CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 020955

PHARMACOLOGY REVIEW(S)

Strongjin

NDA 20,955

REVIEW # 1

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REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

DRUG: Ferrlecit/Ferric sodium gluconate, injection
62.5 mg/5 ml, ampoules

The precise structural and molecular formula are not known.

MW = 100,000 to 500,000

CATEGORY: Hematinic for iron deficiency anemia.

Related INDs: IND [redacted]

Marketing Indications and Dose: Ferrlecit is indicated for treatment of iron deficiency anemia in renal hemodialysis patients on supplemental human erythropoietin. The recommended i.v. dose of Ferrlecit is 62.5 mg over 30 minutes or 125 mg over 1 hour during or after dialysis. Ferrlecit should be given three times per week. For maintenance use, Ferrlecit should be administered at 62.5-125 mg once per week to maintain the target level.

[redacted]
APPEARS THIS WAY ON ORIGINAL

PRECLINICAL STUDIES AND TESTING LABORATORIES:

Type of Study	Study #	Lot #	Lab	Page #
Pharmacology				3-5
Absorption, Distribution, Metabolism and Excretion (ADME):				
Rats & rabbits	----	----	1	5-7
Acute Toxicity Study				8-9
I.V. mice	Journal No. T 1248	----	2	
I.V. rats	Journal No. T 1247	----	2	
I.V. rabbits			1	
I.V. dogs	Journal No. T 1249	----	2	
Subacute to Subchronic Toxicity Study				
4-week i.v. in rats	----	72/001	3	9-11
12-week i.v. in rats	----	73/020 ----	45	11-13
3-month i.v. in rabbits	----	----	1	13-14
Special Toxicity Study				
Local i.v. & i.a. tolerance test in dogs	----	22480-12	3	14-15
Reproductive Toxicity Study				
Segment II i.v. teratology study in mice	95996	50131	6	18-25
Segment II i.v. teratology study in rats	----	----	5	15-17
Segment II i.v. teratology study in rabbits	----	----	1	17
Segment III i.v. pre and postnatal reproductive toxicity study in rats	95997	50131	6	25-26
Mutagenicity				
Ames test	17298-0-409	50131	7	27
In vitro chromosome aberration test in Chinese hamster ovary cells	17298-0-437	50131	7	28-31
In vivo rat micronucleus test	17298-0-454	50131	7	31-32

[redacted]

PHARMACOLOGY:

Pharmacology studies were previously reviewed on August 9, 1995 under IND [redacted] This review is attached below.

Ferrlecit, ferric sodium gluconate, is currently used for intravenous administration in patients with iron deficiency anemia in foreign countries and has been marketed since 1951. Its therapeutic effects were demonstrated in experimental models of anemia in rats.

Primary Activity

1. Effects on Iron Deficient Anemia:

Therapeutic effects of Ferrlecit were examined in an experimental model of anemia induced by iron deficient milk in Wistar rats. In this model, the rats were on the milk at weaning. Treatment with Ferrlecit started when hemoglobin decreased by ~50% and red blood cells (RBC) were ~3.4-4.8 X 10^{12}/Liter. Intravenous administration of either Ferrlecit at 1.25 and 2.5 mg/kg/day or distill water (control) was given to these rats for 28 days. At the end of the study, the RBC count, hematocrit and hemoglobin levels were markedly higher in the treatment group than in the control in a dose dependent manner. They were 51-142% and 103-164% (RBC count), 77-145% and 197-219% (hematocrit) and 83-97% and 102-120% (hemoglobin) higher in the low and high dose treated groups, respectively, than in the control.

In another experimental model of anemia induced by iron deficient milk in Sprague Dawley rats, the rats were on the iron deficient milk (cow's milk) at weaning for total 120 days. Sixty days after on this milk, hemoglobin was markedly reduced by ~55-57% compared to the initial values and the treatment started with either Ferrlecit at 1.88 mg/kg/day (i.p.) or vehicle control. At the end of the study (day 120), hemoglobin was returned to ~77% of the initial values in the treatment group, whereas it continued to reduce to ~28% of the initial value in the control group. The RBC counts were virtually unchanged 60 days after on the iron deficient milk in both groups. However, at the end of the study, RBC counts were ~74% and 132% of the initial value in the control group and treatment groups, respectively. Iron levels in the muscle, liver and brain were ~43%, 424% and 26% higher in the treatment group than the control, respectively. These results suggest that treatment with Ferrlecit can overcome the anemia induced by iron deficient milk.

2. Effects on Hemolytic Anemia:

The therapeutic effects of Ferrlecit were also examined in a hemolytic anemia model induced by acetylphenylhydrazine (50 mg/kg, i.p.) in rats. The hemolytic anemia was indicated by reduction of RBC count. Immediately following the treatment with acetylphenylhydrazine, these rats were treated intraperitoneally with either vehicle control or Ferrlecit at 1.88 mg/kg/day for 12 days. On day 3 after the treatment, RBC counts were reduced by 42% in the control group and 40% in the treatment group compared to the values before induction of anemia. At the end of the treatment (day 12), RBC counts were ~85% and 97% of the initial values in the control and treatment groups, respectively, suggesting that Ferrlecit improves the anemia condition slightly better than the control.

Secondary Activity:

1. Neuropharmacology: The neuropharmacological effects of Ferrlecit were evaluated in mice and rats. Intravenous administration of Ferrlecit at doses up to 31.25 mg/kg did not produce any significant alterations in the light barrier test, sleep time induced by hexobarbital, electroshock, revolving wheel test and horizontal rotating rod test in mice and the motility test in rats. Intravenous administration of Ferrlecit at doses up to 31.25 mg/kg had no anticonvulsant, analgesic and anticholinergic activities.

2. Respiratory Pharmacology: Intraperitoneal administration of Ferrlecit at doses up to 12.5 mg/kg had no effects on the histamine-induced asthma in guinea pigs.

3. Cardiovascular Pharmacology: The cardiovascular effects of Ferrlecit were evaluated in both *in vitro* and *in vivo* preparations. In isolated arteries from rabbit ear, the effects of Ferrlecit on the perfusion rate, measured by a drop counter, were studied. Ferrlecit at concentrations up to 1.25×10^{-2} mg/ml did not significantly alter the perfusion rate. In the Langendoff heart preparation (guinea pig), undiluted Ferrlecit was given via a feeding tube into aorta. In this preparation, Ferrlecit significantly increased the coronary flow rate and heart rate and decreased the force of contraction in a dose dependent manner. The ED_{50} for the flow rate, heart rate and force of contraction were 3, 5.1 and 2.3 mg, respectively. Intravenous administration of Ferrlecit at doses up to 3.95 mg/kg had no significant effects on the systolic and diastolic blood pressures, central venous pressure, heart rate and peripheral flow in anesthetized cats. At 12.5 mg/kg, Ferrlecit slightly decreased the systolic (6.1%) and diastolic (23%) blood pressures and increased the peripheral blood flow (25%).

4. Renal Pharmacology: Intravenous administration of Ferrlecit at doses up to 31.25 mg/kg had no significant effects on the diuresis and urine excretion of K^+, Na^+ and Cl^- in rats.

5. Other Effects:

A. Metabolic Effects: Effects of Ferrlecit on the total serum lipids, triglycerides, cholesterol, free fatty acid and blood sugar were evaluated in rats. Ferrlecit was intravenously administered at 1.25, 12.5, 31.25 and 62.5 mg/kg. Ferrlecit significantly decreased total serum lipids (23-61%), triglycerides (23-47%), cholesterol (12-27%), free fatty acid (31-61%) and blood sugar (11-19%). These were seen at all tested doses but the dose-response effects were not obvious.

B. Effects on Bile Flow: Intravenous administration of Ferrlecit significantly increased the biliary secretion by ~19%, 20%, 11% and 8.4% at 1.25, 12.5, 31.25 and 62.5 mg/kg, respectively, in anesthetized rats.

C. Anti-inflammatory Effects: Intravenous administration of Ferrlecit at doses up to 31.25 mg/kg did not produce any significant effects on the carrageenin-induced edema in the rat paw.

In summary, the animal studies revealed that intravenous or intraperitoneal administration of Ferrlecit at doses of 1.25, 1.88 and 2.5 mg/kg/day for 28 or 60 days can significantly improve the experimental anemia induced by iron deficient milk in rats. This was indicated by increases in the RBC counts, hematocrit and hemoglobin. Intraperitoneal administration of Ferrlecit at 1.5 mg/kg/day for 12 days slightly improves the hemolytic anemia induced by acetylphenylhydrazine in rats.

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME):

ADME studies were previously reviewed on August 9, 1995 under IND [redacted]. This review is attached below.

In a healthy human subject, loss of iron from the body is matched by absorption of iron from the intestine. For example, the iron absorption is increased when the body iron stores are depleted or when erythropoiesis is accelerated and decreased in the states of iron overload or erythroid hypoplasia. Therefore, the total body iron content remains relatively stable. The total body iron is about 50-55 mg/kg in normal men and 35-50 mg/kg in normal women. The body iron is mainly associated with hemoglobin (~70-73%), myoglobin (~10-11%) and apoferritin (~16-21%). Apoferritin is an intracellular iron carrier found in many tissues and plays an

important role in the iron storage. Apoferritin can combine with iron to form ferritin and deposit in tissues and the deposits are called hemosiderin. Ferritin is also found in the plasma but most iron in the plasma is bound to transferrin, an iron-transporting polypeptide. The normal plasma iron level is ~130 μg/dl in men and 110 μg/dl in women and this accounts for ~0.2% of the total body iron. Metabolism of iron is mainly controlled by its role in hemoglobin synthesis. Iron moves from the plasma to erythroid precursor cells in the bone marrow in which hemoglobin is synthesized. The red blood cells are then released into the circulation and ingested by macrophages at the end of the 120-day lifespan. The iron is extracted from hemoglobin and returned to the plasma and utilized again. Iron deficiency anemia occurs when the body iron stores become inadequate for the needs of normal erythropoiesis and thus iron supplements are required. The intravenous administration of iron is needed when oral supplement is not sufficient.

