

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
22-180

PHARMACOLOGY REVIEW(S)



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-180
SERIAL NUMBER:	001
DATE RECEIVED BY CENTER:	10/30/2008
PRODUCT:	Ferumoxytol
INTENDED CLINICAL POPULATION:	Patients with Chronic Kidney Disease
SPONSOR:	AMAG Pharmaceuticals, Inc.
DOCUMENTS REVIEWED:	Electronic submission in EDR
REVIEW DIVISION:	Medical Imaging and Hematology Drug Products (HFD-160)
PHARM/TOX REVIEWER:	David E. Bailey, Ph.D.
PHARM/TOX SUPERVISOR:	Adebayo A. Laniyonu, Ph.D.
DIVISION DIRECTOR:	Rafel Dwaine Rieves, M.D.
PROJECT MANAGER:	Hyon-Zu Lee, Pharm.D.

Date of review submission to Division File System (DFS): December 16, 2008.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number:	22-180
Review number:	02/08
Sequence number:	N 001 30-October-2008
Information to sponsor:	No
Sponsor and/or agent:	AMAG Inc., Cambridge, MA 02138
Manufacturer for drug substance:	Not indicated
Reviewer name:	David E. Bailey, Ph.D.
Division name:	Medical Imaging and Hematology Products
HFD #:	160
Review completion date:	December 12, 2008
Drug:	
Trade name:	Ferumoxytol
Generic name:	Code 7228
Chemical name:	
CAS registry number:	
Molecular formula/molecular weight:	
Relevant INDs/NDAs/DMFs:	IND #62,745
Drug class:	Anti anemia
Route of administration:	Intravenous
Intended clinical population:	Patients with anemia as a result of Chronic Kidney Disease
Clinical formulation:	Mannitol 44 mg/mL in water for injection with 30 mg Fe/mL as Ferumoxytol®.
Clinical Dose:	510 mg Fe as a single IV 1- hour infusion followed by a second 510 mg Fe dose no sooner than 7 days later.

b(4)

Background:

The sponsor submitted the original NDA 22-180 for oral Ferumoxytol (Code 7228) on December 19, 2007 and the division action letter was sent to the sponsor on October 17, 2008. The sponsor submitted their complete response on October 30, 2008.

In the nonclinical primary review for the original NDA, there were no outstanding issues expressed. A battery of nonclinical studies had been conducted to assess the risk of a hypersensitivity type reaction. None of these studies revealed anaphylactic or hypersensitivity type responses in rats, rabbits or guinea pigs.

Even though there were no nonclinical responses, a single anaphylactic response in a clinical trial raised concern in the Medical Officer's review. The sponsor was asked to revisit this issue, and address the possibility of the carbohydrate coating containing residual amounts of dextran. The carbohydrate coating is chemically derived from a dextran, which had several chemical modifications to eliminate the reactive functional groups that induced the anaphylactic responses by dextran.

BRIEF DESCRIPTION OF NONCLINICAL STUDIES

These studies were conducted to evaluate the potential of Ferumoxytol to elicit antigenic, immunologic, hypersensitivity of anaphylactic responses in rats, rabbits and guinea pigs. Full study description can be found in DFS in the original NDA review.

Rat Paw Edema Study

Since the carbohydrate coating on the surface of Code 7228 is derived from a dextran, this study was designed to assess the intravenous potential of Code 7228 to induce edema in the rat paw. Groups of rats received an intravenous injection of 3 different lots of Code 7228 containing 100 mg Fe/kg, following a 4 day washout period between injections. A positive control group of 3 animals received injections of 100 mg/kg of Dextran T70. Prior to each injection and 45 minutes after treatment, the volume of each hind paw was measured by plethysmometry. Edema scores of 0 – 4 for normal, slight, moderate and severe edema, respectively were recorded for each animal at each time period.

Conclusion: The rat paw edema study is known to be a sensitive indicator of the anaphylactoid response of dextran in mammals. In this study the positive control material Dextran T70 produced severe edema as expected. None of the lots of Code 7228 yielded tissue responses or paw edema, therefore, tissue response to Code 7228 (Ferumoxytol) was negative.

Passive Cutaneous Assay (PCA): On day 35 sera was collected for the PCA and PHA assays. Each naïve guinea pig received intradermal injections of diluted serum for passive sensitization. Four hours later, each animal was challenged with intravenous injections of either Code 7228, positive control BSA or negative control mannitol. Animals were examined 30-45 minutes after challenge for passive cutaneous tissue response.

Passive Hemagglutination Assay (PHA): This in vitro assay was conducted to determine whether challenge treatments of Code 7228 and positive control BSA would induce the formation of antigen specific antibodies in the serum. Serial dilutions of guinea pig sera collected during the PCA assay, were added to the microtiter plates, followed by suspensions of either unconjugated SRBC or SRBC conjugated with Code 7228, positive and negative control. Contents of the plates were incubated for 1-3 hours. Plates were evaluated for hemagglutination with the lowest dilution of sera with agglutination being considered the antibody titer.

Systemic Anaphylaxis Assay (SAA): The last group of animals from the PCA were challenged intravenously on Day 36 with Code 7228, mannitol, or BSA. Animals were observed for 4 days after challenge for signs of hypersensitivity or anaphylactic response.

Results of PCA, PHA and SSA: Positive and negative control animals/samples responded as expected. In the animals challenged with Code 7228 there were no positive results indicating antigenicity, hypersensitivity or anaphylactic responses in these studies.

Reviewer's Conclusion:

A battery of studies was conducted to evaluate the potential of ferumoxytol to elicit tissue responses in rats, rabbits and guinea pigs. The rat paw edema study was negative when comparing the response of ferumoxytol to the severe edema observed with the IV administration of the positive control Dextran T70. The administration of ferumoxytol to rabbits by the subcutaneous, perivascular and intravenous routes resulted in no antigenicity, immunotoxicity or hypersensitivity type tissue reactions. After a 35 day induction period, guinea pigs and rats receiving IV or SC injections of Ferumoxytol exhibited no antigenic, hypersensitivity, immunotoxic or anaphylactic responses in the Rat Paw Edema, Passive Cutaneous Assay, Passive Hemagglutination Assay or the Systemic Anaphylaxis Assay following induction and challenge. Although using a battery of animal studies as described here is generally quite predictive of risk of human anaphylactoid responses, animal studies are not always completely accurate in predicting human response.

Recommendations

Recommendation on approvability: Approval.

Recommendation for nonclinical studies: No further nonclinical studies are recommended.

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this page is the manifestation of the electronic signature.**

/s/

David Bailey
12/16/2008 07:17:28 AM
PHARMACOLOGIST

Adebayo Laniyonu
12/16/2008 07:21:26 AM
PHARMACOLOGIST



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
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PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-180
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	12/19/2007
PRODUCT:	Ferumoxytol
INTENDED CLINICAL POPULATION:	Patients with Chronic Kidney Disease
SPONSOR:	AMAG Pharmaceuticals, Inc.
DOCUMENTS REVIEWED:	Electronic submission in EDR
REVIEW DIVISION:	Medical Imaging and Hematology Drug Products (HFD-160)
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Date of review submission to Division File System (DFS): October 03, 2008.

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EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: Approval.
- B. Recommendation for nonclinical studies: No further nonclinical studies are recommended.
- C. Recommendations on labeling:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no studies of ferumoxytol in pregnant women. In animal studies, ferumoxytol caused decreased fetal weights and fetal malformations at maternally toxic doses of 13-15 times the human dose. Ferumoxytol should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

In rats, administration of ferumoxytol at maternally toxic doses during organogenesis, i.e., daily doses approximately 2 times the recommended 510 mg human dose (on a mg/m^2 basis) for 12 days caused a decrease in fetal weights. The cumulative animal exposure was approximately 13 times the human therapeutic course of 1.02 g (on a mg/m^2 basis). In rabbits, administration of ferumoxytol at maternally toxic doses during organogenesis, i.e., daily doses approximately 2 times the recommended 510 mg human dose (on a mg/m^2 basis) for 14 days caused decreased fetal weights and external and/or soft tissue fetal malformations. The cumulative animal exposure was approximately 15 times the human therapeutic course of 1.02 g on a mg/m^2 basis [See 13.3 *Reproductive and Developmental Toxicology*].

8.3 Nursing Mothers

It is not known whether ferumoxytol is present in human milk. Caution should be exercised in ferumoxytol is administered to a nursing woman.

10.1 Nonclinical Data

No macroscopic or microscopic signs of toxicity and no changes in the clinical pathology data related to toxicity were observed following single intravenous doses of ferumoxytol up to 450 mg iron/kg in rats (approximately 10 times the recommended 510 mg human dose on a mg/m^2 basis) and in dogs (approximately 33 times the recommended 510 mg human dose on a mg/m^2 basis).

12.1 Mechanism of Action

Ferumoxytol consists of a superparamagnetic iron oxide nanoparticle that is surrounded by a carbohydrate shell, which functions to isolate the bioactive iron from plasma components until the iron-carbohydrate complex enters the reticuloendothelial system macrophages of the liver, spleen and bone marrow. The iron is released from the iron-carbohydrate complex within vesicles in the macrophages. Iron then either enters the intracellular storage iron pool (eg. ferritin) or is transferred to plasma transferrin for transport to erythroid precursor cells for incorporation into hemoglobin.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Ferumoxytol was not tested for carcinogenic effects. In standard genotoxicity tests, ferumoxytol showed no evidence of mutagenic activity in an *in vitro* Ames test or clastogenic activity in either an *in vitro* chromosomal aberration assay or an *in vivo* micronucleus assay.

No adverse effects on fertility or general reproductive performance were noted in animal studies. Ferumoxytol had no effect on male or female fertility or general reproductive performance in rats.

13.2 Animal Toxicology and Pharmacology

Animal studies demonstrate that the plasma half-life of ferumoxytol increased with increasing dose. The highest tissue concentrations of ferumoxytol were found in the liver, spleen, and central lymph node pool; administered radiolabeled ferumoxytol (^{59}Fe) was predominantly found in the red blood cell fraction by 24 hr. Studies with radiolabeled drug product demonstrated that renal elimination of the iron in ferumoxytol was insignificant, while the carbohydrate coating was significantly excreted in the urine and feces.

Repeat-dose toxicity studies with ferumoxytol up to 12 mg Fe/kg/day for 13 weeks in rats (cumulative exposure is approximately 12 times the anticipated exposure of a human therapeutic course of 1.02 g of ferumoxytol on mg/m² basis) and dogs (cumulative exposure is approximately 40 times the anticipated exposure of a human therapeutic course of 1.02 g of ferumoxytol on mg/m² basis) demonstrated dose-dependent decreases in body weight gain and food consumption, and increases in pigmentation intensity. No systemic toxicity or immunotoxicity was observed at the relevant clinical doses. Changes in red blood cell counts, hemoglobin and serum iron, increases in liver and spleen weight, and the accumulation of iron-positive pigmentation in various organs were observed as expected with the administration of iron-containing agents.

13.3 Reproductive and Developmental Toxicology

In rats, there were no maternal or fetal effects of ferumoxytol at daily doses of 31.6 mg Fe/kg during organogenesis for 12 days, approximately 1 time the recommended human dose of 510 mg (on a mg/m² basis). The cumulative animal exposure was approximately 5 times the human therapeutic course of 1.02 g (on a mg/m² basis). Administration of ferumoxytol during organogenesis at maternally toxic doses of 100 mg Fe/kg/day (daily exposure is approximately 2 times the recommended 510 mg human dose on a mg/m² basis) for 12 days (cumulative exposure is approximately 13 times the human therapeutic course of 1.02 g on a mg/m² basis) caused a decrease in fetal weights.

In rabbits, there were no maternal or fetal effects of ferumoxytol at daily doses of 16.5 mg Fe/kg during organogenesis for 14 days, approximately 1 time the recommended human dose of 510 mg (on a mg/m² basis). The cumulative animal exposure was approximately 7 times the human therapeutic course of 1.02 g (on a mg/m² basis). Administration of ferumoxytol during organogenesis at maternally toxic doses of 45 mg Fe/kg/day (daily exposure is approximately 2 times the recommended 510 mg human dose on a mg/m² basis) for 14 days (cumulative exposure is approximately 15 times the human therapeutic course of 1.02 g on a mg/m² basis) caused decreased fetal weights and external and/or soft tissue fetal malformations.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Pharmacodynamics: Intravenous administration of Ferumoxytol was found to correct anemia in rats. Diet induced anemia in rats resulted in Hb values of 5.1 g/dL compared to 13.2 g/dL in control animals. A single IV dose of 30 mg Fe/kg resulted in an increased Hb value of 11.2 g/dL. Other parameters including RBC, Hct, serum iron and iron binding capacity returned to near normal values after the one treatment.

Safety Pharmacology: Heart rate, systolic, diastolic and mean arterial blood pressure were unaffected by intravenous administration of Ferumoxytol in conscious dogs and in anesthetized dogs receiving the cardiac stress agent dipyridamole. ECG parameters and cardiac rhythm were also unaffected by Ferumoxytol intravenous administration in these studies.

Nonclinical QTc studies were evaluated by a board certified veterinary cardiologist, without detectable prolongation observed. In in vitro cardiac electrophysiologic hERG studies in human kidney cells (HEK293), potassium channel blocking was not observed.

