

Females: The high-dose group exhibited a 35% increase in serum alanin aminotransferase at week 6. This enzyme was elevated in both dose groups at week 13 by as much as 21%. Iron levels in females are summarized in the following table.

Treatment	Iron (µg/dl)		
	Week -2	Week 6	Week 13
Control	162	136	116
6.5 mg Fe/kg/week	198	206	200*
30 mg Fe/kg/week	250	232*	253**

*p ≤ 0.05 **p ≤ 0.01

The significant increase in iron levels was probably unrelated to treatment, since pre-study iron levels in treatment groups were elevated relative to control animals.

Urinary protein was increased by 44-48% in males and females of the high-dose group at week 13. Urine volume was increased by 55% in females of the high-dose group.

Vital Signs/Physical Examination/Ophthalmic Examination: There was no effect on heart rate during weeks 4, 8, and 12. Systolic and diastolic pressure, measured pre- and post-infusion, was not affected on weeks 4, 8, and 12. One female in the low-dose group exhibited premature ventricular contractions, judged to be unrelated to treatment. Respiration was monitored on weeks 4, 8, and 12. Males in both treated groups showed a 23% reduction in breathing rate at midpoint of infusion on week 12, compared to 30 breaths/minute in control males. Females in the high-dose group exhibited a 30% reduction in respiration during and after infusion on week 4, compared to 40 breaths/minute in control females, during and after infusion. These effects were not considered as treatment related. Two dogs in the high-dose group had conjunctivitis, judged not to be treatment related.

Organ Weights: Liver weight in both sexes increased by 30% at 30 mg Fe/kg/week. Kidney weight in females increased by 15% and 20% at low and high doses, respectively (adjusted to bodyweight).

Gross Pathology: Yellow discoloration of heart valves was observed in 7/8 dogs treated with 30 mg Fe/kg/week. Brown or yellow discoloration was observed at injection sites of most treated animals. Gray discoloration in various sections of the GI tract was noted in 3 dogs in the high-dose group. Tissue discoloration was correlated with iron detected histologically.

Histopathology: Major iron deposits were observed in liver, spleen, kidneys and lymph nodes. Brown pigments in macrophages, confirmed as iron deposits by Perl's stain, were found in various organs and tissues in both treated groups as listed below.

6.5 mg Fe/kg/week - cervical lymph nodes, spleen, liver (Kupffer cells).

30 mg Fe/kg/week - lungs, heart, thymus, cervical and mesenteric lymph nodes, spleen, liver, gall bladder, pancreas, kidney, uterus, prostate, thyroid, parathyroid, adrenals, GI tract (stomach to rectum), marrow (sternum).

Mucoid degeneration of the pulmonary (artery) valve was found in 1/8 and 3/8 dogs in the low- and high-dose groups, respectively. This condition is characterized by the conversion of connective tissue to mucoid or gelatinous material. Mucoid degeneration of the aortic valve was observed only in the 30 mg Fe/kg/week group (4/8 dogs). Granulomatous inflammation was found in liver in all groups. Liver from both treated groups contained clumps of sinusoidal/phagocytic cells with iron pigment. Brown pigment was also found in interstitial cells in kidney.

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. Large iron deposits were found in liver, spleen, kidney and lymph nodes. These organs should be considered as potential target-organs of toxicity. Iron was localized in mononuclear phagocytic cells. Mucoid degeneration of the aortic and pulmonary valves was dose-related, but was described as minimal. The toxicological significance of this finding is not apparent. The tolerated dose was 30 mg Fe/kg/week. A no-effect dose was not established.

Addendum: The data described in the "Organ Weights" section refer to the relative organ weights (g/100 g bodyweight).

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

Testing Laboratory: _____

Study #: LPL 002/992102

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

GLP Compliance: A statement of compliance was included.

Animals: Beagle dogs, age 24-32 weeks, 6.4-11.5 kg

Methods: Dogs were treated with intravenous infusion of Venofer® (batch # 807119) for 13 weeks. Animals were treated with 0 (saline), 3, 10, or 30 mg Fe/kg/dose, 3 doses/week (4 dogs/sex/group), using dose volumes of 30, 3, 10, or 30 ml/kg, respectively. Venofer® was diluted with saline to a concentration of 1 mg Fe/ml and infused into the cephalic vein of either forelimb or the saphenous vein of either hindlimb during a 1-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. Dogs were restrained and conscious during infusion. Hematology, clinical chemistry, and urinalysis were performed before the treatment period, and on weeks 6 and 13 of treatment. Electrocardiography and blood pressure measurement was performed prior to the start of treatment, and on weeks 5, 6, 12, and 13. Dogs 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, spleen, testes, thymus, thyroids (with parathyroids), and uterus (with cervix). Histopathological evaluation was performed on the following organs/tissues for all groups: adipose tissue, adrenals, aorta (thoracic), brain, cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, femur (with marrow), gall bladder, heart, ileum, injection sites, jejunum, kidneys, liver, lungs (with bronchi), lymph nodes (mandibular, mesenteric, tracheobronchial, mediastinal, lumbar, inguinal, cisternal), mammary area, nictitans glands, optic nerves, ovaries, pancreas, parathyroids, pituitary, prostate, rectum, salivary glands, sciatic nerve, skeletal muscle (thigh), spinal cord, sternum (with marrow), stomach, spleen, 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 the detection of iron.

Results:

Observed Effects: Brown discoloration of mucous membranes (eyes and gums) was observed in all animals in the high-dose group beginning on week 12. This change was considered to be the result of iron accumulation in these tissues. Occasionally, animals in the middle- and high-dose groups exhibited a 'pain' reaction with vocalization and aggressive behavior upon the attempt at insertion of the needle. One middle-dose male was

not dosed on one occasion due to aggressive behavior. Two high-dose females were not dosed on 4 or 5 occasions due to difficulty in finding a suitable vein. Non-treatment related signs included swelling and/or bruising at the injection sites, scabs, vomiting, and liquid feces.

Mortality: No deaths occurred.

Bodyweight/Food Consumption/Water Consumption: Bodyweight gain was reduced by 31%, 35%, and 56% in the middle-dose females, high-dose males, and high-dose females, respectively. Similar changes were observed in the low-dose group, but were not statistically significant. Control males and females weighed 9.4 and 7.5 kg, respectively, at the beginning of the study, and 11.5 and 9.1 kg, respectively, at termination. The combined bodyweight gain for males and females was significantly reduced in the low-, middle, and high-dose groups by 28%, 33%, and 44%, respectively. No change in food consumption was observed. Water consumption was not monitored.

Hematology/Coagulation/Bone Marrow: Anemia was observed in the middle- and high-dose groups. Reductions in RBC count, hematocrit, and hemoglobin were observed in the high-dose group on week 6. RBC count measured on week 13 was reduced by 12% and 23% in middle- and high-dose males, respectively, and by 19% and 33% in middle- and high-dose females, respectively. Hematocrit and hemoglobin were similarly reduced in the middle- and high-dose groups on week 13. Reticulocyte number was reduced by up to 80% in middle- and high-dose females, on weeks 6 and 13. Neutrophil count was reduced by 49% and 53% in the middle- and high-dose males, respectively, on week 6, and by 28% in high-dose males on week 13. There were no significant changes in APTT or PT. However, two males and one female in the high-dose group exhibited a prolonged APTT (30-40% increase). Deposition of brown pigment (presumably iron) was observed in bone marrow macrophages in the middle- and high-dose groups. Marrow cytology was not performed.

Clinical Chemistry/Urinalysis: Serum albumin was reduced by 17% and 28% in middle- and high-dose females, respectively, and by 18% in the high-dose males on week 13. Inorganic phosphorus levels were reduced by up to 44% in the middle- and high-dose groups on weeks 6 and 13. Changes in iron concentrations are summarized for males and females in the following table.

Treatment	Iron ($\mu\text{g}/\text{dl}$)		
	Week -2	Week 6	Week 13
Males			
Control	121	91	159
3 mg Fe/kg/dose	120	162	205
10 mg Fe/kg/dose	102	180*	267**
30 mg Fe/kg/dose	110	282**	306**
Females			
Control	142	133	233
3 mg Fe/kg/dose	109	151	209
10 mg Fe/kg/dose	80	157	243
30 mg Fe/kg/dose	121	255**	279

*p < 0.05 **p < 0.01 Williams test

Serum iron was increased above the baseline concentrations measured on week -2. However, significant changes were less frequent in females, due to higher levels of iron in the control group. Total iron binding capacity (TIBC) was reduced by 18-33% in high-dose females on weeks 6 and 13. No changes of clinical significance were observed in urinalysis.

Vital Signs/Physical Examination/Ophthalmic Examination:

Changes observed in ECG recordings were not clinically significant (e.g. slight increase in Q amplitude in middle-dose females on week 5). Blood pressure was unaffected by treatment. No treatment-related effects were observed in ophthalmic examination.

Organ Weights: Absolute liver weight was increased by 48% and 110% in the middle- and high-dose males, respectively, and by 40% and 111% in the middle- and high-dose females, respectively. One male treated with 3 mg Fe/kg/dose (low dose) exhibited a 33% increase in absolute liver weight. Absolute spleen weight was increased by 61% and 48% in the high-dose males and females, respectively. Other changes included the following: 32% increase in absolute thyroid weight in high-dose males; absolute kidney weight increased by 13% and 25% in middle- and high-dose females, respectively. The changes in organ weights correlated with iron deposition observed in histopathological examination. Relative organ weights were not reported.

Gross Pathology: Yellow and/or brown discoloration was observed in many organs/tissues, primarily in the high-dose group. Discoloration was observed in the following organs: eyes, gums, salivary glands, thymus, lymph nodes (middle- and high-dose groups), heart valves (1/8 and 6/8 in middle- and high-dose groups, respectively), pancreas, esophagus, stomach, small and large intestine, adrenals, kidneys, nictitans glands, and conjunctiva. Enlargement of liver and spleen, and congested

lymph nodes were observed in the middle- and high-dose groups. All treated groups exhibited signs of local irritation, including hemorrhage, yellow/brown discoloration, and thickened area at injection sites. The incidence of local irritation was greatest in dogs treated with 30 mg Fe/kg/dose (high dose).