Rats and Rabbits:

Methods: To study the serum level and the organ distribution of iron, Ferrlecit was given to rats or rabbits intravenously at 12.5 mg/kg. The serum levels of iron and the iron contents in the erythrocytes, muscle, liver, brain and kidney were determined using a Leitz colorimeter at 0, 30 and 120 minutes after dosing.

Results: In rats, the serum level of iron increased ~39% at 30 minutes after dosing and fell back to about the initial level at 120 minutes. The iron content of erythrocytes were slightly elevated (~7.4%) at 30 minutes after dosing and stayed at about that level at 120 minutes. The iron levels were markedly elevated at 30 minutes in the brain (4.6%) and at 120 minutes after dosing in the muscle (~37%) and liver (~18%). The iron level in the kidney was unchanged within 120 minutes.

In rabbits, the serum level of iron increased ~35% at 30 minutes after dosing and fell back to about the initial level at 120 minutes. The iron content of erythrocytes was slightly elevated (~5%) at 30 minutes after dosing and reduced to about the initial level at 120 minutes. The iron levels were markedly elevated at 120 minutes after dosing in the muscle (~21%), liver (~37%) and brain (11.5%). The iron level in the kidney was unchanged within 120 minutes.

The iron levels in the serum, erythrocyte, muscle, liver, brain and kidney from both species are summarized in the following table.

Iron levels in the serum, erythrocyte, muscle, liver, brain and kidney.

	Rats			Rabbits		
	0 min	30 min	120 min	0 min	30 min	120 min
Serum[1]	395	547	409	400	540	396
Erythrocyte[2]	42	45.1	44.9	42.1	44.2	42.3
Muscle[3]	6.35	6.79	8.69	8.09	8.24	9.76
Liver[3]	68.84	72.09	80.92	52.83	59.48	72.32
Brain[3]	8.62	9.02	8.47	33.80	35.15	37.68
Kidney[3]	12.34	12.47	12.41	24.39	24.59	24.53

1 = μg/100 ml, 2 = mg/100 ml blood, 3 = mg/100 g dry tissue

In summary, the pharmacokinetic studies were conducted in rats and rabbits following intravenous administration of Ferrlecit at 12.5 mg/kg. The results revealed that in both species, serum iron level was markedly elevated (35-39%) at 30 minutes after dosing and the elevated serum iron level was returned to about the initial level within 120 minutes, suggesting that the serum iron is quickly eliminated. In both species, the iron levels in the muscle (21-37%) and liver (18-37%) were markedly increased and these were seen at 120 minutes after dosing. In both species, slight increases of the iron level in the erythrocytes (~5-7%) and brain (5-12%) were also found and the iron level in the kidney was not changed within 120 minutes.

TOXICITY:

The toxicity studies including acute and subacute and subchronic toxicity studies submitted to IND [redacted] were previously reviewed on August 9, 1995 and this review is attached below.

All toxicity studies submitted in this submission were conducted prior to the promulgation of the GLP regulations in 1979. Many details required by the GLP regulations were not adhered to by the investigators. Major deviations included lack of information on the study start and complete dates, quality assurance inspections, identity of the study director and stability of the test compound. The active ingredient (ferric) of Ferrlecit is a stable compound and unlikely degraded. Therefore, the stability of the test compound is not a concern. Other minor deviations are not considered to affect the interpretation of the results of these studies. The deficiencies in terms of the dose selection and study design will be discussed in each individual study below.

ACUTE TOXICITY:

Testing Laboratory [redacted]

Study Start and Completion Dates: Report dates or months: January 1976 (mice), March 1976 or December 16, 1967 (rats), December 16, 1967 (rabbits) and August 1973 (dogs).

GLP and QAU Compliance Statement: Not applicable.

Methods: To assess the acute toxicity of Ferrlecit, CF-I mice (Journal No. T 1248), SPF-Wistar rats (Journal no. T 1247); and mixed-breed dogs (Journal No. T 1249) were employed in the acute toxicity studies. A single intravenous dose of Ferrlecit was given to mice at 99.3, 125, 157.5 and 198.8 mg/kg, to rats at 62.5, 78.8, 98.8, 125 and 157.5 mg/kg and to dogs at 125, 157.5, 198.8 and 250 mg/kg. These animals were observed for signs of toxicity for total 14 days after dosing. At termination, body weights were recorded and all animals were necropsied and the observations were recorded. The acute i.v. toxicity of Ferrlecit was also assessed in separate studies in Sprague-Dawley rats and rabbits. In these studies, Ferrlecit was given intravenously to rats at 187.5, 225, 262.5 and 300 mg/kg and to rabbits at 62.5, 87.5 and 112.5 mg/kg. The animals were observed for 30 days and the minimal lethal doses and LD_{50}s were determined.

Results: In mice, the following clinical signs were observed mainly in all treatment groups immediately following administration of Ferrlecit: decreased activity, tremor and involuntary movement, clonic and tonic convulsions, staggering, ataxies, exophthalmus and increases in the respiratory rate and body temperature. These clinical signs were more severe in the higher dose levels. Most animals appeared normally by 24 hours after dosing. Body weight gains were not markedly changed. There were no treatment related pathological changes. The minimal lethal dose was 125 mg/kg. LD_{50} was 155-158.8 mg/kg.

In Wistar rats, the following clinical signs were observed in all treatment groups immediately following administration of Ferrlecit: decreased activity, staggering, ataxia, piloerection and increase in the respiratory rate. These clinical signs were more severe in the higher dose levels. Most animals appeared normally by 24 hours after dosing. Body weight gains were not markedly changed. There were no treatment related pathological changes. The minimal lethal dose was 78.8 mg/kg. LD_{50} was 102.5 mg/kg. In Sprague-Dawley rats, the minimal lethal dose was 187.5 mg/kg. LD_{50} was identified at 274 mg/kg.

In rabbits, the minimal lethal dose was 62.5 mg/kg. LD_{50} was identified at 70.4 mg/kg.

In dogs, the following clinical signs were observed in all treatment groups at ~4 hours following administration of Ferrlecit: decreased activity, salivation, diuresis and diarrhea, staggering, ataxies and decrease in body temperature. These clinical signs were more severe at higher dose levels. Most animals appeared normally by 24 hours after dosing. The terminal body weights were reduced in the mid and high dose groups by ~2-9% compared to the initial values. In contrast, the animals in the control and low dose groups gained weights ~1-2%. There were no treatment related pathological changes. There were no deaths in the control, low and mid dose groups. The minimal lethal dose was 250 mg/kg. LD_{50} was 262.5 mg/kg.

SUBACUTE/SUBCHRONIC TOXICITY:

4-Week Toxicity of Ferrlecit in Sprague-Dawley Rats During Intravenous Administration

Testing Laboratory: [redacted]

Study Start and Completion Dates: Report date: July 1, 1974

GLP and QAU Compliance Statement: Not applicable.

Animals: Males (100-105 g, 38 days old)
Females (100-105 g, 42 days old)
Sprague-Dawley rats

Methods: To assess the repeated dose toxicity of Ferrlecit in rats, intravenous administration of Ferrlecit was given to rats at 41.25 mg/kg/day 7 days per week for 4 weeks. This was the only dose used in this study. Clinical sign of toxicity was observed daily. Body weights were determined weekly. Water and food consumptions and feces were observed daily. Hematology, clinical chemistry and urinalysis were determined before the study started and at termination. Ophthalmology examination and auditory test were conducted at termination. All animals were necropsied at termination and organ weights were determined.

Results:

1. Clinical Signs: The pain reactions (increased aggressiveness) were noted 2-5 minutes after dosing in the treated animals. At the injection sites, hemorrhages with reddish black coloration, thrombosis and necrosis were seen in the treated but not in the control animals.

2. Mortality: There were no deaths in this study.

3. Body Weight: The mean terminal body weight gains were significantly retarded by ~44-47% (males) and ~23-24% (females) compared to the control.

4. Water and Food Consumption: There were no markedly treatment related changes during the study.

5. Hematology: The numbers of leukocyte (51-67%), neutrophil granulocyte (64-136%) and segmented neutrophil granulocyte (47-91%) were markedly increased in the treated animals at termination compared with those before treatment started.

6. Clinical Chemistry: The serum iron level was significantly increased in both male (83-109%) and female (89-97%) treated animals compared to the control. The capacity of ferropexy was increased in both male (23-29%) and female (16-20%) treated animals compared to the control.

7. Urinalysis: There were no treatment related changes during the study.

8. Ophthalmology Examination: There were no treatment related changes during the study.

9. Auditory Test: There were no treatment related changes during the study.

10. Organ Weights: The relative organ weight to body weight was not markedly changed.

11. Gross Pathology: There were no treatment related changes in the gross pathological examination except that there were hemorrhages with reddish black coloration, thrombosis and necrosis at the injection sites.

12. Microscopic Pathology: Microscopic examination revealed that there were iron deposits in the heart, liver, spleen, kidney, small intestine, bone marrow and at injection sites and deposit of siderophilic pigments in the gonads and lymph nodes in the treatment group. At the injection sites, thrombosis and necrosis were also seen.

