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APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
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CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Applicant's letter date:	August 6, 2013
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Product name:	KRX-0502 (Ferric Citrate)
Indication:	Control of serum phosphorus levels (b) (4) n patients with chronic kidney disease (CKD) on dialysis
Applicant:	Keryx Biopharmaceuticals, Inc.
Review Division:	Cardiovascular and Renal Products
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1 Executive Summary

1.1 Introduction and Clinical Rationale

Keryx Biopharmaceuticals, Inc. (Keryx) has submitted the New Drug Application (NDA 205874) using the 505(b)(2) regulatory mechanism for the use of ferric citrate as a phosphate binder to control (b) (4) the serum phosphate level in chronic kidney disease (CKD) patients on dialysis. Ferric citrate has been approved in the United States (US) as a food supplement and is listed in 21 Code of Federal Regulations (CFR) 184.1298 as generally recognized as safe (GRAS). Ferric citrate (KRX-0502, a coordination complex) as submitted by the Sponsor differs from the GRAS ferric citrate as:

(b) (4)

(b) (4)

End-stage renal disease (ESRD) develops when chronic kidney disease (CKD) has worsened to the point at which kidney function is less than 10% of normal and the kidneys are no longer able to function at a level necessary to maintain life. ESRD Patients need dialysis or a kidney transplant for life support. A decreased capacity for phosphate excretion in the urine of CKD patients may increase the phosphate level in serum, to the point of hyperphosphatemia. The ferric citrate, when orally administered, precipitates phosphate as ferric phosphate in the GI tract, is excreted in the stool and lowers the amount of total phosphate in serum. Excess amount of ferric (Fe^{+3}) iron is reduced to ferrous iron (Fe^{+2}) and taken into the enterocytes via the divalent metal transporter (DMT 1) for storage.

The poor absorption of ferric iron allows its binding with phosphate in the gut, thereby preventing absorption of the phosphates (Hsu 1999). Clinical studies (Study 304, Study 305, and Study PBB00101) have shown a dose-dependent decrease in serum phosphate levels following the oral administration of ferric citrate at a dose of 4.5 and 6 g/day up to 12 g/day for 4 weeks (Study 201 titrated to 11.3 g/day, and Study 202 titrated to 12 g/day). Based on the data from pre-clinical and clinical studies, Sponsor has proposed a maximum dose of ~12 g/day (60 kg body weight) ferric citrate to control the increased serum phosphate levels in CKD patients on dialysis.

Ferric citrate ($C_6H_5FeO_7$) differs from the Keryx drug substance (KRX-0502) [redacted]. The KRX-0502 drug substance, [redacted] is formulated as a [redacted] containing 210 mg ferric iron and [redacted] citrate [redacted] of drug substance. A maximum human recommended dose (MHRD) of 12 g/day (200 mg/kg/day, 60 kg body weight) , that provides [redacted] g ferric iron/day [redacted] mg ferric iron/kg/day) and [redacted] g citrate/day [redacted] mg citrate/kg/day), is proposed by Sponsor to treat hyperphosphatemia in CKD patients on dialysis.

1.2 Brief Discussion of Nonclinical Findings

Keryx-sponsored 28-day repeat dose oral toxicology study in rats has shown a relatively reduced systemic bio-availability of iron when complexed with citrate. Animals exposed to lethal doses of iron compounds developed congestion and hemorrhagic necrosis of the GI tract, decreased activity, weakness, decreased muscular control, prostration, urination, bowel obstruction, gastroenteritis (including diarrhea and vomiting leading to dehydration, and electrolyte imbalance), rapid and shallow respiration, convulsions, coma, respiratory failure, and cardiac arrest (Shanas and Boyd 1969).

No treatment related deaths were observed in Keryx - sponsored rat and dog studies. Results of 90-Day repeat dose oral toxicity study in rats have shown a dose dependent iron deposits visible as black material in the cecum and colon mucosa, and in the spleen and liver of males and females at all dose levels indicating the liver and the GI tract as primary target organs for iron deposition. Pigmented macrophages were seen in the stomach, intestine, colon, and iron granules were present in epithelial cells. The colon was severely affected in male rats and colonic goblet cell hyperplasia and/or hypertrophy was observed even at the lowest dose of ≥500 mg/kg/day (108 mg Fe/kg/day). Females in the low dose group (500 mg/kg/day) showed complete recovery from increased lymphocyte infiltration, while partial recovery was seen in the mid and high dose groups. A partial recovery was also observed in pigmented macrophage aggregates in mediastinal lymph nodes, but not in mesenteric lymph nodes. This reviewer considers the NOAEL in rat studies to be 500 mg/kg/day, rather than 2800 mg/kg/day dosage as determined by the Sponsor.

A significant ($p<0.01$) increase in serum ferritin level with a concomitant rise in serum iron level was seen in male (↑113%) and female (↑67%) beagle dogs in the high dosage group (2800 mg/kg/day) in 16-Week repeat dose oral toxicology study when compared with controls. Increased liver weight, bile duct hyperplasia, GI tract and liver injury in high dose-treated animals can be attributed to iron overload and to correlate with clinical findings. An unscheduled death of a beagle dog in the high dosage group (2000 mg/kg/day) at week 40 of ferric citrate treatment was attributed to liver injury as a result of iron overload and correlated with histopathologic findings and clinical pathology. The NOAEL dose was established at 400 mg/kg/day (89 mg Fe/kg/day) in oral toxicity study in dogs.

Ferric citrate did not show any mutagenic or clastogenic potential and was not considered genotoxic. Ferric citrate and other iron containing compounds were not carcinogenic in prior

mice and rat studies. Data from prior reproductive and developmental toxicological studies did not show adverse effects of iron containing compounds on reproductive functions, or any their teratogenic effects. Slight to moderate toxic effects of iron compounds were observed on GI tract, liver and kidney, and on the cardiovascular, endocrine and immune systems, most likely due to iron overloading and featuring the presence of brown pigment. The GI tract is one of the most common targets for iron toxicity, and is associated with the presence of pigmented macrophages.

1.3 Recommendations

1.3.1 Approvability

Approvable

1.3.2 Additional Non Clinical Recommendations

Sponsor has proposed to conduct juvenile studies in rats and to monitor the extent of iron overloading by using iron -specific Perl staining.

1.3.3 Labeling

Reproductive, developmental and carcinogenic studies were not carried out by the Sponsor to evaluate the toxic effects of KRX-0502 (ferric citrate) on reproductive and developmental system and its carcinogenic potential. The results from published studies have not shown any teratogenic effects of iron compounds on chick embryo, CD-1 mice or Wistar-rats.

Data from lifetime carcinogenic studies of iron- containing compounds in mice (38.5 mg/kg/day) and rats (186.8 mg/kg) suggest that ferric citrate and other orally administered iron - containing compounds are not tumorigenic. However, Sponsor did not address the effects of the overdosing of ferric citrate (KRX-0502), characterized as hemochromatosis in pregnant women and infants (Papanikolaou et al 2004). (b) (4).
The proposed labeling should be revised as below *(italicized)*.

8.1 Pregnancy

Pregnancy Category B: There are no adequate and well controlled studies (b) (4) in pregnant women. *Ferric citrate* (b) (4)

The effect of *Ferric citrate* on the absorption of vitamins and other nutrients has not been studied in pregnant women. Requirements for vitamins and other nutrients are increased in pregnancy. (b) (4)

8.2 Labor and Delivery

The effects of *Ferric citrate* on labor and delivery are not *known*.

13.1 Nonclinical Toxicology

Carcinogenesis, Mutagenesis, and Impairment of Fertility

(b) (4)

Ferric citrate was neither mutagenic *in vitro* in the bacterial reverse mutation assay (Ames test), nor clastogenic in the chromosomal aberration test in Chinese hamster fibroblasts.

(b) (4)

(b) (4)

2 Drug Information

2.1 Drug — Ferric Citrate

CAS Registry Number:	2338-05-8
Generic Name:	Ferric Citrate
Proposed human dose (Max):	12 g/day (body surface area)
Code Name:	KRX-0502 (Ferric Citrate coordination complex)
Chemical Name:	Iron (+3), *x* (1, 2, 3-Propanetricarboxylic acid, 2-hydroxy-), *y* (H2O)
Chemical Formula:	Fe • *x* ($C_6H_4O_7$) • *y*H2O; *x*=0.70-0.87, *y*=1.9–3.3
Molecular Weight ($FeC_6H_5O_7$):	244.9447 g/mole (Anhydrous)

Structure or Biochemical Description:

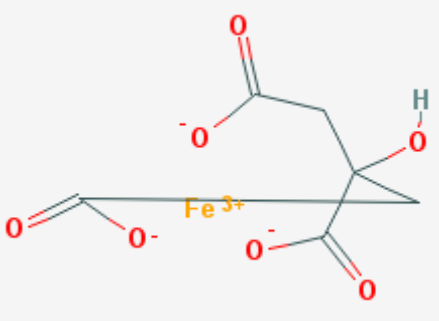


Figure1: Ferric Citrate (2-D Structure, NCBI)

Pharmacologic Class: Phosphate Binder

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 52868 (Keryx Biopharmaceuticals Inc.)

2.3 Drug Formulation

Ferric citrate is a coordination complex consisting of [redacted] Ferric citrate is listed as generally regarded as safe (GRAS) in 21CFR 184.1298. Potential impurities or degradants, and proposed specifications are listed in Table 1.

Table1. Ferric Citrate : Potential impurities (Sponsor's table)

[redacted]

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradation products

[redacted] -identified impurities are listed below in Table 2. The relative response factors (RRF) in relation to [redacted] were determined to enable reporting these substances as %w/w in the [redacted] impurities method. [redacted]

Table 2: Citrate related substances in Ferric Citrate (Sponsor's table)

Impurity	Relative Response Factor (RRF)
(b) (4)	

(b) (4), a process related non-toxic impurity, has also been found in (b) (4) ferric citrate batches when monitored by (b) (4) (b) (4) may be present (b) (4)% to (b) (4)% w/w) and is also monitored in the drug substance. (b) (4) have determined the (b) (4) in drug substance (Table 3) as specified by the Sponsor. *The oral permitted daily exposure (OPDE) values supplied by the Sponsor for* (b) (4) *and* (b) (4) *do not meet the criteria set by the draft* (b) (4). *However, the amounts present in the dug substance are not considered to pose any hazardous problem.*

Table 3: Class (b) (4) and (b) (4) (b) (4) in Drug substance (Sponsor's table)

(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

The CKD patients on dialysis (hemodialysis or peritoneal), having serum phosphate increased to ≥6 mg/dL are the proposed population for this indication. The starting dose of KRX-0502 (Ferric Citrate) will be (b) (4) 6 g/day (b) (4) 2 (b) (4) The dose of KRX-0502 may be titrated up or down

by 1 to 2 (b) (4) per day with meals at 2 to 4 week intervals up to a maximum dose of 12 g/day, to maintain the serum phosphate levels within the desired range of 3.5 to 5.5 mg/dL according to NKF-KDOQI guidelines. KRX-0502 should be (b) (4)

2.7 Regulatory Background

To explore the potential of ferric citrate as a phosphate binder, Dr. Chen Hsu of the University of Michigan opened an Investigational New Drug (IND 52,868) in 1997 based on animal data obtained from his laboratory. The IND was transferred to 'Panion' in 2002 and BF Biotech Inc. of Taiwan and then to Keryx Biopharmaceuticals, Inc. in 2006. Keryx sub-licensed the rights for KRX-0502 to Japan Tobacco, Inc. (JT) and its subsidiary, Torii Pharmaceutical Co., Ltd. in 2007. Following the pre-NDA meeting (08/19/2012), Keryx submitted this NDA using the 505(b) (2) regulatory mechanism.

3 Studies Submitted

Following is the list of studies conducted by the Sponsor (Table 4).

Table 4: Key Nonclinical Studies Submitted by the Sponsor (Sponsor's table)

Study Type and Duration	Route of Administration	Duration	Study Report	GLP Status
Sprague-Dawley Rat				
A 28-Day Oral (Dietary) Range-finding Toxicity Study in Rats	Oral diet	4 weeks	06-2965	Non-GLP
A 90-Day Oral (Dietary) Toxicity Study in Rats with a 30-Day Recovery Period	Oral diet	13 weeks 30 day recovery	07-2038	GLP
A 32-Week Oral (Dietary) Toxicity Study in Rats with a 1-Month Recovery Period	Oral diet	32 weeks 1 month recovery	09-2120	GLP
Beagle Dog				
A 28-Day Oral Range-finding Toxicity Study in Dogs	Oral diet	4 weeks	06-3186	Non-GLP
An Escalating Dose/Maximum Tolerated Dose Oral Toxicity Study in Dogs (33 days)	Oral diet	Phase 1: escalating doses for 5 to 9 days each Phase 2: 7 days	07-3274	Non-GLP
A 16-Week Oral Toxicity Study in Dogs with a 30-Day Recovery Period	Oral diet	16 weeks dosing (dose escalated up over first 4 weeks) 30 day recovery	07-3296	GLP
A 42-Week Oral Toxicity Study in Dogs with a 60-Day Recovery Period	Oral diet	42 weeks dosing (dose escalated up over first 4 weeks) 60 day recovery	09-3386	GLP

GLP=Good Laboratory Practice; JT=Japan Tobacco Inc.

3.1 Studies Reviewed

1. KRX0502 (Ferric Citrate): A 42-Week oral (dietary) toxicity study in dogs with a 60-Day recovery period
2. KRX0502 (Ferric Citrate): A 16-Week oral (dietary) toxicity study in dogs with a 30-Day recovery period

3. KRX0502 (Ferric Citrate): A 32-Week oral (dietary) toxicity study in rats with a one month recovery period
4. KRX0502 (Ferric Citrate): A 90-Day oral (dietary) toxicity study in rats with a 30-Day recovery period

3.2 Studies Not Reviewed

1. *In Vitro* drug-drug interaction study of ferric Citrate (JTT-751) in simulated gastric fluid
2. *In Vitro* drug-drug interaction study of ferric citrate (JTT-751) in simulated gastric fluid-2
3. Analysis report for the in-vitro drug-drug interaction studies of KRX-0502 (ferric citrate)
4. KRX0502 (Ferric citrate): A 28-Day oral (dietary) range finding toxicity study in dogs (Non-GLP)
5. KRX0502 (Ferric Citrate): A 28-Day oral (dietary) range finding toxicity study in dogs (Non-GLP)

3.3 Previous Reviews Referenced

None

4 Pharmacology

Ferric citrate reacts with the phosphate in the gastrointestinal (GI) tract, precipitates as ferric phosphate and is excreted out in the stool. The poor absorption of ferric iron allows it to persist and to bind phosphates in the gut, and prevent absorption of the phosphates (Hsu 1999). Ferric iron is converted to ferrous iron by ferric reductase and duodenal cytochrome B (Dcytb), and taken into the enterocytes as ferrous ion via the divalent metal transporter (Koury and Ponka 2004). The uptake of iron into the enterocyte and the passage from the enterocyte into the blood are highly regulated by the intracellular iron regulatory proteins (IRPs) and the plasma protein (hepcidin), increasing and maintaining the stores of iron in body. Citrate is absorbed systemically for its use in the citric acid cycle for ATP production.

4.1 Primary Pharmacology

***In Vitro* Studies**
Binding studies carried out with ferric citrate hydrate *in vitro* , and using a filter-based method to measure the binding of phosphorus in the filtrate, reveal the binding of 229 mg phosphate/g drug substance at pH 2, 12 mg phosphate/g drug substance at pH 7, and 143 mg phosphate/g drug substance at pH 2 (1 hour incubation), followed by another 1-hour incubation at pH 7 (Iida et al 2013a). Data from an independent study show that dietary administration of ferric citrate in rats binds about 59 to 61 mg phosphate/g ferric citrate (Hsu et al 1999).

