highest Nonlethal	
	Dose Range (mg Fe/kg)	Minimum Lethal Dose (mg Fe/kg)		Dose (mg Fe/kg)	Time of Death
Mice (males)	1000-2000	1200	1390	1000	1-5 days
Mice (females)	1000-2000	1200	1570	1000	1-3 days

Conclusion: Iron sucrose is substantially less lethal (5-fold) when administered by subcutaneous injection, compared to intravenous injection.

**SUBACUTE/SUBCHRONIC TOXICITY:**

7-Day Intravenous Toxicity Study in Rats: Preliminary Study for Subacute Toxicity Test.

Testing Laboratory: \_\_\_\_\_

GLP compliance and study dates were not indicated.

Animals: Male Sprague-Dawley rats, age 6 weeks, 200 g.

Methods: Rats were given i.v. bolus injections of 0 (saline), 4, or 40 mg Fe/kg/day (Hippiron®) for 7 days (5-6 rats/group). Histopathological evaluation was performed on liver, kidneys, spleen and pancreas.

**Results:**

**Observed Effects:** None of the animals exhibited any change in physical condition or behavior.

**Mortality:** There were no deaths.

**Body Weight/Food Consumption/Water Consumption:** Weight gain was reduced in both treated groups, as described in the table below.

Treatment	%Increase in Bodyweight
Control	22.2
4 mg Fe/kg/day	13.0
40 mg Fe/kg/day	8.3

Food and water consumption was not monitored.

**Hematology/Coagulation/Bone Marrow:** There were no effects on hematocrit or hemoglobin levels, as described below. Bone marrow was not examined, and coagulation parameters were not measured.

Treatment (mg Fe/kg/day)	Hct(%)	Hb(g/dl)	Serum Iron (µg/ml)
0	43.8	13.4	261
4	44.7	13.7	152
40	46.5	14.4	386

**Clinical Chemistry/Urinalysis:** Serum iron was increased in the high dose group, as listed in the table above. Occult blood in urine was observed in 1/5 rats at 4 mg Fe/kg/day, 4/6 rats at 40 mg Fe/kg/day.

**Vital Signs/Physical Examination/Ophthalmic Examination:** No observations were reported.

**Organ Weights:** Liver weight was increased by 10% in the 40 mg Fe/kg/day group.

**Gross Pathology:** Spleen enlargement was observed in the control group. Brown discoloration of liver and yellow discoloration of intestines was observed in the 40 mg Fe/kg/day group.

Histopathology:

4 mg Fe/kg/day - brown pigmentation of liver;

40 mg Fe/kg/day - brown pigmentation of liver, kidneys and spleen.

Conclusion: The brown pigmentation of liver, kidneys and spleen was likely the result of iron deposition. Therefore, these organs should be considered as potential target organs of toxicity. Adverse reactions included the impairment of weight gain, and occult blood in urine. A no-effect dose was not established. However, the tolerated dose was 40 mg Fe/kg/day.

13-Week IV Infusion Study in Rats with Weekly Dosing.

Testing Laboratory: \_\_\_\_\_

Study #: VFR 3/951491

Study Dates: 1/5/95-12/20/95

GLP compliance statement is included.

Animals: Crl:CD®BR rats, age 7 weeks, 165-260 g

Methods: Rats were given weekly infusions of Venofer® (Batch # 330109A2). The following dose groups were used: 0 (saline), 6.5 and 30 mg Fe/kg/week (10/sex/group), with dose volumes of 30, 6.5 and 30 ml/kg, respectively. Iron sucrose was infused in restrained and conscious animals during a single 4-hour session per week (27 and 125 µg Fe/kg/min for the low- and high-dose groups, respectively). All rats were acclimated to the restraint procedure in the pre-dosing period. Dosing was performed for 13 weeks, with one dose per week. Hematology, clinical chemistry, and urinalysis were performed on week 6 and 13. For the control and 30 mg Fe/kg/week groups, histopathological evaluation was performed on adrenals, bone marrow (sternum), brain, cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, heart, ileum, jejunum, kidneys, liver, lungs, mammary glands, mesenteric lymph nodes, ovaries, pancreas, parathyroids, pituitary, prostate, rectum, salivary glands, spinal cord, spleen, stomach, testes, thymus, thyroids, trachea, urinary bladder, uterus, and vein from injection site. Kidney, liver and spleen from all groups were stained with Perl's stain and examined for confirmation of iron deposition.