In summary, in the 4-week i.v. toxicity study in rats, Ferrlecit was given to rats intravenously at 42.25 mg/kg/day for 4 weeks. This was the only dose tested. The signs of toxicity were pain reactions, indicated by the increase in the aggressiveness, and the local reactions including hemorrhages with reddish black coloration, thrombosis and necrosis at the injection sites seen in the treated but not in the control animals. There were no deaths

in this study. The mean terminal body weight gains were significantly retarded in the treated animals (~44-47% in males and ~23-24% in females). The serum iron level was significantly increased in both male (83-109%) and female (89-97%) treated animals compared to the control. Histopathological examination revealed that there were iron deposits in multiple organs including the heart, liver, spleen, kidney, small intestine and bone marrow in the treatment group. However, there were no functional changes associated with the iron deposits in these organs, suggesting that the iron deposits did not cause any organ damage. No effect dose and the target organs of toxicity cannot be identified.

12-Week Toxicity of Ferrlecit in Wistar Rats Following Intravenous Administration

Testing Laboratory [redacted]

Study Start and Completion Dates: Report date: January 31, 1975

GLP and QAU Compliance Statement: Not applicable.

Animals: Males (137-186 g, 7.5-8 weeks old)
Females (112-154 g, 7.5-8 weeks old)
Wistar rats

Methods: To assess the repeated dose toxicity of Ferrlecit in rats, intravenous administration of Ferrlecit was given to rats at 2.5, 6.25 and 12.5 mg/kg/day five days per week for 12 weeks. Clinical sign of toxicity, mortality and feces were observed daily. Body weights and food consumptions were determined weekly. Water consumption was measured during the 6th and 12th weeks. Hematology, clinical chemistry and urinalysis were determined before, at 6 and 12 weeks after the study started. Ophthalmology examination and auditory test were conducted at termination. All animals were necropsied at termination and organ weights were determined.

Results:

1. Clinical Signs: No clinical signs of toxicity were observed.

2. Mortality: One control animal died and the cause of the death was not clear.

3. Body Weight: The mean terminal body weight gains were retarded by ~6.3-11% and ~10-19% in the mid and high dose groups, respectively, compared to the control.

4. Water and Food Consumption: There were no markedly treatment related changes during the study.

5. Hematology: There were no treatment related changes during the study.

6. Clinical Chemistry: The serum iron level was significantly increased by 47-59%, 150-173% and 140-234% in the low, mid and high dose groups, respectively, compared to the control. The iron-binding activity was not affected. Other changes were sporadic. For example, alkaline phosphatase was significantly increased ~121-155% in the high dose group. There were increases in the total lipids (25-35% and 49-52% in the mid and high dose groups, respectively) and total cholesterol (54-115% in all treated males and 52% in the high dose females). These findings were opposite of those described under Metabolic Effects of Secondary Activity in the pharmacology section in which there were decreases in the total lipids and cholesterol.

7. Urinalysis: Protein was detected in the urine collected from the mid (16.5 mg/100 ml) and high dose males (4.5 mg/100 ml) and all treated females (6-10 mg/100 ml). A small amount of protein (1.5 mg/100 ml) was also detected in the urine in the control male rats (none in the control female and low dose male rats).

8. Ophthalmology Examination: There were no treatment-related changes during the study.

9. Auditory Test: There were no treatment related changes during the study.

10. Organ Weights: The relative liver weight to body weight was significantly increased by ~44% and 63% in the mid and high dose male groups, respectively, and ~56% in the high dose female group. The relative spleen weight to body weight was also significantly increased by ~27-47% and 58-76% in the mid and high dose groups, respectively.

11. Gross Pathology: There were light to dark brown coloration observed in the following organs: pancreas, spleen, liver, adrenals, intestine and subcutaneous tissue.

12. Microscopic Pathology: Deposits of iron-containing pigments were mainly found in the liver, spleen, kidney, lymph nodes and adrenals in all treatment groups in a dose dependent manner.

In summary, in the 12-week i.v. toxicity study in rats, Ferrlecit was given to rats intravenously at 2.5, 6.25 and 12.5 mg/kg/day for 12 weeks. There were no clinical signs of toxicity observed during the study. The mean terminal body weight gains were retarded by ~6.3-11% and 10-19% in the mid and high dose

groups, respectively. The serum iron level was significantly increased by 47-59%, 150-173% and 140-234% in the low, mid and high dose groups, respectively compared to the control. Alkaline phosphatase was significantly increased ~121-155% in the high dose group. Urine protein was increased in the mid (16.5 mg/100 ml) and high dose males (4.5 mg/100 ml) and all treated females (6-10 mg/100 ml) as compared with control (1.5 mg/100 ml in males and 0 mg/100 ml in females). Histopathological examination revealed that there were deposits of iron-containing pigments in the liver, spleen, kidney, lymph nodes and adrenals in all treatment groups. The intensity of the iron deposits was dose dependent. No effect dose was not identified. Based on the findings in clinical chemistry and iron deposit in the liver, the liver was the target organ of toxicity. Ferrlecit was tolerated at doses up to 12.5 mg/kg/day.

3-Month Toxicity of Ferrlecit in Rabbit Following Intravenous Administration

Testing Laboratory: [redacted]

Study Start and Completion Dates: Report date: December 16, 1967

GLP and QAU Compliance Statement: Not applicable.

Animals: Young rabbits (0.745 kg, age not specified).

Methods: To assess the repeated dose toxicity of Ferrlecit in rabbits, intravenous administration of Ferrlecit was given to 10 rabbits at 1.88 mg/kg for 3 months. This was the only dose tested. There was a control group (10 rabbits). There were no data available for clinical signs of toxicity, mortality, food consumptions and urinalysis in this submission. Body weights were determined weekly. Hematology and clinical chemistry were determined before the study started and at termination. All animals were necropsied at termination and organ weights were determined. Microscopic examination was performed in the following organs: liver, heart, pancreas, duodenum, lungs, adrenals, spleen, kidneys, brain and stomach.

Results:

1. Clinical Signs: Clinical signs of toxicity were not described in the study.

2. Mortality: Information on mortality was not provided.

3. Body Weight: The mean terminal body weight gains were retarded by ~14% in the treated animals compared to the control.

4. Food Consumption: Information on food consumption was not given.

5. Hematology: At termination, hemoglobin was increased in both control (75%) and treatment (85%) groups compared to the initial values.

6. Clinical Chemistry: Glutamic-pyruvic transaminase (SGPT) was increased in the treatment group by ~150% compared to the control. Time for bromsulphthalein to appear in the bile (liver function test) was not markedly affected by the treatment.

7. Urinalysis: Urinalysis was not performed.

8. Organ Weights: The relative lung (50%) and heart (21%) weights to body weight were higher in the treatment group than in the control group. The relative liver weight to body weight was slightly lower (17%) in the treatment group than in the control.

9. Gross Pathology: There were no treatment related changes in this study.

10. Microscopic Pathology: There were no treatment related changes in this study. There were no iron deposits in the tissues examined.

In summary, in the 3-month i.v. toxicity study in rabbits, Ferrlecit was given to rabbits intravenously at 1.88 mg/kg/day for 3 months. Information on clinical sign of toxicity, mortality, food consumptions and urinalysis were not given in this study. The mean terminal body weight gains were retarded in the treated animals (14%). SGPT was increased in the treatment group by ~150% compared to the control. There were no treatment related histopathological changes. There were no iron deposits in the tissues examined. This was not a complete conventional toxicity study. Based on the available data, the liver was the target organ of toxicity since SGPT was increased and Ferrlecit was tolerated at the tested dose (1.88 mg/kg/day).

SPECIAL TOXICITY:

The following study was previously reviewed on August 9, 1995 under IND [redacted]. This review is attached below.

On The Local Tolerability of Ferrlecit Administered Parenterally to Dogs

Testing Laboratory: [redacted]

Study Start and Completion Dates: Report date: August, 1973

GLP and QAU Compliance Statement: Not applicable.

Animals: Males & females (14.7 - 21.8 kg, age not specified)
Mongrel dogs.

Methods: To study local tolerance of Ferrlecit in dogs, Ferrlecit was given either intravenously or intra-arterially to dogs at 62.5 mg/animal. The local reactions were observed at 1, 2, 4, 24, 48 and 96 hours after injection. The histological examinations were performed at injection sites at 24, 48 and 96 hours after injection.

Results: There were no treatment related changes at the injection sites. Slight local reactions including slight bleeding, peri-vascular edema and porous inflammatory infiltration were observed in both control and treatment groups.

REPRODUCTIVE TOXICITY:

Following reproductive studies submitted to IND [redacted] were reviewed on August 9, 1995: Segment II i.v. teratological reproductive toxicity studies in rats and rabbits. The review is attached below.

Teratological Examination of Ampules of Ferrlecit VIA Intravenous Application in Rats

Testing Laboratory: [redacted]

Study Start and Completion Dates: Report date: September 18, 1970

GLP and QAU Compliance Statement: Not applicable.

Animals: Female rats (193-209 g, age not specified)
Wistar rats.

Methods: Pregnant rats were treated with Ferrlecit by intravenous injection at 4 and 20 mg/kg/day on gestation days 6 through 15. These animals were sacrificed on gestation day 20. The body weights and water and food consumptions of the dams were recorded during the entire period of pregnancy. The following observations were made: number of fetuses and their positions in the uterus, dead and live fetuses, sites of implantation, resorption nodes, anomalies, weights at birth. Fetuses were also examined to detect defects in the viscera and anomalies of bones.