Histopathology: The most frequent change noted was the deposition of brown pigment, mostly limited to macrophages. All treated groups exhibited brown pigment deposition in macrophages in lymph nodes (mandibular and mesenteric), spleen (red pulp), liver (Kupffer cells, sinusoidal/phagocytic cells), adrenals, and injection sites. Brown pigment deposits in macrophages were also observed in the following organs/tissues in the middle- and high-dose groups: alveolar septae, heart, gall bladder, uterus, ovaries, prostate, tongue, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, brain (choroid plexus), bone marrow, and nictitans glands. Deposition of brown pigment in macrophages was observed in the following organs/tissues in the high-dose group only: aorta (thoracic), aortic valve, pulmonary valve, thymus, pancreas, urinary bladder, cervix, vagina, testes (interstitial cells), pituitary, salivary glands, esophagus, oral cavity, and eyes (corneoscleral junction and conjunctiva). Pigment deposits in kidneys were localized to glomerular mesangial cells and interstitial cells in the middle- and high-dose groups, and cortical tubular epithelium and macrophages in the high-dose group.

The use of Perl's stain in liver, kidneys, and spleen confirmed that the deposition of brown pigment corresponded to iron deposits. Iron was detected in Kupffer cells and sinusoidal/phagocytic cells in liver sections from all Venofer[®]-treated animals. Iron deposits in hepatocytes were detected in 2/8, 6/8, and 8/8 dogs in the low-, middle-, and high-dose groups, respectively. Iron deposition in glomerular mesangial cells was detected in all low-dose males, and in all animals in the middle- and high-dose groups. Iron deposits in kidney interstitial cells were observed in all Venofer[®]-treated animals, whereas iron deposits in cortical tubular epithelium occurred in middle-dose males and all high-dose animals. Both control and treated animals exhibited iron deposits in spleen macrophages (in red pulp). However, greater amounts of iron were observed in the treated groups. The magnitude of iron deposition, as indicated by Perl's stain, increased in a dose-dependent manner in liver, kidney, and spleen.

Extramedullary hemopoiesis in spleen was observed in all animals in the middle- and high-dose groups. The frequency and severity of extramedullary hemopoiesis in liver was increased in the middle- and high-dose groups. Perivascular fibrosis in liver was observed in three low-dose animals, and all of the middle- and high-dose animals. Hepatocyte necrosis was noted in one male and two females in the high-dose group. Abnormalities in epididymides were observed in 3/4 and 2/4 males in the middle- and high-dose groups, respectively. These changes included absent or reduced number of spermatazoa, spermatocele granuloma, and abnormal spermatogenic cells in ducts. It is noteworthy that deposits of brown pigment (i.e. iron) were not found in the epididymides. Other changes, such as pituitary cysts and dilatation of brain ventricles, occurred with similar frequency in the control and treated groups.

Various signs of irritation at the injection sites were observed with similar frequency in all groups. These included hemorrhage, inflammation, subcutaneous fibrosis, vasculitis, vascular intimal proliferation, subcutaneous and vascular necrosis, and epidermal hyperplasia.

Conclusions: Predictably, the major treatment-related change in dogs treated intravenously with Venofer® (3, 10, or 30 mg Fe/kg/dose, 3 doses/week) was the deposition of iron in many organs and tissues. The accumulation of iron was attributed to the absence of an efficient excretion mechanism for iron. For most organs/tissues where iron accumulation was observed, the iron deposits were limited to macrophages, and no other changes were observed in histopathological examination. Liver (all treated groups), kidneys (all treated groups), and spleen (high-dose group) were considered to be target organs of toxicity, based on the extensive iron accumulation in these organs. Large increases in the weight of liver and spleen correlated with the extent of iron deposition, as indicated by Perl's stain. All treated groups exhibited hepatic lesions, including perivascular fibrosis and hepatocyte necrosis, in addition to extensive iron deposition. The only change observed in heart was the accumulation of iron in macrophages, which occurred in the aortic, pulmonary, and atrio-ventricular valves, and in other regions. Iron deposition was also noted in macrophages in the thoracic aorta. There was no sign of iron deposition in the cardiac parenchyma. The occurrence of extramedullary hemopoiesis in liver and spleen correlated with anemia in the middle- and high-dose animals. Abnormalities such as reduced number of spermatazoa were observed in epididymides in the middle- and high-dose males. Since there was no evidence of iron accumulation in this tissue, it is uncertain whether this

effect was caused by Venofer[®] treatment. A no-effect dose was not established in this study, due to changes in liver, kidney, and bodyweight gain in all treated groups. Hepatocyte necrosis was observed in the 30 mg Fe/kg/dose group only. Therefore, the tolerated dose was 10 mg Fe/kg/dose.

Whereas one dose per week was used in the initial 13-week dog study (0, 6.5, or 30 mg Fe/kg/dose), 3 doses per week were used in the present study (0, 3, 10, or 30 mg Fe/kg/dose). Although slight increases in serum ALT (21-35%) were observed in the 1-dose/week study, no change in ALT was observed in the present study. In contrast, hepatic lesions (i.e. fibrosis and necrosis) other than iron deposits were observed only in the 3-dose/week study. Liver weight was increased by 30% in the 1-dose/week study, and by 110% in the 3-dose/week study. In addition, an increase in spleen weight was observed only in the 3-dose/week study. Mucoid degeneration of the aortic and pulmonary valves was observed in the 1-dose/week study only. Iron distribution occurred in a greater number of organs and in more cell types in the 3-dose/week study. Iron deposits were limited to macrophages in dogs treated with 1 dose/week, whereas iron was observed in macrophages, glomerular mesangial cells, renal tubular epithelium, renal interstitial cells, and hepatocytes in dogs treated with 3 doses/week. Liver, kidney, and spleen were considered to be potential target organs of toxicity in the 1-dose/week study, whereas stronger evidence of toxicity in these organs was found in the present study.

REPRODUCTIVE TOXICOLOGY:

Segment I Fertility and Early Embryonic Study in Rats.

Testing Laboratory: _____

Study #: LPL 003/992500

Study Dates: 2/15/99-9/1/99

GLP Compliance: A statement of compliance was included.

Animals: Crl:CD[®]BR (Sprague-Dawley) rats
Female: age 10-11 weeks*, 225 g
Male: age 8-9 weeks*, 340 g
* age at beginning of treatment; males and females were the same age

Methods: Rats were treated intravenously with 0 (saline), 3, 6.5, or 15 mg Fe/kg/dose, 3 doses/week, in a volume of 15, 3, 6.5, or 15 ml/kg, respectively (24 rats/sex/group). The basis of dose selection was not stated. Venofer® (batch # 807119) was diluted with saline to a concentration of 1 mg Fe/ml, and infused for 1 hr in all dose groups. Rats were restrained and conscious during infusion. Males were dosed 3 times per week throughout the study. Treatment of males commenced at 4 weeks before pairing and continued until necropsy was completed on all females (day 15-16 of gestation). Females were dosed 3 times per week starting at 2 weeks before cohabitation, during cohabitation, and on days 0 (unless dosing occurred on previous day), 3, and 7 of pregnancy. Males and females were paired in a 1:1 ratio until mating was confirmed by observation of spermatazoa in vaginal smear or the presence of a copulatory plug (day 0 of gestation). The maximum duration of pairing was not stated. C-sections and necropsy were performed on day 14 of pregnancy. The contents of the reproductive tract were recorded. Males were sacrificed following the completion of necropsies in females. The following organs were weighed: testes, epididymides, prostate, and seminal vesicles.

Results:

Observed Effects: No treatment-related signs were observed. Signs related to the dosing procedure, such as wet, stained, or soiled fur, were observed in all groups. Correct dosing was inhibited in a dose-dependent manner, presumably due to the occurrence of local irritation. This occurred mostly in males (2-7/group). The incidence of dosing difficulty was more frequent towards the end of the treatment period.

Mortality: Three deaths occurred, none of which were considered as treatment related. One male from the low-dose group died on week 4 of treatment (before pairing). One male and one female in the control group died during the mating period.

Bodyweight/Food Consumption: Weight gain in high-dose females with live fetuses was reduced by 11-14% on days 10, 12, and 14 of pregnancy. The bodyweight of control females with live fetuses was 268 g on day 0, and 346 g on day 14. Bodyweight gain was reduced by 23% in males in the high-dose group after 7 weeks. Males in the control group weighed 339 g at the start of treatment and 482 g after 7 weeks. Food consumption was reduced by 6-13% in the middle- and high-dose females throughout pregnancy.

Necropsy Observations: Small testes were observed in the low- (2/24) and high-dose (2/24) groups. This was not considered as treatment-related, since this change occurs spontaneously in this strain of rats. The relative prostate weight was reduced by 11% in the high-dose males. Sperm count and sperm viability in epididymides was not measured.

Mating and Fertility: Mating and fertility observations are summarized in the following tables.

Dose (mg Fe/kg)	Number Paired	Number Mating	Number Achieving Pregnancy	Mating Index (%)	Conception Rate (%)	Fertility Index (%)
Males						
Control	24	24	24	100	100	100
3.0	23	23	23	100	100	100
6.5	24	24	24	100	100	100
15.0	24	24	23	100	96	96
Females						
Control	24	24	24	100	100	100
3.0	24	24	24	100	100	100
6.5	24	24	24	100	100	100
15.0	24	24	23	100	96	96

Dose (mg Fe/kg)	Number of Animals	Pre-coital Interval (days)					
		n (%)	1-4	5-8	9-12	13-16	17-21
Control	24	n (%)	24 (100)	0	0	0	0
3.0	24	n (%)	24 (100)	0	0	0	0
6.5	24	n (%)	24 (100)	0	0	0	0
15.0*	24	n (%)	18 (75)	3 (13)	2 (8)	1 (4)	0

n = Number of animals in category

* = $p \leq 0.05$ for distribution of pre-coital interval (Mann-Whitney test; one-sided)

There was a significant prolongation of the pre-coital interval in the high-dose group. However, the authors concluded that this was due to an unusually high frequency (100%) of short pre-coital intervals in the control group. No other effects on mating and fertility parameters were observed. No changes were observed in estrous cycles.