***In Vivo* Studies**
Data from studies performed during the 1930s and 1940s have shown that dietary iron sufficient to bind ~50% or more of the phosphates in the intestine lead to a significant decreases in serum phosphate, softening of bones and development of rickets in rats within 3 weeks of treatment. An early study (Brock and Diamond 1934) on young rats has shown that a diet containing an ~ 9.14

g of ferric iron as ferric chloride when fed to rats for 22 to 27 days binds ~ 84% of the dietary phosphate and affects the body weight, serum phosphate and bones (Table 5). Based on the fact that rats, on average, consumed 22.5 g of their diet each day, they consumed ~1290 mg Fe3+/kg/day which is higher than the NOAEL established in rat toxicology studies (Keryx-sponsored 90-day rat toxicology study and the JT-sponsored 32-week). Results of this study have shown that ferric chloride prevents the absorption of dietary phosphates.

Table 5: Effects of Dietary Iron Salts on Body Weight, Serum Phosphate and Formation of Rickets in Rats (Sponsor's table)

Diet	N	Mean Body Weight Change (g)	Serum Phosphorus (mg %)	Degree of Rickets Observed
Day 22				
Control	5	+5.2	7.3	None
Rachitogenic diet	4	+1.8	3.7	Severe active
Ferric chloride	5	+2.0	2.5	Severe active; quantitatively more severe than positive control
Ammonium chloride	5	+4.6	7.2	None
Days 26-27				
Control	5	+9.8	5.7	None
Rachitogenic diet	4	+4.0	2.6	Severe active
Ferrous chloride	5	+0.4	2.7	Moderately severe active
Ferric chloride + phosphate	5	-0.5	6.2	None
Ammonium chloride	5	+10.8	7.8	None
Ferric ammonium citrate	5	-1.8	3.8	Moderately severe active
Reduced iron	4	-0.4	3.6	Moderately severe active
Ferric glucamate	4	-12.8	5.6	None

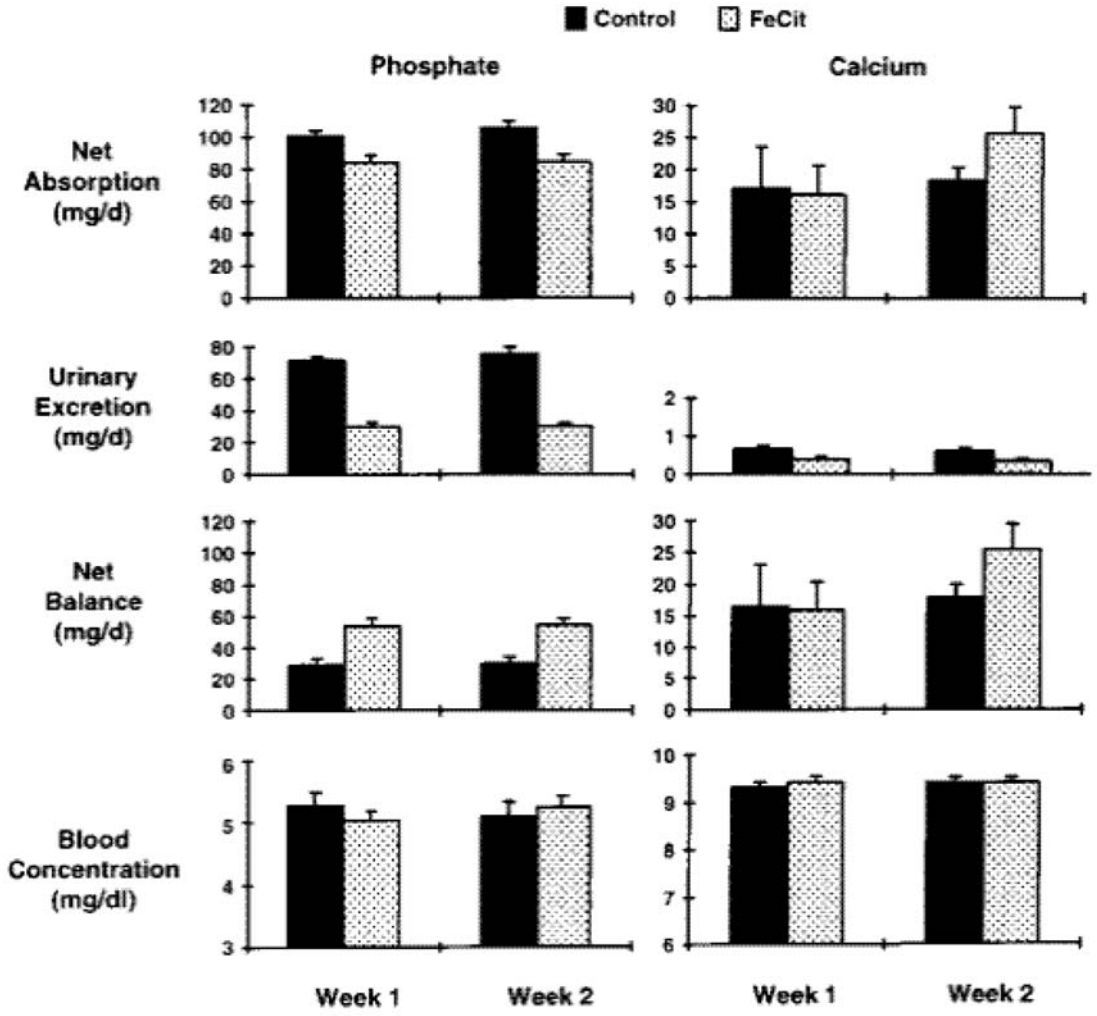
Source: Brock and Diamond 1934, Table II.

Rats receiving dietary ferric chloride diet sufficient to bind 50% of the phosphate consumed have shown significant reductions ($p<0.01$) in total body phosphorus (20.0% and 25.3%) and in total body ash (20.0% and 25.5%) when compared to controls (Rehm and Winters 1940). It has been shown that guinea pigs and rabbits fed diets containing ferric salts have lowered phosphate and calcium levels in blood, and calcium and phosphate in their bone ash (Cox et al 1931). Development of rickets has also been observed in White Leghorn chicks fed diets containing ferric sulfate at levels sufficient to bind ≥ 50% phosphate in the diet as $FePO_4$.

In Vivo Study of Phosphate Levels in a Renal Failure Model

Effects of iron salts on phosphate absorption were studied in normal and renal failure models (subtotal nephrectomy and adenine administration). Male Sprague-Dawley rats (n=6/treatment group) were maintained on a 1.02% phosphorus and 0.95% calcium diet alone or supplemented with approximately 0.95% Fe3+ as ferric citrate for 2 weeks in the control rats or as ferric citrate, ferric chloride or ferric ammonium citrate for 4 weeks in the renal failure model rats (Hsu et al 1999). The intestinal absorption of phosphate ($p<0.005$) and urinary excretion ($p<0.0001$) were lower in the ferric citrate group than in the control group while no differences were observed in body weight, growth rate, urinary creatinine excretion or creatinine clearance, PTH, calcitriol,

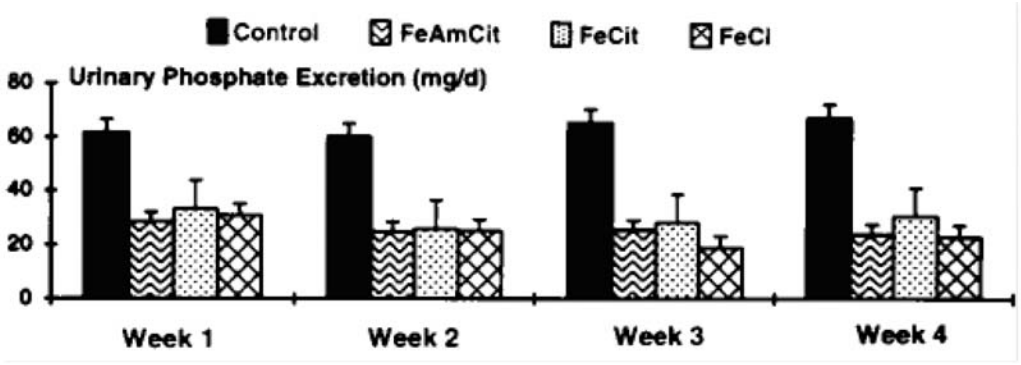
plasma iron, or hematocrit between the control and ferric citrate treated normal rats or renal failure model rats (Fig. 2).



Source: Hsu et al 1999, Figure 1.
Note: Phosphate (left side) and calcium (right side) metabolism in rats with normal renal function. Average measurements are shown for the 1- and 2-week periods. Black bars indicate control animals and stippled bars indicate ferric citrate-treated animals.
FeCit=ferric citrate.

Figure 2: Effect of Dietary Ferric Citrate on Phosphate and Calcium Levels in Normal Male Sprague-Dawley Rats (Sponsor's figure)

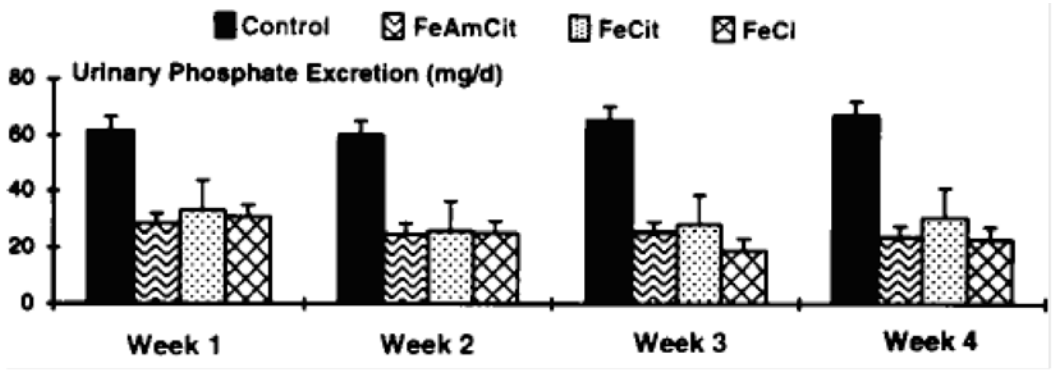
In the renal failure model, fecal excretion of phosphate was higher in the ferric citrate - treated groups compared to the control and the net intestinal absorption of phosphate was lower in the ferric-treated animals than in the control group throughout the experiment (Figure 3).



Source: Hsu et al 1999, Figure 3.
FeAmCit=ferric ammonium citrate; FeCit=ferric citrate; FeCl=ferric chloride.

Figure 3: Effects of Dietary Ferric Salts on Urinary Phosphate Excretion in Azotemic Sprague-Dawley Rats (Sponsor's figure)

In another rat model of renal failure where CKD was induced by administration of 0.75% adenine for 28 days (Lida et al 2013), a significant decrease ($p<0.01$) in serum phosphate concentration was observed in ferric treated animals when compared to controls (Fig. 4). PTH levels were significantly higher in serum of the adenine control group as compared with controls ($p<0.01$).



Source: Hsu et al 1999, Figure 3.
FeAmCit=ferric ammonium citrate; FeCit=ferric citrate; FeCl=ferric chloride.

Figure 4: Effects of Dietary Ferric Salts on Urinary Phosphate Excretion in Azotemic Sprague-Dawley Rats (Sponsor's figure)

4.2 Secondary Pharmacology

Iron is an essential component of hemoglobin, myoglobin, heme enzymes, metalloflavoprotein enzymes, and mitochondrial enzymes, and is used as a supplement in the prevention and/or treatment of anemia (Valerio 2007). Dietary iron supplements are generally used as ferrous, gluconate or sulfate due to the greater efficiency of absorption of ferrous ion, and contain between 10 and 65 mg of elemental iron. Intravenous (IV) iron is administered as single doses of 60 to 510 mg of elemental iron in CKD patients on dialysis to replace the iron lost during blood loss.

4.3 Safety Pharmacology

Sponsor has initiated studies showing that ferric citrate (3500 and 1000 mg/kg/day/28 days) is poorly absorbed in rats and dogs. Sponsor did not conduct any acute cardiovascular or other safety studies. However published nonclinical and clinical studies have documented the effects of high dose iron on target organ systems such as CNS, cardiovascular, GI, hepatic/biliary, endocrine, immune, and skeletal systems in animals and humans.

Central Nervous System

Iron deposits were observed in brain during iron treatment and such residues may be implicated in the pathogenesis of neurodegenerative disorders (Parkinson's disease and Alzheimer's disease). Long-term effects of iron are seen when it is administered at or after the post-natal period that is critical for normal brain development. In rats, the maximum iron uptake in brain was observed in 15-day old pups while in humans, the first year from the third trimester of pregnancy is very critical. During this time period, the brain grows rapidly and essential brain structure/functions are established. Studies showing the effects of iron on rat brain are presented below in Table 6 (de Lima et al 2005).

Table 6: Effects of Iron dosing on CNS Following Oral Administration of Iron to Post-natal, Weanling and Adult Rats (Sponsor's table)

Iron Form	Species	Age; Gender (No.)	Dose[a] (mg Fe/ kg/ day)	Duration	Findings[b]	Reference
Carbonyl iron	Sprague-Dawley Rats	Weanling M (n=10-18 /group)	52.5, 525, 3000 (in diet)	12 weeks	Dose-dependent decrease in body weight and increase in liver non-heme iron 20000 ppm (3000 mg Fe/kg/day) Increase in mean brain non-heme iron (43 µg Fe/g tissue) versus controls (37.3 µg Fe/g tissue) Decreased motor activity, habituation, reflex startle, and conditioned avoidance response Enhanced pre-pulse modulation of startle	Sobotka et al 1996
Iron succinate	Wistar Rats	Post-natal days (n=10-14/ group); 5-7 12-14 19-21 Weaned Day 28 (n=3-5/ group) 30-32	10 (oral)	3 days	At 3 to 5 months of age, short-term (90 minutes) and long-term (24 hours) memory object recognition tests conducted. Also determined brain oxidative stress levels. For iron dosed at all post-natal times, impairment in long-term adult memory recorded. When iron dosed on post-natal Days 12-14, complete long-term memory block. Since exploration time the same, no effects on locomotion, anxiety or motivation noted. Post-natal iron increased adult brain oxidative damage.	de Lima et al 2005
Ferrous sulfate	Wistar Rats	Adult M (n=10/group)	67.5 (in diet)	10 weeks	Increases in serum iron (2X) and zinc (4X). Decrease in serum chromium (8X). Decreases (2X) in brain serotonin and dopamine. Increase (2X) in brain lipid peroxidation. Brain histopathology: meningeal hemorrhage, congestion and edema; choroid plexus of ventricles dilated, hemorrhagic focal areas of encephalomalacia with congested cerebral blood vessels. In cortex, degenerated neurons, satellitosis and neuronophagia noted.	Elseweidy and Abd El-Baky 2008

Iron Form	Species	Age; Gender (No.)	Dose[a] (mg Fe/ kg/ day)	Duration	Findings[b]	Reference
Not Specified	Sprague-Dawley Rats	Adult M (n=8/group)	12, 24, 48 (in diet)	7 days	Psychological stress induced with a communication box. No effects of psychological stress or iron on bodyweight gain and food intake. Psychological stress alone decreases serum iron levels. Iron reduced psychological stress-mediated decrease in serum iron. Iron alone induced dose-dependent increase in total iron content of all brain regions (except brainstem). Iron and psychological stress treatment further increased total iron content of hippocampus, cortex, and striatum. Brain oxidative stress levels increased.	Yu et al 2011

[a] Doses were converted to represent the amount of iron present in each iron salt form when this dose was not provided by the publication. Doses in mg Fe/kg/day were calculated based on the assumption that rats consume approximately 22.5 g of food per day and reference body weight for rats (0.15 kg) given in the FDA Guidance for Industry, Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, was used.
[b] All changes listed are statistically significant ($p<0.01$ or $p<0.05$) as compared with the control group.
FDA=Food and Drug Administration; M=male.