Results: The terminal body weight gains of the dams were significantly retarded by -26% in the high dose group compared to the control. The total food consumption of the dams was significantly lower (21%) in the high dose group than in the control. Water consumption was increased in the treatment groups (13-22%) compared to the control but the changes were not statistically significant. Fertility index was not affected. Gestation index was 10% lower in the high dose group than in the control. Normal live fetuses were 99.5%, 100% and 88.6% in the control, low and high dose groups, respectively. Total 19 resorptions were observed in the high dose group and there were none in the control and low dose groups. The litter size was slightly lower (7.75) in the high dose group compared to the control (9.5). The birth weight was significantly lower (6-10%) in the high dose group than in the control. There were no treatment related anomalies except that there was retardation in the ossification of the cranial bones in 32 high dose fetuses. The summary data are presented in the sponsor's table on page 174 in Volume 1.5 and this table with minor modification is attached below.

Parameter	Control	4 mg/kg	20 mg/kg
# animals mated	20	20	20
# animals pregnant	20	20	20
Fertility index	100%	100%	100%
# of litter	20	20	18
Gestation index	100%	100%	90%
Total fetuses	190	201	175
# normal live	189 (99.5%)	201 (100%)	155 (88.6%)
# normal dead	1 (0.5%)	0 (0%)	1 (0.5%)
# resorptions	0 (0%)	0 (0%)	19 (10.9%)
Litter size	9.5 ± 1.9	10.1 ± 2.5	7.8 ± 3.7
Fetal weight (g) male female	 3.43 ± 0.4 3.32 ± 0.4	 3.46 ± 0.4 3.35 ± 0.3	 3.22 ± 0.3** 2.99 ± 0.4**
Sex ratio (f/m)	0.88	1.18	1.04
Variations: Retardation of ossification of cranial bones	0	0	32

** = P <0.01

In summary, in the Segment II teratological toxicity study in rats, Ferrlecit was given to rats at 4 and 20 mg/kg/day during gestation days 6 through 15. Animals were sacrificed on gestation day 20. In the high dose dams, the terminal body weight gains was retarded by ~26% and food consumption was reduced by 21%. Ferrlecit at 20 ~~mg/kg/day slightly reduced~~ the gestation index (10%), live fetuses (11%), the birth weight (6-10%) and litter size (18%) and produced retardation in the ossification of the cranial bones. Ferrlecit was not teratogenic in this study.

A Segment II Teratological Study of Ferrlecit in Rabbits

Testing Laboratory: [redacted]

Study Start and Completion Dates: Report date: December 16, 1967

GLP and QAU Compliance Statement: Not applicable.

Animals: Female rabbits (1800±100 g, age not specified).

Methods: Pregnant rabbits were treated with Ferrlecit by intravenous injection at 1.88 mg/kg/day on gestation days 1 through 23 or until the day of parturition. These animals were either sacrificed on gestation day 25 or allowed to deliver spontaneously. The following observations were made: number and weight of dead and live fetuses, number of reabsorbed and deformed fetuses and abnormalities, number and weight of dead and live neonates, and number of deformed neonates and abnormalities.

Results: The numbers and weights of dead and live fetuses and neonates were not affected. There were no deformed fetuses and neonates. No malformations were observed in this study. Ferrlecit was not teratogenic in this study.

In summary, in the Segment II teratological toxicity study in rabbits, Ferrlecit was given to rabbits at 1.88 mg/kg/day during gestation days 1 through 23 or until delivery. Animals were sacrificed on gestation day 25 or allowed to deliver spontaneously. There were no treatment related alterations in fetal weight, morphology and viability. Ferrlecit was not teratogenic in this study.

Following reproductive studies submitted to this NDA are reviewed below: a Segment II i.v. teratological reproductive toxicity study in mice and a modified Segment III i.v. reproductive toxicity study in rats.

An Intravenous Segment II Teratology Study of Ferrlecit in Pregnant Mice
(Project #95996)

Testing Laboratory: [redacted]

Study Start and Completion Dates: December 19, 1995 and June 12, 1996

GLP and QAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Females (25-31 g, 9-10 weeks old)
Crl:CD-1(ICR)BR female mice

Methods: To study the potential teratological effects of Ferrlecit in mice, Ferrlecit was given intravenously to pregnant female mice (25/group) at 0, 5, 30 and 100 mg/kg/day during gestation days 6 through 15. The dose selection was based on the dose ranging study (Project # 95999). In this study, pregnant mice were treated with Ferrlecit at 0, 2.5, 5, 15 and 30 mg/kg during gestation days 6 to 15 and the only treatment related change was local reaction at the injection site at the doses of 15 mg/kg or higher. Since there was no evidence of maternal toxicity at doses up to 30 mg/kg/day, dose of 100 mg/kg/day was used as high dose in the main study. All animals were observed for clinical signs of toxicity and mortality. Body weights and food consumptions were also determined. Pregnant females were necropsied on day 18 of gestation. The pregnant status, number and/or status of corpora lutea, implantation and fetus were determined. Fetal weights and sex were determined. All fetuses were examined externally for abnormalities and then sacrificed. Approximately 50% of fetuses were examined for visceral alterations. The remaining fetuses were eviscerated and examined for skeletal alterations.

Results: Two control and 3 treated (high dose) animals died during the study. The major treatment related clinical signs of toxicity were local irritation at the injection site (black or blue discoloration) in the mid and high dose groups and decreased activity and red vaginal discharge in the high dose group. Body weight gain from gestation days 6 to 18 was decreased by ~21% in the high dose group as compared to the control. Food consumption was slightly lower in the high dose group (5.7 g/animal/day) as

compared to the control (6.8 g/animal/day) during gestation days 6 to 9. Gross pathological examination revealed splenic enlargement and hepatic pallor. The incidence of these changes were 2, 1, 8 and 17 (splenic enlargement) and 1, 0, 6 and 3 (hepatic pallor) in the control, low, mid and high dose groups, respectively. Numbers of corpora lutea, implantation sites, dead fetuses, sex ratio and preimplantation losses were not affected. Early resorption was significantly increased in the high dose group (1.7) as compared to the control (0.9). Three dams in the high dose group had total litter loss as compared to 1 dam in the control group. Fetus weight was significantly decreased in the high dose group (1.06 g) as compared to the control (1.41 g). This information was summarized in tables 1, 8 and 9 on pages 24, 36-39 in volume 1.9. These tables are attached below.

TABLE NO. 1 GROUP MATERNAL PERFORMANCE PROJECT NO. 95996

	GROUP 1 SALINE CONTROL	GROUP 2 FERRLECIT 5 MG/KG/DAY	GROUP 3 FERRLECIT 30 MG/KG/DAY	GROUP 4 FERRLECIT 100 MG/KG/DAY
NO. OF MICE MATED	25	25	25	25
NO. OF MICE PREGNANT	18	21	19	22
NO. OF MICE PREGNANT BY AMMONIUM SULPHIDE STAINING	0	0	0	1
NO. OF MICE DYING/SACRIFICED DUE TO POOR CONDITION	2	0	0	1
NO. OF MICE WITH TOTAL RESORPTION/ALL DEAD FETUSES	1	0	0	3
NO. OF MICE WITH LIVE LITTERS AT CESAREAN SECTION	11	18	17	12
NO. OF MICE LITTERING PRIOR TO CAESAREAN	4	3	2	5
PREGNANCY RATE (%)	72.0	84.0	76.0	88.0

TABLE NO. 8

GROUP UTERINE FINDINGS

PROJECT NO. 95996

		TOTAL NO. OF CORPORA LUTEA MEAN (S.D.)	TOTAL IMPLAN-TATION SITES MEAN (S.D.)	MALE FETUSES MEAN (S.D.)	FEMALE FETUSES MEAN (S.D.)	SEX RATIO % MEAN (S.D.)	LIVE FETUSES MEAN (S.D.)	DEAD FETUSES MEAN (S.D.)
GROUP 1 - VEHICLE CONTROL		12.7 (3.24)	12.0 (2.37)	4.3 (2.35)	5.0 (2.09)	44.9 (14.41)	9.3 (3.82)	1.2 (3.43)
GROUP 2 - FERRLECIT 5 MG/KG/DAY		12.3 (2.97)	11.0 (2.50)	5.5 (1.65)	4.8 (2.07)	54.8 (16.68)	10.3 (2.24)	0.1 (0.24)
GROUP 3 - FERRLECIT 30 MG/KG/DAY		14.8 (2.17)	12.6 (1.46)	5.7 (2.31)	5.6 (1.80)	49.2 (16.85)	11.4 (2.06)	0.2 (0.75)
GROUP 4 - FERRLECIT 100 MG/KG/DAY	A	13.8 (1.67)	12.2 (1.11)	3.5 (2.45)	4.2 (2.65)	44.5 (13.87)	7.7 (4.27)	1.0 (2.61)
	B	13.8 (1.72)	12.1 (1.13)	3.7 (2.33)	4.5 (2.47)	44.5 (13.87)	8.2 (3.88)	1.1 (2.69)

A - Including animal with total resorption
B - Excluding animal with total resorption