C-Section Observations: C-section observations are summarized in the following table.

Dose (mg Fe/kg)	No. of Litters	Corpora Lutea	Implantations	Live Young	Resorptions			Implantation Loss (%)	
					Early	Late	Total	Pre-	Post-
Control	23	16.5	15.9	15.0	0.9	0.0	0.9	3.8	5.7
3.0	24	16.0	15.4	14.5	0.6	0.3	0.9	4.1	5.3
6.5	24	15.3	14.7	13.9	0.5	0.2	0.8	3.7	5.3
15.0	23	15.5	15.0	13.8	1.0	0.3	1.2	3.3	8.1

There were no effects on the number of corpora lutea, implantations, live young, or resorptions.

Conclusions: Mating and fertility were unaffected in rats treated with Venofer®. However, the results are incomplete, since sperm count and sperm viability were not characterized in epididymides or testes, as recommended in the ICH guidelines (ICH, Detection of Toxicity to Reproduction for Medicinal Products, 1994). Although mating time was prolonged in the rats treated with 15 mg Fe/kg/dose, the authors concluded that this was due to unusually short mating times in the control group. Bodyweight gain was slightly reduced in males and pregnant females treated with 15 mg Fe/kg/dose, whereas weight gain was unaffected at the lower doses. Therefore, the selection of 15 mg Fe/kg/dose as the highest dose level appeared to be justified. The reduction in weight gain in females was correlated with reduced food consumption.

Segment II Intravenous Teratogenicity Study in Rats.

Testing Laboratory: _____

Study #: VFR 16/974254

Study Dates: 6/11/97-12/24/97

GLP compliance is indicated.

Animals: Female Crl:CD®BR rats, age 8-9 weeks, 178-260 g

Methods: Females were cohabitated with males of the same strain. The day of mating (day 0 of pregnancy) was confirmed by the presence of sperm in vaginal smear or the presence of a vaginal plug. Pregnant rats were treated with 0 (saline), 6.5, 13 or 20 mg Fe/kg/day i.v. on days 6 to 17 of gestation (35 rats in control group, 25 rats in both 6.5 and 13 mg Fe/kg/day groups). Rats were restrained and conscious during the 4-hr infusion of Venofer® (lot # 676109, 692109A1) or saline in lateral tail vein. Dose volume was 20/13, 6.5, 13, and 20 ml/kg for the control, low-, middle-, and high-dose groups, respectively. Dosing was incomplete on a few occasions due to dislodgement of needle, with the following frequency: 5/35 in controls; 4/25 in 6.5 mg Fe/kg/day; 11/25 in 13 mg Fe/kg/day.

After 3 deaths in the 20 mg Fe/kg/day group, this dose was reduced to 13 mg Fe/kg/day. Rats treated with 13 or 20 mg Fe/kg/day are two distinct groups. Dams were sacrificed on day 20 and c-sections were performed. Half of fetuses in each litter were stained with alizarin red for skeletal examination, or Bouin's solution for visceral examination. Fetuses from 20 mg Fe/kg/day group were processed but not evaluated.

Results:

Observed Effects: Three deaths occurred in the 20 mg Fe/kg/day group. Adverse reactions in this group included pale extremities and shallow breathing. Marked enlargement of liver and spleen were found in one rat that died on day 10. The spleen had ruptured, causing bleeding into the abdomen. Congested cisternal lymph nodes, enlarged adrenals, hemorrhagic depressions in stomach mucosa, and complete litter loss were found in the other two rats, sacrificed in extremis on day 18. No deaths occurred in the 6.5 or 13 mg Fe/kg/day groups.

Wet fur was observed in all groups, frequently during infusion. Red periorbital staining, brown perinasal staining, and soft feces were noticed in all groups. These effects are thought to be secondary to the stress induced by the restraining procedure. Dark tails were observed in 3 rats in the 13 mg Fe/kg/day group. Hair loss and red/brown vaginal discharge was observed in treated groups.

Body Weight/Food Consumption: Weight gain (relative to weight on day 6) was reduced by 50% over days 6-10, by 31-34% over days 6-14, and by 10-14% over days 6-18 in the 6.5 and 13 mg Fe/kg/day groups. The average bodyweight of pregnant control dams on day 2 and day 20 of pregnancy was 231 and 357 g, respectively. Food consumption over days 6-17 was reduced by 12% in the 13 mg Fe/kg/day group. The average food consumption for control dams was 298 g/rat over days 6-17.

Litter Observations: Frequency of resorptions or fetal deaths, litter size, fetal weight, and placental weight were unaffected in the 6.5 and 13 mg Fe/kg/day groups. A 2-fold increase in the number of in utero deaths was observed in the 20 mg Fe/kg/day group, along with a slight decrease in mean fetal weight.

One fetus with major skeletal abnormalities was found in the 13 mg Fe/kg/day group. Anomalies in this fetus included cleft palate, fused nasal bones, oligodactyly, brachymelia, interrupted costal cartilage, and distorted ribcage. These malformations are extremely rare in this strain of rat. For

example, the frequency of palatine anomalies was reported to be 0.009% for fetal incidence and 0.095% for litter incidence (Middle Atlantic Reproduction and Teratology Association, Midwest Teratology Association, 1992-1994). Although minor skeletal anomalies were found in all groups, a higher incidence of medially thickened/kinked ribs was apparent in the 6.5 and 13 mg Fe/kg/day groups. There was also a slightly higher incidence of incompletely ossified cranial centers in both treated groups.

Minor visceral anomalies observed in all groups included undescended thymus, thin diaphragm with protruding liver, and dilated ureters. Hepatic hemorrhage occurred with a slightly greater frequency in treated groups: 2/200 in controls; 8/161 at 6.5 mg Fe/kg/day; 7/160 at 13 mg Fe/kg/day.

C-section observations and the incidence of visceral and skeletal anomalies, and major malformations are summarized in the following tables. Gross anomalies were not reported.

C-Section Parameter	Dose (mg Fe/kg/day)			
	0	6.5	13.0	20.0
# Pregnant rats	33	25	25	7
Corpora lutea	14.1	15.1	14.1	13.4
Implantations	12.9	13.3	13.1	11.7
Early resorptions	0.6	0.5	0.2	0.7
Late resorptions	0.1	0.1	0.2	0.6
Live pups	12.2	12.8	12.7	10.4
Sex Ratio (%males)	47.4	53.6	51.7	—*
Litter weight (g)	46.1	47.1	48.6	35.0
Fetal weight (g)	3.8	3.7	3.8	3.5

* fetuses were not examined

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Minor Visceral Abnormalities	Dose (mg Fe/kg/day)		
	0	6.5	13.0
	Fetuses/Litters	Fetuses/Litters	Fetuses/Litters
Number examined	200/33	161/25	160/25
Number affected	45/24	37/23	34/19
Eyes:			
Dilated orbital sinus	0	1/1	0
Lenticular irregularity	0	0	1/1
Thymus: undescended	1/1	3/3	2/2
Interventricular septal defect	0	1/1	1/1
Diaphragm: thin with protruding liver	5/5	9/7	4/3
Liver: thin with additional lobe	2/2	1/1	1/1
Kidneys: absent papilla	2/2	1/1	1/1
Ureters: dilated	29/14	8/6	5/3
Bladder: umbilical artery	0	1/1	3/3
Testes: displaced	3/3	1/1	5/5
Hemorrhages:			
Brain	5/5	9/8	2/2
Eye/surrounding tissue	1/1	1/1	1/1
Subcutaneous	0	0	1/1
Intra-abdominal	0	1/1	2/2
Liver	2/2	8/6	7/6
Kidneys	0	0	1/1

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ON ORIGINAL

Minor Skeletal Abnormalities	Dose (mg Fe/kg/day)		
	0	6.5	13.0
	Fetuses/Litters	Fetuses/Litters	Fetuses/Litters
Number examined	203/33	158/25	156/25
Cranial: bone plaque	0	0	1/1
Vertebral: bipartite	0	4/4	3/3
Ribs: thickened/kinked	1/1	13/7	11/8
Sternebrae:			
Offset	0	2/2	0
Bipartite	1/1	2/1	0
Misshapen	0	1/1	0
Scapula: bent	0	1/1	1/1
Humerus: misshapen	0	1/1	1/1
Total affected by 1 or more of the above	2/2	18/11	14/10
Rib and vertebral configuration			
Cervical rib	1/1	3/3	0
Short 13 th rib	1/1	1/1	0
13/14 or 14/14 ribs	6/4	12/7	9/7
Incomplete ossification			
Cranial centers	9/6	19/9	20/12
Vertebrae:			
cervical	2/2	3/2	6/4
thoracic/lumbar	6/5	2/1	7/6
sacrocaudal	7/6	27/8	9/5
Sternebrae:			
5 th and/or 6 th	104/28	90/22	82/21
other	3/3	9/5	4/2
Total	105/28	90/22	82/21
Ischial bones:	2/2	8/4	4/2
Pubic bones:	2/2	7/4	5/4
Metacarpals/metatarsals	0	3/1	1/1
Precocious ossification			
Cervical vertebral centra	8/7	9/4	6/4

Major Abnormalities	Dose (mg Fe/kg/day)		
	0	6.5	13.0
	Fetuses/Litters	Fetuses/Litters	Fetuses/Litters
Number examined	403/33	319/25	317/25
Number affected	0	0	1/1
Anteriorly fused nasal bones, cleft palate, oligodactyly, brachymelia, interrupted costal cartilages and ribcage	0	0	1/1

Conclusion: A no-effect dose was not established for dams or fetuses. The higher incidence of thickened/kinked ribs and incompletely ossified cranial centers in fetuses of treated groups were correlated with signs of maternal toxicity, which included impaired weight gain and reduced food consumption. No major skeletal or visceral abnormalities were positively associated with Venofer® treatment. However, one fetus in the 13 mg Fe/kg/day group exhibited major malformations that are reported to occur with extremely low frequency in this strain. Because only one fetus exhibited these malformations, it is unclear whether this is indicative of teratogenicity for the test article.