Neurologic and behavioral parameters evaluated in the functional observational battery were unaffected by the intravenous administration of Ferumoxytol.

Pharmacokinetics: Plasma concentrations of ^{59}Fe in the nanoparticles and ^{14}C in the carbohydrate coating in rat and dog showed a rapid decrease after administration of Ferumoxytol. ^{59}Fe was initially found in the liver, spleen and lymph nodes but was found to entirely be accounted for in the RBC by 11 days. ^{14}C in the coating was eliminated in urine and feces with 92% recovery by day 84. Pharmacokinetic parameters for AUC, C_{max} , $t_{1/2}$, Cl and V_z seen in the nonclinical studies were similar to those seen in the Phase 1 studies in the clinic.

Single Dose Toxicity: Single dose IV administration of Ferumoxytol at doses up to 450 mg Fe/kg did not induce toxicity in rats (10 X MHD BSA) or dogs (33 X MHD BSA).

Repeat Dose Toxicity: Rats and dogs were unaffected by intravenous 13-week repeat daily doses of Ferumoxytol at 12 mg Fe/kg/day, the highest dose given. The only effects observed were a consequence of chronic iron overload, including high serum iron levels, increased liver and spleen weights, and accumulation of iron positive pigment in Kupffer's cells in multiple organs and tissues. The NOAEL for both rats and dogs is 12 mg Fe/kg/day for 13 weeks. The daily dose is 0.3 and 0.9 X MHD for rats and dogs respectively, and the cumulative dose is 12 and 40 X MHD for rats and dogs respectively based on BSA.

Genotoxicity: Ferumoxytol was negative in the Ames Reverse Mutation Assay, Chromosomal Aberration Assay in Chinese Hamster Ovary cells and was not clastogenic in the in vivo Mouse Micronucleus test.

Carcinogenicity: 104-Week studies with Ferumoxytol are currently underway in mice and rats.

Reproductive Toxicity: In 3 studies, Ferumoxytol did not affect fertility and general reproductive performance in male and female rats or female rabbits, and did not interfere with embryo-fetal development in offspring at doses that were not maternally toxic.

Hypersensitivity: A battery of studies was conducted to evaluate the potential of ferumoxytol to elicit tissue responses in rats, rabbits and guinea pigs. The rat paw edema study was negative when comparing the response of ferumoxytol to the severe edema observed with the IV administration of the positive control Dextran T70. The administration of ferumoxytol to rabbits by the subcutaneous, perivascular and intravenous routes resulted in no antigenicity, immunotoxicity or hypersensitivity type tissue reactions. Guinea pigs were given IV and SC injections of ferumoxytol over a 35 day induction period, followed by challenge IV doses of ferumoxytol. There were no hypersensitivity or anaphylactic responses observed from the administration of ferumoxytol in the Passive Cutaneous Assay, Passive Hemagglutination Assay or the Systemic Anaphylaxis Assay following induction and IV challenge with ferumoxytol. Although using a battery of animal studies as described here is generally predictive of risk of human anaphylactoid responses, animal studies are not always completely accurate in predicting human response.

In Conclusion: Ferumoxytol is a relatively nontoxic product in a wide variety of nonclinical studies and species, and thus requires a very high dose to induce a toxic response. The initial pharmacological effects are those found in chronic iron overload which always precede any overt signs of toxicity. The findings are consistent with pharmacological effects of high doses of iron compounds administered intravenously.

- B. Pharmacologic activity: Ferumoxytol is a superparamagnetic iron oxide nanoparticle with a polyglucose sorbitol carboxymethylether coating indicated as iron replacement therapy for patients with chronic kidney disease (CKD). It is readily distributed and iron is incorporated into body stores and hemoglobin. Iron is minimally excreted with the carbohydrate coating excreted in the urine and feces.
- C. Nonclinical safety issues relevant to clinical use: None

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-180
Review number: 01/08
Sequence number: N 000 18-December-2007

Information to sponsor: No
Sponsor and/or agent: AMAG Inc., Cambridge, MA 02138
Manufacturer for drug substance: Not indicated

Reviewer name: David E. Bailey, Ph.D.
Division name: Medical Imaging and Hematology Products
HFD #: 160

Review completion date: September 19, 2008

Drug:
Trade name: Ferumoxytol
Generic name: Code 7228
Chemical name:
CAS registry number:
Molecular formula/molecular weight:

Relevant INDs/NDAs/DMFs: IND #62,745
Drug class: Anti anemia
Route of administration: Intravenous

Intended clinical population: Patients with anemia as a result of Chronic Kidney Disease

Clinical formulation: Mannitol 44 mg/mL in water for injection with 30 mg Fe/mL as Ferumoxytol®.

Clinical Dose: 510 mg Fe as a single IV 1- hour infusion followed by a second 510 mg Fe dose /days later. **b(4)**

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-180 are owned by AMAG Pharmaceuticals, Inc. or are data for which AMAG Pharmaceuticals, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 22-180 that AMAG Pharmaceuticals, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that AMAG Pharmaceuticals, Inc. does not own or is from FDA reviews or summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 22-180.

Studies reviewed within this submission:

Correction of iron deficiency anemia following a single intravenous injection of Code 7228 in iron deficient rats.

Effects on general activity and behavior in the mouse following intravenous administration of Ferumoxytol.

Hemodynamic response in the anesthetized rat following IV injection of Code 7228.

The hemodynamic response in the anesthetized male guinea pig following intravenous injection of Code 7228.

A safety pharmacology assessment of Code 7228 on cardiovascular and respiratory parameters in anesthetized dogs.

Effects of Ferumoxytol on Cloned hERG potassium channels expressed in human embryonic kidney cells.

Single dose intravenous toxicity study with Code 7228 in rats.

Single dose intravenous toxicity study with Code 7228 in dogs.

13-Week intravenous injection toxicity and toxicokinetic study with ferumoxytol in rats.

13-week intravenous toxicity and toxicokinetic study with Ferumoxytol in dogs.

Mutagenic test with Code 7228 in Salmonella and E. coli/mammalian-Microsome reverse mutation assay with confirmatory assay

Mutagenic test on Code 7228 measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells.

Mutagenic test with Ferumoxytol (Code 7228) in the in vivo mouse micronucleus assay.

Study of fertility and early embryonic development to implantation in rats

Rat developmental toxicity study with Code 7228 (SEG II)

Rabbit Developmental Toxicity Study with Code 7228 (SEG II)

Acute Intravenous/Perivenous/Intraarterial tolerance study with Code 7228 (Ferumoxytol) in rabbits.

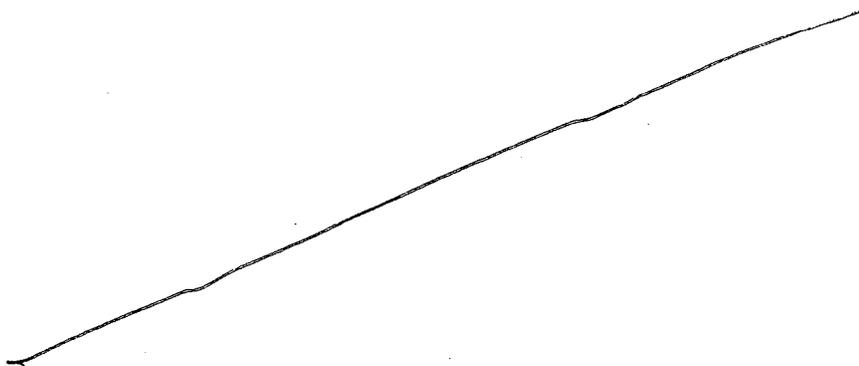
Evaluation of rat paw edema following intravenous injection of Code 7228

Evaluation of the possible immunological effects of Code 7228 in guinea pigs.

Effects of Ferumoxytol on Cloned hERG potassium channels expressed in human embryonic kidney cells.

Pharmacokinetics in the rat after intravenous injection of ^{59}Fe and ^{14}C labeled Code 7228.

Studies not reviewed within this submission:



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2.6.2 PHARMACOLOGY

A. 2.6.2.1 Brief summary

Ferumoxytol is a superparamagnetic iron oxide nanoparticle with a polyglucose sorbitol carboxymethylether coating indicated as iron replacement therapy for patients with chronic kidney disease (CKD). It is readily distributed and the iron is incorporated into body stores and hemoglobin.

2.6.2.2 Primary pharmacodynamics

Study Title: Correction of iron deficiency anemia following a single intravenous injection of Code 7228 in iron deficient rats.

Report Number; HHB-175

Study design: Designed to evaluate over a 7 week period whether Ferumoxytol would be available and incorporated in hemoglobin in rats with anemia induced by a low iron diet. Sprague Dawley male rats 4 weeks old were assigned to one of 3 groups with 6 male animals/group. Group 1 received normal rat chow with 380 ppm Fe, Group 2 and Group 3 received a low iron diet with 7 ppm Fe. After 4 weeks, Group 3 also received an IV injection of Ferumoxytol providing 30 mg Fe/kg. Animals were observed daily, and weighed pretest and weekly. Blood for hematology was collected pretest and at weeks 2, 4 and 7. Parameters included Hgb, Hct, RBC, serum iron and serum total iron binding capacity (TIBC).

Results: After 4 weeks Hgb in rats of the iron deficient diet groups was 3.9 g/dL and for control animals 13.2 g/dL. At 7 weeks Hgb for controls was 14.0 g/dL and 6.9 g/dL for Group 2 and 11.1 g/dL for Group 3 that received the IV Ferumoxytol at week 4. RBC, Hct, serum iron and TIBC were likewise increased in the Group 3 animals, but still not as high as the Control group, with parameters in the Group 2 animals still significantly lower (Hgb 6.9 g/dL) than Control values.

Conclusion:

Intravenous administration of Ferumoxytol was found to correct anemia in rats. Diet induced anemia in rats resulted in Hb values of 3.9 g/dL compared to 13.2 g/dL in control animals after 4 weeks. A single IV dose of 30 mg Fe/kg given at Week 4 resulted in increased Hb value of 11.2 g/dL by week 7. Other parameters including RBC, Hct, serum iron and iron binding capacity returned to near normal values after the one treatment.

Mechanism of action:

Ferumoxytol is a of a superparamagnetic iron oxide nanoparticle that is surrounded by a polyglucose sorbitol carboxymethylether coating, which functions to isolate the bioactive iron from plasma components until the iron-carbohydrate complex enters the reticuloendothelial system macrophages of the liver, spleen and bone marrow. The iron is released from the iron-carbohydrate complex within vesicles in the macrophages. Iron then either enters the intracellular storage iron pool as ferritin or is transferred to plasma transferrin for transport to erythroid precursor cells for incorporation into hemoglobin.

2.6.2.3 Secondary pharmacodynamics**2.6.2.4 Safety pharmacology**Neurological effects:

Study Title: Effects on general activity and behavior in the mouse following intravenous administration of Ferumoxytol.

Report Number: 2598/001

Study Design: This study was designed to evaluate the effects of Ferumoxytol on general behavioral, autonomic and motor activity in mice using the Irwin's method. This study was conducted by _____

Four groups of 6 male Crl:CD-1(ICR)BR mice 5 weeks old and weighing 22-29 g received a single IV dose of vehicle, or Ferumoxytol at a dose of either 100, 300 or 1000 mg Fe/kg. Irwin observations were conducted at 0-5, 15, 30, 60 and 120 minutes after administration. Animals were maintained for 7 days after treatment and observed for survival and signs of toxicity.

The Irwin scoring system involves; Cage: 8 criteria, Arena: 16 criteria, Handling: 11 criteria, General Scores: 16 criteria. Each criteria can be graded as either Normal, Absent or Present.

Results: The only effect observed was an orange to brownish tinge to the skin and extremities in animals of the 300 and 1,000 mg Fe/kg groups. This discoloration remained throughout the 7 day observation period. The scores for Cage, Arena, Handling and General Scores for the animals in the 100, 300 and 1,000 mg Fe/kg groups were comparable to the scores recorded for the Vehicle group.

Conclusion: Administration of Ferumoxytol at doses of 100, 300 and 1,000 mg Fe/kg had no effect on survival or general behavioral, autonomic and motor activity when evaluated by Irwin's method.

Cardiovascular and Pulmonary effects:

The 3 studies below were designed to assess the cardiovascular and pulmonary effects of IV injections of Ferumoxytol (Code 7228) in rat, guinea pig and dog.

Study Title: Hemodynamic response in the anesthetized rat following IV injection of Code 7228.

Report Number: HHB-156

Design: Two groups of Charles River rats weighing 261- 361 were evaluated for changes in mean arterial blood pressure (MABP). Systolic and diastolic blood pressures were recorded directly using cannulated carotid artery for 30 minutes after dosing. Pulse pressure (PP) and MABP were calculated. A saline control group was used for comparison to a group that received Code 7228 at a dose of 120 mg Fe/kg.

Results: Control animals showed a minimal reduction in MABP (<5%) which is considered insignificant. There was no measurable change in MABP for animals of the Code 7228 animals.

Conclusion: Intravenous Code 7228 at a dose of 120 mg Fe/kg had no effect on mean arterial blood pressure in CD-1 mice.