TABLE NO. 8

GROUP UTERINE FINDINGS

PROJECT NO. 95996

		GRAVID UTERUS WEIGHT (G) MEAN (S.D.)	LITTERS WITH 0 RESORPTIONS NO.	%	LITTERS WITH 1 RESORPTION NO.	%	LITTERS WITH ≥ 2 RESORPTIONS NO.	%	LITTERS WITH ≥ 3 RESORPTIONS NO.	%
GROUP 1 - VEHICLE CONTROL		19.22 (3.227)	3	25.0	4	33.3	5	41.7	1	8.3
GROUP 2 - FERRLECIT 5 MG/KG/DAY		18.29 (3.166)	10	55.6	5	27.8	3	16.7	1	5.6
GROUP 3 - FERRLECIT 30 MG/KG/DAY		20.19 (3.223)	7	41.2	5	29.4	5	29.4	2	11.8
GROUP 4 - FERRLECIT 100 MG/KG/DAY	A	13.03 c (3.857)	4	25.0	0	0.0	12	75.0	10 B	62.5
	B	13.55 c (3.354)	-	-	-	-	-	-	-	-

A - Including animal with total resorption
B - Excluding animal with total resorption
SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: c P<0.001 (MANN WHITNEY)
B P<0.01 (FISHER'S)

TABLE NO. 8 GROUP UTERINE FINDINGS PROJECT NO. 95996

		EARLY RESORP-TIONS MEAN (S.D.)	MIDDLE RESORP-TIONS MEAN (S.D.)	LATE RESORP-TIONS MEAN (S.D.)	TOTAL RESORP-TIONS MEAN (S.D.)	PRE-IMPLAN-TATION LOSS (%) MEAN (S.D.)	POST IMPLAN-TATION LOSS (%) MEAN (S.D.)
GROUP 1 - VEHICLE CONTROL		0.9 (1.44)	0.3 (0.45)	0.3 (0.65)	1.5 (1.62)	5.5 (5.44)	21.7 (28.26)
GROUP 2 - FERRLECIT 5 MG/KG/DAY		0.7 (0.91)	0.0 (0.00)	0.0 (0.00)	0.7 (0.91)	9.7 (8.06)	6.0 a (7.48)
GROUP 3 - FERRLECIT 30 MG/KG/DAY		0.5 (0.87)	0.4 (0.61)	0.1 (0.33)	1.0 (1.06)	13.9 (9.24)	10.2 (11.00)
GROUP 4 - FERRLECIT 100 MG/KG/DAY	A	1.7 (1.89)	0.7 (1.08)	1.1 (2.28)	3.5 (3.39)	11.3 (9.32)	36.1 (35.29)
	B	1.7 (1.94)	0.5 (0.92)	0.6 (0.91)	2.9 (2.33)	12.1 (9.09)	31.8 (31.99)

A - Including animal with total resorption
B - Excluding animal with total resorption
SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: a P<0.05 (MANN WHITNEY)

TABLE NO. 9. GROUP LITTER MEAN (S.D.) FETAL WEIGHTS (G) PROJECT NO :95996

	MALES	FEMALES	TOTAL
GROUP 1 - SALINE CONTROL	1.43 .167	1.41 .105	1.41 .118
GROUP 2 - FERRLECIT 5 MG/KG/DAY	1.46 .146	1.41 .153	1.43 .148
GROUP 3 - FERRLECIT 30 MG/KG/DAY	1.37 .118	1.33 .114	1.35 .108
GROUP 4 - FERRLECIT 100 MG/KG/DAY	1.10 ** .151	1.09 ** .139	1.06 ** .181

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: **P<0.01 (DUNNETT'S)

Fetal findings were summarized in table 10 on pages 40-46 in volume 1.9 and this table is attached below.

TABLE NO. 10 — GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS, MINOR ANOMALIES AND COMMON VARIANTS — PROJECT NO. 95996

	GROUP 1 SALINE CONTROL		GROUP 2 FERRLECIT 5 MG/KG/DAY		GROUP 3 FERRLECIT 30 MG/KG/DAY		GROUP 4 FERRLECIT 100 MG/KG/DAY	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL (EXT)	11	112	18	185	17	193	13	123
SKELETAL	11	56	18	93	17	97	13	61
TECHNIQUE OF WILSON (WT)	11	56	18	92	17	96	13	62
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
MAJOR MALFORMATIONS (TOTAL)	1	4	3	3	1	2	0	0
HEAD								
Cleft palate (WT)	0	0	0	0	1	1	0	0
ABDOMINAL CAVITY								
Gastroschisis (WT, EXT)	0	0	1	1	0	0	0	0
KIDNEYS								
Kidney(s) absent (WT)	0	0	1	1	0	0	0	0

L/E = Litters examined
F/E = Fetuses examined
L/A = Litters affected
F/A = Fetuses affected

TABLE NO. 10 — GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS, MINOR ANOMALIES AND COMMON VARIANTS — PROJECT NO. 95996

	GROUP 1 SALINE CONTROL		GROUP 2 FERRLECIT 5 MG/KG/DAY		GROUP 3 FERRLECIT 30 MG/KG/DAY		GROUP 4 FERRLECIT 100 MG/KG/DAY	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL (EXT)	11	112	18	185	17	193	13	123
SKELETAL	11	56	18	93	17	97	13	61
TECHNIQUE OF WILSON (WT)	11	56	18	92	17	96	13	62
MAJOR MALFORMATIONS (CONT'D)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
URETER(S)								
Ureter(s) absent (WT)	0	0	1	1	0	0	0	0
LIMBS								
Abnormal flexure of hindlimbs (EXT)	1	4	1	1	1	1	0	0

L/E = Litters examined
F/E = Fetuses examined
L/A = Litters affected
F/A = Fetuses affected

TABLE NO. 10 GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS, MINOR ANOMALIES AND COMMON VARIANTS PROJECT NO. 95996

	GROUP 1 SALINE CONTROL		GROUP 2 FERRLECIT 5 MG/KG/DAY		GROUP 3 FERRLECIT 30 MG/KG/DAY		GROUP 4 FERRLECIT 100 MG/KG/DAY	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL	11	112	18	185	17	193	13	123
SKELETAL	11	56	18	93	17	97	13	61
TECHNIQUE OF WILSON (WT)	11	56	18	92	17	96	13	62
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
MINOR EXTERNAL AND VISCERAL ANOMALIES (TOTAL)	1	1	0	0	2	3	1	4
KIDNEYS								
Hemorrhage; renal pelvis (WT)	1	1	0	0	0	0	0	0
Reduction of the renal papilla(e) (WT)	0	0	0	0	1	1	0	0
URETERS								
Ureter(s) dilated (WT)	0	0	0	0	2	3	1	4
ABDOMINAL CAVITY								
Bladder distended (WT)	0	0	0	0	1	1	0	0

L/E = Litters examined
F/E = Fetuses examined
L/A = Litters affected
F/A = Fetuses affected

TABLE NO. 10 GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS, MINOR ANOMALIES AND COMMON VARIANTS PROJECT NO. 95996

	GROUP 1 SALINE CONTROL		GROUP 2 FERRLECIT 5 MG/KG/DAY		GROUP 3 FERRLECIT 30 MG/KG/DAY		GROUP 4 FERRLECIT 100 MG/KG/DAY	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL	11	112	18	185	17	193	13	123
SKELETAL	11	56	18	93	17	97	13	61
TECHNIQUE OF WILSON	11	56	18	92	17	96	13	62
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
MINOR SKELETAL ANOMALIES (TOTAL)	5	7	12	15	13	31 B	12	40 C
SKULL								
Hyoid bone: Absent	0	0	0	0	0	0	1	1
Hyoid bone: Reduced ossification	1	2	1	1	1	2	2	3
Supraoccipital bone: Irregular ossification	1	1	0	0	1	1	3	7
Supraoccipital bone: Bipartite	0	0	0	0	1	1	2	2

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: B P<0.01 C P< 0.001 (FISHER'S)

L/E = Litters examined
F/E = Fetuses examined
L/A = Litters affected
F/A = Fetuses affected

TABLE NO. 10

GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS
MAJOR MALFORMATIONS, MINOR ANOMALIES AND COMMON VARIANTS

PROJECT NO. 95996

	GROUP 1 SALINE CONTROL		GROUP 2 FERRLECIT 5 MG/KG/DAY		GROUP 3 FERRLECIT 30 MG/KG/DAY		GROUP 4 FERRLECIT 100 MG/KG/DAY	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL	11	112	18	185	17	193	13	123
SKELETAL	11	56	18	93	17	97	13	61
TECHNIQUE OF WILSON	11	56	18	92	17	96	13	62
MINOR SKELETAL ANOMALIES (CONT'D)	**L/A**	**F/A**	**L/A**	**F/A**	**L/A**	**F/A**	**L/A**	**F/A**
STERNEBRAE								
Extra sternebra	1	1	0	0	0	0	0	0
RIBS								
Bilateral ribs on 7th cervical vertebra: extra	0	0	0	0	0	0	1	1
Cervical rib(s): Extra	0	0	0	0	0	0	1	1
LIMBS								
Reduced No. of phalanges in forepaw(s)	2	2	3	3	6	9	6	16 C
Reduced No. of phalanges in hindpaw(s)	0	0	2	2	1	1	6 A	19 C

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: A P<0.05 C P< 0.001 (FISHER'S)