Addendum: Venofer® was not teratogenic in rats. There was no evidence of embryo-fetal toxicity in Venofer®-treated rats.

Segment II Intravenous Teratogenicity Study in Rabbits.

Testing Laboratory: _____

Study #: VFR026/983656

Study Dates: 6/18/98-3/15/99

GLP Compliance: A statement of compliance was included.

Animals: New Zealand White rabbits, virgin females, age 19-26 weeks, 3.3-5.5 kg.

Methods: Females were mated with male New Zealand White rabbits of established fertility. Following mating, all females were injected intravenously with 25 i.u. luteinizing hormone to induce ovulation. The day of mating was designated as day 0 of gestation. On days 1-5 of pregnancy, the animals were acclimated to the restraint procedure used for dosing. Females were randomly assigned to the following dose groups.

Group #	Dose (mg Fe/kg/day)	Number of Females
1	0 (saline)	22
2	3	22
3	6.5	22
4	13	5
4A	13 (alternate days)	19
4B	13 (alternate days)	20

Group 4A: Days 6, 8, 10, 12, 14, 16, 18

Group 4B: Days 7, 9, 11, 13, 15, 17, 19

Dose selection was based on results from a preliminary Segment II rabbit study with Venofer[®], in which rabbits were treated with 13 mg Fe/kg/day i.v. Although Venofer[®] was generally well tolerated, this dose was considered as the maximum tolerated dose because of the severity of local irritation. As a result of injury at the injection site, administration of the drug became difficult towards the end of the treatment period. In the present study, rabbits in groups 1, 2, 3, and 4 were treated daily with intravenous infusion of Venofer[®] (batch # 711119) or saline on days 6-19 of pregnancy. Following completion of treatment of the first 5 animals in group 4, the remaining 17 animals and an additional 22 were assigned to two subgroups designated for treatment on alternate days. This was done in response to the marked local irritation of Venofer[®] in group 4. Group 4A was treated on days 6, 8, 10, 12, 14, 16, and 18 of gestation. Group 4B was treated on days 7, 9, 11, 13, 15, 17, and 19 of gestation. Venofer[®] was diluted with saline to a concentration of 1 mg Fe/ml and infused in the marginal veins of the right and left ears on alternate days. The rate of infusion was 3.25 ml/kg/hr (equal to 3.25 mg Fe/kg/hr) for all groups, and the dose volumes were 3, 6.5, and 13 ml/kg for the low-, middle, and high-dose groups, respectively. The infusion time was 55 min, 2 hr, and 4 hr for the low-, middle-, and high-dose groups, respectively. The dose volume and infusion time used for the control group was not stated.

Bodyweight and food consumption was monitored throughout the study. Females were sacrificed by intravenous injection of pentobarbitone sodium on day 29 of gestation, and C-sections were performed. Heads from one third of fetuses in each litter were fixed in Bouin's fluid. The torsos and remaining intact fetuses were examined for visceral anomalies and stained with Alizarin-red for skeletal examination.

Results:

Observed Effects: Abortion and mortality was observed in a few animals. These observations are listed in the table below.

Observation	GROUP #					
	1	2	3	4	4A	4B
# Mated	22	22	22	5	19	20
# Killed in extremis	0	1	0	1	0	0
# Aborted	0	0	1	1	2	1
# Not Pregnant	2	1	3	1	5	3
# Pregnant on Day 29	20	20	18	2	12	15
# Delivered Prematurely on day 29	0	0	0	0	0	1

One female in group 3 aborted on day 22 after a short period of bodyweight loss. Four females in groups 4, 4A, and 4B aborted on days 23-28. In these animals, bodyweight loss and reduced consumption of food and water was observed during periods ranging from day 16 to 28. Changes observed in necropsy of females that aborted included accentuated lobular pattern in liver, swelling of spleen, and enlarged kidneys. One female in group 2 was sacrificed for humane reasons on day 13, due to the limited use of the hindlimbs. The cause of this condition was attributed to suspected spinal damage during the restraint procedure. One rabbit from group 4 was sacrificed in extremis on day 25. The moribund condition was preceded by weight loss and a sharp reduction in food intake on days 22-25. Necropsy observations for this animal included red staining on tail and urogenital area, accentuated lobular pattern in liver, slight swelling and brown discoloration of spleen, enlarged kidneys, and hemorrhage in the cecum-colon junction. Eleven late resorptions and two early resorptions were present in uterus. One female in group 4B delivered prematurely on day 29 (9 live fetuses and 2 placentas), following weight loss and reduced food consumption on days 22-29. Most clinical signs in treated females that survived through study termination occurred with similar frequency in control females. However, signs such as loose feces, lack of food consumption, reduced body temperature, and scab formation at the injection site were observed only in treated groups.

Bodyweight/Food Consumption: Among the surviving females that did not abort, no changes in bodyweight gain were observed. Control females weighed 4.39 ± 0.08 kg on day 6, and 4.59 ± 0.07 kg on day 20. Food consumption was reduced by 23-47% in group 4 only. The daily food consumption in control females ranged from 144 to 196 g/animal/day.

C-Section Observations: The contents of the reproductive tracts are listed in the following table.

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Group = 1 2 3 4/4A/4B*

Compound = Control -----Venofer-----

Dosage (mg Fe/kg/day) = 0 3 6.5 13

Group	Number of Pregnant Females		Corpora Lutea	Implan-tations	Live Young			Resorptions			Implantation Loss (%)	
					Males	Females	Total	Early	Late	Total	Pre-	Post-
1	20	Mean	12.4	10.8	4.8	4.8	9.6	0.5	0.8	1.2	13.7	11.7
		SD	2.3	1.8	2.0	1.9	2.3	0.7	0.9	1.1		
2	20	Mean	12.2	10.4	4.7	5.0	9.7	0.4	0.5	0.8	15.2	8.0
		SD	2.2	2.9	1.7	1.8	2.9	0.6	0.7	0.9		
3	18	Mean	12.7	10.8	4.8	5.0	9.8	0.3	0.7	1.0	15.1	8.1
		SD	1.9	2.5	2.4	2.4	1.9	0.5	0.8	1.0		
4	2	Mean	12.0	11.0	4.0	5.0	9.0	0.5	1.5	2.0	8.4	19.2
		SD	1.4	1.4	1.4	1.4	2.8	0.7	1.2	1.4		
4A	12	Mean	11.0	9.8	4.5	4.1	8.6	0.3	0.8	1.1	15.0	10.4
		SD	2.2	2.9	1.9	2.0	2.5	0.5	0.9	1.0		
4B	15	Mean	12.6	10.7	3.5	4.7	8.2	0.9	1.6	2.5a	17.3	23.7b
		SD	3.6	4.1	1.8	2.5	3.3	0.9	1.3	1.6		
Background Control Data (18 studies)												
Mean			12.8	11.0	4.9	4.6	9.5	0.6	0.9	1.5	14.5	13.5
Low			11.7	9.6	3.7	3.9	8.4	0.2	0.3	0.6	9.2	5.8
High			15.0	12.4	5.9	5.5	10.2	1.6	1.6	2.9	21.6	22.1

* Group 4: Treatment Days 6-19.

Group 4A: Days 6, 8, 10, 12, 14, 16, 18.

Group 4B: Days 7, 9, 11, 13, 15, 17, 19.

a - p<0.05; b - p<0.01

There were no effects on the number of corpora lutea, implantations, live offspring, or sex ratio. Although there were significant increases in the number of total resorptions and the percentage of post-implantation loss in group 4B, the number of resorptions was within the historical control range. These changes were not observed in group 4A.

Litter Observations: The results from measurement of fetal and placental weights are summarized in the following table.

Group	=	1	2	3	4/4A/4B*
Compound	=	Control	-----Venofer-----		
Dose (mg Fe/kg/day)	=	0	3	6.5	13

Group		Fetal Weight (g)			Placental Weight (g)
		Male	Female	Total	
1	Mean	43.3	42.4	42.7	5.6
	SD	4.2	4.7	3.9	0.7
	# litters	20	20	20	20
2	Mean	42.0	42.6	42.5	5.7
	SD	3.9	3.9	3.3	0.8
	# litters	20	20	20	20
3	Mean	40.5	39.2	39.5	5.8
	SD	7.7	5.9	6.6	0.8
	# litters	18	18	18	18
4	Mean	35.2	30.3	32.5	5.6
	SD	9.5	10.0	9.9	0.3
	# litters	2	2	2	2
4A	Mean	42.2	41.8	42.0	6.0
	SD	4.6	6.0	5.1	0.6
	# litters	12	12	12	12
4B	Mean	42.4	42.7	43.0	6.3
	SD	5.6	6.4	6.1	1.1
	# litters	14	15	15	15
Background Control Data (18 studies)					
Mean		40.5	41.0	39.8	5.4
Low		37.4	37.7	37.0	4.8
High		43.0	43.7	42.6	5.7

*Group 4: Treatment Days 6-19
 Group 4A: Days 6, 8, 10, 12, 14, 16, 18
 Group 4B: Days 7, 9, 11, 13, 15, 17, 19

There were no significant changes in fetal or placental weight, although there was a tendency towards reduced fetal weight in group 4.

Gross anomalies observed in fetuses are summarized in the following table.