Histopathology was performed on cervical lymph nodes from all groups. The following organs were weighed for all groups: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, thyroid, and uterus.

Results:

Observed Effects: Dose-related brown discoloration of urine was observed, beginning at week 3. Wet fur and red/brown staining of periorbital and/or nasal areas was observed in all groups, including controls. These reactions are thought to be secondary to the stress associated with the restraining procedure.

Mortality: No deaths occurred.

Body Weight/Food Consumption/Water Consumption: The mean body weight of control males and females was 230 and 186 g, respectively, at the beginning of the study and 470 and 285 g, respectively at study termination. Weight gain was reduced by 23% in males and 16% in females in the 30 mg Fe/kg/week group. Weekly food intake was reduced by 8-16% in males in the high-dose group, starting on week 2. Food intake values for control males and females at the beginning of the study were 198 and 141 g/rat/week, respectively. Water consumption was normal.

Hematology/Coagulation/Bone Marrow: Hematocrit in females was reduced by 13% at both doses after 6 weeks. RBCs in females were slightly reduced (6-8%) at 30 mg Fe/kg/week. Platelets were increased by 23% at 30 mg Fe/kg/week after 6 weeks. Neutrophil counts were volatile, with large changes in both directions. Thrombin time was unaffected by treatment. Bone marrow was normal.

Clinical Chemistry/Urinalysis: Minor increases in plasma protein (albumin and globulin), urea nitrogen and alkaline phosphatase were observed in treated rats. Cholesterol was increased by 73% in the high-dose group after 13 weeks. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were elevated by 84% and 67%, respectively, in males of the high dose group.

The following table summarizes the effects of iron sucrose injections on serum iron levels in males and females.

Treatment	Serum Iron ( $\mu\text{g}/\text{dl}$ )			
	Week 1	Week 6	Week 13	
<b>MALES</b>				
Control	269	185	219	
6.5 mg Fe/kg/week	297	242	345**	
30 mg Fe/kg/week	396**	442**	541**	
<b>FEMALES</b>				
Control	319	370	457	
6.5 mg Fe/kg/week	369	392	522*	*p < 0.05
30 mg Fe/kg/week	450**	459**	552**	**p $\leq$ 0.01

Total iron binding capacity was reduced by 10% in females of both treated groups at week 13.

Protein levels in urine were increased in both treated groups by up to 108%. Urine volume was decreased in males during week 13 by 40% and 27% in the 6.5 and 30 mg Fe/kg/week groups, respectively.

Vital Signs/Physical Examination/Ophthalmic Examination:  
Ophthalmic examination was performed on week 13 in the control and high-dose groups. No effects were observed.

Organ Weights: Liver and spleen weights were increased by up to 40% in both treated groups. No change in heart weight was observed.

Gross Pathology: Enlarged cervical lymph nodes, enlarged liver, enlarged adipose tissue, and opaque spleen capsule were common effects in the treated groups.

Histopathology: Major iron deposits, confirmed by Perl's staining, were observed in liver (high-dose group) and spleen (low- and high-dose groups), with minimal quantities detected in kidneys (high-dose group). Iron deposition in liver was detected primarily in sinusoidal/phagocytic cells (both doses), as well as in Kupffer cells (both doses) and hepatocytes (high dose). Trace to minimal amounts of brown pigment deposits, presumably iron, were found in the following organs/tissues of the high-dose group: lungs, heart, thymus, cervical and mesenteric lymph nodes, pancreas, bladder, uterus, cervix, ovaries, prostate, adrenals, pituitary, salivary glands, mammary glands, GI tract, eyes, and bone marrow (low-dose group was not examined). The brown pigment deposits were limited to macrophages. Ferric pigment was also found in renal cortical tubular epithelium and in spleen capsule. Inflammatory cells were found at injection sites in treated rats.