L/E = Litter examined
F/E = Fetuses examined

L/A = Litters affected
F/A = Fetuses affected

TABLE NO. 10

GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS
MAJOR MALFORMATIONS, MINOR ANOMALIES AND COMMON VARIANTS

PROJECT NO. 95996

	GROUP 1 SALINE CONTROL		GROUP 2 FERRLECIT 5 MG/KG/DAY		GROUP 3 FERRLECIT 30 MG/KG/DAY		GROUP 4 FERRLECIT 100 MG/KG/DAY	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
EXTERNAL	11	112	18	185	17	193	13	123
SKELETAL	11	56	18	93	17	97	13	61
TECHNIQUE OF WILSON	11	56	18	92	17	96	13	62
MINOR SKELETAL ANOMALIES (CONT'D)	**L/A**	**F/A**	**L/A**	**F/A**	**L/A**	**F/A**	**L/A**	**F/A**
VERTEBRAL COLUMN								
Ossification center(s) on 1st lumbar vertebra	2	2	3	3	3	5	2	2
Thoracic vertebral centrum: Absent	0	0	0	0	0	0	1	3
Thoracic vertebral centrum: Reduced ossification	0	0	0	0	0	0	1	1
Caudal vertebra(e): Reduced No.	1	1	8	10	10 A	19 C	12 C	36 C

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: A P<0.05 C P< 0.001 (FISHER'S)

L/E = Litter examined
F/E = Fetuses examined

L/A = Litters affected
F/A = Fetuses affected

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TABLE NO. 10 : GROUP INCIDENCE OF FETAL EXTERNAL, VISCERAL AND SKELETAL FINDINGS MAJOR MALFORMATIONS, MINOR ANOMALIES AND COMMON VARIANTS — PROJECT NO. 95996

	GROUP 1 SALINE CONTROL	GROUP 2 FERRLECIT 5 MG/KG/DAY	GROUP 3 FERRLECIT 30 MG/KG/DAY	GROUP 4 FERRLECIT 100 MG/KG/DAY
	AFFECTED FETUSES/LITTER MEAN % (S.D.)	AFFECTED FETUSES/LITTER MEAN % (S.D.)	AFFECTED FETUSES/LITTER MEAN % (S.D.)	AFFECTED FETUSES/LITTER MEAN % (S.D.)
COMMON SKELETAL VARIANTS				
Ribs - total 14th (unilateral and bilateral)	26.7 (23.41)	14.9 (24.86)	24.7 (30.50)	18.6 (28.69)
Sternebrae (reduced/irregular/absent/ semi-bipartite/bipartite)	11.4 (17.59)	10.3 (15.45)	23.3 (18.82)	49.6 b (30.88)

SIGNIFICANTLY DIFFERENT FROM CONTROL (GROUP 1) VALUE: b P<0.01 (MANN-WHITNEY)

In the above tables, there was increased incidence of the reduced numbers of ossified caudal vertebra and phalanges and sternebra variants in the mid and high dose groups. These are considered as delayed ossification not major malformations.

In conclusion, Ferrlecit produced some maternal toxicities in the high dose group. Ferrlecit significantly reduced fetal weight and increased early resorption and the incidence of dam with total litter loss in the high dose group. Ferrlecit was not teratologic at the doses up to 100 mg/kg/day in this test system.

A Modified I.V. Segment III Pre and Postnatal Reproductive Toxicity Study of Ferrlecit in Rats
(Project #95997)

Testing Laboratory: [redacted]

Study Start and Completion Dates: December 15, 1995 and November 21, 1996

GLP and QAU Compliance Statement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Females (212-284 g, ~12 weeks old)
Crl:CD(SD)BR Sprague-Dawley rats

Methods: To study the potential effects of Ferrlecit on reproductive performance in rats, Ferrlecit was given by intravenous injection to pregnant rats (24/group) at 0, 1, 5 and 10 mg/kg/day during gestation day 6 through day 21 post partum. The dose selection was based on the findings from a Segment II i.v. dose ranging study in rats (Project #95998). In this study, pregnant rats were treated with Ferrlecit at 0, 2.5, 5, 15 and 30 mg/kg during gestation days 6 to 17 and severe maternal toxicities were seen at doses of 15 and 30 mg/kg/day. These maternal toxicities included mortality, decreased body weight and food consumption, enlarged lymph nodes and spleen, swollen pancreas and pale areas on the liver. All animals were observed for clinical signs of toxicity and mortality. Body weight and food consumption were recorded. Pregnant females were allowed to deliver spontaneously and to rear their progeny (F1). The offsprings (F1) were examined for external abnormalities, physical development (pinna unfolding, tooth eruption, eye opening, vaginal opening), physiological functions (righting reflex, visual functions, geotaxis test and auricular startle response) and behavior (motor activity, auditory startle habituation and 'E' water Maze). F1 animals were mated and the pregnant females were allowed to deliver spontaneously and to rear their progeny (F2). The offsprings (F2) were examined for general condition and body weight. Any pups (F2) found dead were examined internally and externally.

Results: There were no deaths in this study. Local reaction (blue discoloration) was observed more frequently in the mid and high dose groups. F0 females had body weight loss during gestation days 6-9 in the mid (2.3 g) and high dose groups (3.8 g). Food consumption (F0) was lower during gestation days 6-9 or 15-18 in the high dose group (67 or 88.8 g/animal) as compared to control (77.1 or 101.3 g/animals). Body weight of F1 pups were lower in the mid (8-10%) and high (11-17%) dose groups as compared to the control during days 4-21 post partum. The body weight of F1 animals remained lower in the high dose females from weaning until week 7. Delayed vaginal opening and decreased motor activity (females) were also seen in the high dose group. There were no other adverse effects observed during the study. The reproductive performance of F1 generation was not affected.

In conclusion, Ferrlecit produced body weight retardation on the F0 females and F1 males and females in the mid and high dose groups. Delayed vaginal opening and decreased motor activity was also seen in high dosed females. No other adverse effects on reproductive performance were observed in this study.

MUTAGENICITY:

Mutagenicity Test With Ferrlecit in the Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay
(17298-0-409)

Testing Laboratory: [redacted]

Dates Started and Completed: November 20, 1995 and February 27, 1996

Compliance with GLP and QAU Requirement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Methods: To examine the potential mutagenic effects of Ferrlecit, the reverse mutation assay (Ames test) was conducted using the direct plate incorporation method in four strains salmonella typhimurium (TA98, TA100, TA1535 and TA1537) and one strain of E. Coli (WP2uvrA) in the presence and absence of metabolic activation, S-9 mix from rat liver. The following concentrations of Ferrlecit were tested: 625, 1250, 2500, 3750 and 5000 µg/plate with and without S-9. The highest concentration of Ferrlecit tested in this study was 5 mg/plate and there were no toxicities seen at this concentration. Positive controls (2-nitrofluorene, sodium azide, ICR-191, 4-nitroquinoline-N-oxide, 2-aminoanthracene) were also tested. The result was considered positive if the test substance induced at least two-fold (TA98, TA100 and WP2uvrA) or three fold (TA1535 and TA1537) increases in revertant colonies compared to the control in a concentration dependent manner.

Results: The results indicated that Ferrlecit did not significantly increase the colonies. The positive controls, however, significantly induced increase in the colonies compared to the solvent controls.

In conclusion, the results suggest that Ferrlecit was not mutagenic in this test system.

[redacted]
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Ferrlecit: In Vitro Chromosome Aberration Test in Chinese Hamster Ovary Cells
(17298-0-437)

Testing Laboratory: [redacted]

Dates Started and Completed: November 20,1995 and March 22, 1996

Compliance with GLP and QAU Requirement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Methods: To examine the potential induction of chromosomal aberrations by Ferrlecit, the *in vitro* chromosomal aberration test was conducted in Chinese hamster ovary (CHO) cells in the presence and absence of metabolic activation, S-9 mix from rat liver. The following concentrations of Ferrlecit were tested: 313, 625, 938 and 1250 µg/ml with and without S-9. Cells were exposed to Ferrlecit for 2 hours (with S-9) or 17.8 hours (without S-9). The test drug was then removed and the cells were incubated for ~2 hours (without S-9) or 8 or 18 hours (with S-9). The cells were arrested in metaphase using 0.1 µg/ml colcemid for 2 hours before harvest. The chromosomal aberrations were observed and compared between the control and test groups. Positive controls (cyclophosphamide, 10 or 25 µg/ml and mitomycin C, 0.08 µg/ml) were also tested.

Results: Ferrlecit significantly increased the frequency of the chromosomal aberration in CHO cells with metabolic activation. The chromosomal aberrations mainly included chromatid break, chromatid and chromosome gaps, interstitial deletion, tri and quadri radials and chromosome intrachange. The increased frequency of chromosomal aberration was seen at the high concentration (1250 µg/ml) in the treatment period of 10 hours. This was found at all concentrations tested when the treatment period was extended up to 20 hours. This was not seen in the absence of metabolic activation. The results were summarized in Tables 3 and 4 on pages 80C and 80D in volume 1.15. These tables are attached below. The positive controls, mitomycin and cyclophosphamide, also significantly increased the frequency of the chromosomal aberration in CHO cells.