GROSS OBSERVATIONS												
Group*	Fetuses						Litters					
	1	2	3	4	4A	4B	1	2	3	4	4A	4B
Dose (mg Fe/kg/day)	0	3	6.5	13	13	13	0	3	6.5	13	13	13
Number Examined	192	192	177	18	103	123	20	20	18	2	12	15
Number Affected	2	1	2	-	1	2	2	1	2	-	1	2
MALFORMATIONS												
Cranioschisis	-	-	1	-	-	-	-	-	1	-	-	-
Cranioschisis; forelimb flexure; malrotated hindlimb; ablepharia; umbilical hernia	-	-	-	-	-	1	-	-	-	-	-	1
Wide upper jaw; large sutural bone nasal suture; sutural bone right fronto/parietal suture; enlarged lacrimal fossa; cleft palate; dorsoventral distortion of sternum	-	-	-	-	-	1	-	-	-	-	-	1
Cervical scoliosis	1	-	-	-	-	-	1	-	-	-	-	-
Lumbar scoliosis	-	-	1	-	-	-	-	-	1	-	-	-
Lumbar to caudal spina bifida; flattened cranium and protrusion occipital region; malrotated hindlimb	-	1	-	-	-	-	-	1	-	-	-	-
Forelimb flexure	1	-	-	-	1	-	1	-	-	-	1	-

*Group 4: Treatment Days 6-19

Group 4A: Days 6, 8, 10, 12, 14, 16, 18

Group 4B: Days 7, 9, 11, 13, 15, 17, 19

Gross malformations were observed in treated and control rabbits. However, the low frequency and lack of dose-dependency suggest that these effects were not treatment related.

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Visceral and head observations are listed in the following table.

VISCERAL/HEAD OBSERVATIONS												
Group*	Fetuses						Litters					
	1	2	3	4	4A	4B	1	2	3	4	4A	4B
Dose (mg Fe/kg/day)	0	3	6.5	13	13	13	0	3	6.5	13	13	13
Number of Heads Examined	67	64	60	6	34	40	20	20	18	2	12	15
ANOMALIES (head)												
Head												
Subdural hemorrhage cerebellum	-	1	-	-	1	-	-	1	-	-	1	-
Folded retina	2	-	3	-	2	4	2	-	3	-	2	2
Number of heads affected	2	1	3	0	3	4	2	1	3	0	3	2
VARIATIONS (head)												
	0	0	0	0	0	0	0	0	0	0	0	0
Number of Fetuses Examined	190	191	175	18	102	121	20	20	18	2	12	15
ANOMALIES (viscera)												
Head												
Opaque area eyes	-	-	-	-	-	3	-	-	-	-	-	1
Abdomen												
Fluid in	-	-	1	-	1	5	-	-	1	-	1	2
Gall bladder												
Hemorrhage on	3	5	2	-	-	2	3	5	2	-	-	1
Small	-	-	1	-	-	-	-	-	1	-	-	-
Kidney(s)												
Renal cavitation	1	-	-	-	-	-	1	-	-	-	-	-
Bladder												
Clotted blood around	-	1	-	-	-	-	-	1	-	-	-	-
Umbilical artery												
Dilated	-	-	-	-	2	-	-	-	-	-	2	-
Blood around	-	-	-	-	1	-	-	-	-	-	1	-
VARIATIONS (viscera)												
Stomach												
Gas in	2	1	1	1	1	1	1	1	1	1	1	1
Number of fetuses affected	6	7	5	1	4	8	5	7	4	1	4	4

Note: Individual fetuses/litters may occur in more than one category.

Fetuses with gross malformations were excluded.

*Group 4: Treatment Days 6-19

Group 4A: Days 6, 8, 10, 12, 14, 16, 18

Group 4B: Days 7, 9, 11, 13, 15, 17, 19

Dilation of umbilical artery was observed in group 4A. Treatment-related abnormalities included opacity in eyes (group 4B) and fluid in abdomen (groups 3, 4A, 4B). Other changes were observed with similar frequency in the control and treated groups.

Observations from skeletal examination are summarized in the following tables.

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MINOR SKELETAL ABNORMALITIES/VARIATIONS												
Group*	Fetuses						Litters					
	1	2	3	4	4A	4B	1	2	3	4	4A	4B
Dose (mg Fe/kg/day)	0	3	6.5	13	13	13	0	3	6.5	13	13	13
Number Examined	190	191	175	18	102	121	20	20	18	2	12	15
MINOR ABNORMALITIES												
Cranial												
Fissures/extra sutures	6	2	6	-	3	4	6	2	4	-	3	3
Fused parietal to Interparietal	-	2	-	-	-	-	-	1	-	-	-	-
Unossified area	1	-	-	-	-	-	1	-	-	-	-	-
Bent cornua of hyoid	2	-	4	1	-	1	2	-	3	1	-	1
Vertebrae												
Hemicentric (Total)	2	-	1	1	-	1	2	-	1	1	-	1
Cervical	2	-	-	-	-	1	2	-	-	-	-	1
Thoracic	-	-	-	1	-	-	-	-	-	1	-	-
Lumbar	-	-	1	-	-	-	-	-	1	-	-	-
Bipartite (Thoracic)	-	-	-	1	-	-	-	-	-	1	-	-
Small (Caudal)	1	-	2	-	-	-	1	-	2	-	-	-
Ribs												
Thickened	-	-	1	-	1	1	-	-	1	-	1	1
Branched	1	-	1	-	-	-	1	-	1	-	-	-
Sternebrae												
Additional centre(s)	7	3	8	2	3	5	3	2	6	1	2	3
Offset	1	-	2	-	1	1	1	-	2	-	1	1
Bifurcated	1	-	-	-	-	-	1	-	-	-	-	-
Bipartite	-	-	1	-	-	-	-	-	1	-	-	-
Flattened	1	-	-	-	-	-	1	-	-	-	-	-
Omosternum ossified	-	-	-	-	-	1	-	-	-	-	-	1
Costal cartilage												
Bifurcated xiphoid	1	-	-	-	-	-	1	-	-	-	-	-
Branched	-	-	1	-	-	-	-	-	1	-	-	-
Caudal region												
Minimal kink	-	1	-	-	-	-	-	1	-	-	-	-
VARIATIONS												
Rib and Vertebral Configuration												
Cervical rib	-	2	-	1	1	1	-	2	-	1	1	1
Number with 12/13 or 13/13 ribs	100	119	110	16	43	78	19	18	17	2	11	15
18 thoracolumbar vertebrae	1	-	-	-	-	-	1	-	-	-	-	-
20 thoracolumbar vertebrae	38	37	60	11	16	31	11	14	14	2	6	10
Offset pelvic girdle	11	10	16	1	6	9	8	5	12	1	3	4
Incomplete Ossification												
Enlarged lacrimal fossa	-	-	-	-	-	1	-	-	-	-	-	1
Cranial bones												
Vertebral element												
Cervical	4	1	4	1	1	2	2	1	3	1	1	2
Sternebrae												
5th	5	14	1	-	1	4	3	6	1	-	1	2
Other	2	1	-	-	-	2	2	1	-	-	-	2
Total	7	15	1	-	1	5	4	6	1	-	1	2
<16 ossified caudal vertebrae	-	-	-	-	-	1	-	-	-	-	-	1
Unossified astragali	-	-	1	-	-	-	-	-	1	-	-	-
Unossified epiphyses	-	2	13	4	-	7	-	1	5	1	-	4
Metacarpals/metatarsals	-	12	10	9	1	5	-	7	7	2	1	4
Precocious Ossification												
Small anterior fontanelle	2	5	-	-	-	-	2	3	-	-	-	-
Ossified olecranon processes	20	6	13	1	11	9	8	4	6	1	6	5

Note: Individual fetuses/litters may occur in more than one category.

Fetuses with gross malformations were excluded.

*Group 4: Treatment Days 6-19

Group 4A: Days 6, 8, 10, 12, 14, 16, 18

Group 4B: Days 7, 9, 11, 13, 15, 17, 19

SKELETAL MALFORMATIONS												
Group*	Fetuses						Litters					
	1	2	3	4	4A	4B	1	2	3	4	4A	4B
Dose (mg Fe/kg/day)	0	3	6.5	13	13	13	0	3	6.5	13	13	13
Number Examined	190	191	175	18	102	121	20	20	18	2	12	15
Sternebrae												
Fused centres/Bridge of Ossification	-	1	-	-	-	3	-	1	-	-	-	3
Vertebrae												
Fused (caudal)	-	1	-	-	1	-	-	1	-	-	1	-
Misaligned (Total)	1	1	3	-	-	-	1	1	3	-	-	-
Lumbar	-	-	1	-	-	-	-	-	1	-	-	-
Caudal	1	1	2	-	-	-	1	1	2	-	-	-

Note: Individual fetuses/litters may occur in more than one category.
Fetuses with gross malformations were excluded.

*Group 4: Treatment Days 6-19

Group 4A: Days 6, 8, 10, 12, 14, 16, 18

Group 4B: Days 7, 9, 11, 13, 15, 17, 19

Malformations observed in the Venofer[®]-treated groups included fused sternebrae, fused vertebrae, and misaligned vertebrae. Among these changes, the incidence of fused sternebrae may have been treatment-related. This malformation did not appear to be secondary to maternal toxicity, since bodyweight gain was unaffected in the individual females that delivered the affected fetuses. However, the results are equivocal. Whereas the fetal incidence of fused sternebrae was 3/121 in group 4B (treated on odd-numbered days), there was no incidence (0/102) in group 4A (treated on even-numbered days). Furthermore, there was one occurrence in group 2, but none in group 3. There was no incidence in group 4 (13 mg Fe/kg/day), although the significance of this is uncertain due to the low number (18) of fetuses examined in this group. Thus, the dose-dependency of this effect is questionable. It is noteworthy that the fetal incidence of fused sternebrae in group 4B, 2.48%, is greater than the historical control value of 1.26%, obtained from studies on New Zealand White rabbits performed in the same laboratory (Palmer, In: Pathology of Laboratory Animals: Benirschke et al. (Ed.), 1978).— Each incidence in group 4B was observed in separate litters, and the litter incidence was 20% (3/15).