Study Title: The hemodynamic response in the anesthetized male guinea pig following intravenous injection of Code 7228.

Report Number: HHB 152B

Design: Two groups (9/group) of Hartley guinea pigs were evaluated for changes in mean arterial blood pressure (MABP). Systolic and diastolic blood pressures were recorded directly using cannulated carotid artery for 60 minutes after dosing. Pulse pressure (PP) and MABP were calculated. A saline control group was used for comparison to a group that received Code 7228 at a dose of 120 mg Fe/kg.

Results: Control animals showed a minimal reduction in MABP (<7%) which is considered insignificant. There was no measurable change in MABP for 7 animals of the Code 7228 group animals. In 1 animal there was a mild reduction in MABP that persisted for 50 minutes.

Conclusion: Intravenous Code 7228 at a dose of 120 mg Fe/kg had no effect on mean arterial blood pressure in 8 of 9 Hartley guinea pigs. One animal had reduced MABP that persisted 50 minutes.

Study Title: A safety pharmacology assessment of Code 7228 on cardiovascular and respiratory parameters in anesthetized dogs.

Report Number: E992-920

Design: Groups of Marshall Farms beagle dogs weighing 7-11 kg and 9-11 months of age were evaluated. A pulse oximeter was applied to monitor blood gases. ECGs were recorded with leads in axial lead configuration. The femoral artery and vein were catheterized for continual hemodynamic monitoring. Blood flow was measured by use of a flow probe in the contralateral femoral artery. A Vehicle Control group (mannitol 44 mg/mL) was used for comparison to the groups that received Code 7228 at sequential doses of 4, 40 or 400 mg Fe/kg. There was an 80 min interval between each sequential dose. A 20 minute pre-dose period was monitored just prior to each dose of Code 7228.

Hemodynamic and respiratory parameters were monitored continuously and recorded for 4 hours by an electronic data system. Arterial blood pressure, (systolic, diastolic and mean), heart rate, femoral artery blood flow, left ventricular pressure, with calculated \pm dP/dtmax, respiration and ECG parameters were recorded electronically. Data for each animal was recorded every 5 minutes for 20 min predose, and for 60 minutes after each IV administration. Blood (0.2 mL) was collected from femoral artery predose and at 30 and 60 minutes after each dose for pO₂, pCO₂ and pH. Blood for serum creatinine was collected about midpoint of each urine collection. Urine was collected for 20 min predose and at 30 and 60 min after each dose.

Results: Diastolic, systolic and mean blood pressure were unaffected by treatment with either vehicle or Ferumoxytol. There were no changes in heart rate, femoral artery blood flow, respiratory rate, pulmonary volume or pulmonary flow after vehicle or Ferumoxytol doses when compared to baseline values. With doses of up to 400 mg Fe/kg of Ferumoxytol and with vehicle, there were no changes observed in ECG waveforms or QTc prolongation. Increased urine flow was noted with both Vehicle and Ferumoxytol treatment, which is considered a consequence of volume of infused liquid. GFR as indicated by creatinine clearance, did not change following Vehicle or Ferumoxytol treatment. Urinary sodium, potassium and chloride excretion fluctuated sporadically, but were insignificant when compared to pretest baseline values.

Conclusion: Intravenous Code 7228 at a dose of up to 400 mg Fe/kg had no effect on cardiovascular or respiratory parameters when compared to pretest control values. ECG waveforms and QTc duration were unaffected by Vehicle and Ferumoxytol infusion. Urinary flow was increased by infusion of both Vehicle and Ferumoxytol as a result of the volume of liquid infused. Fluctuations in excretion of urinary electrolytes were insignificant when compared to pretest values.

Cardiovascular electrophysiology

Study Title: Effects of Ferumoxytol on Cloned hERG potassium channels expressed in human embryonic kidney cells.

Report Number: 060321.BEN

Design: This study was designed to evaluate the in vitro effects of Ferumoxytol on the hERG potassium channel current. Decreased action potential duration causes prolongation of the QT interval and has been associated with a dangerous ventricular arrhythmia. Ferumoxytol in 3 cells at each concentration of 100 and 500 $\mu\text{g Fe/mL}$ were compared to Vehicle control (mannitol, 44 mg/mL) and positive control (60nM terfenadine).

Results: Ferumoxytol at concentrations of 100 and 500 $\mu\text{g Fe/mL}$ did not decrease action duration. The Vehicle control material had no effect on action potential duration. The positive control material, terfenadine, inhibited hERG potassium current by $72.1 \pm 1.8\%$.

Conclusion: This study demonstrates that IV Ferumoxytol does not result in decreased duration of action potential, causing prolongation of the QT interval.

2.6.2.5 Pharmacodynamic drug interactions

None studied.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary:

Plasma concentrations of ^{59}Fe in the nanoparticles and ^{14}C in the carbohydrate coating in rat and dog showed a rapid decrease after administration of Ferumoxytol. ^{59}Fe was initially found in the liver, spleen and lymph nodes but was found to entirely be accounted for in the RBC by 11 days. ^{14}C in the coating was eliminated in urine and feces with 92% recovery by day 84.

2.6.4.2 Methods of Analysis

2.6.4.3 Absorption

Since Ferumoxytol is administered intravenously, absorption was not studied.

2.6.4.4 Distribution

Study title: Pharmacokinetics in the rat, guinea pig and rabbit after intravenous injection of ^{59}Fe labeled Code 7228.

Study Number: HHB-160
Report Date: June 14, 2007
Laboratory: Advanced Magnetics, Cambridge, MA

In-life Dates: December 1998 – January 1999
GLP compliance: No
QA report: No

Design:

This study was designed to evaluate the half-life of radio labeled Code 7228 in rats, guinea pigs and rabbits after a single IV injection. Groups of 3 animals of each species received 2.2 mg Fe/kg and 3.8 $\mu\text{Ci}/\text{mg}$ by IV injection.

Blood samples were collected at 15, 30, 60, 90, 120 and 240 minutes after administration for rats, with guinea pigs and rabbits also collected at 360 minutes. A measured amount of each sample was counted on a gamma counter for ^{59}Fe .

Results:

Half life was found to be as follows:

Rat: 104 ± 8 minutes
Guinea pig: 120 ± 6 minutes
Rabbit: 81 ± 21 minutes

Reviewer's Conclusions:

Ferumoxytol (Code 7228) is rapidly distributed throughout the vascular system and rapidly cleared from the blood with half life of 2 hours or less for all three species.

Study Title: Tissue distribution of Code 7228- ^{14}C following intravenous injection in the rat.

Study Number: HHB-174
Report Date: June 19, 2007
Laboratory: Advanced Magnetics, Cambridge, MA

In-life Dates: June 19 – October 3, 2000
GLP compliance: No
QA report: No

Design:

This study was designed to evaluate the half-life of radio labeled Code 7228 in rats. Post injection sacrifice time periods were 1, 7, 14, 28, 56, and 84 days. Groups of 4 rats for each time period were injected IV with ferumoxytol delivering a dose of 6 mg Fe/kg with 5.41 μCi of ^{14}C . Urine and feces were collected from each animal for each 7 day period. At necropsy tissues were collected for analysis. The following tissues and organs were collected from each animal: blood, liver, spleen, thigh muscle, brain, heart, lungs, adrenal, kidney, small intestine, large intestine, testes, bone marrow, central lymphatic system, and peripheral lymphatic system.

Results:

At 24 hours after dosing the liver (26.5%), spleen (5.6%) and lymph gland pool (2.5%) contained the highest concentration of the radioactivity. Elimination half life was 10 days for liver, 12.7 days for spleen and 15.1 days for central lymph gland pool. Total recovery over the 84 day collection period for administered ^{14}C was 90 ± 3 days with 78% in urine and 10% in feces.

Reviewer's conclusion:

The carbohydrate coating on ferumoxytol is absorbed predominantly into liver, spleen and lymph nodes where it is taken up by the reticuloendothelial system and separated from the iron. The carbohydrate coating is eliminated primarily in urine and feces with half-life of 10 – 15 days.

Other pharmacokinetic and toxicokinetic data was collected in the 13-week toxicology studies in rats and dogs and the data is presented with the review of each of those studies.

2.6.4.5 Metabolism

2.6.4.6 Excretion

2.6.4.7 Pharmacokinetic drug interactions

None studied.

2.6.4.8 Other Pharmacokinetic Studies

2.6.4.9 Discussion and Conclusions

Plasma concentrations of ⁵⁹Fe in the nanoparticles and ¹⁴C in the carbohydrate coating in rat and dog showed a rapid decrease after administration of Ferumoxytol. ⁵⁹Fe was initially found in the liver, spleen and lymph nodes but was found to be entirely accounted for in the RBC by 11 days. ¹⁴C in the coating was eliminated in urine and feces with 92% recovery by day 84. Representative pharmacokinetic parameters for AUC, C_{max}, t_{1/2}, Cl and V_z seen in the nonclinical studies and those seen in the Phase 1 studies in the clinic are shown in the table below.

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Representative rat, dog and human values are shown in the following table.

Species	Dose Level (mg Fe/kg)	Sex	C _{max} (µg/mL)	T _{max} (hr)	AUC ₀₋₁₂ (µg.hr/mL)	t _{1/2} (hr)	CL (mL.hr/kg)	V _{ss} (mL/kg)
Day 1								
Rat	2	M	35.3	0.25	83.0	1.13	22.8	87.8
		F	27.1	0.25	85.7	1.19	21.3	111
Dog	2	M	25.0	0.25	115	1.55	17	168
		F	23.5	0.25	106	1.47	19	184
Human	2	M	62.2		996	11.4	2.18	34.8
		F						

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Ferumoxytol is relatively nontoxic in a wide variety of nonclinical studies and species, and thus requires a very high dose to induce a toxic response. The initial pharmacological effects are those found in chronic iron overload which always precede any overt signs of toxicity. The findings are consistent with pharmacological effects of high doses of iron compounds administered intravenously.

Single dose IV administration of Ferumoxytol at doses up to 450 mg Fe/kg did not induce toxicity in rats or dogs, which is 10 and 33 X MHD BSA for rats and dogs respectively.

Rats and dogs were unaffected by intravenous 13-week repeat daily doses of Ferumoxytol at 12 mg Fe/kg/day, the highest dose given. The only effects observed were a consequence of chronic iron overload. The NOAEL for both rats and dogs is 12 mg Fe/kg/day. The daily dose is 0.3 X MHD and the cumulative dose is 12 X MHD BSA for rats, and 0.9 and 40 X MHD BSA for dogs, respectively.

Genetic toxicology:

Ferumoxytol was negative in the Ames Reverse Mutation Assay, Chromosomal Aberration Assay in Chinese Hamster Ovary cells and was not clastogenic in the in vivo Mouse Micronucleus test.

Carcinogenicity:

104-Week studies with Ferumoxytol are currently underway in mice and rats.

Reproductive Toxicity:

In 3 studies, Ferumoxytol did not affect fertility and general reproductive performance in male and female rats or female rabbits, and did not interfere with embryo-fetal development in offspring at doses that were not maternally toxic.

Local tolerance, antigenicity and immunotoxicity:

Rabbits were unaffected by subcutaneous, perivascular and intravenous administration of Ferumoxytol. Guinea pigs did not develop a hypersensitivity or anaphylactic response following induction and IV challenge with Ferumoxytol. Results of the rat paw edema study were negative.

2.6.6.2 Single-dose toxicity

Study title: Single dose intravenous toxicity study with Code 7228 in rats.

Key study findings:

The findings in this study are consistent with pharmacological effects of high doses of iron compounds administered intravenously. The incidence is dose related and more prominent in Group 4 (450 mg Fe/kg) and included high levels of serum iron, increased liver weights, and the accumulation of iron positive pigment in Kupffer's cells in multiple organs and tissues. The NOAEL for this study is considered to be 450 mg Fe/kg.

Study no.: 6782-103
Volume #, and page #: Module 4 in EDR
Laboratory and location: _____

b(4)

Date of study initiation: September 2, 1998.
In-Life dates: September 15 – 30, 1998.
Report date: February 17, 1999

GLP compliance: Yes
QA report: Yes
Drug, lot and purity: Lot # 98080401, Purity 100%.

Design: This study was designed to evaluate the toxicity of a single intravenous dose of Code 7228 when administered via the lateral tail vein to male and female Sprague-Dawley CrI:CD@BR rats. Study animals were 6-7 weeks of age and males weighed 163-202 g and females 135-179 g at randomization into the study. Harlan Teklad Certified rodent diet #8728C was available ad libitum and water was supplied by an automatic system. Animals were assigned to dose groups and terminated according to the following table.

b(4)

Group	Dosage mg Fe/kg	Dose Vol mL/kg	Number Animals		Number sacrificed					
			Male	Female	Day 1*		Day 4		Day 16	
					M	F	M	F	M	F
1 Control	0	15	13	13	3	3	5	5	5	5
2 Low	4	1.3	10	10	0	0	5	5	5	5
3 Mid	40	1.3	10	10	0	0	5	5	5	5
4 High	450	15	13	13	3	3	5	5	5	5

* 24 hours after treatment.