TABLE 3
CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS
Cells Fixed 10.0 Hours After Treatment

Assay No.: 17298 Trial #: 1 Lab #: CY12155 Metabolic Activation: (+S9)

Compound: Ferriecit (ferric sodium gluconate injection) Date:12/14/95

					NUMBER AND TYPE OF ABERRATION																	
					NOT COMPUTED			SIMPLE		COMPLEX								OTHER	# OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH>1 ABERRATIONS
				CELLS SCORED	TG	SG	UC	TB	SB	ID	TR	QR	CR	D	R	CI	DF	GT			
CONTROLS																						
NEGATIVE:	McCoy's 5a		A	100	4				1			1		1					0.03	3.0	0.0
			B	100	2	1								1			1		0.02	2.0	0.0
			A+B	200	6	1			1			1		2			1		0.03	(2.5)	0.0
POSITIVE:	CP	25.0µg/ml		25	7	1		1	2	2	3	1				4		1	>0.92	44.0*	16.0*
TEST ARTICLE		313µg Fe/ml	A	100	6						1	1									
			B	100	7	1		2				1							0.02	2.0	0.0
			A+B	200	13	1		2			1	2							0.03	3.0	0.0
		625µg Fe/ml	A	100	8	2		2			1	1							0.03	2.5	0.0
			B	100	6			3	1		1	1	3	1					0.04	4.0	0.0
			A+B	200	14	2		5	1		2	2	3	1					0.10	7.0	1.0
		938µg Fe/ml	A	100	11	1		3		1	2	1		1					0.07	5.5	0.5
			B	100	10	1		1						1					0.08	8.0	0.0
			A+B	200	21	2		4		1	2	1		2					0.02	2.0	0.0
		1250µg Fe/ml	A	100	12	1		7	1	1									0.05	5.0	0.0
			B	100	6	2		6			1	3						1	0.19	9.0	2.0
			A+B	200	18	3		13	1	1	1	3						1	0.10	10.0	0.0

* Significantly greater than the negative controls, $p<0.01$

TABLE 4
CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS
Cells Fixed 20.0 Hours After Treatment

Assay No.: 17298 Trial #: 2 Lab #: CY12165 Metabolic Activation: +S9

Compound: Ferlecit (ferric sodium gluconate injection) Date: 12/14/95

					NUMBER AND TYPE OF ABERRATION																	
					NOT COMPUTED			SIMPLE		COMPLEX								OTHER	# OF ABERRATIONS PER CELL	% CELLS WITH ABERRATIONS	% CELLS WITH>1 ABERRATIONS
				CELLS SCORED	TG	SG	UC	TB	SB	ID	TR	QR	CR	D	R	CI	DF	GT			
CONTROLS																						
NEGATIVE:	McCoy's 5a		A	100	2														0.00	0.0	0.0
			B	100	8			1	1	1				1	1				0.05	4.0	1.0
			A+B	200	10			1	1	1				1	1				0.03	2.0	0.5
POSITIVE:	CP	10µg/ml	A	25	8	1		6	1	3	6	6				4			1.04	68.0*	32.0*
TEST ARTICLE		313µg Fe/ml	A	100	16			10	1	4	12	15	6	1		11			0.60	29.0	16.0
			B	100	18	3		7	1	5	8	18	8			6			0.53	27.0	15.0
			A+B	200	34	3		17	2	9	20	33	14	1		17			0.57	28.0*	15.5*
		625µg Fe/ml	A	100	8	2		2		2	1	3	1	2	1				0.12	10.0	2.0
			B	100	6	4		1	1			3	1			1			0.07	5.0	1.0
			A+B	200	14	6		3	1	2	1	6	2	2	1	1			0.10	7.5**	1.5
		938µg Fe/ml	A	100	6			2			4	1							0.07	7.0	0.0
			B	50	9	7		11	1	8	11	15	9	1		8			1.28	62.0	34.0
			A+B	150	15	7		13	1	8	15	16	9	1		8			0.47	25.3*	11.3*
		1250µg Fe/ml	A	100	10	4		16		7	14	32	10		2	6		1	>0.97	40.0	27.0
			B	100	11			3		1	1	2							0.07	5.0	2.0
			A+B	200	21	4		19		8	15	34	10		2	6		1	>0.52	22.5*	14.5*

* Significantly greater than the negative controls, $p<0.01$
** Significantly greater than the negative controls, $p<0.05$

TG = chromatid gap
SG = chromosome gap
UC = uncoiled chromosome
TB = chromatid break
SB = chromosome break
ID = interstitial deletion
TR = triradial
QR = quadri radial
D = dicentric
R = ring
CI = chromosome intrachange
DF = dicentric with fragment
GT = a cell containing more than 10 aberrations

The increased frequency of chromosomal aberration was interpreted by sponsor as a result of production of active oxygen species in the presence of S-9 and ferric ion. The aroclor-1254-induced rat-liver S-9, which was used in this test, can cause chromosomal aberrations in Chinese hamster ovary (CHO) cells (not in human lymphocytes) via production of active oxygen species (Mutation Research, 1989, 214:115-122). Potentiation of the lipid peroxidation or production of active oxygen species by iron is well

documented (Archives of Biochemistry and Biophysics, 1986, 246(2):501-514, J. Biological Chemistry, 1984, 259(23):14354-14356 and W.A. Pryor, Free Radicals in Biology, Vol. V, Academic Press, New York, pp. 1-28, 1982). This would further increase the frequency of chromosomal aberration induced by aroclor-1254-induced rat-liver S-9 in CHO cells. Lipid peroxidation and generation of free radicals are known to be associated with DNA damage (Mutation Research, 1988, 195:137-149 and Archives of Biochemistry and Biophysics, 1986, 246(2):501-514).

In conclusion, Ferrlecit significantly increased the frequency of chromosomal aberrations in the presence of metabolic activation in this test system. The increased frequency of chromosomal aberration was likely due to production of active oxygen species in the presence of S-9 and ferric ion.

Ferrlecit: In Vivo Rat Micronucleus Test
(17298-0-454)

Testing Laboratory: [redacted]

Dates Started and Completed: November 20, 1995 and February 22, 1996

Compliance with GLP and QAU Requirement: Sponsor included a statement of compliance with GLP regulation and a quality assurance statement.

Animals: Male (251-299 g, ~8 weeks old)
Females (181-217 g, ~8 weeks old)
Sprague-Dawley (SD) rat

Methods: To examine the potential mutagenic effect of Ferrlecit, rat bone marrow micronucleus test was conducted. Ferrlecit was given to rats by intravenous injection at 26, 52.1 and 104 mg/kg. Positive (cyclophosphamide, 60 mg/kg) and negative (saline) controls were also tested. Rats were sacrificed 24, 48 and 72 hours after dosing and bone marrow was collected. The frequency of micronucleated polychromatic erythrocytes (PCEs) was then determined. The result is considered positive if a statistically significant dose-related increase in micronucleated PCEs or detection of a reproducible and statistically significant positive response for at least one dose level.

Results: Ferrlecit produced some clinical signs of toxicity such as hypoactive and pale (mid and high doses) and nasal discharge or urine stains (high dose). There were four males found dead (2 in mid dose and 2 in high dose groups), suggesting that males were more sensitive to the test article than females. There were no

statistically significant dose-dependent increases in the micronucleated PCEs. Slight but significant increases in the frequency of micronucleated PCEs were found at 72 hour sampling time in the high dose males (not females). This did not occur at 24 and 48 hours. When combined both males and females, the result was not significant. The results were summarized in Table 1 on page 98 in volume 1.15. This table is attached below.

TABLE 1

MICRONUCLEUS DATA SUMMARY TABLE

SPONSOR: R&D Laboratories, Inc.

TEST ARTICLE: Ferrlecit (ferric sodium gluconate injection)

ASSAY: 17298-0-454

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCEs MEAN OF 1000 PER ANIMAL ± S.E. MALES	FEMALES	TOTAL	RATIO PCE:NCE MEAN ± S.E. MALES	FEMALES
CONTROLS							
VEHICLE	Saline 8.33 ml/kg	24 hr	0.14 ± 0.05	0.22 ± 0.05	0.18 ± 0.04	0.99 ± 0.02	0.80 ± 0.06
POSITIVE	CP 60.0 mg/kg	24 hr	3.44 ± 0.66*	2.02 ± 0.36*	2.73 ± 0.42*	0.56 ± 0.11**	0.38 ± 0.08**
TEST ARTICLE	26.0 mg Fe/kg	24 hr	0.18 ± 0.06	0.22 ± 0.08	0.20 ± 0.05	0.86 ± 0.11	0.89 ± 0.10
		48 hr	0.08 ± 0.04	0.30 ± 0.14	0.19 ± 0.08	0.62 ± 0.10**	0.86 ± 0.06
		72 hr	0.30 ± 0.07	0.40 ± 0.19	0.35 ± 0.10	1.07 ± 0.28	1.04 ± 0.31
	52.1 mg Fe/kg	24 hr	0.20 ± 0.07	0.48 ± 0.27	0.34 ± 0.14	0.83 ± 0.07	0.74 ± 0.13
		48 hr	0.32 ± 0.10	0.14 ± 0.09	0.23 ± 0.07	0.60 ± 0.14**	0.91 ± 0.18
		72 hr	0.38 ± 0.09	0.46 ± 0.29	0.42 ± 0.16	0.79 ± 0.11	0.95 ± 0.13
	104 mg Fe/kg	24 hr	0.44 ± 0.30	0.56 ± 0.26	0.50 ± 0.19	0.86 ± 0.12	0.60 ± 0.11
		48 hr	0.24 ± 0.10	0.44 ± 0.10	0.34 ± 0.07	0.52 ± 0.14**	0.87 ± 0.03
		72 hr	0.58 ± 0.14*	0.36 ± 0.07	0.47 ± 0.08	0.68 ± 0.09	0.64 ± 0.09

* Significantly greater than the corresponding vehicle control, $p<0.05$.
** Significantly lower than the corresponding vehicle control, $p<0.05$.

In conclusion, there were no statistically significant dose-dependent increases in the micronucleated PCEs. The significant increase in the frequency of micronucleated PCEs occurred only at 72 hour sampling time in the high dose males (not females). Therefore, Ferrlecit was not mutagenic in this test system.

LABELING:

The labeling is according to 21 CFR, Subpart B. The following revisions in the labeling are recommended.