**Conclusion:** The frequency of administration in the present study was 1 dose/week, in contrast to the 3 dose/week protocol for the proposed human study. However, the weekly dose levels used were high enough to provide useful information for safety assessment. The major change seen in treated animals was widespread deposition of iron pigment, limited mostly to macrophages. The greatest accumulation of iron was found in liver and spleen, which correlated with increased weights of these organs. Liver and spleen should be considered as potential target organs of toxicity. A small reduction (22%) in bodyweight gain in the 30 mg Fe/kg/week group was observed. The tolerated dose was 30 mg Fe/kg/week. The results are incomplete due to the absence of histopathological evaluation of the 6.5 mg Fe/kg/week group. A no-effect dose was not established.

**Addendum:** Organ weights were reported as the absolute weights only.

13-Week IV Infusion Study in Rats Using 3 Doses Per Week.

Testing Laboratory: \_\_\_\_\_

Study #: LPL 001/992101

Study Dates: 2/2/99-9/1/99

GLP Compliance: A statement of compliance was included.

Animals: Crl:CD®BR rats, age 6 weeks,  
Males: 172-205 g  
Females: 135-182 g

Methods: Rats were treated with intravenous infusion of Venofer® (batch # 807119) for 13 weeks. The following dose groups were used: 0 (saline), 3, 10, or 30 mg Fe/kg/dose, 3 doses/week (10 rats/sex/group), with dose volumes of 30, 3, 10, or 30 ml/kg, respectively. Venofer® was infused in the lateral tail vein of restrained and conscious rats during a one-hour session on Mondays, Wednesdays, and Fridays. The dose rates were 50, 167, and 500 µg Fe/kg/min for the low-, middle-, and high-dose groups, respectively. All rats were acclimated to the restraint procedure in the pre-dosing period. Hematology, clinical chemistry, and urinalysis were performed on weeks 6 and 13. Ophthalmic examination was performed before the treatment period and on week 13. Rats were sacrificed at the end of week 13. The following organs were weighed for all groups: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries,

pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, thyroids, and uterus. Histopathological evaluation was performed on the following organs/tissues for all groups: adrenals, aorta (thoracic), brain, cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, femur (marrow), heart, ileum, injection site (tail), jejunum, Kidneys, lachrymal glands, Harderian glands, liver, lungs, mammary area, lymph nodes (mandibular, mesenteric, pancreatic, cisternal, renal, lumbar, axillary, and inguinal), optic nerves, ovaries, pancreas, parathyroids, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum (with marrow), stomach, subcutis, tail, testes, thymus, thyroids, tongue, trachea, urinary bladder, uterus, and vagina. All tissues were stained with hematoxylin and eosin. Sections of liver, spleen, and kidney were also stained with Perl's stain for detection of iron deposits.

#### Results:

Observed Effects: Hair loss was observed in all treated groups. There was an increased incidence of darkened tail in the middle- and high-dose groups, and an increased incidence of brown staining of fur in all treated groups. Occasionally, dosing could not be performed. This was usually due to difficulty in locating a suitable vein. Failure to dose occurred not more than 5 times in a single animal in the control, low-, and middle-dose groups. Failure to dose occurred with greater frequency in the high-dose females. Six females in the high-dose group could not be dosed on 1, 2, 3, 4, 5, and 10 occasions.

Mortality: There were two deaths in the control group. One male died from an accidental injection of air during the dosing procedure (time of death not stated). Another male was found dead at the end of the dosing procedure on week 7. The cause of death could not be determined. There were no deaths in the Venofer®-treated groups.

Bodyweight/Food Consumption/Water Consumption: Bodyweight gain was reduced by 15% and 32% in males treated with 10 and 30 mg Fe/kg/dose, respectively. Control males and females weighed 188 and 155 g, respectively, at the onset of the study, and 453 and 263 g, respectively, at study termination. Food consumption was reduced by 10-20% in males treated with 10 and 30 mg Fe/kg/dose during most of the treatment period. Water consumption was not monitored.