Misaligned vertebrae were observed in the control and treated groups. However, the frequency was not dose-related. Fused vertebrae occurred only once in each of groups 2 and 4A. The frequency of minor skeletal abnormalities was not affected by treatment. However, a tendency towards delayed ossification in epiphyses, metacarpals, and metatarsals was observed in all treated groups.

Conclusions: Although skeletal malformations were observed in fetuses from the Venofer®-treated females, these results are equivocal. Therefore, Venofer® is not considered to be teratogenic in rabbits. However, an additional Segment II study is needed to provide conclusive evidence relating to this question. Other changes observed in fetuses included delayed ossification of epiphyses, metacarpals, and metatarsals in all treated groups, and opacities in eyes in the high dose/alternate day groups. Only two litters from rabbits treated daily with 13 mg Fe/kg were examined, due to severe local irritation. Therefore, the number of fetuses from this group was insufficient for the evaluation of teratogenicity.

GENETIC TOXICOLOGY:

Bacterial Mutation Assay.

Testing Laboratory: _____

Study #: 96/VFR012/1211

Report Date: 1/17/97 (start date is not stated)

GLP compliance is indicated.

Methods: The mutagenicity of Venofer® (lot# 572109) was evaluated using the Ames test (Ames et al., Mutation Res, 31, 1975). The histidine-dependent Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 were used. Escherichia coli CM891, a tryptophan-dependent strain, was also tested. S9 liver fraction was prepared from CD rats weighing 200 g, pretreated with Aroclor 1254 in cornoil, 500 mg/kg i.p., to induce microsomal enzymes. All tests were performed with or without the S9 liver fraction. The following compounds were tested as positive controls in the indicated strains:

Positive Control	Concentration (µg/plate)	Strain
2-Aminoanthracene ¹	2	TA1535
	10	CM891
9-Aminoacridine ²	80	TA1537
2-Nitrofluorene ²	1	TA98
Benzo[a]pyrene ¹	5	TA98, TA100, TA1537
N-Ethyl-N'-nitro-N-Nitrosoguanidine ²	2	CM891
	3	TA100
	5	TA1535

1 - tested only in presence of S9

2 - tested only in absence of S9

Water was added to control cultures. The criteria for mutagenicity (i.e. % increase in revertant colonies) was not stated.

Results: Two sets of assays were performed. In the first assay, bacterial strains were incubated with Venofer[®], 5-5000 µg of iron sucrose/plate. No toxicity was observed at any concentration, and no mutagenic effects were observed. The second assay was performed using concentrations of 50-5000 µg of iron sucrose/plate. Again, Venofer[®] produced no increase in the number of revertant colonies. Positive controls produced increases in the number of revertant colonies.

Conclusion: Venofer[®] has no mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537, or E. coli CM891.

Bacterial Mutation Assay.

Testing Laboratory: _____

Report #: 265

Study Dates: April 21, 1983 - July 26, 1983

GLP compliance is not indicated.

Methods: The mutagenicity of Hippiron[®] (iron sucrose) was investigated using the Ames test (Ames et al., Mutation Res, 31, 1975). Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 (histidine dependent) and E. coli WP2 uvrA (tryptophan dependent) were used. All assays were performed in the absence and presence of S9 liver fraction. Hippiron[®] was diluted in DMSO, which was used as the solvent control. S9 liver fraction was prepared from Sprague-Dawley rats treated with phenobarbital and benzoflavone. The following compounds were tested as positive controls:

2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide
N-ethyl-N'-nitro-N-nitrosoguanidine
9-aminoacridine
2-nitrofluorene
2-aminoanthracene (requires microsomal enzyme activation)

The criteria for classification as a mutagen (i.e. % increase in revertant colonies) was not indicated.

Results: Hippiron[®] was tested over a concentration range of 0.5-100 µg/plate. It is assumed that the concentration units are µg of iron sucrose/plate, since this was indicated in other studies on mutagenicity. No mutagenic activity was observed. The positive control compounds produced the predicted mutagenic effects.

Conclusion: Hippiron[®] has no mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538, and E. coli WP2 uvrA.

Mammalian Cell Mutation Assay.

Testing Laboratory: _____

Study #: VFR 014/971264

Study Dates: 12/12/96-4/29/97

GLP compliance is indicated.

Methods: The mutagenic activity of Venofer[®] (lot# 572109) was tested in L5178Y mouse lymphoma cells. This cell line is heterozygous for thymidine kinase (TK +/-). The positive controls methyl methanesulfonate (absence of S9) and 20-methylcholanthrene (presence of S9) were tested. The S9 liver fraction was prepared from male Sprague-Dawley rats age 7-8 weeks treated with Aroclor 1254, 500 mg/kg i.p. All tests were performed in the absence and presence of S9.

In the first part of this study, the toxicity of Venofer[®] was evaluated. Cells were treated for 3 hr with Venofer[®] (7.8-2000 µg Fe/ml). Cells were washed once, and grown in fresh media for 48 hr. Toxicity was evaluated by measuring growth inhibition. The concentration which inhibited growth by 80% (LC₈₀) was selected as the maximum concentration for the mutagenicity assay.

Mutagenicity was evaluated in the second part of the study, in two separate tests. Cells were incubated for 3 hr with 62.5-1000 µg Fe/ml, washed once, and incubated in fresh media for 48 hr. 10⁶ cells were plated in selective medium (3 plates/concentration). Mutation frequency was assessed by counting the number of cell colonies of >100 µm diameter after 11-12 days of incubation. Mutation frequency was calculated as the number of mutations per 10⁶ survivors. The criteria for a positive response were the following:

1. A statistically significant increase in mutation frequency in treated cultures of at least 100, relative to control.
2. Evidence of a dose relationship over at least two consecutive doses.
3. An increase in the absolute number of colonies in the treated cultures.

Results: In the preliminary toxicity assay, Venofer® inhibited growth in a dose-related manner. Over a concentration range of 7.8-2000 µg Fe/ml, growth was reduced by 0-99% in the presence of S9, and by 3-99% in the absence of S9. Although increases in mutation frequency were observed at 167 and 250 µg Fe/ml in test 1, and at 300 µg Fe/ml in test 2, these were not considered as biologically significant. The observed increases were close to the upper limit of the historical control range, and occurred only under conditions of severe toxicity.

Conclusion: Venofer® did not exhibit mutagenic activity in the mouse lymphoma L5178Y cell line.

Mouse Micronucleus Test.

Testing Laboratory: _____

Study #: 96/VFR013/1243

Study Dates: 10/21/96-4/8/97

GLP compliance is stated.

Methods: The clastogenic activity of Venofer® (lot# 572109) was investigated in CD-1 mice, age 4-5 weeks, weighing 18-27 g. A preliminary toxicity study of Venofer® was performed using a single intraperitoneal injection of 625, 1250, 2500 or 5000 mg iron sucrose/kg, with a dose volume of 20 ml/kg (2 mice/sex/group). The reason for use of intraperitoneal administration is not stated, and plasma iron concentration was not monitored for evidence of absorption. Mice were sacrificed 72 hr after injection. A dose of 5000 mg iron sucrose/kg was selected for the micronucleus test, in which mice were treated with a single i.p. injection of Venofer® or water. Mitomycin C, 12 mg/kg was administered orally as a positive control. Mice were sacrificed at 24, 48 and 72 hr post-dose (5/sex/group/timepoint). The frequency of micronucleated cells among polychromatic (immature) erythrocytes and mature erythrocytes was measured.

Results:

Toxicity Test: No deaths occurred in the preliminary toxicity test. One male and one female treated with 5000 mg iron sucrose/kg exhibited piloerection, hypoactivity and hunched posture. 5000 mg iron sucrose/kg was selected for use in the micronucleus test.

Micronucleus Test: Transient piloerection and hunched posture was observed in one male. There was no significant increase in micronucleated cells among polychromatic erythrocytes from treated mice at any time. The frequency range of micronucleated cells in mature RBCs in all groups was 0-4.2/1000, suggesting no pre-existing bone marrow abnormalities. Mitomycin C produced a 32-fold increase in frequency of micronucleated cells at 24 hr. The ratio of polychromatic to mature RBCs was unaffected by Venofer® or mitomycin C.

Conclusion: Although no evidence of Venofer®-induced micronucleus formation in mice was found in the present study, the results are inconclusive due to the lack of evidence of absorption. Since Venofer® is intended for intravenous administration, it is unclear why the route of administration used was intraperitoneal injection.

Chromosome Aberration Test in Cultured Human Lymphocytes.

Testing Laboratory: _____

Study #: VFR 4/950317

Study Dates: 12/12/94-6/7/95

GLP compliance is indicated.

Methods: Human blood was collected from healthy male donors. Lymphocytes were separated and washed by repeated centrifugations, and suspended at a concentration of 1×10^6 cells/ml in RPMI 1640 media with 20% fetal calf serum and 175 µg/ml phytohemagglutinin. 5 ml of cell suspension was incubated for 48 hr. S9 liver fraction was added to one set of cultures. Ferrum Hausmann IV® (iron sucrose, lot# 330109A2) was then added to both sets of cultures (with and without S9). Cultures without S9 were incubated for 18 hr with 0 (water), 39.1, 78.1, 156.3, 312.5, 625, 1250, 2500 or 5000 µg/ml Ferrum Hausmann IV®. It assumed that the concentration units refer to

iron sucrose, since this was indicated in other studies on mutagenicity. Cells that were incubated with S9 were treated for 3 hr with Ferrum Hausmann IV[®] using the same concentrations, followed by centrifugation and resuspension in fresh media, and 15 hr of incubation. Ethylmethanesulfonate (500 and 750 µg/ml, without S9) and cyclophosphamide (10 and 15 µg/ml, with S9) were used as positive controls. Cells were arrested in metaphase 2 hours before harvesting by the addition of colchicine (0.25 µg/ml). Cells were harvested, fixed, and mounted on slides. The proportion of mitotic cells per 1000 cells was recorded. The concentration which reduced the mitotic index by 50% was selected for metaphase analysis.