Cardiovascular System

Effects of iron overload have been seen on the development of the heart in young suckling and adult rats and on ischemia/reperfusion injury. Hemochromatosis and congestive heart failure can be lethal expressions of iron toxicity in humans. To determine the effect of iron on the cardio-vascular system, male weanling Sprague-Dawley rats were fed carbonyl iron-supplemented diets containing 35 (control), 350, 3500, and 20000 µg Fe/g of food (n=8 to 10/group) for 12 weeks (0.0035%, 0.035%, 0.35%, and 2% Fe providing 5.25, 52.5, 525, and 3000 mg Fe/kg/day, respectively)(Whittaker and Chanderbhan 2001). No effects were seen on serum iron and TIBC at diets of up to 2% Fe, while the mean non-heme iron concentrations in the heart increased significantly ($p<0.001$) with dose. Similarly, the heart/body weight ratio increased ($p<0.001$) with dietary iron content (0.37% in the control animals vs. 1.03% in the rats fed the 2% Fe diet). Cardiomyopathy was observed in rats receiving 525, and 3000 mg Fe/kg/day. Foci of degenerated myofibers with variable numbers of mononuclear and polymorphonuclear inflammatory cells and a few fibroblasts were observed. Signs of fibrosis near these degenerative or necrotic lesion as well as disruption of the ventricular or atrial walls and thrombi formation were noted. Seven animals (2/8 animals at 0.35% dietary Fe; 5/10 animals at 2% dietary Fe) died before the termination of the study. Five of these animals had heart damage which included iron in the cytoplasm of the myocardial fibers, hemorrhagic necrosis, epicardial damage, and clot formation.

To determine the effects of iron supplementation on the development of the heart, suckling male Sprague-Dawley rats were administered ferrous sulfate (~20 mg/kg/day) by gastric feeding tube twice a day from the age of 3 days to 20 days and the gross morphological and histological effects were compared with littermate controls (n=16/group) (Neffgen and Rakušan 1975). A decrease in hemoglobin level was observed (↓16.2% to 7.9%) during the first 20 days of life whereas in iron-treated animals, the hemoglobin level declined to a lesser extent (12%). No differences in body weight between the controls and treated animals were observed. The hearts of animals treated with iron were significantly ($p<0.001$) smaller (based on weight) than controls as was myocardial fiber size; whereas fiber density (F/mm2) was correspondingly higher. To

determine the effects of iron treatment on heart response to ischemic/reperfusion injury, female Sprague-Dawley rats were administered iron for 4 months at dietary contents of 15, 35, 150, and 300 µg Fe/g (calculated to provide approximately 2.3, 5.3, 22.5, and 45 mg Fe/kg/day). In normothermic rats ischemic/ reperfusion cardiac arrest was induced in the heart at the end of the study in an ex vivo perfusion model. Ischemia/reperfusion injury was increased in rats fed the diet containing 150 and 300 µg Fe/g as reflected by the levels of low molecular weight iron and malonaldehyde and formation of hydroxyl radicals (van Jaarsveld and Schulenburg 1997).

Respiratory System
Sponsor's submitted published literature showed no effects of iron dosing on respiratory system.

5 Pharmacokinetics/ADME/Toxicokinetics

Iron is an essential component of human body, and present in hemoglobin, myoglobin, various enzymes, circulating in the blood as transferrin or stored as ferritin in liver and other iron stores (Fig. 5). Therapeutic doses of iron when administered regulate the homeostatic processes and its storage/circulation (Papanikolaou and Pantopoulos, 2005).

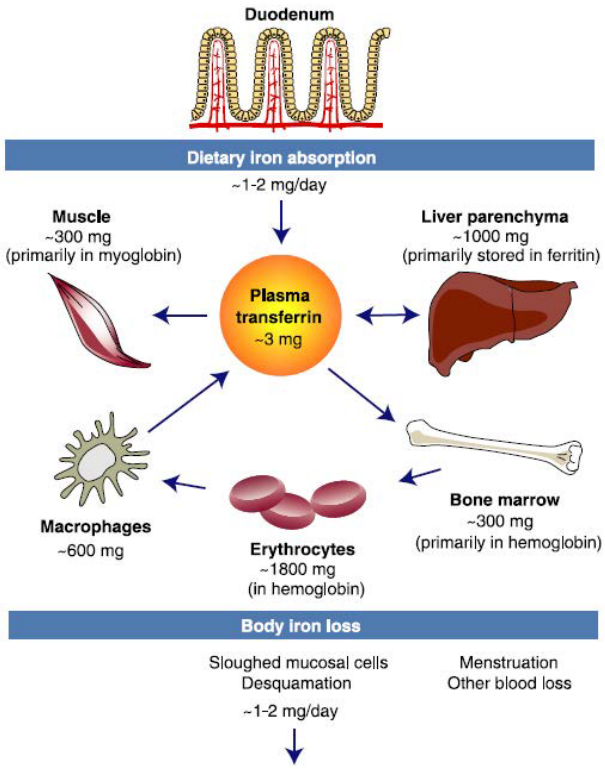


Figure 5: Iron Homeostasis in Humans (Sponsor's Figure)

In view of the small amount of circulating iron in serum compared to total body stores, typical pharmacokinetic data based on serum levels (maximum plasma concentration; area under the plasma concentration time curv half-life [$t_{1/2}$]) were not generated. Rather, standard iron parameters (serum or plasma iron, ferritin, total iron binding capacity [TIBC], and transferrin

saturation [TSAT]) that reflect the total iron status of the animal are reported by the Sponsor. No increases in serum iron were observed in normal rats fed 4% ferric citrate in the diet for 2 weeks (Hsu et al 1999). Azotemic rats (5/6 nephrectomized) fed a diet containing 4% ferric citrate for 4 weeks demonstrated a 67% higher concentration of serum iron than azotemic rats fed a control diet. No effect on serum iron parameters was observed after 28 days on a diet providing 3500 mg/kg/day in normal rats and 1000 mg/kg/day in normal dogs) conducted by the Sponsor. Statistically significant changes in iron parameters, which are indicative of increased iron absorption and overloading, are observed in rats at intakes of 2800 mg/kg/day following 90 days of dosing. In dogs, these effects are observed at ≥1200 mg/kg/day following 16 weeks of dosing.

The extent to which different forms of iron are absorbed varies depending on the salt form and oxidation state of the metal. Studies have shown that ferrous iron absorption is 2 to 7 times greater than the absorption of ferric iron e.g., when it is givenwith concomitant administration of vitamin C. The poor absorption of ferric iron allows ferric iron to bind phosphates in the gut, thereby preventing absorption of the phosphates (Hsu 1999). Ferric citrate was used in all toxicology studies performed by the Sponsor. Data from published literature reported herein are limited to oral formulations of ferric (Fe^{3+}) and ferrous (Fe^{2+}) iron salts and elemental iron (as carbonyl or electrolytic iron) as these are most pertinent to the proposed indication. Differences in absorption due to the form of iron administered (ferric versus ferrous as well as elemental iron) in rats and dogs are given in table 7.

Table 7: Iron Absorption and Elimination in Rats and Dogs (Sponsor's table)

	Rat (N=14 males)[a]	**Dog (N=6 males)[a]**
Estimated iron intake via diet	11.9 mg Fe/kg/day	7.9 mg Fe/kg/day
^{55}Fe (i.v.) as ferric citrate (20 moles citrate/mol iron)	Single dose 1 µCi/kg (specific activity approximately 15 1 µCi/µg)	
Duration of measurement of radiolabel in RBCs[b]	223 days	734 days
Mean half-life ($t_{1/2}$) of ^{55}Fe[c]	182±25 days[d]	552±92 days
Estimated body iron loss	0.171 mg Fe/kg/day	0.075 mg Fe/kg/day
Nutritional growth requirements	0.128 mg Fe/kg/day	0
Absorption (loss plus growth requirements)	0.35 mg Fe/kg/day	0.075 mg Fe/kg/day
Human equivalent dose (adjusted for body surface area)[e]	0.060 mg Fe/kg/day	0.045 mg Fe/kg/day

Source: Finch et al 1978
[a] The mean weights of the rats and dogs used in the study were approximately 0.47 and 12.5 kg, respectively.
[b] Analyzed using liquid scintillation counting.
[c] Calculated (based on 6 concentrations after a single exponential rate of decrease in radioactivity had been established in order to account for initial excessive loss of radiolabeled iron through the gastrointestinal tract) as follows (adjusted for blood volume changes due to growth and blood sampling).
[d] Corrected for blood volume and hemoglobin change.
[e] Body weight and body surface area adjustments calculated based the FDA Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers.
FDA=Food and Drug Administration; Fe=iron; i.v.=intravenous; N=number; RBC=red blood cell.

As shown in table 7, absorption of iron varies from species to species, and is greater in rat than dogs. Humans absorb approximately 0.012 (males) to 0.024 (females) mg Fe/kg/day. Thus, iron absorption from the diet is approximately 2.5- to 5-fold greater in the rat and 2- to 4-fold greater in the dog compared to humans, after adjusting for body surface area. This difference is due to a greater capacity for rats and dogs to excrete iron through intestinal mucosal cells.

5.1 ADME

Absorption

Prior to absorption, Fe^{3+} is reduced to Fe^{2+} either non-enzymatically and enzymatically by gastric juice, ascorbic acid or is duodenal cytochrome-b (Dcyt-b) on the apical surface of the enterocyte. Iron is absorbed through the duodenum, proximal small intestine, and proximal jejunum and then transported into the enterocyte via the divalent metal transporter (DMT1). The uptake of iron via DMT1 is dependent upon the intracellular iron concentration within the enterocyte. When intracellular iron concentrations are high, iron regulatory proteins (IRPs) destabilize DMT1 messenger ribonucleic acid (mRNA), decreasing the expression of DMT1 protein.

IRPs stabilize the mRNA message and expression of DMT1 is increased when intracellular iron concentrations are low. Iron is stored within the enterocyte as ferritin and is exported from cells via ferroportin. When iron intake is high, iron export from the enterocyte into systemic circulation is decreased due to an increased expression of hepcidin, which binds directly to ferroportin and induces its degradation. Hepcidin, a hormone produced in the liver in response to increases in iron storage and transferrin saturation (TSAT), inhibits the passage of iron from cells through ferroportin-1. High iron stores are due to an increased hepcidin levels, which decrease ferroportin levels and consequently limit systemic iron absorption.

Bioavailability

Published studies have reported that $FeSO_4$ and Ferric chloride are absorbed equally well from the diet, and at a rate 2 to 16 times less than that of ferrous salts (Palacios 2011). Carbonyl iron (Fe^0) and electrolytic iron (Fe^0) are less bioavailable and have higher median lethal dose (LD_{50}) values than ferrous and ferric salts (Table 8).

Table 8: Bioavailability of $FePO_4$ and Electrolytic Iron as Compared to $FeSO_4$ (Sponsor's table)

Iron Source	Bioavailability Relative to $FeSO_4$	
	Hemoglobin Repletion Experiment	Radiolabeled Iron Experiment
$FePO_4$	0.30	0.25
Electrolytic iron	0.72	0.55

Source: Forbes et al 1989, Table 3, Table 6, and Table 7
Note: The same experiment was performed at 2 to 3 laboratories using the same method; mean results across the different laboratories are presented as the results were similar.
$FeSO_4$=ferrous sulfate; $FePO_4$=ferric orthophosphate.

The reduced bioavailability of carbonyl iron and electrolytic iron is thought to be due to the requirement that these relatively insoluble forms of iron be converted to Fe^{2+} by gastric acid prior to absorption (Table 8, Whittaker et al 2002).

Composition of diet and the amount of iron present in basal diet have been shown to affect the absorption of iron. Phosphates, carbonates, oxalates, and tannates are known to inhibit iron absorption; whereas, ascorbic acid, tricarboxylic acids, amino acids, and sugars increase iron absorption (WHO 1983). The basal amount of iron (38µg/g) in rodent diet (AIN-76A) was almost 6-fold less than the amount of iron (220µg/g) in the diet used in applicant's-sponsored studies in rats; however, both diets were otherwise similar in total protein, fat, fiber, carbohydrate, and mineral content.

The supplemental iron used in sponsored toxicological studies was not adjusted for the basal amount of iron present in the diet. The percentage of iron contribution from the diet decreased with higher doses of ferric citrate administered as determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Variations in base line differences in iron parameters were also noted in rats, dogs, and humans. Females were found to have slightly higher baseline serum iron compared to male rats while differences were not apparent in dogs (as below):

Base line Serum Iron, Ferritin, TIBC and TSAT in Rats, Dogs & Humans (Sponsor's table)

	Iron Parameters				
Species	**Serum Iron (µg/dL)**	**Ferritin (ng/mL)**	**TIBC (µg/dL)**	**TSAT (%)**	**Source**
Rat (Sprague Dawley)	82-222 (M) 90-350 (F)[a]	NA	NA	NA	Charles River Technical Bulletin 1984
Dog (strain not specified)	125-225	525-1100	225-325	NA	Roth-Johnson 2011 in Clinical Veterinary Advisor: Dogs and Cats
Human	50-150	20-250 (M) 15-150 (F)	250-370	22-46	Powell 2008 in Harrison's Principles of Internal Medicine

[a] Ranges reported are the 95% confidence intervals (2×SD) obtained from data for 20 animals of each sex at 19 to 21 weeks of age.
F=female; M=male; NA=not available; SD=standard deviation; TIBC=total iron binding capacity; TSAT=transferrin saturation.

Distribution

Under normal conditions, most of the non-heme iron (0.1%) is bound as ferric iron to transferrin receptors, TrfR1 and TrfR2 (600 nM; 35.52 ng/mL) with high affinity (10^{23} M^{-1} at pH 7.4) to deliver iron to erythroid precursors and iron-requiring cells in the liver, pancreas, heart, and muscle.

The majority of the iron in humans (60% to 70%) is bound to hemoglobin in RBCs (1800 mg) and in erythroid precursors in the bone marrow (300 mg), whereas a small amount (7.5% to 15%) is present in myoglobin and in several essential enzymes (Fig. 5).

The rest is stored in liver parenchymal cells (about 1000 mg), bone marrow, spleen, and muscles in the form of ferritin (4500 iron atom/molecule) in cytoplasm. Approximately 30 mg iron is required per day for erythropoiesis and is provided by recycling of iron via reticuloendothelial macrophages.

Data from iron distribution studies in rats (Table 9) have shown that iron is predominantly accumulated in the liver followed by GI tract, heart, liver, kidney, spleen, and pancreas after dietary administration of carbonyl (elemental) iron for 12 weeks.

Table 9: Dose Response of Non-Heme Liver and Heart Iron Content Following Oral Administration of Carbonyl Iron in Sprague Dawley and Fischer 344 (F344) Rats
(Sponsor's table)

Daily Iron Dose		Non-Heme Fe (Mean [SE])		
µg Carbonyl iron/g diet/day	mg Carbonyl iron/kg body weight/day (estimated)[a]	Liver		Heart
		Sprague Dawley Rat	F344 Rat	Sprague Dawley Rat
		µg non-heme iron/g tissue	µg non-heme iron/g tissue	g non-heme iron/g tissue
35 (control)	5.25	112 (5)	262 (37)	34.4 (0.7)
350	52.5	234 (21)	--	39.2 (2.0)
1500	225	--	685 (31)	--
3500	525	911 (45)	934 (29)	40.5 (2.4)
5000	750	--	979 (29)	--
10000	1500	--	1848 (97)	--
20000	3000	3501 (163)	--	44.4 (1.1)

Source: Whittaker and Chanderbhan 2001, Table 2 and Whittaker et al 1997, Table V
[a] Doses converted to mg/kg/day based on the assumption that rats consume 22.5 g/day (~ 10% to 15% of their body weight per day) and that the rats weighed on average 150 g. Mean body weights in the control Sprague Dawley rats were 43 g at the start of the study and 385 g at the end. Mean body weights in the Sprague Dawley rats in the 3500 µg carbonyl iron/g diet group were 42 g at the beginning of the study and 295 g at the end.
Fe=iron; F344=Fischer 344; SE=standard error.