Hematology/Coagulation/Bone Marrow: On week 13, there was evidence of macrocytic anemia. Slight but significant reductions in red blood cell (RBC) count were observed in the middle- and high-dose groups (reductions of 6-11% in either sex). In the middle- and high-dose groups, mean corpuscular volume (MCV) was increased by 4-8%, and mean corpuscular hemoglobin (MCH) was increased by 5-10% (both changes were significant). Reticulocyte levels were increased by 88% and 70% in high-dose males and females, respectively. Slight but significant increases in MCV and MCH were also observed on week 6 in the middle- and high-dose groups. Changes in white blood cell (WBC) levels were also observed on week 13. Neutrophil count was increased by 91% and 108% in middle- and high-dose males, respectively, and by 53% in high-dose females. Monocyte level was increased by 115% and 50% in the high-dose males and females, respectively. Similar effects on WBC counts were observed on week 6.

Activated partial thromboplastin time (APTT) was reduced by 15% and 23% in males treated with 10 and 30 mg Fe/kg/dose, respectively. A slight but significant reduction in prothrombin time (PT) also occurred in the middle- and high-dose males. Changes in coagulation parameters were observed only on week 13. Deposits of brown pigment in bone marrow macrophages were observed in middle-dose males, and in high-dose males and females. Marrow cytology was not characterized.

Clinical Chemistry/Urinalysis: After 13 weeks, males treated with 30 mg Fe/kg/dose exhibited a 6.6-fold increase in serum ALT. A 2.5-fold increase in ALT was observed in one male treated with 10 mg Fe/kg/dose. AST was increased by 4-fold in the high-dose males, and by 52-137% in 3 females in the high-dose group. Alkaline phosphatase was increased by 68% and 50% in the high-dose males and females, respectively. A 2-fold increase in bilirubin occurred in high-dose males. Cholesterol and triglyceride levels were increased in the middle- and high-dose groups. Urea nitrogen was increased by 21% and 36% in males treated with 10 and 30 mg Fe/kg/dose, respectively. Slight changes in  $K^+$  (increase),  $Ca^{2+}$  (increase), and phosphorus (decrease) were measured in the high-dose males. Alpha<sub>1</sub>, alpha<sub>2</sub>, and gamma globulins were slightly increased in males treated with 10 and 30 mg Fe/kg/dose, and beta globulins were increased by 23% in the 30 mg Fe/kg/dose group. Consequently, the A/G ratio was reduced by approximately 20% in the middle- and high-dose males. On week 6, changes in alpha and beta globulins, urea nitrogen, cholesterol, and triglycerides were similar to those observed on week 13. Serum iron concentration is summarized in the following table.

Treatment	Iron ( $\mu\text{g}/\text{dl}$ )	
	Week 6	Week 13
<b>Males</b>		
Control	144	185
3 mg Fe/kg/dose	147	233*
10 mg Fe/kg/dose	302**	412**
30 mg Fe/kg/dose	488**	559**
<b>Females</b>		
Control	248	323
3 mg Fe/kg/dose	271	429
10 mg Fe/kg/dose	422**	484**
30 mg Fe/kg/dose	463**	506**

\*p < 0.05 \*\*p < 0.01 Williams test

As expected, serum iron concentration was increased in a dose-dependent manner. Total iron binding capacity, an indirect measure of serum transferrin, was increased by 30% in the high-dose males. No clinically significant changes were observed in urinalysis.

Vital Signs/Physical Examination/Ophthalmic Examination: No abnormalities were observed in the ophthalmic examination.

Organ Weights: Absolute liver weight was increased in a dose-related manner in all treated groups (18-72% increase in males and 9-54% increase in females). Absolute spleen weight was increased by 46% and 120% in middle- and high-dose males, respectively, and by 63% in high-dose females. Absolute kidney weight was increased by 10-20% in the middle- and high-dose groups. Absolute lung weight was reduced by up to 24% in males in all treated groups. Absolute epididymides weight was reduced by 14-21% in all treated groups. Relative organ weights were not reported.