Observations:

<u>Mortality:</u>	Twice daily cage check
<u>Clinical signs:</u>	Daily
<u>Body weights:</u>	Pretest and weekly
<u>Food consumption:</u>	Weekly
<u>Ophthalmoscopy:</u>	Pretest, 1 hour after first dose and just prior to termination
<u>Hematology:</u>	RBC, WBC and differential, HGB, HCT, platelet count, blood cell morphology, and calculated M:E ratio, MCV, MCH and MCHC
<u>Coagulation:</u>	aPTT and PT
<u>Clinical chemistry:</u>	AST, APT, LDH, GGT, AP, P, Ca, Cl, K, Na, BUN, glucose, creatinine, total protein, albumin, globulin, A/G ratio, cholesterol, triglycerides, serum Fe, total bilirubin
<u>Gross pathology:</u>	All animals. Some 45 tissues and organs were examined and retained in fixative.
<u>Organ weights:</u>	Adrenals, brain, kidneys, liver and testes with epididymides
<u>Histopathology:</u>	Gross lesions, lungs, lymph nodes, liver and spleen were examined microscopically for all animals.

Results:

<u>Mortality:</u>	All animals survived to scheduled termination.
<u>Clinical signs:</u>	Limb swelling and discoloration in all Code 7228 groups.
<u>Body weights:</u>	NNF (No Noteworthy Findings)
<u>Food consumption:</u>	NNF
<u>Ophthalmoscopy:</u>	NNF
<u>Hematology:</u>	NNF
<u>Coagulation:</u>	NNF
<u>Clinical chemistry:</u>	Serum was noticeably darker in color. Group 4 animals had lower AST and LDH values, with increased GGT, neutrophil counts, monocyte counts, and serum iron values at the Day 4 and Day 16 samplings.

- Organ Weights: Group 4 liver weights increased in males and females at Day 4 and Day 16 necropsies.
- Gross pathology: Dark colored skin, lymph nodes, serosa of intestines, and liver in Day 4 and Day 16 necropsies of Groups 3 and 4 animals.
- Histopathology: Accumulation of brown intravascular pigment with apparent alteration of vascular permeability characterized by edema and extracellular pigment in the dermis observed at the Day 1 (24 hour) sacrifice in all groups receiving Code 7228.
- Microscopic findings in tissues from the Day 4 and Day 16 sacrifices included primarily an accumulation of brown pigment in the cytoplasm of macrophages/Kupffer's cells in the liver, spleen, lung, lymph nodes, thymus, testes, ovaries, intestine and skin of Group 4 animals of both sexes. The brown pigment stained positive for iron and was somewhat less in the Day 16 tissues.
- Reviewer's Conclusions: The findings in this study are consistent with pharmacological effects of high doses of iron compounds administered intravenously. The incidence is dose related and more prominent in Group 4 (450 mg Fe/kg) and included high levels of serum iron, increased liver weights, and the accumulation of iron positive pigment in Kupffer's cells in multiple organs and tissues. The NOAEL for this study is considered to be 450 mg Fe/kg as a single intravenous dose.

Study title: Single dose intravenous toxicity study with Code 7228 in dogs.

Key study findings:

The findings in this study are consistent with pharmacological effects of high doses of iron compounds administered intravenously. The incidence is dose related and more prominent in Group 4 (450 mg Fe/kg) and included high levels of serum iron, increased liver weights, and the accumulation of iron positive pigment in Kupffer's cells in multiple organs and tissues. The NOAEL for this study is considered to be 450 mg Fe/kg.

Study no.: 6782-104 b(4)
Volume #, and page #: Module 4 in EDR
Laboratory and location: _____

Date of study initiation: October 21, 1998.
In-Life dates: November 2-17, 1998.
Report date: April 26, 1999

GLP compliance: Yes
QA report: Yes
Drug, lot and purity: Lot # 98080401, Purity 100%.

Design: This study was designed to evaluate the toxicity of a single intravenous dose of Code 7228 when administered via the cephalic vein to male and female purebred Beagle dogs from _____. Study animals were 5-6 months of age and males weighed 7.3-11.9 kg and females 6.7-10.2 kg at randomization into the study. Harlan Teklad Certified Canine Diet #8727C was available ad libitum and water was supplied by an automatic system. Animals were assigned to dose groups and terminated according to the following table. b(4)

Group	Dosage mg Fe/kg	Dose Vol mL/kg	Number Animals		Number sacrificed			
			Male	Female	Day 4		Day 16	
					M	F	M	F
1 Control	0	15	6	6	3	3	3	3
2 Low	4	0.13	6	6	3	3	3	3
3 Mid	40	1.3	6	6	3	3	3	3
4 High	450	15	6	6	3	3	3	3

Observations:

<u>Mortality:</u>	Twice daily cage check
<u>Clinical signs:</u>	Daily
<u>Body weights:</u>	Pretest and weekly
<u>Food consumption:</u>	Weekly
<u>Ophthalmoscopy:</u>	Pretest, 1 hour after first dose and just prior to termination

Physical and Neurobehavioral Examinations: Prior to treatment and 1 hr after first dose on Day 1 by staff veterinarian and included respiration rate, rectal temperature, evaluation of reflexes, gait, responsiveness and papillary reflex.

Hematology: RBC, WBC and differential, HGB, HCT, platelet count, blood cell morphology, and calculated M:E ratio, MCV, MCH and MCHC

Coagulation: aPTT and PT

Clinical chemistry: AST, APT, LDH, GGT, AP, P, Ca, Cl, K, Na, BUN, glucose, creatinine, total protein, albumin, globulin, A/G ratio, cholesterol, triglycerides, serum Fe, total bilirubin

Urinalysis: Appearance, volume, specific gravity, pH. Qualitative protein, glucose, ketones, bilirubin, blood and microscopic sediment

Gross pathology: All animals. Some 45 tissues and organs were examined and retained in fixative.

Organ weights: Adrenals, brain, kidneys, liver with gallbladder, and testes with epididymides and thyroid/parathyroid

Histopathology: Gross lesions, brain, kidney, lungs, lymph nodes, liver with gallbladder, testes with epididymides, thymus, ovary and spleen were examined microscopically for all animals.

Results:

Mortality: All animals survived to schedule termination.

Clinical signs: Brown discoloration in mucous membranes, especially gums of animals in 450 mg Fe/kg group.

Physical Exams: NNF (No Noteworthy Findings)

Neurobehavioral Exams: NNF

- Body weights: NNF (No Noteworthy Findings)
- Food consumption: NNF
- Ophthalmoscopy: Brown discoloration of sclera and palpebral conjunctiva in all 450 mg Fe/kg animals
- Hematology: Animals in the 450 mg Fe/kg group exhibited increased MCHC on Day 3 only. These parameters as well as all others were considered NNF on Day 16.
- Coagulation: Animals in the 450 mg Fe/kg group exhibited increased aPTT on Day 3 only. All others NNF.
- Clinical chemistry: Serum was noticeably darker in color and serum iron values were higher in animals of the 40 and 450 mg Fe/kg groups. The group receiving 450 mg Fe/kg exhibited increased total cholesterol, AST, bilirubin, P and LDH values. Other parameters for Day 4 and Day 16 samplings in Code 7228 groups were NNF.
- Urinalysis: NNF
- Organ Weights: Increased liver weights in males and females of the 450 mg Fe/kg group at Day 4 and Day 16 necropsies.
- Gross pathology: Dark colored skin, lymph nodes, serosa of intestines, and liver in Day 4 and Day 16 necropsies of animals receiving 40 and 450 mg Fe/kg.
- Histopathology: Accumulation of brown intracytoplasmic pigment of macrophages/Kupffer's cells was observed in liver, spleen, lung, lymph nodes, kidney, thymus, gallbladder, testes, ovary, and choroid plexus of the brain in animals of the 450 mg Fe/kg group on Day 4 and was still present in the Day 16 animals. The pigment stained strongly for the presence of iron.
- Reviewer's Conclusions: The findings in this study are consistent with pharmacological effects of high doses of iron compounds administered intravenously. The incidence is dose related and more prominent in Group 4 (450 mg Fe/kg) and included high levels of serum iron, increased liver weights, and the accumulation of iron positive pigment in Kupffer's cells in multiple organs and tissues. The NOAEL for this study is considered to be 450 mg Fe/kg.

2.6.6.3 Repeat-dose toxicity

Study Title: 13-Week intravenous injection toxicity and toxicokinetic study with ferumoxytol in rats.

Key study findings:

The findings in this study are consistent with pharmacological effects of high doses of iron compounds administered intravenously. The incidence is dose related and more prominent in the 12 mg Fe/kg and included high levels of serum iron, increased liver and spleen weights, and the accumulation of iron positive pigment in macrophages/Kupffer's cells in multiple organs and tissues. Toxicokinetic values increased with dose and with time. The NOAEL for this study is considered to be 12 mg Fe/kg.

Study no.: 6782-114
Volume # and page #: Module 4 in EDR
Laboratory and location: _____

Date of study initiation: September 16, 2004.
In-Life dates: September 27– December 29, 2004.
Report date: September 21, 2007.

GLP compliance: Yes
QA report: Yes
Drug, lot and purity: Lot # 03080501, Purity 100%.
Control solution: Mannitol 44 mg/ml in water for injection.

b(4)

Design: This study was designed to evaluate the toxicity of repeat doses of ferumoxytol when administered daily via the lateral tail vein for 13 weeks. Male and female Sprague-Dawley Crl:CD@BR rats were 6-7 weeks of age and males weighed 163-202 g and females 135-179 g at randomization into the study. Harlan Teklad Certified rodent diet #8728C was available ad libitum and water was supplied by an automatic system. Separate animals were assigned for toxicokinetic studies.

Animals were assigned to dose groups and terminated according to the following table.

Group	Dosage mg Fe/kg	Dose Vol mL/kg	Number Animals		Number sacrificed			
			Male	Female	Day 86		Day 93-94	
					M	F	M	F
Toxicity animals								
1 Control	0	2	10	10	0	0	10	10
2 Low	2	0.67	10	10	0	0	10	10
3 Mid	6	1	10	10	0	0	10	10
4 High	12	2	10	10	0	0	9*	10
Toxicokinetics animals								
5 Control	0	2	9	9	9	9	0	0
6 Low	2	0.67	9	9	9	9	0	0
7 Mid	6	1	9	9	9	9	0	0
8 High	12	2	9	9	9	9	0	0

* On day 31 one male was found dead.

Observations:

Mortality: Twice daily cage check

Clinical signs: Daily

Body weights: Pretest and weekly

Food consumption: Weekly

Ophthalmoscopy: Pretest and just prior to termination

Hematology: RBC, WBC and differential, HGB, HCT, platelet count, reticulocyte count, blood cell morphology, and calculated M:E ratio, MCV, MCH and MCHC

Urinalysis: Appearance, volume, specific gravity, pH, Na, K, Cl. Qualitative protein, glucose, ketones, urobilinogen, bilirubin, blood and microscopic sediment

Coagulation: aPTT and PT

Clinical chemistry: AST, ALT, LDH, GGT, AP, P, Ca, Cl, K, Na, BUN, glucose, creatinine, total protein, albumin, globulin, A/G ratio, cholesterol, triglycerides, serum Fe, total bilirubin, serum protein electrophoresis, percent iron saturation, unsaturated iron binding capacity, total iron binding capacity.

Gross pathology: All animals.

- Organ weights: Adrenals, brain, heart, kidneys, liver lung, ovaries, pituitary, prostate, salivary gland, seminal vesicles, spleen, and testis with epididymis, thymus, thyroid/parathyroid and uterus.
- Tissue preservation: Adrenal (2), brain, cecum, cervix, colon, duodenum, epididymus, esophagus, eye (2), femur/bone marrow, gross lesions, Harderian gland, heart, ileum, injection site, jejunum, kidney (2), larynx, liver, lung/bronchi, lymph node (mesenteric, cervical, popliteal, mandibular), mammary gland (females), optic nerve (2), ovary (2), pancreas, Peyer's patch, pituitary, prostate, rectum, salivary gland [mandibular (2)], salivary gland [parotid (2)], sciatic nerve, seminal vesicle (2), skeletal thigh muscle, skin, spinal cord (3), spleen, sternum/marrow, stifle joint, stomach, testis (2), thymus, thyroid/parathyroid (2), tongue, trachea, urinary bladder, uterus and vagina.
- Histopathology: Gross lesions were examined microscopically for all animals. All collected tissues listed above were examined microscopically for all animals in the control and 12 mg Fe/kg groups.
- Adrenals, liver, spleen, kidneys, lymph nodes, lungs, duodenum, jejunum and ileum were stained with Perl's stain for iron and examined from all animals.
- Toxicokinetics: Animals were maintained for blood drawing on Day 1 and Day 86 to determine toxicokinetic parameters. The first 3 rats/sex/group were bled at 15 and 90 minutes post-dose, the second 3 rats/sex/group at 30 minutes and 3 hours post-dose, and the last 3 rats/sex/group at 60 minutes and 12 hours post-dose. Immediately after the last blood drawing on Day 86, all toxicokinetic animals were euthanized and discarded without necropsy.