Excretion

Based on a Fe^{55} study, the $t_{1/2}$ for iron excretion in rats and dogs is 182 and 552 days, respectively. In addition, a significant loss of iron (approximately 0.171 mg/kg/day in rats and 0.075 mg/kg/day in dogs) was observed through RBCs entering the gut lumen or bleeding. Iron is also lost when cells are shed from the skin and GI tract (Table 7). In contrast, the loss of iron in human is low 0.016 to 0.033 mg/kg/day (Finch et al 1978, Papanikolaou and Pantopoulos 2005), except during menstruation (1.6 mg Fe/day). Other sources of iron excretion include sloughing off of intestinal cells, and dermal epithelial cells and excretion in the bile and urine.

Drug Interaction

A ferric citrate drug interaction study conducted by the Sponsor in vitro at pH 2.0, pH 4.5, and pH 6.8 (Study Report P15226.01), has shown that precipitates were observed with alendronate sodium (pH 2.0), benserazide HCl (pH 2.0), ciprofloxacin HCl (pH 2.0 and 4.5), doxycycline hyalite (pH 2.0, 4.5, and 6.8), levodopa (pH 2.0), levofloxacin HCl (pH 2.0, 4.5, and 6.8), methotrexate (pH 2.0), sertraline (pH 2.0 and 4.5), valproate sodium (pH 4.5), and vancomycin HCl (pH 2.0). Precipitates were not observed when attempted with adenovir dipivoxil, amlodipine mesylate, atenolol, carvedilol, cetirizine dihydrochloride, clonidine HCl, clopidogrel bisulfate, donepezil HCl, doxazosin mesylate, enalapril, famotidine, fluoxetine HCl, gabapentin, haloperidol, ibandronate sodium, ibuprofen sodium, isosorbide mononitrate, losartan potassium, loxoprofen sodium, memantine HCl, metoprolol tartrate, nicardipine HCl, nicorandil, nizatidine, paroxetine HCl, penicillamine, pravastatin sodium, propranolol HCl, rimantadine HCl, theophylline, or tramadol HCl.

Sponsor did not conduct any pharmacokinetic studies of citrate in nonclinical species.

6 General Toxicology

6.1 Single-Dose Toxicity

Single dose toxicity studies were not carried out by the Sponsor. However, the data submitted from published reports have shown the toxicity (median lethal dose: LD_{50}) of individual components of iron containing compounds: iron and citrate.

Based on the oral toxicity data (Table 10) of ferrous iron, mice ($LD_{50,}$ 31.5 to 630 mg/kg) seem to be more sensitive than rats ($LD_{50,}$ 255 to 2329 mg/kg) and dogs ($LD_{50,}$ 464 to 600 mg/kg).

Table 10: Acute Toxicity of Ferrous Iron following Oral Administration (Sponsor's table)

Test Article	Test Species / Strain	Fed / Fasted	LD_{50} (mg Fe/kg)[a]	Citation
Elemental Iron (Fe^0)				
Elemental iron	Guinea pig / --	--	20000	ChemIDplus 2013a
Elemental iron	Rat / --	--	30000	
Elemental iron	Rat / -- (albino rat)[b]	--	98600	Shanas and Boyd 1969
Ferrous (Fe^{2+}) Forms				
Ferrous fumarate	Mouse / GFF[b]	--	630	Berenbaum et al 1960
Ferrous fumarate	Mouse / WAG[b]	--	580	Berenbaum et al 1960
Ferrous fumarate	Mouse / Swiss-Webster	Fed[c]	516	Weaver et al 1961
Ferrous fumarate	Rat / Wistar	Fed[c]	2329	Weaver et al 1961
Ferrous gluconate	Mouse / GFF[b]	--	320	Berenbaum et al 1960
Ferrous gluconate	Mouse / Swiss-Webster	Fed[c]	457	Weaver et al 1961
Ferrous gluconate	Rat / Wistar	Fed[c]	865	Weaver et al 1961
Ferrous gluconate	Dog / Mongrel[d]	--	>46.4	Weaver et al 1961
Ferrous sulfate	Mouse / GFF	--	230	Berenbaum et al 1960
Ferrous sulfate	Mouse / Fairfield Webster	Fasted[e]	42.5	Eickholt and White 1965
Ferrous sulfate	Mouse / B_4BC[b]	Fasted[e]	31.5	
Ferrous sulfate	Mouse / Swiss-Webster	Fed[c]	305	Weaver et al 1961
Ferrous sulfate	Rat / Wistar	Fed[c]	780	
Ferrous sulfate	Rat / Sprague-Dawley	Fed[c]	1100	Whittaker et al 2002
Ferrous sulfate	Rat / Sprague-Dawley	Fasted[c]	255	Toblli et al 2008
Ferrous sulfate	Dog / Mongrel[d]	--	23.5	Weaver et al 1961
Ferric (Fe^{3+}) Forms				
Ferric ammonium citrate	Mouse / --	--	1000	Somers 1947
Ferric chloride	Mouse / --	--	500	Somers 1947
Ferric chloride	Mouse / --	--	308[f]	ChemIDplus 2013b
Ferric chloride	Rat / --	--	155[f]	ChemIDplus 2013b

[a] Doses were converted to represent the amount of iron present in each iron salt form when this dose was not provided by the publication.
[b] Additional details regarding strain not provided in publication.
[c] Iron containing solutions were administered to rats and mice given free access to food and water, except during the period of testing.
[d] Intravenous administration.
[e] Mice were fasted for at least 18 hours and not more than 24 hours prior to dosing.
[f] Doses provided in ChemIDplus entry were assumed to be the total dose of the salt (ferric chloride) and were therefore converted to represent the dose of iron.
--=not specified; LD_{50}=median lethal dose.

Results have shown that fasted mice are more sensitive to ferrous iron toxicity ($LD_{50,}$ 31.5 to 42.5 mg/kg) than fed mice mice ($LD_{50,}$ 305 to 516 mg/kg). Toxicity of ferric iron is lower than ferrous iron when administered orally to mice (308 to 1000 mg/kg) and rats (155 mg/kg), probably due to relatively poor absorption of the ferric form.

Decreased activity, weakness, decreased muscular control, prostration, urination, bowel obstruction, gastroenteritis (including diarrhea and vomiting leading to dehydration, hemoconcentration, and electrolyte imbalance), rapid and shallow respiration, convulsions, coma, respiratory failure, cardiac arrest, congestion and hemorrhagic necrosis of the gastrointestinal (GI) tract are the toxic effects observed following the oral administration of iron in mice, rats and dogs (Shanas and Boyd 1969). A low toxicity of citrate, expressed as physiological disturbances (acidosis and calcium deficiency) has been reported in mice and rats when orally administered at LD_{50} doses (Table 11).

Table 11: Acute Toxicity of Citrate following Oral Administration (Sponsor's table)

Test Species	LD_{50} (mg/kg)	Effects	Citation
Mouse	5400	Physiological disturbances (acidosis and calcium deficiency); "high"[a] doses caused nervous system effects as well as severe damage to the stomach mucosa	OECD SIDS 2001
Rat	3000		
	5000		
	12000		

[a] Doses at which nervous system effects and damage to the stomach mucosa were observed are not specified.
LD_{50}=median lethal dose.

6.2 Repeat-Dose Toxicity

The 7- repeated-dose toxicity studies consisted of two 28-day dose range-finding studies (rat, dog), a 33-day maximum tolerated dose (MTD) study in the dog, 90-day study in the rat, a 16-week study in the dog, and 2 chronic studies: 32 weeks in the rat, and 42 weeks in the dog. Only 4 repeated-dose toxicity studies of GLP standards were reviewed here. The iron and citrate content of dietary doses administered in repeated dose studies are given below:

Iron and Citrate dosages provided by dietary ferric citrate ingested by Rats and Dogs
(Sponsor's table)

Study Type (Study Number)	Ferric Citrate Dose (mg/kg/day)	Iron Dose (mg/kg/day)[a]	Citrate Dose (mg/kg/day)
Rat			
A 28-Day Oral (Dietary) Range-finding Toxicity Study in Rats (Study Report 06-2965)	0, 500, 2000, 3500	0, 108, 432, 756	0, 271, 1084, 1897
A 90-Day Oral (Dietary) Toxicity Study in Rats With a 30-Day Recovery Period (Study Report 07-2038)	0, 500, 1400, 2800	0, 108, 302, 605	0, 271, 759, 1518
A 32-Week Oral (Dietary) Toxicity Study in Rats With a 1-Month Recovery Period (Study Report 09-2120)	0, 500, 1000/1400, 2000/2800	0, 112, 223/312, 446/624	0, 268, 536/750, 1072/1501
Dog			
A 28-Day Oral Range-finding Toxicity Study in Dogs (Study Report 06-3186)	0, 500, 1000	0, 108, 216	0, 271, 542
An Escalating Dose/Maximum Tolerated Dose Oral Toxicity Study in Dogs (Study Report 07-3274)	1000, 2000, 2500, 3000, 3500	216, 432, 540, 648, 756	542, 1084, 1355, 1626, 1897
A 16-Week Oral Toxicity Study in Dogs With a 30-Day Recovery Period (Study Report 07-3296)	0, 500, 1200, 2800	0, 108, 259, 605	0, 271, 650, 1518
A 42-Week Oral Toxicity Study in Dogs With a 60-Day Recovery Period (Study Report 09-3386)	0, 400, 1000, 2000	0, 89, 223, 446	0, 214, 536, 1072

[a] Doses presented for iron and citrate are based on measured results from the drug substance batches that are presented in Module 2.6.7, Table 2.6.7.4A and Table 2.6.7.4B and Module 3.2.S.4.4.
JT=Japan Tobacco Inc.

6.2.1 JTT-751 (Ferric Citrate): A 90-Day Oral (Dietary) Toxicity Study in Rats with a 30-Day Recovery Period

Conducting laboratory and location:	(b) (4)
Study No.:	07-2038
Date of study initiation:	14 January 2008
Drug/lot No:	Ferric Citrate/30654
Dosage:	500, 1400 and 2800 mg/kg/day
Species/strain:	Sprague-Dawley Rats
Number/sex/group	15 /sex/group; 10/sex in low-dose group
Route:	Oral (Dietary mixture)
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

Results of 90-day toxicity study in rats have shown dosage -dependent iron deposition in the cecum, colon, spleen, and liver of males and females treated at all dose levels. Decrease in urinary phosphorus, increase in calcium levels and altered serum iron parameters, returned to control levels by the end of 30-Day recovery period. Pathological changes in the cecal mucosa (mucosal thickening, basophilia, and increased mixed inflammatory cell infiltrate) and colonic epithelium (goblet cell hyperplasia, increased size of colonic glands) at mid and high dosage (1400 and 2800 mg/kg/day) were also returned to control levels by the end of recovery period. Effects were considered to be adaptive responses to ferric citrate treatment, and the NOAEL was not determined.

Purpose

The present study was conducted to assess the toxicity of ferric citrate (KRX0502) in rats to select the doses for long-term studies.

Methods

On the basis of a 28-day dosage ranging study in Sprague-Dawley (Crl: CD SD) rats (Study No. 06-2965), the ferric citrate was administered to rats (15/sex/day) via dietary mixture for 90-Day at doses of 0, 500, 1400, or 2800 mg/kg/day followed by 30-Day recovery period (Table 12). The maximum dose 2800 mg/kg/day is 14 times equivalent to maximum proposed human dose 12 gram/day dose in a 60 kg human (200 mg/kg) on a mg/kg basis, and 2.27 times the human dose on the basis of mg/body surface area. Animals were observed twice daily for mortality, general condition, clinical signs, appearance, activity, behavior, skin, fur, eyes, nose, oral cavity, abdomen, external genitalia, ophthalmology, body weights, food consumption, clinical pathology, organ weights, macroscopic observations. Blood and urine samples were collected from 10 animals/sex/group during week 13 and from 5 animals/sex/group (control and mid- and high-dose groups) during week 17. Animals were fasted overnight prior to blood collection.

Table 12: Experimental outline (Sponsor's table)

Group	Dose[a] (mg/kg/day)	Number of Animals											
		Total		Clinical Pathology				Necropsy				Microscopic Pathology	
				Week 13		Week 17		End of Dose		Recovery			
		M	F	M	F	M	F	M	F	M	F	M	F
1	0[b]	15	15	10	10	5	5	10	10	5	5	15	15
2	500	10	10	10	10	0	0	9	10	0	0	10	10
3	1400	15	15	10	10	5	5	9	10	5	5	15	15
4	2800	15	15	10	10	5	5	10	10	5	5	15	15

[a]Doses represent the weight of KRX 0502 (ferric citrate) without any corrections.
[b]Control animals received untreated diet.

The first day of dosing was defined as Day 1 of the study.

Blood samples, collected into EDTA tubes as anticoagulant, were analyzed for hematological parameters: hemoglobin concentration, hematocrit, erythrocyte count, platelet count, mean platelet volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, total leukocyte count, reticulocyte count, differential leukocyte count. Peripheral blood smears were prepared. Coagulation parameters were analyzed using blood samples collected into sodium citrate as anticoagulant.

The **clinical chemistry** parameters: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea nitrogen, creatinine, cholesterol, triglycerides, total protein, albumin, total bilirubin, sodium, potassium chloride, calcium, inorganic phosphorus, gamma-glutamyl transferase, ferritin, iron, and unsaturated iron binding were analyzed in serum or plasma using the Hitachi 917, Roche Corporation Automatic Analyzer. Globulin, albumin/globulin ratio, transferrin saturation, total iron binding capacity were also evaluated.

Urinalysis was performed with samples collected into ice-chilled containers overnight for: protein, glucose, ketones, occult blood, pH, bilirubin, urobilinogen, creatinine, calcium, Inorganic phosphorus, appearance, specific gravity, volume, calcium/creatinine ratio and phosphorus/creatinine ratio. Protein results were verified using a three percent sulfosalicylic acid test while bilirubin results were confirmed via Ictotest reagent tablets (Henry, 1991).

Histopathology was performed on weighed organs (Table 13) embedded in paraffin using the hematoxylin stain while Prussian blue (Perl) and von Kossa stains were used for ferric ions and calcium ions, respectively.

Bone marrow slides from control dogs (Animal Nos. 1391 and 1891) and high-dose dogs (Animal Nos. 4391 and 4892) sacrificed at the end of dosing (Week 43) were stained with Prussian blue for detection of ferric iron in the pigment deposits.

Statistical Analysis
Experimental group parameters (body weight, body weight change from interval to interval,

cumulative body weight change from baseline, food consumption, coagulation, clinical chemistry, urinalysis and urine chemistry, organ weights) were compared to mean value for the control group using Bartlett's test for variance homogeneity (Bartlett, 1937), Williams' test (Williams', 1971, 1972), and Dunnett's test (Dunnett, 1955, 1964) for statistically significant differences. With 75% of the values for a clinical pathology parameter, Fisher's Exact Test (Fisher, 1973) was performed followed by Mantel's test (Mantel, 1963).