Gross Pathology: Enlarged liver was observed in 4/10 males and 10/10 females in the high-dose group. Enlarged spleen occurred in 17/20 rats in the high-dose group. Brown discoloration was observed in pancreas, small intestine, cecum, and subcutis in the high-dose group. Brown discoloration was also observed in adrenals in the middle- and high-dose groups, and in lymph nodes in all treated groups. Reduced adipose tissue was noted in the middle- and high-dose groups. Alopecia was observed in all treated groups.

**Histopathology:** Deposition of brown pigment, most often in macrophages, was the most frequently observed change. In all treated groups, deposition of brown pigment in macrophages was observed in liver (Kupffer cells), spleen (red pulp), adrenals, lymph nodes (mandibular, mesenteric, pancreatic, lumbar), pancreas, stomach, and uterus. For the 10 and 30 mg Fe/kg/dose groups only, macrophage accumulation of brown pigment was observed in lungs, heart (mostly in valves), thymus, kidneys, cervix, ovaries, epididymides, testes, thyroids, duodenum, jejunum, cecum, eyes, bone marrow, and injection site. Brown pigment deposition in macrophages was also observed in trachea, urinary bladder, vagina, prostate, seminal vesicles, parathyroids, pituitary (males), ileum, colon, and subcutis in the high-dose group only. In liver, brown pigment deposits were also found in hepatocytes (high-dose group), sinusoidal/phagocytic cells (all treated groups), and vascular endothelium (all treated groups). Deposits of brown pigment in kidney were also observed in glomerular mesangial cells (all treated groups), cortical tubular epithelium (increased frequency in all treated groups), medullary tubular epithelium (middle- and high-dose females), and vascular endothelium (middle- and high-dose groups). Other sites of brown pigment deposition included the vascular endothelium in lungs (middle- and high-dose groups), zona glomerulosa in adrenals (middle- and high-dose groups), and spleen capsule (middle- and high-dose groups).

The presence of iron deposits in liver, kidney, and spleen was confirmed by the use of Perl's stain. Iron deposits in liver were observed in hepatocytes (low-dose females, middle- and high-dose groups), Kupffer cells (all treated groups), sinusoidal/phagocytic cells (all treated groups), and vascular endothelium (all treated groups). In kidneys, iron deposits were observed in glomerular mesangial cells (all treated animals), cortical tubular epithelium (increased frequency and amount in all treated groups relative to controls), medullary tubular epithelium (all treated groups), and vascular endothelium (all treated groups). Iron deposits in spleen were localized to macrophages in red pulp in all control and treated animals, with greater amounts observed in the treated groups. Iron was also observed in the spleen capsule. There was a strong correlation between the localization of iron deposits and brown pigment deposits in liver, kidney, and spleen.

Aside from iron deposits, few changes were observed. Loss of hepatocytes associated with large clumps of pigmented phagocytes was found in 2/20 high-dose animals. Hepatocyte necrosis was observed in 1/20 control rats and 2/20 high-dose rats. Given the low frequency of these lesions, it is uncertain whether

these were treatment related. Pancreatic acinar cell atrophy was observed in 2/10 high-dose males, but not in females. Germ cell degeneration/depletion occurred in 3/10 low-dose males. Degeneration of nerve fibers in the optic tract was observed in 1-2 females in each of the treated groups. There was an increase in the frequency and severity of vascular fibrosis at the injection site in treated groups, suggestive of local irritation. Subcutaneous fibrosis was observed in all treated groups.