Results:

- Mortality:** All animals survived to scheduled termination except one 12 mg Fe/kg male. Cause of death was pyelonephritis, and unrelated to drug treatment.
- Clinical signs:** Thin appearance, discoloration of tail and rough hair coat in the 6 and 12 mg Fe/kg Ferumoxytol groups.
- Body weights:** Reduced (statistically significant) in males and females of 12 mg Fe/kg group.
- Food consumption:** Reduced (statistically significant) in males and females of 12 mg Fe/kg group.
- Ophthalmoscopy:** NNF
- Hematology:** RBC, HGB, HCT, WBC increased in 6 and 12 mg Fe/kg groups with increased neutrophil and monocyte counts.
- Coagulation:** NNF
- Clinical chemistry:** Serum was noticeably darker in color. Animals of the 12 mg Fe/kg group had lower ALT, AST and LDH values, with increased GGT and serum iron values.
- Organ Weights:** Liver and spleen weights increased significantly in males and females of the 6 and 12 mg/kg group.
- Gross pathology:** Dark colored skin, lymph nodes, serosa of intestines, and liver at necropsies of animals in the 6 and 12 mg Fe/kg groups.
- Histopathology:** Microscopic findings included primarily an accumulation of brown pigment in the cytoplasm of macrophages/Kupffer's cells in the adrenals, liver, spleen, kidneys, lung, lymph nodes and GI tract of animals of both sexes in the 6 and 12 mg Fe/kg groups. The brown pigment stained positive for iron.
- Toxicokinetics:** Exposure increased with time and with increasing doses. The increase was greater than dose proportional suggesting an accumulation. Distribution was limited to the plasma and extracellular fractions in tissues of both male and female rats. Differences in values for C_{max} and AUC_{0-12} were not of statistical significance for males and females.

Representative values are shown in the following table.

Group	Dose Level (mg Fe/kg)	Sex	C _{max} (µg/mL)	T _{max} (hr)	AUC ₀₋₁₂ (µg.hr/mL)	t _{1/2} (hr)	CL (mL.hr/kg)	V _{ss} (mL/kg)
Day 1								
6	2	M	35.3	0.25	83.0	1.13	22.8	87.8
		F	27.1	0.25	85.7	1.19	21.3	111
7	6	M	102	0.25	293	2.82	19.7	58.7
		F	110	0.25	282	2.75	20.5	58.9
8	12	M	214	0.25	723	2.83	15.8	50.7
		F	277	0.25	643	2.62	18.0	50.3
Day 86								
6	2	M	39.0	0.25	96.5	1.43	20.0	79.2
		F	34.9	0.25	84.5	1.43	22.6	106
7	6	M	173	0.25	511	3.18	11.0	40.9
		F	131	0.25	359	3.27	15.6	56.4
8	12	M	344	0.50	1431	5.10	7.12	45.0
		F	334	0.25	975	4.18	10.8	57.6

Reviewer's Conclusions:

The findings in this study are consistent with pharmacological effects of high doses of iron compounds administered intravenously. The incidence is dose related and more prominent in the 12 mg Fe/kg group and included high levels of serum iron, increased liver and spleen weights, and the accumulation of iron positive pigment in macrophages/Kupffer's cells in multiple organs and tissues. Ferumoxytol is distributed in plasma and extracellular fractions. The NOAEL for this study is 12 mg Fe/kg/day for 93 consecutive daily doses. The cumulative dose is 1,116 mg Fe/kg.

Study Title: 13-week intravenous toxicity and toxicokinetic study with Ferumoxytol in dogs.

Key study findings:

The findings in this study are consistent with pharmacological effects of high doses of iron compounds administered intravenously. The incidence is dose related and more prominent in the 12 mg Fe/kg group and included high levels of serum iron, increased liver weights, and the accumulation of iron positive pigment in macrophages/Kupffer's cells in multiple organs and tissues. The NOAEL for this study is 12 mg Fe/kg/day for 93 days which is a cumulative dose of 1,116 mg Fe/kg.

Study no.: 6782-115
Volume # and page #: Module 4 in EDR
Laboratory and location: _____

b(4)

Date of study initiation: September 7, 2004
In-Life dates: September 21 – December 23, 2004
Report date: September 21, 2007

GLP compliance: Yes
QA report: Yes
Drug, lot and purity: Lot # 03080501, Purity 100%.

Design: This study was designed to evaluate the 13-week toxicity of repeated daily doses of Ferumoxytol when administered via the cephalic vein to male and female purebred Beagle dogs from _____ . Study animals were 4-5 months of age and males weighed 5.6 - 7.8 kg and females 5.1 - 6.5 kg at randomization into the study. Harlan Teklad Certified Canine Diet #8727C was available ad libitum and water was supplied by an automatic system.

b(4)

Animals were assigned to dose groups and terminated according to the following table.

Group	Dosage mg Fe/kg	Dose Vol mL/kg	Number Animals		Number sacrificed	
			Male	Female	Day 93 - 94	
					M	F
1 Control	0	0.4	3	3	3	3
2 Low	2	0.067	3	3	3	3
3 Mid	6	0.2	3	3	3	3
4 High	12	0.4	3	3	3	3

Observations:

Mortality: Twice daily cage check

Clinical signs: Daily

Body weights: Pretest and weekly

Food consumption: Weekly

Ophthalmoscopy: Pretest, 1 hour after first dose and just prior to termination

Electrocardiogram: 10 lead pretest, week 8 and week 14, conducted and evaluated staff veterinarian.

Physical and Neurobehavioral Examinations: Prior to treatment and 1 hr after first dose on Day 1 by staff veterinarian and included respiration rate, rectal temperature, evaluation of reflexes, gait, responsiveness and papillary reflex.

Hematology: Pretest, week 8 and 14 for RBC, WBC and differential, HGB, HCT, platelet count, reticulocytes count, blood cell morphology, and calculated M:E ratio, MCV, MCH and MCHC

- Coagulation: aPTT and PT
- Clinical chemistry: AST, ALT, LDH, GGT, AP, P, Ca, Cl, K, Na, BUN, glucose, creatinine, total protein, albumin, globulin, A/G ratio, cholesterol, triglycerides, serum Fe, total bilirubin, serum protein electrophoresis, percent iron saturation, unsaturated iron binding capacity, and total iron binding capacity.
- Urinalysis: Appearance, volume, specific gravity, pH, Na, K, Cl. Qualitative protein, glucose, ketones, bilirubin, urobilinogen, blood and microscopic sediment
- Gross pathology: Tissues and organs were examined and retained in fixative for all animals.
- Organ weights: Adrenals, brain, heart, kidneys, liver, lung, ovaries, pituitary, prostate, salivary gland, seminal vesicles, spleen, and testis with epididymis, thymus, thyroid/parathyroid and uterus.
- Tissue preservation: Adrenal (2), brain, cecum, cervix, colon, duodenum, epididymus, esophagus, eye (2), femur/bone marrow, gallbladder, gross lesions, Harderian gland, heart, ileum, injection site, jejunum, kidney (2), larynx, liver, lung/bronchi, lymph node (mesenteric, cervical, popliteal, mandibular), mammary gland (females), optic nerve (2), ovary (2), pancreas, Peyer's patch, pituitary, prostate, rectum, salivary gland [mandibular (2)], salivary gland [parotid (2)], sciatic nerve, seminal vesicle (2), skeletal thigh muscle, skin, spinal cord (3), spleen, sternum/marrow, stifle joint, stomach, testis (2), thymus, thyroid/parathyroid (2), tongue, trachea, urinary bladder, uterus and vagina.
- Histopathology: All collected tissues were stained with H & E and examined microscopically for all animals. In addition, adrenals, liver, spleen, kidneys, lymph nodes, lungs, duodenum, jejunum and ileum were stained with Perl's stain for iron and examined from all animals.
- Toxicokinetics: Blood samples from all animals were drawn at 0.25, 1, 4, 8, 12 and 24 hours after dosing on Day 1 and Day 86 to determine toxicokinetic parameters.

Results:

<u>Mortality:</u>	All animals survived to scheduled termination.
<u>Clinical signs:</u>	Brown discoloration in mucous membranes, especially gums of animals in the 6 and 12 mg Fe/kg groups after day 70.
<u>Physical Exams:</u>	NNF (No Noteworthy Findings)
<u>Neurobehavioral:</u>	NNF
<u>Body weights:</u>	NNF
<u>Food consumption:</u>	NNF
<u>Ophthalmoscopy:</u>	Yellow to brown discoloration of sclera and palpebral conjunctiva in all 12 mg Fe/kg animals
<u>Electrocardiogram:</u>	NNF for heart rate and waveforms.
<u>Hematology:</u>	NNF
<u>Coagulation:</u>	NNF
<u>Clinical chemistry:</u>	All parameters NNF except for serum which was noticeably darker in color and serum iron values were higher in animals of the 6 and 12 mg Fe/kg groups.
<u>Urinalysis:</u>	NNF
<u>Organ Weights:</u>	Increased liver weights in males and females of the 12 mg Fe/kg group.
<u>Gross pathology:</u>	Dark colored lymph nodes, serosa of intestines, and liver in 12 mg Fe/kg.
<u>Histopathology:</u>	Accumulation of brown intracytoplasmic pigment of macrophages/Kupffer's cells was observed primarily in liver, spleen and lymph nodes of the all animals receiving ferumoxytol. The intensity and frequency was dose related and increased with increasing dose. The pigment stained strongly for the presence of iron. There were no degenerative changes observed that were associated with the pigment in any organ or tissue.

Toxicokinetics: Exposure increased with time and with increasing doses. The increase was greater than dose proportional suggesting an accumulation. Distribution was limited to the plasma and extracellular fractions in tissues of both male and female rats. Values for C_{max} and AUC_{0-24} were not statistically different between males and females.

Representative values are shown in the following table.

Group	Dose Level (mg Fe/kg)	Sex	C_{max} ($\mu\text{g/mL}$)	T_{max} (hr)	AUC_{0-12} ($\mu\text{g}\cdot\text{hr/mL}$)	$t_{1/2}$ (hr)	CL ($\text{mL}\cdot\text{hr/kg}$)	V_{ss} (mL/kg)
Day 1								
2	2	M	25.0	0.25	115	1.55	17	168
		F	23.5	0.25	106	1.47	19	184
3	6	M	93.7	0.25	266	1.78	22	118
		F	83.7	0.25	227	1.28	26	139
4	12	M	195	0.25	619	2.71	19	72
		F	159	0.50	564	2.47	21	77
Day 86								
2	2	M	31.7	0.25	131	1.44	15.3	146
		F	34.1	0.25	125	1.40	16.1	135
3	6	M	82.4	0.50	318	3.45	19.5	105
		F	76.6	0.25	278	3.75	22.6	149
4	12	M	151	0.50	632	4.31	19.2	118
		F	219	0.25	799	3.19	15.6	77

Reviewer's Conclusions:

The findings in this study are consistent with pharmacological effects of which doses of iron compounds administered intravenously. The incidence is dose related and more prominent in the 12 mg Fe/kg group and included increased liver weights, and the accumulation of iron positive pigment in Kupffer's cells in liver, spleen and intestinal serosa. The NOAEL for this study is considered to be repeated doses of 12 mg Fe/kg/day for 13 weeks.

Tissues evaluated in the repeat dose toxicity studies are shown in the table on the following page.

Histopathology inventory:

Study	Single dose		13-week	
	Rat	Dog	Rat	Dog
Adrenals	X*	X*	X*	X*
Aorta	x	x	x	
Bone (femur, marrow)	x	x	x	x
Brain	X*	X*	X*	X*
Cecum	x	x	x	x
Colon	x	x	x	x
Duodenum	x	x	x	x
Epididymis	x	x	x	x
Esophagus	x	x	x	x
Eye/optic nerve	x	x	x	x
Gall bladder		x		x
Gross lesions	x	x	x	x
Harderian gland	x	x	x	x
Heart	X*	X*	X*	X*
Ileum	x	x	x	x
Jejunum	x	x	x	x
Kidneys	X*	X*	X*	X*
Larynx	x	x	x	x
Liver	X*	X*	X*	X*
Lungs	X*	X*	X*	X*
Lymph nodes	x	x	x	x
Mammary Gland	x	x	x	x
Ovaries	X*	X*	X*	X*
Pancreas	x	x	x	x
Peripheral nerve	x	x	x	x
Pituitary	X*	X*	X*	X*
Prostate	X*	X*	X*	X*
Salivary gland	X*	X*	X*	X*
Sciatic nerve	x	x	x	x
Seminal vesicles	X*	X*	X*	X*
Skeletal muscle	x	x	x	x
Skin	x	x	x	x
Spinal cord	x	x	x	x
Spleen	X*	X*	X*	X*
Sternum	x	x	x	x
Stomach	x	x	x	x
Testes	X*	X*	X*	X*
Thymus	X*	X*	X*	X*
Thyroid/parathyroid	X*	X*	X*	X*
Trachea	x	x	x	x
Urinary bladder	x	x	x	x
Uterus	X*	X*	X*	X*
Vagina	x	x	x	x

X, histopathology performed
 *, organ weight obtained

2.6.6.4 Genetic toxicology

Study title: Mutagenic test with Code 7228 in Salmonella and E. coli/mammalian-Microsome reverse mutation assay with confirmatory assay

Key findings: Ferumoxytol (Code 7228) was negative for inducing reverse mutations based on assay criteria at concentrations of 100-5000 ug Fe/plate, with and without metabolic activation.