Table 13: Tissues examined for histopathology (Sponsor's table)

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY[a]
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (rib)		X[b]	
bone (sternum, distal femur)		X	X
bone marrow (sternum, femur)		X	X[c]
brain (medulla, pons, cerebrum and cerebellum)	X	X	X
epididymides	X	X	X
esophagus		X	X
eyes		X	X
Harderian gland		X	X
heart	X	X	X
kidneys	X	X	X[d]
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X[d]
liver	X	X	X[d]
lungs (with mainstem bronchi)		X	X
lymph nodes (mesenteric, mediastinal)		X	X[d]
mammary gland (inguinal)		X	X
nerve (sciatic)		X	X
optic nerve		X	
ovaries	X	X	X
pancreas		X	X
pituitary gland	X[e]	X	X
prostate gland/seminal vesicles	X	X	X
salivary glands (submandibular)		X	X
skeletal muscle (*rectus femoris*)		X	X
skin		X	X
small intestine (duodenum, ileum, jejunum)		X	X
spinal cord (cervical, thoracic, lumbar)		X	X
spleen	X	X	X[d]
stomach		X	X

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY[a]
testes	X	X	X
thymus	X	X	X
thyroid/parathyroid glands	X[e]	X	X
tongue		X	X
trachea		X	X
urinary bladder		X	X
uterus (body/horns) with cervix	X	X	X
vagina		X	X
tissues with macroscopic findings including tissue masses		X	X

[a]These tissues were examined for all animals from Groups 1 and 4 at the end of the dosing period and for animals in Groups 2 and 3 that died prior to study termination.
[b]Bone marrow smears were prepared only at the scheduled necropsies from the ribs of animals in Groups 1and 4.
[c]Qualitative examination (no differential count).
[d]The kidneys, liver, spleen, colon, cecum and mesenteric lymph nodes were also examined for all animals in Groups 2 and 3 at the end of the dosing period, and for all animals in Group 3 at the end of the recovery period.
[e]Weighed post-fixation.

Results

Mortality

One male rat (No. 3010) died in mid dosage group (1400 mg/kg/day) at day 28, and one (No. 2004) in low dosage group (500 mg/kg/day) at Day 59. Cause of their death could not be determined, however, as per Sponsor's note, death was not considered to be test article- related.

There were no clinical signs of concern and no ocular findings at the end of dosing period.

The mean body weight and the mean body weight gains were slightly lower (4% to 9%) in females and males (7% to 11%) in high dosage group (2800 mg/kg/day) when compared to controls, and were statistically non-significant (Figs. 6/7)

The mean food consumption, was significantly increased ($p<0.05$) in females at 500 mg/kg/day (6%), 1400 mg/kg/day (10%), 2800 mg/kg/day (16%) and in males at1400 mg/kg/day (9%), and 2800 mg/kg/day (21%) when compared to controls at week 13 (Fig. 6). A normalizing trend was seen in the mean food consumption of rats during the recovery period in comparison to control, however, the percent recovery was slow in males (7%) and females (10%) of high dosage group at the recovery week 1 (Fig. 7).

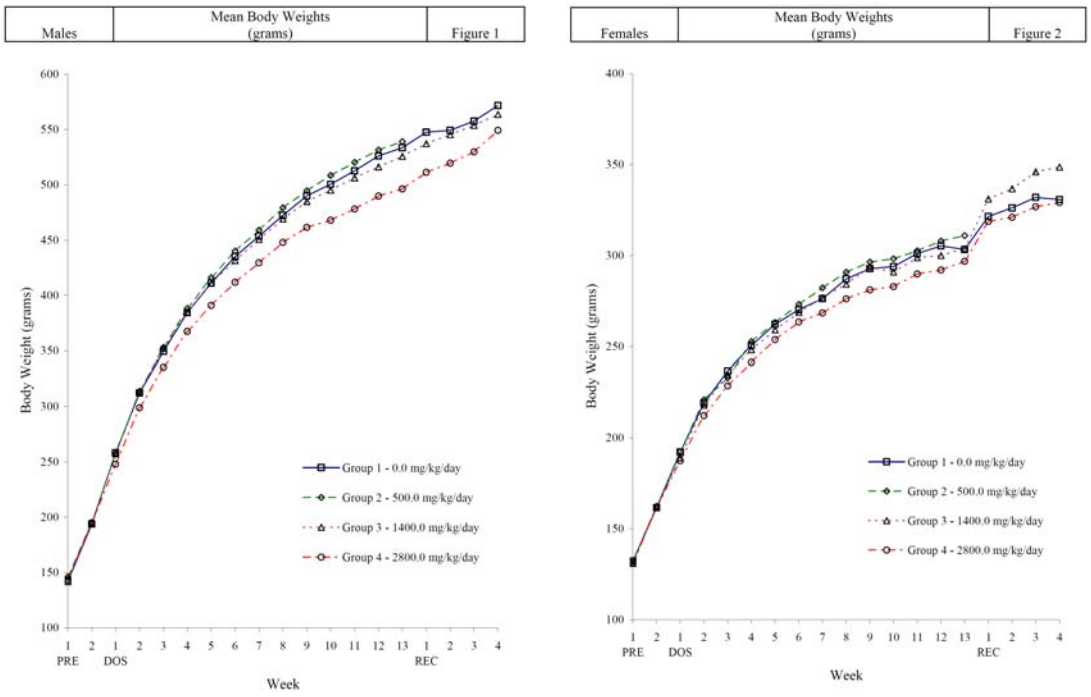


Figure 6: Change in the mean body weights in male and female rats during the dosing period
(Sponsor's Figure)

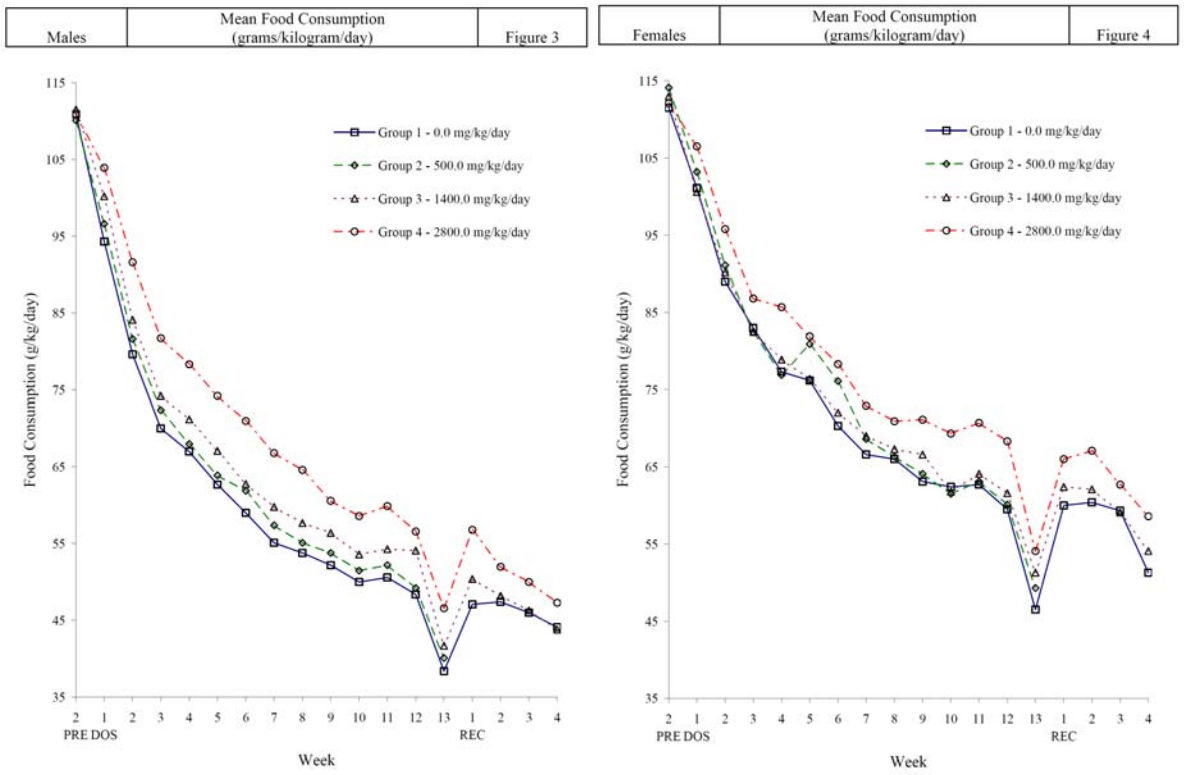


Figure 7: Change in the food consumption in male and female rats during the dosing period
(Sponsor's Figure)

Hematology
A significant increase ($p<0.01$) in hemoglobin (10%), hematocrit (7%), and reticulocytes (41%) was observed in males at high dosage (2800 mg/kg/day) of ferric citrate. In addition, a consistent increase in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was noted in this group of animals. An increase in reticulocytes (35%) and platelets (22%) was also reported in females in the high dosage group (2800 mg/kg/day).

Significant increases were observed in neutrophils (59% and 64% in males and females, respectively) and eosinophils (273% and 170%, respectively) in high dosage group of animals. Most of the changes observed during the treatment period have shown a normalizing trend during the recovery phase except some that were considered to reflect normal variability.

There were no changes observed in coagulation parameters. However, prothrombin time was slightly decreased (< 1 second) in males tat mid and high dosages (1400 and 2800 mg/kg/day), and returned to normal values by the end of recovery period.

Clinical Chemistry
Changes observed in clinical chemistry parameters were more significant in males receiving 2800 mg/kg/day of iron citrate in comparison to females (Table 14).

Table 14: Changes in Serum Iron Parameters relative to controls (Sponsor's table)

	Males	Females
Dose (mg/kg/day)	**2800**	**2800**
Iron	+41%[a]	+6%
Ferritin	+42%[a]	+27%
Transferrin Saturation (TSAT)	+80%[a]	+16%
Total Iron –Binding Capacity (TIBC)	-18%[a]	-10%[a]
Unsaturated Iron-Binding Capacity (UIBC)	-44%[a]	-44%

[a]Absolute values are statistically significantly different from control.

There were increases in mean serum levels of iron and ferritin (water-soluble iron-protein complex) and in saturation of transferrin (TSAT) in males. Consistent with the increased saturation of transferrin was a decrease in total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC).

Significant increases ($p<0.05$) in serum phosphates were recorded in males at all dosage levels (10% to 18% higher than control), and in females at 2800 mg/kg/day (21% higher than control). Decreases ($p<0.01$) in serum albumin were observed in females at 1400 and 2800 mg/kg/day (8% and 11% lower than controls, respectively); and in parathyroid hormone in males at 2800 mg/kg/day (41% lower than control).

A decrease in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in females was observed at all dosage levels (23% to 29% lower than control), and a slight decrease in ALT

in males was observed at 2800 mg/kg/day. In view of lack of histopathologic findings, these changes were not considered to be adverse, and iron parameters were comparable to controls following a 30-Day recovery period.

Urinalysis
Urinary phosphorus/creatinine levels were decreased ($p<0.05$) at mid and high dosage (1400 and 2800 mg/kg/day) of ferric citrate, and accompanied by an increase in urine calcium/creatinine levels in males and females (Table 15).

Table 15: Urinary Calcium/Creatinine and Phosphorus/Creatinine Concentration (Sponsor's table)

Dose (mg/kg/day)	Males		Females	
	Ca/Creat	P/Creat	Ca/Creat	P/Creat
0	0.02	0.92	0.08	1.66
500	0.03	0.73	0.12	1.39
1400	0.05[b]	0.25[b]	0.07	0.73[b]
2800	0.31[b]	0.01[b]	0.49[b]	0.03[b]

[a]Values are the means of the ratios for individual animals.
[b]Values are statistically significantly increased or decreased as compared to control values.
Ca = calcium; P = phosphorus; Creat = creatinine

The calcium concentration and pH of the urine were significantly increased at the mid and high dosages of ferric citrate treated animals (Table 16). However, the pH and levels of calcium and phosphorus were comparable to that of the controls by the end of the 30-Day recovery period.

Table 16: Change in pH values during the treatment period (Sponsor's table)

Dose (mg/kg/day)	Males		Females	
	Mean pH (n = no. of animals)	No. of animals with pH>9.0	Mean pH (n = no. of animals	No. of animals with pH>9.0
0	7.1 (n = 9)	0	6.5 (n = 10)	0
500	7.4 (n = 9)	0	6.7 (n = 10)	0
1400	7.9* (n =6)	3	7.0** (n = 10)	0
2800	8.5 (n = 1)	9	8.1** (n = 6)	4

Organ weights
No changes were observed in organ weights following the treatment of ferric citrate.

Macroscopic and Microscopic Findings
There were no macroscopic findings of concern except the presence of black material in cecum, jejunum, ileum, colon and rectum/low colon of the males and females in all treated groups, (Table 17) and attributed to iron deposition. Dose related microscopic findings were the present as brown/blue granular material in the cecal lumen and/or glandular lumens (≥500 mg/kg/day) and thickening of the cecal mucosa in animals that received1400 or 2800 mg/kg/day of ferric citrate.

Table 17: Presence of black material in GI Tract (Sponsor's table)

	Males				Females			
Ferric citrate (KRX 0502) (mg/kg/day)	0	500	1400	2800	0	500	1400	2800
Number of animals examined	10	10	10	10	10	10	10	10
Jejunum	0	1	0	0	0	0	0	1
Ileum	1	4	1	1	0	0	0	2
Cecum	0	9	9	9	0	10	9	9
Colon	0	2	0	0	0	4	1	1
Rectum/Low Colon	0	1	0	0	0	4	1	2

Goblet cell hyperplasia and/or increased size of the colonic glands were found in in the colonic epithelium of most of the animals (Table 18) at all dosage levels. Inflammatory cell infiltrate was observed in a few animals at mid and high dosage levels.

Table 18: Microscopic findings in Colon (Sponsor's table)

	Males				Females			
KRX 0502 (Ferric citrate) (mg/kg/day)	0	500	1400	2800	0	500	1400	2800
Number of animals examined	10	10	10	10	10	10	10	10
Macrophages with brown pigment	0	2	9	6	0	2	5	10
Goblet cell hyperplasia/increased gland size	0	2	6	10	0	0	0	0
Mucosal cells: basophilia	1	0	1	1	0	0	0	0
Increased mixed inflammatory cell infiltrate	0	0	1	2	0	0	0	1

Pathological findings observed in colon and caecum of treated animals returned to normal levels during the recovery period. However, the iron deposits in the colon, mesenteric lymph node, liver, spleen, and kidneys persisted.

Conclusion

Results of 90-day study have shown dosage -dependent iron deposits in the cecum, colon, spleen, and liver of males and females treated at 1400 (females only) and 2800 mg/kg/day. Urinary phosphate level decreased, blood calcium level increased and serum iron parameters were altered. The altered parameters returned to control levels by the end of 30-Day recovery period. There were pathological changes in the cecal mucosa (thickening, basophilia, and increased mixed inflammatory cell infiltrate) and colonic epithelium in males at all dosages (goblet cell hyperplasia, increased size of colonic glands) but pathology was absent in treated rats at the end of recovery period. Effects were considered to be adaptive responses to ferric citrate treatment, and the NOAEL was not determined.

6.2.2. JTT-751 (Ferric Citrate): A 32 Week Oral (Dietary) Toxicity Study in Rats with a 1-Month Recovery Period

Conducting laboratory and location:	(b) (4)
Study No.:	09-2120
Date of study initiation:	10 March 2009
Drug/lot No:	Ferric Citrate/34867, 34868, & 34972
Dosage:	500, 1000/1400 and 2000/2800 mg/kg/day
Species/strain:	Sprague-Dawley CD® Rats
Number/sex/group	20/sex/group
Route:	Oral (Dietary mixture)
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

Dosage dependent microscopic finding of brown pigmentation and macrophage infiltration was evident at all dosage levels due to overloading of iron in liver, colon and lymph nodes. Pigmentation did not fully reverse by the end of 4-Week recovery period, however, no tissue damage was associated. *This reviewer does not agree with the NOAEL dose of 2800 mg/kg/day as determined by the Sponsor and suggest lowering the NOAEL to 500 mg/kg/day due to treatment related persistent pigmentation and presence of macrophages at mid and high dosage levels.*

Purpose

The present study was conducted to assess the toxicity of orally administered ferric citrate in rats for 32 Weeks.