S9 liver fraction was prepared from male Sprague Dawley rats age 7-8 weeks, treated with 500 mg/kg i.p. Aroclor 1254. Rats were sacrificed 5 days after treatment.

Results:

Effects on Mitosis:

- S9 Mitotic index was increased by 30-85% in a non-dose-related manner.
- +S9 38% reduction in mitotic index at 5000 µg iron sucrose/ml.

Metaphase Analysis: Since a 50% reduction was not achieved in either set of cultures, a low, middle, and high dose were selected for metaphase analysis.

- S9 1250, 2500, 5000 µg iron sucrose/ml:
No change in frequency of chromosomal aberrations.
- +S9 625, 2500, 5000 µg iron sucrose/ml:
No change in frequency of chromosomal aberrations.

Both positive control compounds produced large increases in the frequency of chromosomal damage.

Conclusion: The present study showed no evidence of clastogenic activity for Ferrum Hausmann IV[®] (iron sucrose).

APPEARS THIS WAY
ON ORIGINAL

SPECIAL TOXICOLOGY STUDIES:

Perivenous Tolerance in Rabbits Following a Single Injection.

Testing Laboratory: _____

Study #: VFR 2/951737

Study Dates: 1/3/95-1/15/96

GLP compliance is indicated.

Methods: - New Zealand White rabbits age 11-12 weeks, weighing 2.2-3 kg (3 males and 3 females) were injected once with 0.2 ml Venofer® (lot# 330109A2) in lateral vein of right ear. Injections were administered for 10-15 sec. Saline was injected in left ear as a control. Local irritations were evaluated over a period of 4 days, and scored on a 0-4 scale. Rabbits were sacrificed at 4 days after injection, and the injection sites were fixed and evaluated.

Results:

Venofer® Injection Sites: No clinical or behavioral signs were observed. Very slight to well-defined erythema was found at the Venofer® injection sites. Brown discoloration and slight to moderate bruising was observed at or beyond the Venofer® injection sites. Scabs and minimal to moderate hemorrhage were found at injection sites. Minimal to moderate dermal edema and focal ulceration were also observed.

Saline Injection Sites: White discoloration and bruising were noted around the saline injection sites. Dermal inflammation, dermal hemorrhage and scab formation were also noted.

Conclusion: Intravenous Venofer® injection produced a treatment related reaction, with edema, hemorrhage, inflammation, overlying focal ulceration, and scab formation present in the majority of injection sites.

Intra-arterial Tolerance in Rabbits Following a Single Injection.

Testing Laboratory: _____

Study #: VFR 1/951736

Study Dates: 12/16/94-1/15/96

GLP compliance is indicated.

Methods: New Zealand White rabbits (3 males and 3 females) age 10-13 weeks, 2.2-3 kg, were injected with 0.5 ml Venofer® (lot # 330109A2) in median artery of right ear, and saline was injected in left ear as a control. Local irritation was rated on a 0-4 scale. Rabbits were sacrificed on the fourth day after injection. Auricular tissue with the injection sites was fixed and evaluated.

Results: Slight weight loss was observed in one male. Otherwise, no clinical signs or behavioral changes were noted. Starting at 1 day after dosing, very slight to well defined erythema was observed in both Venofer® and saline injection sites. Hematoma, slight to moderate bruising, slight swelling and scabs were observed around both injection sites. Trace hemorrhage in arterial wall, and dermal hemorrhage were observed in Venofer® injection sites.

Conclusion: Most of the changes observed after intra-arterial Venofer® injection were also found in saline injection sites, and are therefore considered as secondary to the injection procedure. However, dermal hemorrhage and hemorrhage in the arterial wall appears to be treatment-related.

PROPOSED TEXT OF THE LABELING FOR VENOFER®.

Several changes should be incorporated, as described below.

1. Carcinogenesis, Mutagenesis, Impairment of Fertility:

Sponsor's Version:

Evaluation: The text is not in accord with 21 CFR 201.50, Subpart B, (April 1, 1999). The statement, _____ should be deleted since a completed Segment I fertility study in rats was submitted in this application. A brief summary of the results of this study should be included.

Proposed Version:

2. Pregnancy:

Sponsor's Version:

Evaluation: The text is not in accord with 21 CFR 201.50, Subpart B, (April 1, 1999). Teratogenicity studies in rats and rabbits were included in this application, but were not cited. The results of these studies should be summarized. No effects were observed in the rat study. However, the results from the rabbit study were equivocal. Although skeletal malformations (fused sternebrae) were observed in fetuses from Venofer[®]-treated females, the dose-dependency of this effect was not conclusively demonstrated. Therefore, Venofer[®] was not considered to be teratogenic in rabbits, although an additional teratogenicity study is necessary to provide conclusive evidence. This section should be changed to comply with the requirements for the "Pregnancy" subsection.

Proposed Version: "Pregnancy Category B:"

3. Nursing Mothers:

Sponsor's Version:

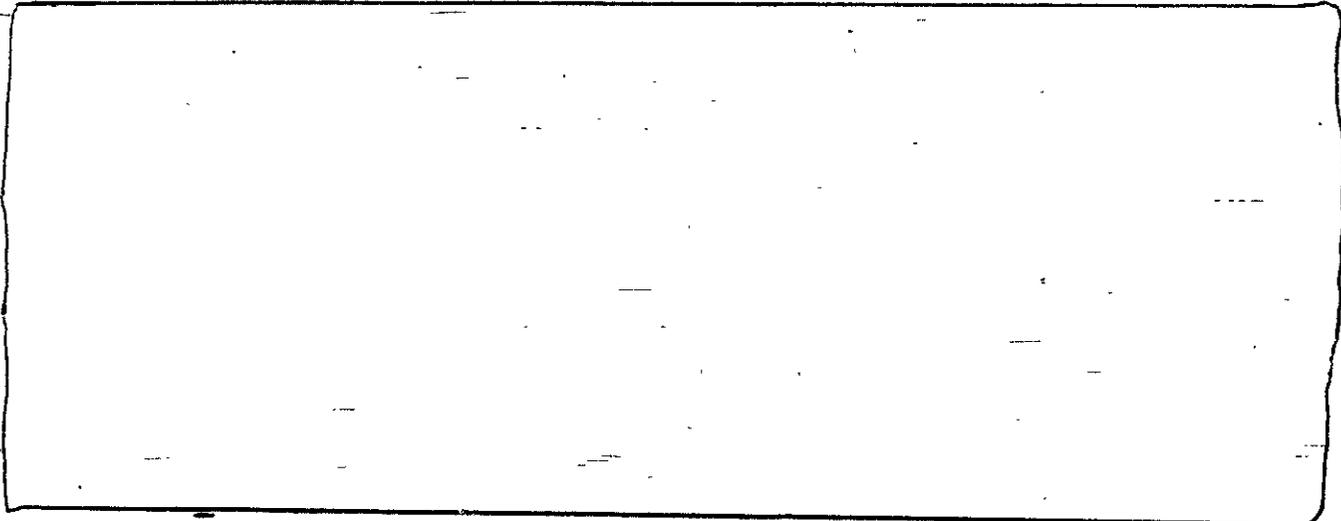
Evaluation: The text is not in accord with 21 CFR 201.50, Subpart B, (April 1, 1999). This section should be changed to comply with requirements for the "Nursing mothers" subsection.

Proposed Version: "Venofer® is excreted in milk of rats. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Venofer® is administered to a nursing woman."

4. Overdosage:

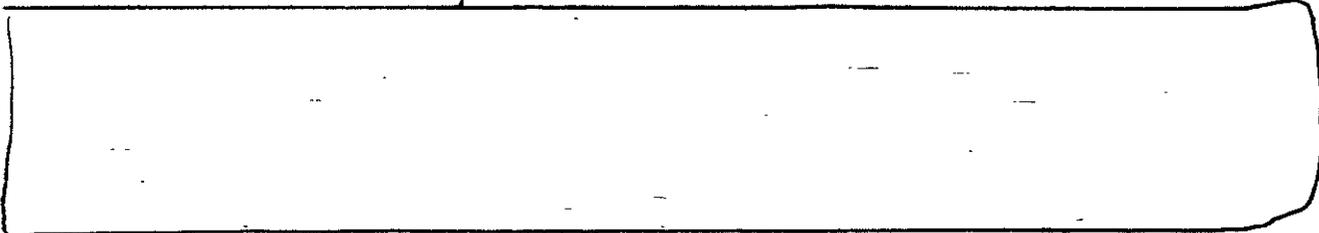
Sponsor's Version:

"Dosages of Venofer® in excess of iron needs may lead to accumulation of iron in storage sites leading to hemosiderosis. Periodic monitoring of iron parameters such as serum ferritin and transferrin saturation may assist in recognizing iron accumulation. Venofer® should not be administered to patients with iron overload and should be discontinued when serum ferritin levels _____ Particular caution should be exercised to avoid iron overload where anemia unresponsive to treatment has been incorrectly diagnosed as iron deficiency anemia.



Symptoms associated with overdosage or infusing Venofer® too rapidly included hypotension, headache, vomiting, nausea, dizziness, joint aches, paresthesia, abdominal and muscle pain, edema, and cardiovascular collapse. Most symptoms have been successfully treated with IV fluids, hydrocortisone, and/or antihistamines. Infusing the solution as recommended or at a slower rate can also alleviate symptoms.

Preclinical Data



Evaluation: The text is not in accord with 21 CFR 201.50, Subpart B, (April 1, 1999). The minimum lethal dose in rodents should be stated to provide more information on potentially lethal dose levels. The statement that indicates



The statement should be revised using the Human Equivalent Dose, which is calculated based on body surface area.

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ON ORIGINAL**

Proposed Version:



SUMMARY AND EVALUATION:

Venofer[®] (iron sucrose injection) is a brown, aqueous solution containing iron(III)-hydroxide sucrose complex, and is intended for intravenous administration. The following indications for Venofer[®] are stated in the Sponsor's draft labeling: treatment of dialysis-associated anemia;

The recommended dose of Venofer[®], assuming a 50-kg bodyweight, is 2 mg iron/kg/dose in adults, administered one to three times per week for a total dose of 20 mg iron/kg.