Study no.: 19860-0-409-OECD
Volume and page: Electronic submission in EDR
Conducting laboratory: _____
Date of study initiation: August 21, 1998
Experimental start date: August 28, 1998
Experimental complete date: September 17, 1998
Report date: November 30, 1998
GLP compliance: Yes
QA report: Yes
Drug: Lot # 98080401, Purity 100%:

Methods

Strains/species/cell line: S. typhimurium: TA98, TA100, TA1535, TA1537
E. coli: WP2uvrA

Doses used in definitive study: 3 plates/dose at doses of 100, 333, 1000, 3330, 5000 ug/plate, with and without Aroclor induced S9 rat liver enzyme.

Basis of dose selection: Preliminary assay. Ten doses ranging from 6.67 – 5000 ug/plate were tested in the presence and absence of S9 mix.

Negative controls: Mannitol 44 mg/mL in water for IV injection

Positive controls: benzo(a)pyrene, 2-nitrofluorene, 2-aminoanthracene, sodium azide, ICR-191, 4-nitroquinoline-N-oxide.

Incubation and sampling times: 24-52.4 hours

Results:

Study validity : The study is valid and followed the protocol that incorporated all of the criteria necessary for a valid study. Five doses were included in the confirmatory study with 3 plates per dose in the presence and absence of an exogenous metabolic activation system of mammalian microsomal enzymes derived from Aroclor-induced rat liver S9 mix. Positive control materials induced reverse mutations as expected.

Study outcome: Interpretation is based on the generally accepted criteria described in the study protocol defining a positive test. The results of the *S. typhimurium*/*E. coli* mammalian reverse mutation assay with confirmatory assay indicate that under the conditions of this study, Code 7228 did not cause a significant increase in the number of revertants per plate of any of the tester strains either in the presence or absence of S9 mix microsomal enzymes including the top dose of 5000 ug Fe/plate. Code 7228 is considered negative in this study

Study title: Mutagenic test on Code 7228 measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells.

Key findings: Ferumoxytol (Code 7228) was negative for inducing chromosomal aberrations in CHO cells based on assay criteria at concentrations of 34.9-5100 ug Fe/mL, with and without metabolic activation.

Study no.: 19860-0-437-OECD
Volume and page: Electronic submission in EDR
Conducting laboratory: _____

b(4)

Date of study initiation: August 21, 1998
Experimental start date: September 03, 1998
Experimental complete date: November 5, 1998

Report date: November 24, 1998
GLP compliance: Yes
QA report: Yes
Drug: Lot # 98080401, Purity 100%:

Methods

<u>Strains/species/cell line:</u>	Chinese hamster ovary cells (CHO-WBL) from the permanent cell line of Dr. S. Wolff, University of California, San Francisco. The cell line has average cycle time of 12-14 hours with a modal chromosome number of 21.
<u>Basis of dose selection:</u>	Preliminary assay, replicates of 15 doses ranging from 34.9 – 5100 ug Fe/mL were tested in the presence and absence of S9 mix.
<u>Doses used in definitive study:</u>	3 replicates/concentration at doses ranging from 34.9 – 5100 ug Fe/mL were incubated for 3 hours with cultures harvested at 20 hours. The doses of 1750, 2500, 3750 and 5100 ug Fe/mL, with and without Aroclor induced S9 rat liver enzyme were analyzed for chromosomal aberrations.
<u>Negative controls:</u>	Mannitol 44 mg/mL
<u>Positive controls:</u>	Mitomycin C and cyclophosphamid. Cells arrested in metaphase with Colcemid
<u>Incubation and sampling times:</u>	Treatment period of 3 hours with cells harvested after 20 hours.

Results:

Study validity : The study is valid and followed the protocol that incorporated all of the criteria necessary for a valid study. Four doses were included in the confirmatory study with 3 plates per dose in the presence and absence of an exogenous metabolic activation system of mammalian microsomal enzymes derived from Aerochlor-induced rat liver S9 mix. Positive control materials induced chromosomal aberrations as expected.

Study outcome: Interpretation is based on the generally accepted criteria described in the study protocol defining a positive test. The results of the Chinese Hamster Ovary chromosomal aberration assay with confirmatory assay indicate that under the conditions of this study, Code 7228 did not cause a significant increase in the number of cells with chromosomal aberrations, polyploidy or endoreduplication either in the presence or absence of S9 mix, including the top dose of 5100 ug Fe/mL. Code 7228 is considered negative for inducing chromosomal aberrations in this study

Study title: Mutagenic test with Ferumoxytol (Code 7228) in the in vivo mouse micronucleus assay.

Key study findings: Ferumoxytol was not clastogenic in mouse bone marrow cells.

Study no.: 19860-0-455-OECD
Report date: December 10, 1998
Report Location: Module #4 in EDR

Conducting laboratory and location: _____

b(4)

Date of study initiation: August 21, 1998
In-Life Dates: September 1 – October 7, 1998
GLP compliance: Yes
QA report: Yes
Drug lot # 98080401
Purity: Assumed to be 100%.

Design: This study was designed to assess the ability of Code 7228 to induce in vivo clastogenic effects by detecting micronuclei in polychromatic erythrocyte stem cells in mouse bone marrow. Test animals were described as adult male and female mice CRL:CD-1(ICR)BR from ' _____ They were 7-8 weeks old and weighed 23-38 grams. Three male and three female mice were assigned to each dose group in the preliminary screen with 6 males assigned to each dose group at each time point in the definitive study.

b(4)

In the preliminary screen, Code 7228 was dosed by intravenous injection at dose levels of 0, 200, 500, 800, 1000 or 1500 mg Fe/kg body weight. The dose of 1500 mg Fe/kg was selected as the maximum tolerated dose and used as the top dose in the definitive study.

In the definitive study, 12 male mice received mannitol at 44 mg/mL in sterile water for injection, which served as negative control vehicle. Six mice received cyclophosphamide at 60 mg/kg and volume of 10 mL/kg by oral intubation which served as positive control. Three groups with 12 male mice each received Code 7228 at 375, 750 or 1500 mg Fe/kg and 6 mice from each group were serially sacrificed at 24 and 48 hrs after treatment. The positive control cyclophosphamide group was sacrificed at 24 hrs after treatment. All intravenous injections of negative control and Code 7228 were at the volume of 50 mL/kg.

Mice were euthanized at 24 or 48 hours after dosing for extraction of the bone marrow. Femur bone marrow smears were prepared and stained with May-Gruenwald and Giemsa solutions. The stained slides were examined for the presence of micronuclei in 1000 erythrocytes and the ratio of polychromatic to normochromatic erythrocytes calculated.

The criteria for a positive response consisted of a statistically significant increase in micronucleated polychromatic erythrocytes in at least one Code 7228 treated group.

Results: All animals survived in the prescreen study including the top dose of 1500 mg Fe/kg. Some animals of both sexes in the 1000 and all mice in the 1500 mg Fe/kg groups exhibited hypoactivity and lethargy during the first 2 hrs after dosing and rough hair coat for 24 hours after dosing. All animals at the 1500 mg Fe/kg and some at 1000 mg Fe/kg exhibited orange to brown discoloration of the ears, eyelids, tail and digits resulting from systemic distribution of the drug.

In the definitive study, all vehicle and positive control animals appeared normal throughout the study observation period. All mice in the 1500 mg Fe/kg group exhibited hypoactivity and lethargy during the first 2 hrs after dosing and rough hair coat for 24 hours. All animals at the 1500 mg Fe/kg exhibited orange to brown discoloration of the ears, eyelids, tail and digits resulting from systemic distribution of the drug.

Code 7228 did not induced significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls at any harvest time ($p \leq 0.05$, Kastenbaum-Bowman Tables). There was no statistically significant dose related increase in the incidence of micronucleated polychromatic erythrocytes in male mice at doses of 0, 375, 750 or 1500 mg Fe/kg at the 24 or 48 hour collection periods and the positive control, cyclophosphamide, produced statistically significant increases in micronucleated polychromatic erythrocytes ($p \leq 0.05$, Kastenbaum-Bowman Tables).

Reviewer's Conclusion: The results indicate that Code 7228 (Ferumoxytol) did not induce a statistically significant increase in micronucleated polychromatic erythrocytes in mouse bone marrow and therefore is not considered clastogenic in the in vivo mouse micronucleus test. Code 7228 (Ferumoxytol) is negative in this study.

2.6.6.5 Carcinogenicity

Studies of 104-week duration are underway in rats and mice.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Study of fertility and early embryonic development to implantation in rats

Key study findings: 18 mg Fe /kg (135 X MHD for males and 45 X MHD for females) of Code 7228 is the NOAEL for toxicity, male and female fertility, reproductive performance and fetal effects.

Study No.: 6782-113
Report Date: July 21, 2003
Report Location: Module #4 in EDR

Conducting laboratory: _____
Date of study initiation: December 17, 2002
In-Life Dates: December 27, 2002 – March 14, 2003

GLP compliance: Yes
QA report: Yes
Drug: Lot # 01062601, purity assumed 100%

Design: This study was designed to assess the effects of Code 7228 on fertility and general reproductive performance on male and female rats. Males were treated for 28 days prior to cohabitation and for a total period of 10 weeks. Females were treated 14 days prior to cohabitation and through presumed gestation day 7 and then sacrificed for caesarean sectioning. Male and female rats were treated daily by IV injection and assigned to respective groups according to the following design.

GROUP	1		2		3		4	
COMPOUND	Mannitol Control (44.4 mg/mL)		Ferumoxytol®		Ferumoxytol®		Ferumoxytol®	
DOSE (mg Fe/kg/day)	0.0		6		12		18	
VOLUME (mL/kg)	3.0		3.0		3.0		3.0	
No. Animals/Group	20 M	20 F	20 M	20 F	20 M	20 F	20 M	20 F
Cumulative Dose (mg Fe/kg)	0	0	450	150	900	300	1350	450

Eighty Sprague Dawley, ~~_____~~ Crl:CD(SD)IGS BR male rats from ~~_____~~ weighing 267-324 g and 19 weeks of age, and the same number, age and strain of female rats weighing 193 - 254 g at the time of cohabitation, were equally distributed among 4 treatment groups, and administered daily intravenous doses via the tail vein.

Dosages used were 0 (Mannitol Control), 6, 12 and 18 mg Fe/kg/day. All dose concentrations were adjusted to provide the appropriate amount of material in 3.0 mL/kg/dose. All doses were injected at a rate of 1-2 mL/minute.

Males were treated 28 days prior to mating, during two 21-day cohabitation periods for a minimum of 70 days. Females were treated from 14 days prior to cohabitation, a maximum of two 21 day cohabitation periods, and through day 7 of presumed gestation for a maximum total of 63 days.

Survival and clinical signs were recorded daily.

Body weights and food intake were recorded twice weekly for the males. Female body weights and food intake were recorded twice weekly until they were impregnated, and thereafter they were weighed on days 0, 7 and 13 of presumed gestation.

At the end of the 70-day treatment period the males were sacrificed by CO₂ asphyxiation and reproductive organs including prostate, left testis, left epididymus and left vas deferens along with any gross lesions retained in fixative. For each male, the right deferens was dissected and immediately used for sperm motility assessments. The right epididymus was frozen on dry ice and subsequently used for the determination of sperm count and sperm morphology.

On day 13 of presumed gestation, the females of each group were sacrificed by CO₂ asphyxiation and uterine contents examined. The thoracic, abdominal and pelvic viscera were examined and gross lesions along with ovaries and uterus preserved in formalin.

The number and distribution of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses were recorded. All fetuses were counted, examined externally and euthanized by intraperitoneal injection of Euthasol[®] and discarded. Indices for pre-implantation loss, post-implantation loss, male fertility and female fertility were calculated.

Results: All animals survived to scheduled termination except 1 female in the Control group whose pregnancy was mistimed and delivered early, and the animal was immediately sacrificed. With the exception of the 1 female that delivered early, there were no clinical signs observed in any group of parent animals, except rough hair coat and orange to dark brown pigmentation of tail, skin, ears, and extremities of animals in the 12 and 18 mg Fe/kg/day groups.

There were no drug or dose related effects on body weight, body weight gains or food intake for any animals of any group.

There were no drug or dose related effects seen at gross necropsy for males or females of any of the Code 7228 treated groups when compared to mannitol control group animals.

Summary of female and litter reproductive parameters.

PARAMETER	TREATMENT GROUP			
	1 Control Mannitol 44 mg/mL	2 Code 7228 6 mg Fe/kg	3 Code 7228 12 mg Fe/kg	4 Code 7228 18 mg Fe/kg
M/F Assigned	20/20	20/20	20/20	20/20
Dam Deaths	1	0	0	0
No. Pregnant	17	18	20	19
No. Delivered Early (Mis-timed pregnancy)	1	0	0	1
Total Live Fetuses	220	286	310	280
Mean Litter Corpora Lutea	16.3	17.3	16.8	17.6
Live Litters (#)	16	18	20	19
Mean Litter Implants	14.5	16.4	16.4	15.5
Mean Litter Resorption	0.8	0.6	0.8	0.8
Mean Fetal Death	0	0	0	0
Mean Litter Live Pups	13.8	15.9	15.5	14.7
Pre-implantation Loss (%)	11.7	9.3	9.7	8.6
Post-implantation Loss (%)	6.4	3.4	5.2	5.0
Female Fertility Index	85	90	100	100

The number and distribution of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses were unaffected by treatment. Indices for pre-implantation loss, post-implantation loss, male fertility and female fertility were also unaffected by treatment.