1. Sponsor's Version:

[redacted]

Evaluation: The statement is not adequate and the results of mutagenic studies were not included.

[redacted]

2. Sponsor's Version:

[redacted]

Evaluation: The results of the reproductive studies were not included.

Suggested Version:

[redacted]

3. Sponsor's Version:

[redacted]

[redacted]

Evaluation: This information should be conveyed in the section of Carcinogenesis, Mutagenesis, Impairment of Fertility.

Suggested Version: The entire section should be removed.

4. Sponsor's Version:

Overdose:

Second Paragraph:

[redacted]

Evaluation: The statement was not adequate and the results of the acute toxicity studies were not included.

[redacted]

SUMMARY AND EVALUATION:

Ferrlecit, ferric sodium gluconate, has been marketed in foreign countries since 1951. It is currently used for intravenous administration in patients with iron deficiency anemia. Animal studies revealed that intravenous administration of Ferrlecit at doses of 1.25, 1.88 and 2.5 mg/kg/day can significantly improve the anemic conditions in experimental anemia induced by iron deficient milk in rats. This was indicated by increases in the RBC counts, hematocrit and hemoglobin.

In the present NDA, sponsor is asking for approval to market Ferrlecit for treatment of iron deficiency anemia in renal hemodialysis patients on supplemental human erythropoietin. The recommended i.v. dose of Ferrlecit is 62.5 mg of Ferrlecit over 30 minutes or 125 mg over 1 hour during or after dialysis. Ferrlecit should be given three times per week. For maintenance use, Ferrlecit should be administered at 62.5-125 mg once per week to

maintain the target level. In support of this NDA, following preclinical studies were submitted: pharmacological studies, pharmacokinetic studies in rats and rabbits, i.v. toxicity studies: acute toxicity studies in mice, rats, rabbits and dogs, 4-week and 12-week toxicity studies in rats and a 3-month toxicity study in rabbits, a special i.v./i.a. local tolerance study in dogs, i.v. reproductive toxicity studies: Segment II teratological studies in mice, rats and rabbits and a modified Segment III study in rats, mutagenicity studies: Ames test, in vitro chromosome aberration test in Chinese hamster ovary cells and in vivo micronucleus test in rats.

The pharmacokinetic studies were conducted in rats and rabbits following intravenous administration of Ferrlecit at 12.5 mg/kg. The results revealed that in both species, serum iron level was markedly elevated (35-39%) at 30 minutes after dosing and returned to about the initial level within 120 minutes, suggesting that the serum iron is quickly eliminated. In both species, the iron levels in the muscle (21-37%) and liver (18-37%) were markedly increased and these were seen at 120 minutes after dosing. In both species, slight increases of the iron level in the erythrocytes (~5-7%) and brain (5-12%) were also found and the iron level in the kidney was not changed within 120 minutes.

In the acute toxicity studies, decreased activity, staggering and ataxia were observed in all species tested. The following major signs of toxicity were also seen: tremor, involuntary movement, clonic and tonic convulsions, exophthalmus and increases in the respiratory rate and body temperature in mice, piloerection and increase in the respiratory rate in rats, decrease in the body temperature, salivation, diuresis and diarrhea in dogs. These clinical signs were observed immediately after dosing in mice and rats or 4 hours after dosing in dogs. Most animals appeared normally by 24 hours after dosing. The body weights at termination (2 weeks after dosing) in dogs were reduced by ~2-9% compared to the initial values in the 198.8 and 250 mg/kg dose groups. The minimal lethal doses were 125 mg/kg in mice, 78.8 mg/kg in rats, 62.5 mg/kg in rabbits and 250 mg/kg in dogs.

In the 4-week i.v. toxicity study in rats, Ferrlecit was given to rats intravenously at 41.25 mg/kg/day (7 days per week) for 4 weeks. This was the only dose tested. The signs of toxicity were pain reactions (indicated by the increase in the aggressiveness) and hemorrhages with reddish black coloration, thrombosis and necrosis at the injection sites. There were no deaths in this study. The mean terminal body weight gains were significantly retarded in the treated animals (-44-47% in males and -23-24% in females). The serum iron level was significantly increased in both male (83-109%) and female (89-97%) treated animals compared to the

control. Histopathological examination revealed that there were iron deposits in the heart, liver, spleen, kidney, small intestine and bone marrow. The iron deposits did not cause any organ damage. No effect dose and the target organs of toxicity cannot be identified.

In the 12-week i.v. toxicity study in rats, Ferrlecit was given to rats intravenously at 2.5, 6.25 and 12.5 mg/kg/day (5 days/week) for 12 weeks. There were no clinical signs of toxicity and treatment related deaths in this study. The mean terminal body weight gains were retarded by ~6.3-11% and 10-19% in the mid and high dose groups, respectively. The serum iron level was significantly increased by 47-59%, 150-173% and 140-234% in the low, mid and high dose groups, respectively compared to the control. Alkaline phosphatase was significantly increased ~121-155% in the high dose group. Urine protein was increased in the mid (16.5 mg/100 ml) and high dose males (4.5 mg/100 ml) and all treated females (6-10 mg/100 ml) as compared with control (1.5 mg/100 ml in males and 0 mg/100 ml in females). Histopathological examination revealed that there were deposits of iron-containing pigments in the liver, spleen, kidney, lymph nodes and adrenals in all treatment groups. The intensity of the deposits was dose dependent. No effect dose was not identified. Based on the findings in clinical chemistry and iron deposit in the liver and kidney, the liver and kidney were the target organs of toxicity. Ferrlecit was tolerated at doses up to 12.5 mg/kg/day.

In the 3-month i.v. toxicity study in rabbits, Ferrlecit was given to rabbits intravenously at 1.88 mg/kg/day for 3 months. This subclinical dose was the only dose tested in this study. Information on clinical signs of toxicity, mortality, food consumptions and urinalysis were not given in this study. The mean terminal body weight gains were retarded in the treated animals (14%). SGPT was increased in the treatment group by ~150% compared to the control. There were no treatment related histopathological changes. There were no iron deposits in the tissues examined. This was not a complete conventicnal toxicity study. Based on the available data, the liver was the target organ of toxicity since SGPT was increased and Ferrlecit was tolerated at the tested dose (1.88 mg/kg/day).

In Segment II teratological toxicity study in mice, Ferrlecit was given intravenously to pregnant female mice at 0, 5, 30 and 100 mg/kg/day during gestation days 6 through 15. Ferrlecit produced some maternal toxicities in the high dose group. Ferrlecit significantly reduced fetal weight and increased early resorption and the incidence of dam with total litter loss in the high dose group. Ferrlecit also increased the incidence of delayed ossifications. Ferrlecit was not teratologic at the doses up to 100 mg/kg/day in this test system.

In the Segment II teratological toxicity study in rats, Ferrlecit was given to rats at 4 and 20 mg/kg/day during gestation days 6 through 15. Animals were sacrificed on gestation day 20. In the high dose dams, the terminal body weight gain was retarded by -26% and food consumption was reduced by 21%. Ferrlecit at 20 mg/kg/day reduced the gestation index (10%), live fetuses (11%), the birth weight (6-10%) and litter size (18%) and produced retardation in the ossification of the cranial bones. Ferrlecit was not teratogenic in this study.

In the Segment II teratological toxicity study in rabbits, Ferrlecit was given to rabbits at 1.88 mg/kg/day during gestation days 1 through 23 or until delivery. This subclinical dose was the only dose tested in this study. Animals were sacrificed on gestation day 25 or allowed to deliver spontaneously. There were no treatment related alterations in fetal weight, morphology and viability. Ferrlecit was not teratogenic in this study.

In the Segment III pre and postnatal reproductive toxicity study in rats, Ferrlecit was given by intravenous injection to pregnant rats at 0, 1, 5 and 10 mg/kg/day during gestation day 6 through day 21 post partum. Ferrlecit produced body weight retardation on the F0 females and F1 males and females in the mid and high dose groups. Delayed vaginal opening and decreased motor activity was seen in the high dose females. No other adverse effects on reproductive performance were observed in this study.

Ferrlecit was negative in the Ames test and rat micronucleus test. Ferrlecit significantly increased the frequency of chromosomal aberrations in the presence of metabolic activation in *in vitro* chromosome aberration test in Chinese hamster ovary cells. This was likely due to production of active oxygen species in the presence of aroclor-1254-induced rat-liver S-9 and ferric ion.

Iron deposits were observed in the heart, liver, spleen, kidney, small intestine, lymph nodes, adrenals and bone marrow in the 4-week and 13-week i.v. toxicity studies in rats. There were no severe organ toxicities associated with the iron deposits. In the 3-month i.v. toxicity study in rabbits, only one subclinical dose (1.88 mg/kg/day) was tested. There is no valid non-rodent toxicity study. Ferrlecit has not been tested for effects on fertility. In the pre-IND (September 21, 1995) and pre-NDA (September 16, 1997) meetings, sponsor and the Division agreed that sponsor will conduct the following phase IV studies: 13-week i.v. toxicity study in dogs and Segment I fertility and reproductive performance study in rats.

RECOMMENDATION:

From a preclinical standpoint, this NDA is approvable. Sponsor should be asked to revise the labeling as recommended.

/S/ [redacted]

Ke Zhang, Ph.D. 4/7/98

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang
HFD-345/Dr. Viswanathan

/S/ [redacted]

4/8/98

R/D Init.: J. Choudary 3/5/98

KZ/hw/3/23/98, 3/27/98 & 4/7/98
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