The pharmacology of Venofer[®] has been characterized only in humans. Venofer[®] acts by replenishing body iron stores in patients with iron deficiency, and maintains body iron stores in patients with continuing blood loss. The therapeutic activity of Venofer[®] is manifested as increases in hemoglobin concentration (15.7 and 13.8 g/dl in normal males and females, respectively), hematocrit (0.46 and 0.40 in normal males and females, respectively), serum ferritin (20-200 µg/l in normal individuals), and transferrin saturation (20-50% in normal individuals).

Plasma kinetics studies revealed that iron was rapidly cleared from serum following intravenous administration of Venofer[®] in rats ($t_{1/2}$ = 1.15 hr) and minipigs. In contrast, the plasma $t_{1/2}$ values measured in two human studies using healthy volunteers were 9.31 ± 6.77 hr and 5.3 ± 1.6 hr (Krzysko et al., Zbl Pharm, 123, 1984; Danielson et al., Arzneim-Forsch/ Drug Res, 46, 1996). The major sites of iron distribution in Venofer[®]-treated rats and minipigs were RBCs (red blood cells), liver, and bone marrow, with low levels present in spleen and kidneys. The extensive accumulation of iron in RBCs in Venofer[®]-treated animals is consistent with results from clinical studies (Beshara et al., Br J Haem, 104, 1999). 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. In a human study, urinary excretion during 24 hr post-administration was negligible (Danielson et al., *Arzneim-Forsch/Drug Res*, 46, 1996).

The acute toxicity of Venofer® was characterized in rats and mice. Death occurred following i.v. bolus injection of doses as low as 100 mg Fe/kg in rats and 150 mg Fe/kg in mice. Adverse reactions included pale eyes, sedation, hypoactivity, and hemorrhagic lesions in stomach, intestines, and lungs. Dark reddening of liver, kidney, and spleen was also observed.

A 13-week intravenous toxicity study of Venofer® in rats using one dose per week was performed. Rats were treated with 6.5 or 30 mg Fe/kg/dose. There were no deaths. Iron accumulation was found in most of the organs and tissues examined. Iron deposits were present in tissue macrophages, hepatocytes, and renal tubular epithelium. Liver and spleen were considered as potential target organs of toxicity, due to the high accumulation of iron. The tolerated dose was 30 mg Fe/kg/dose. The results of this study are incomplete due to the absence of histopathological evaluation of the 6.5 mg Fe/kg/dose group.

A 13-week intravenous toxicity study of Venofer® in rats using three doses per week was performed. Rats were treated with 3, 10, or 30 mg Fe/kg/dose. Iron accumulation was observed in most of the organs and tissues examined. Iron deposits were present in tissue macrophages, hepatocytes, renal tubular epithelium, glomerular mesangial cells, and vascular endothelium in lungs and adrenals. The only change observed in heart was the accumulation of iron in macrophages, which was mostly limited to valves. There was no sign of hemosiderin deposition in cardiac parenchyma, which is associated with iron overload (Goyer, In: Casarett & Doull's Toxicology, Fifth Edition, Klaassen (Ed.), 1996). Liver, kidney, and spleen were considered to be target organs of toxicity, based on the extensive accumulation of iron. Liver toxicity was further evidenced by marked increases in serum ALT and AST levels. The tolerated dose in this study was 3 mg Fe/kg/dose.

A 13-week intravenous toxicity study of Venofer® in dogs using one dose per week was performed. Dogs were treated with 6.5 or 30 mg Fe/kg/dose. No deaths occurred. Iron deposits were present in many organs and tissues, but the cellular distribution was limited to macrophages. Liver, spleen, kidneys, and lymph nodes were considered as potential target organs of toxicity, due to the high accumulation of iron. Mucoid degeneration of the pulmonary valve was observed in the

low- and high-dose groups (1/8 and 3/8, respectively). This condition is characterized by the conversion of connective tissue to mucoid or gelatinous material. Mucoid degeneration was also found in the aortic valve in the high-dose group (4/8). This change was not observed in the control groups. All observations of mucoid degeneration were described as minimal. Furthermore, this effect was not observed in the 13-week dog study using three doses per week, or in either of the 13-week rat studies. The toxicological significance of this effect in the present study is unclear. The tolerated dose was 30 mg Fe/kg/dose.

A 13-week intravenous toxicity study of Venofer® in dogs using three doses per week was performed. Dogs were treated with 3, 10, or 30 mg Fe/kg/dose. There were no deaths. Iron accumulation in macrophages in various organs/tissues was the most common effect. Iron deposits were also present in hepatocytes, glomerular mesangial cells, and renal tubular epithelium. The target organs of toxicity were liver, kidney, and spleen. This conclusion was based on the presence of heavy iron deposits in each of these organs, in addition to fibrosis and necrosis in liver. The only change observed in heart was the accumulation of iron in macrophages, which occurred in the aortic, pulmonary, and atrio-ventricular valves, and in other regions. There was no sign of iron deposition in the cardiac parenchyma. The tolerated dose was 10 mg Fe/kg/dose.

No changes in fertility and mating performance occurred in male and female rats treated intravenously with 3, 6.5, or 15 mg Fe/kg/dose Venofer®, 3 doses/week. The dose selection appeared to be appropriate, since a slight reduction in weight gain was observed in both sexes in the high-dose group only.

A teratogenicity study of Venofer® was performed on rats using dose levels of 6.5 and 13 mg Fe/kg/day i.v. Venofer® did not produce teratogenic effects or embryo-fetal toxicity. Weight gain in females was reduced in both treated groups. The high dose was reduced from 20 to 13 mg Fe/kg/day after study initiation, due to three deaths and severe toxicity.

A teratogenicity study of Venofer® was conducted on rabbits using dose levels of 3, 6.5, or 13 mg Fe/kg/day i.v., and 13 mg Fe/kg/dose on even- or odd-numbered days. Daily dosing with 13 mg Fe/kg/day was discontinued and changed to alternate day dosing, due to severe local irritation. As a result, only 18 fetuses were examined in the 13 mg Fe/kg/day group. A low incidence of fused sternbrae in fetuses from Venofer®-treated females was observed, but was limited to the groups treated with 3 mg Fe/kg/day and 13 mg Fe/kg/dose on odd-numbered days. This

malformation was not observed in the following groups: control, 6.5 mg Fe/kg/day, 13 mg Fe/kg/day, and 13 mg Fe/kg/dose on even-numbered days. Therefore, the dose-dependency of this effect is uncertain. It is noteworthy that the incidence of fused sternebrae among fetuses in the 13 mg Fe/kg/dose (odd-numbered days) group was greater than the historical control value reported by the same laboratory for the same strain of rabbits (Palmer, In: Pathology of Laboratory Animals, Benirschke et al. (Ed.), 1978). Furthermore, this malformation was not associated with maternal toxicity. Given the equivocal nature of these results, Venofer® was not considered to be teratogenic in rabbits. However, an additional teratogenicity study is necessary to provide conclusive evidence regarding this question.

A Segment III reproductive study in rats will be submitted by the Sponsor as a Phase IV study, as agreed to by the Division of Gastrointestinal and Coagulation Drug Products in a written communication dated April 28, 1999. The protocol for this study was reviewed in IND _____

A series of genetic toxicology studies was performed with Venofer®. No evidence of mutagenicity was observed in two studies using the Ames bacterial mutation assay, or in the mouse lymphoma cell (L5178Y) forward gene mutation (TK +/-) assay. There was no evidence of clastogenicity in the mouse micronucleus test using intraperitoneal administration, or in the chromosome aberration test with human lymphocytes.

Local irritation was observed in rabbits following a single intravenous injection with Venofer®. The treatment-related reaction included edema, hemorrhage, inflammation, focal ulceration, and scab formation. A single intra-arterial injection of Venofer® in rabbits produced dermal hemorrhage and hemorrhage in the arterial wall.

The proposed human dose of Venofer® is 2 mg Fe/kg/dose i.v. administered one to three times per week, for a cumulative dose of 20 mg Fe/kg in 10 doses. Depending on the amount of dose and frequency of administration, the duration of treatment could range from 2 to 10 weeks. Since Venofer® may be administered up to three times per week, the 13-week intravenous toxicity studies on rats and dogs using 3 doses/week are the most relevant studies for evaluation of preclinical toxicity. The tolerated dose in rat was 3 mg Fe/kg/dose, with target-organ toxicity observed in liver, kidney, and spleen. The tolerated dose in dogs was 10 mg Fe/kg/dose, with target-organ toxicity observed in liver, kidney, and spleen. The preclinical toxicity studies of Venofer® submitted by the Sponsor are satisfactory.

An important factor related to Venofer®-induced toxicity is that this drug will be used in iron deficient patients, whereas the preclinical toxicity studies were performed using animals with normal iron stores. Presumably, Venofer® will be less likely to produce toxicity resulting from iron overload in the intended patient population.

The label is not according to 21-CFR, 201.50 Subpart B (April 1, 1999), and changes are needed as described in the review section.

RECOMMENDATIONS:

1. From a preclinical viewpoint, the application is recommended for approval with a provision that labeling be changed as described in the review section.
2. The Sponsor should submit a Segment III prenatal and postnatal study in rats as a Phase IV study, as previously agreed.
3. Given the equivocal results obtained in the Segment II teratogenicity study in rabbits, the Sponsor may be asked to submit another Segment II study in rabbits using appropriate dose levels as a Phase IV study.

ISI

David B. Joseph, Ph.D.
Pharmacologist

4/13/00
Date

Comment: *① Concur ② Sponsor met all the previous commitments under IND ③ A Team Leader memorandum on labeling will follow.*

Jasti B. Choudary, B.V.Sc., Ph.D. Date

ISI 4/18/00

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 12/13/99
DBJ/hw/2/3/00, 2/8/00, 3/16/00, 3/20/00, 3/29/00 & 4/12/00