When male rats were administered 6, 12 or 18 mg Fe/kg/day for at least 70 days, there were no drug or dose related effects that were observed in any group on sperm motility, caudal epididymal sperm count or sperm morphology when compared to control groups.

A summary of sperm analysis parameters is shown in the table below.

GROUP	1	2	3	4
COMPOUND	Mannitol Control (44 mg/mL)	Code 7228	Code 7228	Code 7228
DOSE (mg Fe/kg)	0.0	6.0	12.0	18.0
VOLUME (mL/kg)	3.0	3.0	3.0	3.0
No. Males Examined	20	20	20	20
Motility (%)	89	94	86	93
Epididymal Count (10 ⁶ Sperm/Gram)	921.5	860.6	960.9	906.0
Sperm Morphology (% Abnormal)	1.1	0.9	1.1	1.0
Male Fertility Index	85	90	100	100

Report Conclusion: The NOAEL for general toxicity, male and female fertility, and reproductive performance is the highest dose given at 18 mg Fe/kg/day (29 X MHD BSA for males and 10 X MHD BSA for females). The NOAEL for fetal effects is also 18 mg Fe/kg/day.

Reviewer's Comment: Agree.

APPEARS THIS WAY
ON ORIGINAL

Embryofetal development

Study title: Rat developmental toxicity study with Code 7228 (SEG II)

Key study findings: The NOAEL for maternal toxicity is 10 mg Fe/kg/day, with decreased food intake, body weight and body weight gain observed in the two top dose groups. The NOAEL for reproductive function in dams is 100 mg Fe/kg/day. The NOAEL for embryo-fetal development of offspring is 31.6 mg Fe/kg/day.

Study no.: 6782-111
Report Date: January 27, 2002
Report Location: Module #4 in EDR
Conducting laboratory: _____
Date of study initiation: July 26, 2001
In-Life Dates: August 3 - 24, 2001
GLP compliance: Yes
QA reports: Yes
Drug: lot # 99042101, purity assumed 100%.

b(4)

Design: This study was designed to assess the maternal and fetal effects of Code 7228 (Ferumoxytol) when administered to pregnant rats. One-hundred-twenty predated female _____ Sprague Dawley rats (CrI:CD@[SD]) from _____ weighing 189-264 g and 11-12 weeks of age, were randomly distributed among 4 treatment groups according to the following design.

b(4)

Group	Treatment	Dose (mg Fe/kg)	Dose Volume (mL/kg/day)	Females Assigned
1	Mannitol Control, 44 mg/mL	0	3.33	25
2	Code 7228	10	0.33	25
3	Code 7228	31.6	1.05	25
4	Code 7228	100	3.33	25

Females were administered daily intravenous doses of either Mannitol IV water at 44 mg/mL (Control), or Code 7228 on days 6-17 of presumed gestation. Dosages of 0, 10, 31.6 and 100 mg Fe/kg/day of Code 7228 were used. Dosages were selected based on results of a range finding study where doses of 6, 18, 50 and 100 mg Fe/kg were given. In the range finding study, maternal toxicity was observed in the 50 and 100 mg Fe/kg groups and all rats survived in the top dose group, which was considered the maximum tolerated dose.

All rats in this definitive study were observed daily for survival and clinical signs. Body weights were recorded on Days 0, 2, 4, 6, 8, 10, 14, 19, and 20 of presumed gestation.

Feed consumption values were recorded for the same intervals. Dams were sacrificed by lethal injection on day 20 of presumed gestation. The thoracic, abdominal and pelvic viscera were examined and gross lesions were preserved in formalin. The number and distribution of corpora lutea, implantations, early and late resorptions, and live and dead fetuses were recorded. All fetuses were weighed and evaluated for external alterations and sex recorded. Half of the fetuses were fixed in Bouin's fixative for visceral examination and the other half were fixed in isopropyl alcohol and stained with Alizarin red S for skeletal evaluation. Indices for pre-implantation loss and post-implantation loss were calculated.

Results: This study was initiated with 25 presumed pregnant dams per group. There were no deaths or premature deliveries in any of the groups.

Clinical signs in the dams included orange to dark brown pigmentation of tail, ears and extremities in the 31.6 and 100 mg Fe/kg groups. This is considered a result of the intravenous distribution of the drug.

The NOAEL for maternal toxicity in pregnant dams was considered to be 10 mg Fe/kg/day, with the effects of decreased food intake, body weight and body weight gain observed in the two top dose groups.

Even with the maternal toxicity observed in the dams of the two top dose groups, the only fetal effects observed were insignificantly decreased fetal body weights in the 100 mg Fe/kg group, and an increase in the delayed ossification of ribs and sternebra in the 31.6 and 100 mg Fe/kg groups. There was no effect on numbers of corpora lutea, implants, resorptions, live and dead fetuses, external, visceral and skeletal alterations. Pre-implantation loss and post-implantation loss values were unaffected by treatment when compared to controls.

Results of the study are shown in the following summary table.

PARAMETER	Treatment Groups			
	Mannitol Control	Code 7228 10 mg Fe/kg	Code 7228 31.6 mg Fe/kg	Code 7228 100 mg Fe/kg
Dams Assigned	25	25	25	25
Dams Died	0	0	0	0
Aborted	0	0	0	0
Pregnancies (%)	25 (100)	25 (100)	25 (100)	25 (100)
Live Litters	25	25	25	25
Corpora Lutea Mean Litter	15.2	16.0	15.9	15.1
Implants Mean Litter	13.5	13.9	14.3	12.8
Total Live Fetuses	324	336	342	300
Live Fetuses Mean Litter	13.0	13.4	13.7	12.0
Total Resorbed Fetuses	13	12	15	21
Total Dead Fetuses	0	0	0	0
Pre-Implant Loss (%) ^A	11.4	12.7	19.6	14.5
Post-implant loss (%) ^B	3.8	3.5	3.9	6.7
Gravid Uterine Weights (g)	77.4	78.8	79.5	67.8 ^C
Fetal Wts (M/F)	3.80/3.63	3.73/3.57	3.81/3.55	3.61/3.43
Sex Ratio (M/F)	0.98	1.02	0.98	1.04
Corrected dam wt. (g)	303.4	298.0	296.3	270.3 ^D
Soft Tissue Malformations	1	1	0	1
Skeletal Variations	121	114	118	109
Skeletal Malformations	0	0	1	1

^APre-implant Loss: No. of Corpora Lutea - No. Implants/No. Corpora Lutea x 100

^B Post-implant Loss: Number Implants- Number of Live Fetuses/Number Implants x 100

^C Significant difference at P≤0.05.

^D Significant difference at P≤0.01.

Reviewer's Conclusion: The NOAEL for general toxicity in the dams is 10 mg Fe/kg/day with decreased food intake, body weights and body weight gains in the two top dose groups receiving Code 7228. The NOAEL for reproductive function in dams is 100 mg Fe/kg/day. The NOAEL for embryo-fetal development of offspring is 31.6 mg Fe/kg/day.

Study title: Rabbit Developmental Toxicity Study with Code 7228 (SEG II)

Key study findings: The NOAEL for reproductive function in dams and for embryo-fetal development of offspring is 16.5 mg Fe/kg/ day for 14 days.

Study no.: 6782-110
Report Date: June 27, 2002
Report Location: Module #4 in EDR

b(4)

Conducting laboratory: _____
Date of study initiation: July 9, 2001
In-Life Dates: July 10 – August 9, 2001

GLP compliance: Yes
QA reports: Yes
Drug Lot: #9904210104, Purity assumed 100%.

Design: This study was designed to assess the maternal and fetal effects of Code 7228 (Ferumoxytol) when administered to pregnant rabbits. Rabbits were supplied by _____
 _____ Eighty-eight predated New Zealand White,
 — (NZW)SPF, female rabbits weighing 3.0-4.1 kg and 6 - 9 months old, were randomly assigned to groups according to the following design.

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Group	Treatment	Dose (mgFe/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg/day)	Number Females
1	Control Mannitol 44 mg/mL	0	0	1.51	20
2	Ferumoxytol	6	0.1	0.2	20
3	Ferumoxytol	16.5	0.5	0.55	20
4	Ferumoxytol	45.3	2.5	1.51	20

Females were administered daily intravenous doses of either Mannitol, 44 mg/mL in water for injection (control), or Ferumoxytol on days 7-20 of presumed gestation. Dosages of 0, 6, 16.5 and 45.3 mg Fe/kg/day were used.

Dosages were selected based on results of a pilot rabbit study with doses of 6, 18, 50 and 100 mg Fe/kg/day. The highest dose of 100 mg Fe/kg/day exceeded the minimum lethal dose with maternal deaths. The dose of 50 mg Fe/kg/day exceeded the maximum tolerated dose with abortions and significantly decreased dam body weight. The lowest dose of 6 mg Fe/kg/day was the maternal and fetal NOAEL.

All rabbits were observed daily for survival and clinical signs. Body weights were recorded on Days 0, 2, 6, 8, 10, 14, 19, 20, 22, 24 and 29 of presumed gestation. Feed consumption values were recorded for the same intervals. Dams were sacrificed by lethal injection on day 29 of presumed gestation. The thoracic, abdominal and pelvic viscera were examined and gross lesions were preserved in formalin. The number and distribution of corpora lutea, implantations, early and late resorptions, and live and dead fetuses were recorded. All fetuses were weighed and evaluated for external, visceral and skeletal alterations and sex recorded. Indices for pre-implantation loss and post-implantation loss were calculated.

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Results: Caesarean section parameters are shown in the following summary table.

PARAMETER	Treatment			
	Mannitol Control	Ferumoxytol 6 mg Fe/kg	Ferumoxytol 16.5 mg Fe/kg	Ferumoxytol 45.3 mg Fe/kg
Dams Assigned	20	20	20	20
Dams Died	0	0	0	0
Aborted	0	0	0	2
Pregnancies (%)	19 (95)	20 (100)	20 (100)	20 (100)
Live Litters	19	19	20	16
Litters All Dead or Resorbed	0	0	0	2
Corpora Lutea Mean Litter	11.3	11.0	10.6	11.6
Implants Mean Litter	8.7	8.7	8.8	8.9
Total Live Fetuses	156	162	169	130
Live Fetuses Mean Litter	8.2	8.1	8.4	8.1
Total Resorbed Fetuses	10	12	6	9
Total Dead Fetuses	0	0	0	3
Pre-Implant Loss (%) ^A	22.5	19.2	14.8	21.6
Post-implant loss (%) ^B	5.1	10.6	3.0	7.9
Gravid Uterine Weights (g)	494	461	504	458
Fetal Wts (M/F)	40.9/40.0	38.1/37.9	40.4/39.9	35.2/34.5
Sex Ratio (M/F)	0.82	0.96	1.12	0.89
Corrected DamWts (kg)	3.53	3.48	3.57	3.57
External Malformations	0	0	0	9
Soft Tissue Malformations	0	0	0	7
Skeletal Variations	124	127	141	101
Skeletal Malformations	6	1	2	2

^APre-implant Loss: No. of Corpora Lutea – No. Implants/No. Corpora Lutea x 100

^B Post-implant Loss: Number of Implants- Number of Live Fetuses/Number Implants x 100

The study was initiated with 20 presumed pregnant dams per group and there were no maternal deaths during the study. Two premature deliveries occurred in the 45.3 mg Fe/kg group during the treatment period. There was a decrease in body weights and food intake noted prior to the aborted delivery by both dams. The dose of 45.3 mg Fe/kg exceeded the maternal maximum tolerated dose.

Maternal body weights, body weight gains and food intake were reduced in the top dose group of 43 mg Fe/kg, but were unaffected in the 6 and 16.5 mg Fe/kg groups.

There were no treatment related effects on pregnancy rates or number of live litters in pregnant dams of the 6 and 16.5 mg Fe/kg groups.

For the 6 and 16.5 mg Fe/kg groups:

Numbers of corpora lutea, implant sites, live and dead fetuses, and resorption sites were unaffected by treatment. Rates of pre-implant loss and post-implant loss were similar with no evidence of drug or dose related effects.

Fetal and gravid uterine weights were unaffected by treatment as were group fetal sex ratios.

The incidence of variations and malformations in these 2 groups was comparable to control values.

In the 43 mg Fe/kg group:

This dose exceeded the maximum tolerated dose and was maternally toxic as evidenced by decreased body weight, body weight gain and food intake.

Two dams aborted and 2 other dams had all fetuses dead or resorbed.

As compared to the control group, there was an increase in the number of external and soft tissue malformations observed.

These fetal effects observed in this top dose group are not considered a direct fetal effect but rather a consequence of maternal toxicity.

Reviewer's Conclusion: Groups of presumed pregnant female rabbits were administered Code 7228 at daily doses of 0, 6, 16.5, or 43.5 mg Fe/kg on days 7-20 of presumed gestation. The NOAEL for maternal toxicity, female reproductive performance and embryo-fetal development of offspring is 16.5 mg Fe/kg/day for 14 days.