Methods

Ferric citrate was orally administered to Sprague-Dawley rats at the highest selected dosage of 2800 mg/kg/day. That dosage is based on 90-day toxicity studies in rats, and is 14 times the human equivalent dosage of 200 mg/kg (12 gram/day i/ 60 kg human) and is 2.27 times the human dosage on the basis of mg/body surface area. Dosages of 500 and 1400 mg/kg/day were selected for the low and mid doses to determine the dose relationship of test article effects per protocol described in Table 19.

Animals were observed twice daily for signs of toxicological response (abnormalities in general condition, appearance, activity, behavior, respiration). Animals in extremely poor health or in a possible moribund condition were identified for further monitoring and possible euthanasia.

Observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration and palpation for tissue masses were conducted.

Table 19: Experimental Outline (Sponsor's table)

The test article was administered in the diet to rats for at least 32 weeks.

		Number of Animals											
				Clinical Pathology				Necropsy					
		Total		Weeks 13 and 33		Recovery (Week 37)		End of Dose (Week 33)		Recovery (Week 37)		Microscopic Pathology	
Group	Dose[a] (mg/kg/day)	M	F	M	F	M	F	M	F	M	F	M	F
1	0[b]	20	20	15	15	5	5	15	15	5	5	20	20
2	500	20	20	15	15	5	5	15	15	5	5	20	20
3	1000/1400[c]	20	20	15	15	5	5	15	15	5	5	20	20
4	2000/2800[c]	20	20	15	15	5	5	15	15	5	5	20	20

[a]Doses represent the weight of JTT-751 (ferric citrate) without any corrections.
[b]Control animals received untreated diet.
[c]The test article was administered for 6 weeks at doses of 1000 and 2000 mg/kg/day. Beginning Week 7, the dose levels were adjusted to 1400 and 2800 mg/kg/day based on Sponsor request per comments received from the U.S. FDA.

The first day of dosing was defined as Day 1 of the study.

Ophthalmological examination
Lids, lacrimal apparatus and conjunctiva were examined visually. The cornea, anterior chamber, lens, iris, vitreous humor, retina and optic disc were examined by indirect ophthalmoscopy.

Body Weight
Animals were weighed twice weekly at pretest, during treatment and recovery (after fasting). Terminal, fasted body weights were obtained just prior to necropsy.

Food Consumption
Unrestricted food supply was available to all animals (7 days/week). Food consumption was measured weekly (5/6 days), beginning one week prior to treatment.

Calculation
Grams of food consumed/day (g/day) = grams of food consumed/ 5/6 days

Clinical Pathology
Animals were fasted overnight and blood samples were collected from 15animals/sex/group at Week-13 (middle), Week-33 (termination), and from 5animals/sex/group at Week37, the end of recovery period.

Hematology parameters analyzed in blood samples containing K2EDTA as the anticoagulant and using the ADVIA 120 Hematology analyzer included: hemoglobin concentration, hematocrit, erythrocyte count, platelet count, mean platelet volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell, distribution width, total leukocyte count, reticulocyte count, and differential leukocyte count. Sodium citrate was used as anticoagulant to collect the blood samples for coagulation studies.

Clinical Chemistry
Blood samples for clinical chemistry were collected without any anticoagulant to obtain the serum, and analyzed for following parameters using Hitachi 917, Roche Corporation Automatic Analyzer: aspartate aminotransferase , alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, glucose, cholesterol, triglycerides, total protein , albumin, total bilirubin, sodium, potassium, chloride, calcium, inorganic phosphorus, ferritin, iron, unsaturated iron binding capacity, globulin, albumin/globulin ratio, transferrin saturation (TSAT) total iron binding capacity (TIBC), and parathyroid hormones (PTH).

Urinalysis was performed using the urine samples collected into ice-chilled containers overnight (~16 hours), and included: protein, glucose, ketones, nitrites, pH, bilirubin, urobilinogen, creatinine, calcium, inorganic phosphorus, appearance, specific gravity and volume. Microscopic examination was performed on urine samples manually (Henry, 1991) using the sulfosalicylic acid test for the determination of turbidity and precipitation.

Organ weights
Overnight fasted animals from 32 Weeks main study (15animals/sex/group) and recovery period (5animals/sex/group) were euthanized; organs were weighed and subjected to macroscopic examination. Paired organs were weighed together. Slides were made from specified organs for histopathological examination (Table 20). Prussian blue was used on selected tissues to stain for iron.

Table 20: List of organs for macroscopic and microscopic examination (Sponsor's table)

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY[a] (Groups 1 and 4)
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (femur)		X	
bone (sternum, distal femur)		X	X
bone marrow (sternum, femur)		X	X[b]
brain (medulla, pons, cerebrum and cerebellum)	X	X	X
epididymides	X	X	X
esophagus		X	X
eyes		X	X
Harderian gland		X	X
heart	X	X	X
kidneys[c]	X	X	X
lacrimal glands		X	X
large intestine (cecum[c], colon[c], rectum[c])		X	X

liver[c]	X	X	X
lungs (with mainstem bronchi)		X	X
lymph nodes (mesenteric[c], mediastinal[c])		X	X
mammary gland		X	X
nerve (sciatic)		X	X
optic nerve		X	
ovaries[c]	X	X	X
pancreas		X	X
pituitary gland	X[d]	X	X
prostate gland/seminal vesicles	X	X	X
salivary glands (submandibular)		X	X
skeletal muscle (*rectus femoris*)		X	X
skin		X	X
small intestine (duodenum[c], ileum[c], jejunum[c])		X	X
spinal cord (cervical, thoracic, lumbar)		X	X[e]
spleen[c]	X	X	X
stomach[c]		X	X
testes	X	X	X
thymus	X	X	X
thyroid/parathyroid glands	X[d]	X	X
tongue		X	X
trachea		X	X
urinary bladder		X	X
uterus (body/horns) with cervix	X	X	X
vagina		X	X
gross lesions		X	X

[a]Tissues were examined microscopically for animals in Group 3 that died prior to study termination

[b]Qualitative examination (no differential count).

[c]Kidney, liver, spleen, colon, cecum, stomach, duodenum, jejunum, ileum, rectum, ovaries, and mediastinal and mesenteric lymph nodes were also examined for all animals in Groups 2 and 3 at the end of dosing and all groups at the end of the recovery period.

[d]Weighed post-fixation.

[e]Only the cervical region was examined.

Statistical analysis

Body weight, food consumption, hematology, coagulation, clinical chemistry, urinalysis, organ weights, organ/body weight and organ/brain weight ratios were subjected to statistical analyses using the Bartlett's test for variance homogeneity (Bartlette, 1937) and William's test for a monotonic trend as applicable (Williams, 1971, 1972).

Results

Mortality

Two males treated at mid-dosage died spontaneously, one male in high dosage group (Week 5) was euthanized due to snout trauma and not considered to be treatment- related.

Clinical Observation
Dark feces were found in all animals from the start of week 2 to the end of dosing at week 32, and attributed to the unabsorbed ferric citrate in the GI tract. A decrease in dark feces noticed by the end of recovery period at week 4 indicated reversal of the effect.

Ophthalmology
No ophthalmic abnormalities were noted at the end of dosing.

Body weights
A dose -dependent decrease was observed in body weight and body weight gain in males at all dose levels, and in females, only at the 500 mg/kg/day at intervals between Weeks 17 and 32 when compared to controls (Table 21). However, the body weight decreases were not significant.

Table 21: Decreases in Mean Body Weight and Body Weight Gain in Males at Week 32 (Sponsor's table)

	Dose mg/kg/day	Mean Body Weight	Mean Body Weight Gain
Males	500	-6%[b]	-9%[b]
	1000/1400	-9%[b]	-14%[b]
	2000/2800	-12%[b]	-18%[b]

[a]Percent change = (Mean value of treated group – Mean value of control group) ÷ Mean value of control group
[b]Absolute values are statistically significantly different from control values.

A significant increase in mean male body weight of approx. 47 g was observed in the high dose group by the end of 4-week recovery period, as compared to a loss of 1.4 grams for the controls. During same time period, male in the mid dosage group gained ~16.5 grams compared to the controls (1.5 grams). No apparent changes were seen in the body weight increase and body weight gains in ferric citrate treated females. The male weight loss during treatment was reversed during the recovery.

Food consumption
A dose dependent increase was seen in mean food consumption of males at mid and high dose levels and predominantly at all dose levels in females (Table 22).

Table 22: Increase in Mean Food Consumption during the Dosing period (Sponsor's table)

	Dose (mg/kg/day)	Food Consumption (grams/day)
Males	500	-
	1000/1400	+7%
	2000/2800	+11%[b]
Females	500	+10%
	1000/1400	+14%[b]
	2000/2800	+23%[b]

[a]Percent change = (Mean value of treated group – Mean value of control group) ÷ Mean value of control group
[b]Absolute values are statistically significantly different from control values.

The intake of test article (ferric citrate) was found to be close to the nominal dosage at proposed dosage levels (Table 23).

Table 23: Daily Intake of Ferric Citrate (Sponsor's table)

	Weeks 1 to 6		Weeks 7 to 32		Weeks 1 to 32
	Nominal Dose (mg/kg/day)	Actual Dose (% Nominal)	Nominal Dose (mg/kg/day)	Actual Dose (% Nominal)	Actual Dose (mg/kg/day)
Males	500	101.2%	500	102.3%	510.4
	1000	98.3%	1400	102.4%	1349.4
	2000	100.3%	2800	101.8%	2692.4
Females	500	98.5%	500	102.7%	509.5
	1000	102.0%	1400	105.0%	1385.5
	2000	102.2%	2800	103.9%	2746.8

Hematology

Dose- dependent changes were seen in hematology parameters in males at mid and high- dosages and in females at all dosages. Mean platelet volume (MPV) in males was increased to 10% and 17%, at mid and high dosage. In females the increase was 4%, 8% and 19% at low, mid and high dosage, respectively (Table 24).

Table 24: Change in Mean Platelet Volume Relative to Control (Sponsor's table)

	Dose	Mean Platelet Volume (MPV)		
	mg/kg/day	Week 13	Week 33	Recovery
Males	500	-	-	-
	1000/1400	+10[b]	+6[b]	+8[b]
	1400/2800	+17[b]	+15[b]	+14[b]
Females	500	+4[b]	+4[b]	-
	1000/1400	+8[b]	+8[b]	+5[b]
	1400/2800	+19[b]	+15[b]	+17[b]

[a]Percent change = (Mean value of treated group – Mean value of control group) ÷ Mean value of control group

[b]Absolute values are statistically significantly different from control values.

No changes were seen hemoglobin, hematocrit and red blood cell counts in males and females at any dosage. Slight, but significant, increases were observed in MCV (2 to 4%), MCH (3 to 8%), and MCHC (2 to 4%) at 13 and/or 33 weeks in males at mid and high dosage when compared with controls. An increase (22%) in reticulocyte count in females was noticed in high dosage group at Week 13 and 33.

A significant increase was observed in white blood cell count in males at high dosage and in females at all dosages. Neutrophils were increased at all dosages levels in females while eosinophils at mid and high dosage. Increase in neutrophils was noticed at high dosage in males at Week 13 (75%) and Week 33 (172%) as shown in Table 25.

Table 25: Change in Mean Neutrophils and Eosinophil Counts (Sponsor's table)

	Dose	Neutrophils			Eosinophils		
	mg/kg/day	Wk 13	Wk 33	Recovery	Wk 13	Wk 33	Recovery
Males	500	-	-	-	-	-	-
	1000/1400	-	-	-	-	-	-
	2000/2800	-	-	-	+75%	+172%[b]	-
Females	500	-	+38%[b]	-	-	-	-
	1000/1400	-	+44%[b]	-	-	+100%[b]	-
	2000/2800	-	+108%[b]	-	-	+386%[b]	-

[a]Percent change = (Mean value of treated group – Mean value of control group) ÷ Mean value of control group

[b]Absolute values are statistically significantly different from control values.

At the end of recovery period, the mean platelet volume (MPV) remained increased in males and females at mid and high dosage while other hematological parameters returned to control values.

There were no changes in coagulation parameters in males and females at any dosage.

Clinical Chemistry

The mean serum ferritin concentration, reflecting an increase in iron overload was increased significantly in males at mid and high dosage groups at Week-13 (28 & 60%) and Week-33 (21 & 68%) when compared to controls. Serum ferritin levels were also increased in females at mid and high dosage at Week-13 (29 % 51%) and Week-33 (18 & 26%) as shown in Table 26.

Table 26: Changes in Iron parameters Relative to controls (Sponsor's table)

	Dose	Iron		Ferritin		TSAT	
	mg/kg/day	Wk 13	Wk 33	Wk 13	Wk 33	Wk 13	Wk 33
Males	500	-	-	+21%	+20%	-	-
	1000/1400	+30%[b]	+18%	+28%	+21%	+39%[b]	+19%
	2000/2800	+43%[b]	+30%[b]	+60%[b]	+68%[b]	+54%[b]	+26%
Females	500	-	-	-	-	-	-
	1000/1400	-	-	+29%[b]	+18%	-	-
	2000/2800	-16%[b]	+18%[b]	+51%[b]	+26%[b]	-	+22%[b]

[a]Percent change = (Mean value of treated group – Mean value of control group) ÷ Mean value of control group

[b]Absolute values are statistically significantly different from control values.

TSAT = Transferrin Saturation

The mean serum iron level and saturation of transferrin (TSAT) were found to be increased in males at mid and high dosage at Week-13 (39 & 54%) and Week-33 (19 & 26%) compared to controls while in females a 22% increase was observed at high dosage at Week-33. Dosage-dependent increase was observed in serum phosphorus levels in both males and females at Week-13 (6 to 41%) and Week-33 (11 to 46%) in ferric citrated animals with a concomitant decrease of mean parathyroid hormone level in high dosage males at Week-33 (61%).

Urinalysis

The phosphate/creatinine ratio was decreased 19 to 92% in males and 25 to 89% in females in a dosage -dependent manner at all doses at Week 13. The decreases in phosphate levels were less pronounced, with the exception of males at 2800 mg/kg/day by the end of dosing period at Week33. In Week 33, phosphate/creatinine ratios were decreased 42 and 99%, respectively, in males at 1400 and 2800 mg/kg/day and 48% in females at 2800 mg/kg/day suggesting a physiological response to preserve serum phosphate. Increased urine ratios of calcium/creatinine, as compared to control values, were noted at 2800 mg/kg/day in males at Weeks 13 and 33 and in females only at Week 13 suggesting that PTH may be involved in this response (Table 27)

Table 27: Ratios of Urinary Concentrations of Calcium and Phosphorus to Creatinine (Sponsor's table)

	Dose	Ca/Creat			P/Creat		
	mg/kg/day	Wk 13	Wk 33	Recovery	Wk 13	Wk 33	Recovery
Males	0	0.04	0.03	0.05	1.35	1.20	1.17
	500	0.03	0.03	0.11	1.10[a]	1.07	1.23
	1400	0.05	0.04	0.07	0.53[a]	0.70[a]	1.51
	2800	0.23[a]	0.18[a]	0.06	0.11[a]	0.01[a]	1.38
Females	0	0.12	0.21	0.23	2.11	1.71	1.75
	500	0.12	0.20	0.29	1.59[a]	1.74	1.96
	1400	0.16	0.19	0.24	1.16[a]	1.73	2.19
	2800	0.44[a]	0.19	0.19	0.24[a]	0.89[a]	2.04

[a]Values are statistically significantly different from control values.