**Conclusions:** The most frequent histopathological change in rats treated with Venofer<sup>®</sup> was the accumulation of iron in various organs and tissues. Iron was usually localized to macrophages, although - vascular endothelium and parenchymal cells (hepatocytes, renal tubular epithelium, and adrenal zona glomerulosa cells) also contained iron deposits. The accumulation of iron was attributed to the absence of an efficient excretion mechanism for iron. In most organs/tissues, iron deposition was graded as minimal or slight, limited to macrophages and vascular endothelium, and was not associated with histopathological changes in other cells. Liver, kidneys, and spleen were considered to be target organs of toxicity, based on the extensive iron deposition in these organs in the 10 and 30 mg Fe/kg/dose groups. Increased organ weights were observed only in the target organs, presumably due to heavy accumulation of iron. Although the frequency of hepatic lesions was very low, large increases in serum levels of ALT, AST, and bilirubin in rats (mostly in males) treated with 30 mg Fe/kg/dose were indicative of hepatotoxicity and impaired liver function. The increase in serum urea nitrogen in males treated with 10 or 30 mg Fe/kg/dose was suggestive of impaired kidney function, although iron deposition was the only change observed in kidneys. Iron accumulation in heart was limited to macrophages, primarily in the valves. There was no sign of hemosiderin deposition in the cardiac parenchyma, which is associated with iron overload (Goyer, In: Casarett & Doull's Toxicology, Fifth Edition, Klaassen (Ed.), 1996). Other pathological changes, such as pancreatic acinar cell atrophy, occurred with very low frequency and cannot be positively correlated with Venofer<sup>®</sup> treatment. Alopecia and evidence of local irritation was observed in all treated groups. There was evidence of macrocytic anemia in rats treated with 10 or 30 mg Fe/kg/dose, although the changes in RBC count and MCV were small. The tolerated dose was 3 mg Fe/kg/dose.

The present study, in which rats were administered 3 doses per week (0, 3, 10, or 30 mg Fe/kg/dose), yielded results that are similar to observations from the initial 13-week toxicity study in rats using one dose per week (0, 6.5, or 30 mg Fe/kg/dose).

Iron deposition in many of the organs/tissues examined was the major treatment-related change in both studies, although some organs contained iron deposits in the 3-dose/week study only. The cellular distribution of iron deposits was observed in macrophages, hepatocytes, and renal tubular-epithelium in both studies. Iron deposition in glomerular mesangial cells and vascular endothelium in lungs and adrenals occurred only in the 3-dose/week study. Increased serum concentrations of ALT and AST, and increased weight of liver and spleen (correlated with iron deposition) was observed in both studies. However, the magnitude of these changes was greater in the present study. For example, serum ALT was increased by 6.6-fold in the 3-dose/week study, and by 84% in the 1-dose/week study. Furthermore, an increase in kidney weight (correlated with iron deposition) and an increase in serum bilirubin were observed only in the present study. Liver and spleen were considered to be potential target organs of toxicity in the 1-dose/week study, whereas stronger evidence of target-organ toxicity in liver, spleen, and kidney was found in the present study. Other effects observed only in the 3-dose/week study included hair loss in all treated groups, and difficulty in dosing associated with local irritation.

10-Week Intravenous Toxicity Study in Dogs. Brown et al., J Lab Clin Med, 50(6), 362, 1957.

Mongrel dogs weighing 5.9-17.5 kg were treated intravenously with iron saccharate (iron sucrose). The age and sex of the animals was not stated. Dogs were given a cumulative dose of 500 or 1000 mg Fe/kg i.v. over 6-10 weeks of treatment (2 dogs/group). The frequency of dosing and the method of intravenous injection (i.e. bolus injection or infusion) was not stated.

There were no observed effects. No deaths occurred for at least 4 years after the study. Bodyweight was maintained or increased, and food consumption was normal. Fibrosis of veins at the injection site was observed near the end of the treatment period.

Slight increases in RBC count, hemoglobin, and hematocrit were observed at 2-3 weeks. Serum bilirubin was normal and the clearance of bromsulfophthalein was unaffected, suggesting there was no change in liver function. Serum iron increased from 75 to 1000 µg% during treatment, followed by a reduction after the end of treatment. Autopsy was performed at 4-7 years after treatment. No liver lesions were found. Heavy iron deposits in reticuloendothelial cells were observed.