2.6.6.7 Local tolerance

Study title: Acute Intravenous/Perivenous/Intraarterial tolerance study with Code 7228 (Ferumoxytol) in rabbits.

Key study findings: Code 7228 (Ferumoxytol) did not produce a tissue response following intravenous, perivascular or intraarterial injection in rabbits.

Study no.:	6782-105
Report Date:	January 21, 1999
Report Location:	EDR, Module #4
Conducting laboratory and location:	_____
Date of study initiation:	September 18, 1998
In-Life Dates:	September 21 – October 5, 1998
GLP compliance:	Yes
QA reports:	Yes
Drug:	Lot # 98080401, Purity assumed 100%.

b(4)

Design: This study was designed to assess the intravenous, perivascular and intraarterial potential of Code 7228 to induce irritation and tissue responses. Groups of 6 male New Zealand White rabbits received either an intravenous injection of 0.42 mL/kg, perivenous injection of 0.1 mL/kg or intraarterial injection of 0.42 mL/kg of Code 7228 containing 30 mg Fe/mL. Control groups received injections of vehicle material containing 44 mg/mL of mannitol in the right ear of each animal to serve as control injection. Code 7228 was injected into either the marginal ear vein, central ear artery or adjacent to marginal ear vein.

Clinical observations were recorded 4 hours after treatment and daily thereafter, and just prior to sacrifice. Body weights were recorded just prior to treatment and on Days 4 and 15.

Local irritation scores for erythema (0-4) and edema (0-4) for each injection site were recorded 1 and 4 hours after treatment and daily thereafter.

Three animals from each group were serially sacrificed on Day 4 and Day 15, with gross necropsy conducted and vein, artery and subcutaneous tissues collected for microscopic evaluation of test and control sites.

Results: Daily individual clinical signs were considered normal for all animals throughout the study, and all animals gained body weight from day of treatment to terminal sacrifice.

Erythema or edema were not observed at any of the injection sites for control or Code 7228 treated animals. Minute hematomas were observed at several control and Code 7228 sites around both vein and arterial vessels as a consequence of the procedure of vascular puncture, and these effects cleared by the 4th day.

Histopathologically, there were no drug related tissue responses seen in any animal, at any dose, at any interval for intravenous, perivenous or intraarterial injection of either control or Code 7228 injection sites.

Reviewer's Conclusion: There were no drug-related tissue responses at 1 or 4 hrs, or at 2-15 days after administration in animals receiving intravenous, perivenous or intraarterial injections of Code 7228 or the control material. Several control and test sites had minute hematomas around both venous and arterial vessels as a consequence of the procedure of vascular puncture, and these cleared by the 4th day. Drug related tissue responses were not observed as a result of administration of Code 7228 (Ferumoxytol)

2.6.6.8 Special toxicology studies

Study title: Evaluation of rat paw edema following intravenous injection of Code 7228

Key study findings: Based on the criteria for determining a positive test, in this study Code 7228 (Ferumoxytol) was negative and did not produce a tissue response following intravenous injection in rats.

Study no.:	HHB-148B
Report Date:	May 23, 2007
Report Location:	EDR, Module #4
Conducting laboratory and location:	Advance Magnetics, Cambridge, MA
Date of study initiation:	August 10, 1998
In-Life Dates:	August – October 1998
GLP compliance:	No
QA reports:	No
Drug:	Lot # 98080401, Purity assumed 100%.

Design: The coating on the surface of the Code 7228 is derived from dextran. Therefore, this study was designed to assess the intravenous potential of Code7228 to induce edema in the rat hind paw. Groups of 3 male ~~CD@~~ CD@[CrI:CD@ (SD)BR] rats from ~~CD@~~ on 3 different days, received an intravenous injection containing 100 mg Fe/kg following a 4 day washout period between injections of Code 7228. A total of 15 animals in 5 groups received all 3 injections of different lots of Code 7228. A positive control group of 3 animals received injections of 100 mg/kg of Dextran T70. b(4)

Prior to each injection and 45 minutes after treatment, the volume of each hind paw was measured by plethysmometry. Visual scores of 0 – 4 for normal, slight, moderate and severe swelling, respectively were recorded for each animal at each time period.

Results: The positive control material Dextran T70 produced severe swelling as expected. None of the lots of Code 7228 yielded tissue responses or paw edema.

Reviewer's Conclusion: Based on the pre-test criteria established for determining a positive test, in this study Code 7228 (Ferumoxytol) was negative and did not produce a positive tissue response following intravenous injection in rats.

Study title: Evaluation of the possible immunological effects of Code 7228 in guinea pigs.

Key study findings: After induction with Code 7228 a delayed hypersensitivity or anaphylactic response was not observed in guinea pigs when challenged, in the Passive Cutaneous Assay, Passive Hemagglutination Assay or the Systemic Anaphylaxis Assay.

Study no.: 1555
Report Date: May 11, 2004
Report Location: EDR Module 4
Conducting laboratory: _____
Date of study initiation: August 8, 2001
In-Life Dates: August 24 - October 27, 2001
GLP compliance: Yes
QA reports: Yes
Drug: Lot 99042101, Purity assumed 100%.

Design: This study was designed to assess the potential of Code7228 to elicit a delayed hypersensitivity or anaphylactic response in the guinea pig, with either intraperitoneal or subcutaneous applications in the induction phase followed by a single dose challenge phase. The test animals selected were Hartley Guinea Pigs from ' _____

— Sixty females, 5 weeks old and 314-420 grams were assigned to 6 groups. Additional animals were received to serve as naïve animals for the Passive Cutaneous Assay.

Study design:

Group	Number Animals	Induction Treatment (Route)	Antigenic Dose	Number Doses	Challenge Dose
1	10	Code 7228 (IP)	12 mg Fe/kg/day	7	6 mg Fe/kg, Code 7228
2	10	Code 7228 (IP)	60 mg Fe/kg/day	7	6 mg Fe/kg Code 7228
3	10	Code 7228 (SC)	12 mg Fe/kg/day	3	6 mg Fe/kg Code 7228
4	10	Bovine Serum Albumin with Gerbu (SC)	1.0 mg/ animal	3	1.0 mg/ animal BSA
5	10	Mannitol with Gerbu (SC)	19.8 mg/kg	3	17.6 mg/kg Mannitol
6	10	Mannitol (IP)	88 mg/kg/day	7	17.6 mg/kg Mannitol

All animals were observed daily and weighed weekly just prior to each successive dose. The Buehler technique was used for scoring of the incidence and severity of erythema and edema.

In the induction phase each animal received the prescribed dose of each material either intraperitoneally or subcutaneously according to protocol on the specified days. A challenge intravenous injection of Code 7228 was given on day 35 to each animal.

Application sites were depilated and all animals were scored for erythema and edema at 24 and 48 hours after challenge. Upon completion of scoring, all animals were euthanized by injection and carcasses discarded.

Passive Cutaneous Assay (PCA): On day 35, the first 5 animals of each group were bled and the sera used for the PCA and PHA. Serum from each animal was diluted by duplicate two-fold serial dilutions with sterile saline. Eight sites per animal received intradermal injections of 100uL of diluted serum to naïve guinea pigs to sensitize them. Four hours after passive sensitization, each animal was challenged with intravenous injection of either Code 7228 (6 mg Fe/kg), positive control (BSA 1 mg/animal) or negative control (Mannitol 44 mg/mL, 0.4 mL/kg) materials. All solutions contained 1% Evans Blue dye. All animals were examined 30-45 minutes after challenge with a blue spot from the dye of 5 mm diameter or larger considered positive for PCA.

Passive Hemagglutination Assay (PHA): This assay was conducted to determine whether the challenge treatments of Code 7228 and the positive control material BSA would induce the formation of antigen specific antibodies in the serum. This in vitro study was conducted with sheep erythrocytes (SRBC). Code 7228, BSA, and mannitol were incubated with the SRBC for 15 minutes, then washed and resuspended in PBS at concentration of 5% SRBC. Serial dilutions of guinea pig sera (0.1 mL) collected during the PCA assay, were added to the microtiter plates, followed by 0.1 mL of 0.1% suspensions of either unconjugated SRBC or SRBC conjugated with Code 7228, positive and vehicle/negative control. Contents of the plates were gently shaken and incubated at room temperature for 1-3 hours. Digital photos of each plate were evaluated for hemagglutination with the lowest dilution of sera that showed agglutination being considered the antibody titer.

Systemic Anaphylaxis Assay (SAA): The last 5 animals/group were challenged intravenously on Day 36 with 0.4 mL of Code 7228 (15 mg Fe/mL), mannitol (44 mg/mL), or BSA (1 mg/animal). Animals were observed for 4 days after challenge for signs of hypersensitivity or anaphylactic response.

Results: Positive and negative control animals/samples responded as expected. In the animals challenged with Code 7228 there were no positive results observed indicating hypersensitivity or anaphylactic responses in any of the animals/samples.

Reviewer's Conclusion: Induction with Code 7228 by either IP or SC injection did not elicit hypersensitivity or anaphylactic responses in Passive Cutaneous Assay, Passive Hemagglutination Assay or Systemic Anaphylaxis Assay in guinea pigs when challenged with intravenous injections of Code 7228.

2.6.6.9 Discussion and Conclusions

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

The following summary table was extracted from information provided by the sponsor and prepared by the reviewer.

The nonclinical studies completed in support of clinical trials are presented in the table below:

STUDY	SPECIES	DOSE (mg Fe/kg)	Major Findings	NOAEL		
				mg/kg/day	Multiple of MHD, Based on BSA	
					Daily	Cumulative
Correction of anemia	Rat	30	Corrected anemia	30	0.7	0.7
hERG effects	HEK 293 Kidney	100, 500 µg/plate	NNF*	N/A**	N/A**	N/A**
Cardiovascular Safety Pharmacology	Dog	4, 40, 400	NNF*	400	9	9
Neuropharm profile arterial administration	Rat	300, 1000	NNF*	1,000	22	22
Single dose	Rat	4, 40, 450	NNF*	450	10	10
Single dose	Dog	4, 40, 450	NNF*	450	33	33
Repeat dose 13-week	Rat	2, 6, 12	NNF*	12	0.3	12
Repeat dose 13-week	Dog	2, 6, 12	NNF*	12	0.9	40
Ames assay	Staph and E. coli (w/ & w/o S9)	5000 µg Fe/plate	Negative	N/A**	N/A**	N/A**
Chromosomal Aberration Assay	Chinese Hamster (w/ & w/o S9)	5000 µg Fe/ml	Negative	N/A**	N/A**	N/A**
Mammalian Micronucleus Assay	Mice	1500 (24 & 48 hrs)	Negative	1500	30	30
Fertility and General Reproduction	Rat	6, 12, 18	Adult: NNF* Fetal: NNF*	18	0.4	10
Embryo-Fetal	Rat	10, 31.6, 100	Maternal – 100 exceeds MTD***	31.6	1	5
			Fetal – NNF*	31.6	1	5
Embryo-Fetal	Rabbit	6, 16.5, 45.3	Maternal - 45.3 exceeds MTD***	16.5	1	7
			Fetal – NNF*	16.5	1	7
Subcutaneous, IV, perivascular irritation	Rabbit	30	NNF*	N/A**	N/A**	N/A**
Dermal sensitization, System anaphylaxis	Guinea pig	60	Negative	N/A**	N/A**	N/A**
Rat paw edema	Rat	100	NNF*	N/A**	N/A**	N/A**

* NNF = No Noteworthy Findings.

** N/A = Not Applicable.

*** MTD = Maximum Tolerated Dose

OVERALL CONCLUSIONS AND RECOMMENDATIONS**Conclusions:**

Ferumoxytol is a relatively nontoxic product in a wide variety of nonclinical studies and species, and thus requires a very high dose to induce a toxic response. The initial pharmacological effects are always those found in iron overload which always precede any overt signs of general toxicity. The findings are consistent with pharmacological effects of high doses of iron compounds administered intravenously.

A battery of studies was conducted to evaluate the potential of ferumoxytol to elicit tissue responses in rats, rabbits and guinea pigs. The rat paw edema study was negative when comparing the response of ferumoxytol to the severe edema observed with the IV administration of the positive control Dextran T70. The administration of ferumoxytol to rabbits by the subcutaneous, perivascular and intravenous routes resulted in no antigenicity, immunotoxicity or hypersensitivity type tissue reactions. Guinea pigs were given IV and SC injections of ferumoxytol over a 35 day induction period, followed by challenge IV doses of ferumoxytol. There were no hypersensitivity or anaphylactic responses observed from the administration of ferumoxytol in the Passive Cutaneous Assay, Passive Hemagglutination Assay or the Systemic Anaphylaxis Assay following induction and IV challenge with ferumoxytol. Although using a battery of animal studies as described here is generally predictive of risk of human anaphylactoid responses, animal studies are not always completely accurate in predicting human response.

Recommendations: Approval.

Suggested labeling: See Page 3 of this review.

Signatures:

Reviewer: _____

Supervisor: _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

**This is a representation of an electronic record that was signed electronically and
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/s/

David Bailey
10/3/2008 02:37:21 PM
PHARMACOLOGIST

Adebayo Lanionu
10/6/2008 05:24:20 PM
PHARMACOLOGIST