As observed the urine pH increased to alkaline side following the administration of ferric citrate to males and females, and returned to normal values by the end of recovery period (Table 28).

Table 28: Urine pH in Dosing period (Sponsor's table)

	Dose	Mean pH (Number of animals)			Number of pH values ≥ 9.0		
	mg/kg/day	Wk 13	Wk 33	Recovery	Wk 13	Wk 33	Recovery
Males	0	6.8 (15)	6.6 (15)	6.6 (5)	0	0	0
	500	7.3[a](15)	7.0 (15)	6.6 (5)	0	0	0
	1400	7.6[a](12)	6.9 (13)	6.8 (5)	3	0	0
	2800	7.8[a] (8)	7.7[a] (11)	6.6 (5)	6	3	0
Females	0	6.6 (15)	6.1 (15)	6.3 (4)	0	0	0
	500	7.0 (15)	6.3 (14)	6.3 (5)	0	0	0
	1400	7.2[a] (15)	6.7[a] (15)	6.4 (5)	0	0	0
	2800	7.4[a] (14)	6.8[a] (10)	6.9 (5)	1	3	0

[a]Values are statistically significantly different from control values.
Wk = Week

Organ weights

Mean absolute weight of the spleen, spleen/body weight spleen/brain weight ratios increased at 2000/2800 mg/kg/day compared to control, and was correlated with presence of brown pigment. An increase in the mean absolute liver weight and liver/body weight and liver/brain weight ratios was observed in females at 2000/2800 mg/kg/day, and was correlated with microscopic findings of brown pigment (Table 29).

The liver/body weight ratio was significantly increased in females at 1000/1400 mg/kg/day, but the increase in absolute weight was slight (↑10%) and individual values were within the control range for this study.

Table 29: Changes in Mean Absolute Organ Weights in Females at End of Dosing Relative to Control (Sponsor's table)

	Dose (mg/kg/day)	Spleen	Liver	Thyroid/Parathyroid
Females	1000/1400	-	-	+17%[b]
	2000/2800	+21%[b]	+15%[b]	+22%[b]

[a]Percent change = (Mean value of treated group – Mean value of control group) ÷ Mean value of control group

[b]Absolute values are statistically significantly different from control values.

The mean absolute weight of the thyroid gland was significantly increased (↑17% and ↑22%) in females at mid and high dosage, respectively. Thyroid/body weight and thyroid/brain weight were also increased at high dosage and thyroid/brain weight was increased at mid -dosage.

There were no histopathological findings that could be correlated with increases in thyroid/parathyroid weights. In males, the statistically significant increases in thyroid/body weight ratio at mid-dosage and thyroid/body weight and thyroid/brain weight ratios at high-dosage were attributed to lower body weights and/or normal variation. Organ weights in treated groups returned to normal and were comparable to controls by the end of recovery period

Macroscopic Findings

Following 32Weeks of dosing, animals (15/sex/group at 0 and 2000/2800 mg/kg/day) were examined for gross, macroscopic and microscopic findings at necropsy. A total of 3 unscheduled deaths (2 males at mid-dosage and 1 male at high dosage) occurred prior to necropsy.
Liver, kidneys, spleen, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, mesenteric lymph nodes, mediastinal lymph nodes and ovaries were harvested for microscopic evaluation. Black granular material was present in the cecum in virtually all treated animals. Black material was also found in the stomach, duodenum, jejunum, ileum, colon and rectum/low colon of several treated animals (Table 30).

Table 30: Presence of Black Material in the Gastrointestinal Tract (Sponsor's table)

	Males				Females			
Ferric citrate (JTT-751) mg/kg/day	0	500	1000/1400	2000/2800	0	500	1000/1400	2000/2800
No. animals examined	15	15	15	15	15	15	15	15
Stomach	1	0	0	3	0	1	0	0
Duodenum	0	2	0	1	0	0	0	0
Jejunum	0	2	0	1	0	1	1	2
Ileum	1	1	0	1	0	1	2	2
Cecum	1	14	11	13	0	15	14	10
Colon	0	2	1	1	0	1	0	0
Rectum/Low Colon	0	0	1	1	1	0	0	0

Microscopic Findings

There were dosage- related findings of brown pigment (hemosiderin or lipofuschin) in Kupffer cells in the liver, macrophages in the spleen and tubular epithelial cells in the kidneys (Table 31). Brown pigment was still present in the liver and spleen at the end of the recovery period.

Table 31: Presence of Brown Pigment in the Liver, Spleen and Kidneys following 32 weeks of Dosing period (Sponsor's table)

	Males				Females			
Ferric citrate (JTT-751)	0	500	1000/ 1400	2000/ 2800	0	500	1000/ 1400	2000/ 2800
No. animals examined	15	15	15	15	15	15	15	15
Liver								
minimal	2	1	6	8	0	1	2	9
slight	0	0	1	2	0	0	0	1
total	2	1	7	10	0	1	2	10
Spleen								
minimal	5	2	1	1	0	0	0	0
slight	10	6	1	0	4	6	4	0
moderate	0	7	13	2	11	9	11	7
marked	0	0	0	12	0	0	0	8
total	15	15	15	15	15	15	15	15
Kidney								
minimal	3	3	2	9	4	10	10	13
slight	0	0	0	1	0	0	0	1
total	3	3	2	10	4	10	10	14

Macrophages containing brown pigment were found in the wall of the colon of most animals, especially at the high dosage. Pigmented macrophages were also present in the submucosa in the stomach, at the base of Peyer's patches in the small intestine, in the lamina propria and submucosa in the colon and at the base of lymphoid nodules in the colon, cecum and rectum.

Prussian blue (Perl) staining of colon (Animal No. 4015M) confirmed the presence of iron in pigmented macrophages in mucosal epithelial cells (Table 32).

Table 32: Presence of Pigmented Macrophages in the GI tract (Sponsor's table)

	Males				Females			
Ferric citrate mg/kg/day	0	500	1000/ 1400	2000/ 2800	0	500	1000/ 1400	2000/ 2800
No. animals examined	15	15	15	15	15	15	15	15
Stomach	0	0	0	2	0	0	0	0
Duodenum	0	0	2	2	0	0	0	1
Jejunum	0	0	1	2	0	0	3	2
Ileum	0	0	1	4	0	0	0	6
Colon	0	12	14	15	0	13	14	15
Cecum	0	0	1	7	0	0	2	7
Rectum	0	0	1	2	0	0	3	3

In addition to pigmented macrophages, increased mixed inflammatory cell infiltrates, increased goblet cells, and thickening of mucosal/submucosal and muscularis externa were also present in the colon wall of most of the treated animals. Full recovery was seen in the stomach by the end of recovery period. However, pigmented macrophages were still present in the intestinal wall at the end of the recovery period. The pigmented macrophage aggregates were increased in size, number and/or amount of pigment in males and females in a dosage- dependent manner in sinuses (sinus histiocytes), mesenteric lymph nodes (Table 33), and mediastinal lymph nodes (Table 34). Pigmented macrophages were also seen in ovaries and were still present at the end of the recovery phase.

Table 33: Microscopic Findings in Mesenteric Lymph Nodes (Sponsor's table)

	Males				Females			
Ferric citrate mg/kg/day	0	500	1000/ 1400	2000/ 2800	0	500	1000/ 1400	2000/ 2800
No. animals examined	15	15	15	15	15	15	15	15
Increased pigmented macrophage aggregates								
minimal	0	4	4	5	0	7	2	8
slight	0	1	2	4	0	3	1	6
moderate	0	0	3	4	0	1	4	0
total	0	5	9	13	0	11	7	14
Increased lymphocytes								
minimal	0	0	0	0	0	0	0	1
slight	0	3	4	3	0	3	6	6
moderate	0	3	1	0	0	2	1	0
total	0	6	5	3	0	5	7	7
Ectatic/cystic sinuses								
minimal	0	0	1	0	0	0	3	1
slight	0	0	0	0	0	0	0	2
moderate	0	0	0	0	0	0	1	0
marked	0	0	0	0	0	0	0	1
total	0	0	1	0	0	0	4	4

Table 34: Microscopic Findings in Mediastinal Lymph Nodes (Sponsor's table)

	Males				Females			
Ferric citrate mg/kg/day	0	500	1000/ 1400	2000/ 2800	0	500	1000/ 1400	2000/ 2800
No. animals examined	15	15	15	15	15	15	15	15
Increased pigmented macrophage aggregates								
minimal	1	2	9	8	0	5	3	7
slight	0	1	1	2	1	2	9	4
moderate	0	0	1	2	0	0	0	2
total	1	3	11	12	1	7	12	13
Pigmented sinus histiocytes								
minimal	1	3	3	3	3	3	5	5
slight	0	0	2	1	2	1	4	5
moderate	0	0	1	1	0	0	0	1
total	1	3	6	5	5	4	9	11

A complete recovery of increased lymphocytes was observed in animals treated at low dose level (500 mg/kg/day) while a partial recovery was seen in the sinus ectasia/cysts and pigmented macrophage aggregates in mediastinal and mesenteric lymph nodes.

Conclusion

Microscopic findings such as inflammatory cell infiltrates in the stomach and cecum, increased goblet cells in the ileum, cecum and rectum and mucosal thickening in the stomach, duodenum, jejunum, cecum and pigmented macrophage aggregates in the medullary cords were evident at all dosage levels in males and females due to overloading of iron and did not reverse by the end of 4 week recovery period in mid and high dosage groups. *This reviewer does not agree with the NOAEL dose of 2800 mg/kg/day as determined by the Sponsor, and lowered to 500 mg/kg/day in view of effects and findings at this dosage.*

6.2.3. JTT-751 (Ferric Citrate): A 16-Week Oral (Dietary) Toxicity Study in Dogs with a 30-Day Recovery Period

Conducting laboratory and location:	(b) (4)
Study No.:	07-3296
Date of study initiation:	14 January, 2008
Drug/lot No:	KRX 0502 (Ferric Citrate)/30654
Dosage:	500, 1200 and 2800 mg/kg/day
Species/strain:	Beagle dogs
Number/sex/group	4-6 /sex/group
Route:	Oral (Dietary mixture)
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

Enhanced serum ferritin in male (↑64%) and female animals (↑34%) at mid dosage (1200 mg/kg/day) and a ~9 & 15 fold increase at high dosage (2800 mg/kg/day) level was observed when compared with controls in 16-week dog study. The serum iron level was also increased in males (↑113%) and females (↑67%) at high dosage. Increased liver weight, bile duct hyperplasia, GI tract and liver injury observed at high-dosage is considered to be due primarily to iron overload and correlated with histopathological and clinical findings. *In view of findings observed at the 1200 mg/kg/day this reviewer sets the NOAEL at 500 mg/kg/day rather than the mid dosage proposed by Sponsor.*

Purpose

The present study was conducted to assess the effects of orally administered ferric citrate (KRX-0502) to Beagle dogs for a16-weeks.

Methods

Based on a dose- range finding toxicity study (28-Day), KRX-0502 was orally fed to Beagle dogs via dietary admixture for 3 weeks of dose escalation followed by 13 weeks at dosages of 0, 500, 1200, and 2800 mg/kg/day (0, 108, 259, and 605 mg Fe/kg/day; n=6/sex/group in the control and mid- and high-dosage groups; 4/sex in the low-dose group). At the end of the study, 2-animals/sex/group from the control, mid and high dosage groups were euthanized and necropsied after 30-Day treatment -free recovery period.

Animals were assessed for viability, clinical observations, ophthalmology, body weights, food consumption, clinical pathology, organ weights, macroscopic observations and histopathology. The hematological parameters included were: hemoglobin concentration, hematocrit, erythrocyte count, and platelet count, mean platelet volume, mean corpuscular volume mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, total leukocyte count, reticulocyte count, and differential leukocyte count.

The clinical chemistry parameters were analyzed by using the Hitachi 917, Roche Corporation Automatic Analyzer and included: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, cholesterol, triglycerides, total protein, albumin, total bilirubin, sodium, potassium chloride, calcium, inorganic phosphorus, gamma-glutamyl transferase, ferritin, iron, and unsaturated iron binding. Histopathology was performed on organs embedded in paraffin using the hematoxylin stain while Prussian blue (Perl) and von Kossa stains were used for ferric ions and calcium ions, respectively. Table 35 lists the organs and tissues that were harvested:

Table 35: Selected organs for histopathological examinations (Sponsor's table)

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY[a]
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (rib)		X[b]	
bone (sternum, femur)		X	X
bone marrow (sternum, femur)		X	X[c]
brain (medulla/pons, cerebrum and cerebellum)	X	X	X
epididymides	X	X	X
esophagus		X	X[d]
eyes		X	X
gallbladder		X	X[d]
heart	X	X	X
kidneys	X	X	X[d]
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X[d]
liver	X	X	X[d]
lungs (with mainstem bronchi)		X	X
lymph nodes (mesenteric, mediastinal)		X	X[d]
mammary gland (inguinal)		X	X
nerve (sciatic)		X	X
optic nerve		X	
ovaries		X	X
pancreas		X	X[d]
pituitary gland	X	X	X
prostate gland	X	X	X
salivary glands (submandibular)		X	X
skeletal muscle (*rectus femoris*)		X	X
skin		X	X
small intestine (duodenum, ileum, jejunum)		X	X[d,e]
spinal cord (cervical, thoracic, lumbar)		X	X
spleen	X	X	X[d]

Table 35 (contd)

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY[a]
stomach		X	X[d]
testes	X	X	X
thymus	X	X	X[d]
thyroid/parathyroid glands	X	X	X
tongue		X	X
trachea		X	X
urinary bladder		X	X
uterus (body/horns) with cervix		X	X
vagina		X	X
tissues with macroscopic findings including tissue masses		X	X[f]

[a]These tissues were examined for all animals from Groups 1 and 4 at the end of the dosing period.

[b]Bone marrow smears were prepared for animals in Groups 1and 4 only.

[c]Qualitative examination (no differential count).

[d]These tissues were also examined for all animals in Groups 2 and 3 at the end of the dosing period and for all animals at the end of the recovery period.

[e]The duodenum was not examined for animals in Groups 2 and 3 at the end of the dosing period nor for any animal at the end of the recovery period.

[f]In addition, ileocecal and ileocolic junctions with gross findings were examined for all animals in Groups 2 and 3 at the end of the dosing period and lymph nodes (other) with gross findings were examined for all animals at the end of the recovery period.

Urinalysis

Urine samples were collected into ice-chilled containers and analyzed for appearance, specific gravity, volume, calcium/creatinine ratio, phosphorus/creatinine ratio, protein, glucose, ketones, occult blood, pH, bilirubin, urobilinogen, creatinine, calcium and inorganic phosphorus.

Results

Mortality

There were no unscheduled deaths.

Body weight

A 7% decrease in body weight of males and 13% in females was found at the end of dosing period in high dosage group. Lower mean body weight gains were observed in males (33%) and females (53%) at high dosage when compared to controls, and returned to control values by end of recovery period in animals that had received the mid and high dosages.

Food Consumption

A significant increase in food consumption was observed in both males and females at all dosage e levels except in males 4210 and 4213 (40%) and female 4711 (30%), a decrease was noticed at the high dosage. No differences were found in consumption of food during the recovery period.

Clinical Observation

Red exudates (<10 to >20 cm in size) under the cages, discolored watery dark/black or gray stool, and discolored teeth were observed in all KRX-0502-treated animals within the first few

days of dosing and continued throughout the dosing period in most of the males and females at mid and high dosage.. At the end of 30-Day recovery period, discolored stool and exudates under the cage were no longer visible.