This study offers little useful toxicity information, due to the absence of a control group, the low number of animals used, the delay in performing the necropsy, and lack of information regarding the actual daily dose of iron and the frequency of dosing.

13-Week IV Infusion Study in Dogs with Weekly Dosing.

Testing Laboratory: \_\_\_\_\_

Study #: VFR 5/951735

Study Dates: 1/4/95-12/20/95

GLP compliance statement is included.

Animals: Beagle dogs weighing 5-10 kg, age 18-20 weeks.

Methods: Dogs were treated with 0 (saline), 6.5 or 30 mg Fe/kg/week (4 dogs/sex/group) for 13 weeks. Venofer® (Batch # 330109A2) was infused into the cephalic vein of either forelimb or the saphenous vein of either hindlimb during a single 4-hour session per week (27 and 125 µg Fe/kg/min for the low- and high-dose groups, respectively). Dose volume was 30, 6.5 and 30 ml/kg for control, low-dose and high-dose groups, respectively. Dogs were restrained and conscious during infusion. Animals were sacrificed at 7 days after the final dose. Hematology, clinical chemistry, and urinalysis were performed on weeks -2, 6 and 13. Histopathological evaluation was performed in all groups for the following organs/tissues: adrenals, aorta (arch and abdominal), brain, cecum, colon, duodenum, epididymides, esophagus, eyes, femur and joint, gall bladder, heart, ileum, injection sites, jejunum, kidneys, liver, lungs/bronchi, lymph nodes (cervical and mesenteric), mammary gland, optic nerve, ovaries, pancreas, parathyroids, pituitary, prostate, rectum, salivary glands, sciatic nerve, skeletal muscle, skin, spinal cord, sternum (with marrow), stomach, spleen, testes, thymus, thyroids, tongue, trachea, urinary bladder, uterus and vagina. Liver, spleen and kidneys were stained with Perl's stain for confirmation of iron deposition. The following organs from all animals were weighed: adrenals, brain, heart, kidneys, liver, lung, ovaries, pancreas, pituitary, prostate, spleen, testes-(with epididymides) thymus, thyroids and uterus.

**Results:**

**Observed Effects:** Dark stained urine was observed in most animals of the 30 mg Fe/kg/week group, and in 1 animal of the 6.5 mg Fe/kg/week group. Liquid feces was observed in control and treated groups, as well as occasional vomiting.

**Mortality:** No deaths occurred.

**Body Weight/Food Consumption/Water Consumption:** Weight gain, food consumption, and water consumption were not affected by treatment.

**Hematology/Coagulation/Bone Marrow:**

**Males:** Males in both treatment groups showed a slight decrease in MCHC ( $\approx 10\%$ ), and up to 32% reduction in neutrophils at week 6. Lymphocytes were reduced by 26% in the 30 mg Fe/kg/week group at week 13. A 50-70% decrease in eosinophils in both dose groups was found at week 6, which persisted in the high-dose group through week 13. Monocytes were increased by 62% in the high-dose group.

**Females:** Females in the high dose group exhibited a 50% reduction in reticulocytes at week 6, a 21% reduction of WBCs at week 13, and a 49% reduction of monocytes at week 13. A 30% reduction in neutrophils was observed in the same group at week 13, but was not significant.

PT and APTT were not affected by treatment. There were no changes in bone marrow.

**Clinical Chemistry/Urinalysis:**

**Males:** Serum glucose was slightly elevated in both treated groups at weeks 6 and 13. Alkaline phosphatase was increased by 23% in the high-dose group at week 13. Phosphorus levels in both treated groups were reduced by 17% and 19% at weeks 6 and 13, respectively. Iron levels in males are summarized in the following table.

Treatment	Iron ( $\mu\text{g}/\text{dl}$ )		
	Week -2	Week 6	Week 13
Control	149	107	102
6.5 mg Fe/kg/week	158	145	148
30 mg Fe/kg/week	187	227**	197*

\*p  $\leq$  0.05    \*\*p  $\leq$  0.01