Ophthalmic Examination
No ocular abnormalities were found during the dosing period of this study.

Hematology
A significant increase ($p<0.05$) in MCV observed in males at1200 and 2800 mg/kg/day and in MCH, MCV and RDW in females at 2800 mg/kg/day is indicative of increased erythropoiesis. A significant increase ($p<0.01$, ↑43%) was noticed in MPV levels of females at mid dosage and in platelet counts at high dosage. A significant 22% prolongation of the coagulation time was observed in males and females at 2800 mg/kg/day : mean increases of 5 and 6.2 seconds mean activated partial thromboplastin time respectively. All hematological parameters returned to control values by the end of recovery period.

Clinical Chemistry
An increased level of serum ferritin was evident in male (↑64% & 15 fold) and female animals (↑34% & 9 fold) in mid and high dosage groups (Table 36).

Table 36: Serum Iron Parameters in Male Dogs, 16 Week Study (Sponsor's table)

Dose (mg ferric citrate/kg/day)	**0**	**500**	**1200**	**2800**
Dose[a] (mg Fe/kg/day)	**0**	**108**	**259**	**605**
Pre-Test				
N	6[b]	4	6[b]	6[b]
Iron (µg/dL)	113 (27.1)	170 (27.8)	115 (37.6)	128 (49.1)
TSAT (%)	32 (7.3)	47 (6.6)	34 (10.0)	39 (13.8)
Ferritin (ng/mL)	167 (21.4)	166 (29.2)	170 (32.0)	186 (41.0)
TIBC (µg/dL)	352 (43.0)	359 (17.7)	341 (37.8)	329 (53.9)
Week 16				
N	6[b]	4	6[b]	6[b]
Iron (µg/dL)	179 (76.4)	195 (42.4)	113 (33.9)	381** (123.8)
TSAT (%)	47 (19.3)	51 (12.6)	38 (10.2)	54 (--)[c]
Ferritin (ng/mL)	165 (10.8)	219 (19.6)	271* (42.0)	2553** (2614.0)
TIBC (µg/dL)	381 (40.9)	386 (17.0)	300** (27.8)	327 (--)[c]
Recovery[d]				
N	2	--	2	2
Iron (µg/dL)	144 (34.6)	--	88 (4.9)	201 (79.9)
TSAT (%)	38 (5.7)	--	25 (1.4)	53 (20.5)
Ferritin (ng/mL)	183 (3.5)	--	250 (0.0)	1620 (975.8)
TIBC (µg/dL)	373 (33.2)	--	355 (3.5)	380 (2.1)

Source: Study Report 07-3296, Table 12.
[a] Doses presented for iron are based on measured results from the drug substance batches that are presented in Module 2.6.7, Table 2.6.7.4A and Table 2.6.7.4B and Module 3.2.S.4.4.
[b] N=4/group (main cohort [end of dosing]); plus N=2/group (recovery) [Source: Study Report 07-3296, Section 2.3]
[c] N=1
[d] Statistical analyses not conducted when N≤2.
Significant difference relative to control: *p<0.05; **p<0.01.
SD=standard deviation; TIBC=total iron binding capacity; TSAT=transferrin saturation.

At the same time serum iron level was increased to 113 % in males and 67% in females in high dose group (2800 mg/kg/day) with consistent decreases in UIBC and TIBC (Table 37).

Table 37: Serum Iron Parameters in Female Dogs, 16 Week Study (Sponsor's table)

Dose (mg ferric citrate/kg/day)	**0**	**500**	**1200**	**2800**
Dose[a] (mg Fe/kg/day)	**0**	**108**	**259**	**605**
Pre-Test				
N	6[b]	4	6[b]	6[b]
Iron (µg/dL)	173 (51.6)	164 (57.0)	179 (27.1)	208 (24.9)
TSAT (%)	49 (12.3)	47 (11.1)	50 (8.3)	58 (9.7)
Ferritin (ng/mL)	163 (32.4)	184 (28.4)	152 (24.4)	155 (30.2)
TIBC (µg/dL)	352 (26.1)	346 (50.5)	357 (15.9)	362 (39.0)
Week 16				
N	6[b]	4	6[b]	6[b]
Iron (µg/dL)	263 (41.7)	174* (51.9)	137** (27.5)	438** (135.7)
TSAT (%)	72 (8.3)	50** (9.0)	43** (8.1)	86 (--)[c]
Ferritin (ng/mL)	176 (22.7)	215 (14.7)	235* (40.7)	1612** (975.9)
TIBC (µg/dL)	367 (44.3)	345 (48.7)	319 (20.4)	348 (--)[c]
Recovery[d]				
N	2	--	2	2
Iron (µg/dL)	237 (8.5)	--	124 (29.7)	296 (53.0)
TSAT (%)	71 (6.4)	--	32 (8.5)	75 (21.9)
Ferritin (ng/mL)	198 (26.9)	--	300 (89.8)	3240 (608.1)
TIBC (µg/dL)	336 (17.7)	--	391 (9.9)	403 (47.4)

Source: Study Report 07-3296, Table 12.

[a] Doses presented for iron are based on measured results from the drug substance batches that are presented in Module 2.6.7, Table 2.6.7.4A and Table 2.6.7.4B and Module 3.2.S.4.4.

[b] N=4/group (main cohort [end of dosing]); plus N=2/group (recovery) [Source: Study Report 07-3296, Section 2.3]

[c] N=1

[d] Statistical analyses not conducted when N≤2.

Significant difference relative to control: *p<0.05; **p<0.01.

SD=standard deviation; TIBC=total iron binding capacity; TSAT=transferrin saturation.

Increased (p<0.01) AST, ALT and Alkaline Phosphatase levels were seen in male and females treated with the high dosage of test article (2800 mg/kg/day) while total protein, albumin, cholesterol and triglycerides were low, and did not return to normal levels in follow up recovery period (Table 39).

Urinalysis

A significant (p<0.01) decrease (1.2 and 1.6 fold) in phosphate and calcium (2.7 and 2.2-fold) levels (normalized to creatinine) were observed in males and females, respectively, at high dosage and returned to controls by end of the recovery period. These findings are consistent with

physiologic response to conserve serum phosphorus and calcium levels. Values were at control levels by end of the dosing period.

Organ weights

A consistent increase (52%) in liver weight and decrease in thymus (64%), spleen (33%), heart (22%), prostate (60%), and epididymides (35%) associated with histopathological findings is considered to be a result of test article treatment at higher dosage level (Table 38). Except for liver, weight of other organs was returning towards normal by end of the recovery period.

Table 38: Change in absolute organ weights (Sponsor's table)

	Males		Females
Dose (mg/kg/day)	**1200**	**2800**	**2800**
Liver	+32%	+52%	+44%[b]
Thymus	-	-64%[b]	-30%
Spleen	-	-33%[b]	-
Heart	-	-22%[b]	-23%
Prostate	-	-60%[b]	-
Epididymides	-	-35%[b]	-

[a]Percent changes were calculated as [(treated value - control value) ÷ control value] x 100.

[b]Absolute values are statistically significantly different from control values.

Macroscopic and Microscopic Findings

Black or brown discoloration was observed in large intestine and liver. Thickening of the gall bladder correlated microscopically with lymphoid and/or goblet-cell hyperplasia and/or edema were observed at all dosage levels with increased severity at high dosage level. Prussian blue staining confirmed deposition of iron in the cecum, colon, and/or rectum.

Microscopic findings remained positive at the end of the recovery phase. Brown pigmented macrophages, brown pigments indicative of iron deposits and basophilic staining of connective tissue (mostly in lamina propria and/or submucosa) were found in digestive tract, and stained positive for ferric iron with the Prussian blue. A minimal to moderate liver damage as evident by increased liver weight, bile duct hyperplasia, chronic inflammatory foci (brown pigmented) and altered liver specific enzymes (Table 39), were seen in mid and high dosage groups.

Brown discoloration, enlarged size, and irregular surface and shape of the liver correlated with the chronic inflammatory foci, brown pigment (ferric iron) accumulation, and/or bile ductile proliferation observed microscopically. As a result of lower serum albumin, ascites developed in 2 males and 1 female in the high dosage group by the end of dosing. At the recovery phase, the reduced extent of clinical chemistry and hepatic injury parameters and absence of ascites indicated partial or complete recovery, and histopathological changes in liver were not observed at the high dosage. No effects were seen in sternum or femur histomorphology.

Table 39: Serum liver function parameters in high dosage- treated animals relative to controls
(Sponsor's table)

	Males	Females
Dose (mg/kg/day)	**2800**	**2800**
Aspartate Aminotransferase	+185%[b]	+58%[b]
Alanine Aminotransferase	+256%[b]	+171%[b]
Alkaline Phosphatase	+174%[b]	+184%[b]
Total Protein	-19%[b]	-20%[b]
Albumin	-28%[b,c]	-29%[b]
Cholesterol	-25%[b]	-35%[b]
Triglycerides	+61%[b]	-

[a]Percent changes were calculated as [(treated value - control value) ÷ control value] x 100.

[b]Absolute values are statistically significantly different from control.

[c]With decreased albumin/globulin ratio.

Conclusion

Histopathology was evident in liver at doses of 500 and 1200 mg/kg/day and in GI tract at 1200 mg/kg/day at the end of dosing in this 16-Week dog study. Tissue damage as a result of iron overloading was observed in liver and digestive tract of male and females in mid and high dosage and did not reverse during the 30 day post recovery period. *In view of irreversible findings still present during the recovery period at mid and high dosage, the no observed adverse effect dosage level (NOAEL) is considered to be 500 mg/kg/day by this reviewer instead of 1200 mg/kg/day as stated by the Sponsor.*

6.2.4 JTT-751 (Ferric Citrate): A 42-Week Oral (Dietary) Toxicity Study in Dogs with a 30-Day Recovery Period

Conducting laboratory and location:	(b) (4)
Study No.:	09-3386
Date of study initiation:	10 March 2009
Drug/lot No:	Ferric Citrate/34867, 34868, & 34972
Dosage:	400, 1000 and 2000 mg/kg/day
Species/strain:	Beagle dogs
Number/sex/group	7 /sex/group
Route:	Oral (Dietary mixture)
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

An unscheduled death occurring at the high dosage (2000 mg/kg/day) at week 40 was attributed to iron overload – induced liver injury and correlated with histopathological findings and clinical pathology in 42-Week dog study. Slight to moderate histopathological changes were observed in liver at mid dose level while at the low dose changes were limited to brown pigmentation and persisted by the end of 60-Day recovery period. The NOAEL dose was established at 400 mg/kg/day.

Purpose

The present study was conducted to assess the toxicity of 42-Week oral exposure to ferric citrate (KRX-0502) in Beagle dogs.

Methods

Ferric citrate was fed to Beagle dogs (7/sex/day) via dietary admixture for 35 weeks at dosages of 0, 400, 1000, and 2000 mg/kg/day (0, 89, 223, and 446 mg Fe/kg/day) following a dosage escalation period of 7 weeks (Table 40). Animals were observed twice daily for mortality and general condition and assessed for clinical signs, ophthalmology, body weights, food consumption, and clinical pathology including serum clinical chemistries.

At the end of the study 4/sex/group from the main study, and 3/sex/group from the control, mid and high dosage recovery groups (60-day) were euthanized and necropsied for gross pathology and harvesting of organs for weight and histopathology. One male in high dosage group (2000 mg/kg/day) was euthanized in Week 40 (Day 275) due to liver injury as a result of iron overload.

Blood and urine samples were collected prior to dosing and in Weeks 16, 29, and 42 and at the end of the recovery period (Week 51) for evaluation of hematological parameters: hemoglobin concentration, hematocrit, erythrocyte count, platelet count, mean platelet volume, mean corpuscular volume mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, total leukocyte count, reticulocyte count, differential leukocyte count

and urine analysis. Bone marrow smears were prepared from all animals, stained with Wright stain and qualitative evaluation was performed by a clinical pathologist.

Table 40: Oral Administration of Ferric Citrate to Beagle Dogs (Sponsor's table)

Group	Daily Dose[a] (mg/kg/day)	Number of Animals											
		Total		Clinical Pathology				Necropsy				Microscopic Pathology	
				Pretest, Weeks 16, 29 and 42 (End of Dosing)		Week 51 (End of Recovery		Week 43 (End of Dosing)		Week 51 (End of Recovery)			
		M	F	M	F	M	F	M	F	M	F	M	F
1	Weeks 1 to 42: 0 Weeks 43 to 51: Recovery	7	7	7	7	3	3	4	4	3	3	7	7
2	Week 1: 100 Week 2: 200 Week 3: 300 Weeks 4 to 42: 400 Weeks 43 to 51: Recovery	7	7	7	7	3	3	4	4	3	3	7	7
3	Week 1: 175 Week 2: 350 Week 3: 525 Weeks 4 to 6: 700 Week 7: 850 Weeks 8 to 42: 1000 Weeks 43 to 51: Recovery	7	7	7	7	3	3	4	4	3	3	7	7
4	Week 1: 300 Week 2: 600 Week 3: 900 Weeks 4 to 6: 1200 Week 7: 1600 Weeks 8 to 42: 2000 Weeks 43 to 51: Recovery	7	7	7	7	3	3	3	4	3	3	7	7

[a]Doses represent the weight of JTT-751 (ferric citrate) without any correction.
The first day of dosing was defined as Day 1 of the study.

Blood coagulation studies were performed in blood sample collected using sodium citrate as anticoagulant for prothrombin time, activated partial thromboplastin time and fibrinogen.

The **clinical chemistry** parameters comprised: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, cholesterol, triglycerides, total protein, albumin, total bilirubin, sodium, potassium chloride, calcium, inorganic phosphorus, gamma-glutamyl transferase, ferritin, iron, and unsaturated iron binding were analyzed using the Hitachi 917, Roche Corporation Automatic Analyzer.

In addition to this globulin, albumin/globulin ratio, transferrin saturation and total iron binding capacities were also evaluated.

Urinalysis was performed with samples collected into ice-chilled containers overnight for: protein, glucose, ketones, occult blood, pH, bilirubin, urobilinogen, creatinine, calcium, inorganic phosphorus, appearance, specific gravity, volume, calcium/creatinine ratio and phosphate/creatinine ratio.

Histopathology
Organs were weighed and prepared for microscopy. Tissues samples were embedded in paraffin sectioned and stained using hematoxylin and eosin, as well as Prussian blue (Perl) and von Kossa stains for identifying ferric ions and calcium ions, respectively. Organs included those noted in Table 41:

Table 41: Tissues examined for Histopathology (Sponsor's table)

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (rib)		X	X[a]
bone (sternum, femur)		X	X
bone marrow (sternum, femur)		X	X[a]
brain (medulla/pons, cerebrum and cerebellum)	X	X	X
epididymides	X	X	X
esophagus		X	X
eyes		X	X
gallbladder		X	X
heart	X	X[b]	X[b]
kidneys	X	X	X
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X
liver	X	X	X
lungs (with mainstem bronchi)		X	X
lymph nodes (mesenteric, mediastinal)		X	X
mammary gland (inguinal)		X	X
nerve (sciatic)		X	X
optic nerve		X	
ovaries		X	X
pancreas		X	X
pituitary gland	X	X	X
prostate gland	X	X	X

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY
salivary glands (submandibular)		X	X
skeletal muscle (*Rectus femoris*)		X	X
skin		X	X
small intestine (duodenum, ileum, jejunum)		X	X
spinal cord (cervical, thoracic, lumbar)		X	X[c]
spleen	X	X	X
stomach		X	X
testes	X	X	X
thymus	X	X	X
thyroid/parathyroid glands	X	X	X
tongue		X	X
trachea		X	X
urinary bladder		X	X
uterus (body/horns) with cervix		X	X
vagina		X	X
tissues with macroscopic findings including tissue masses		X	X

[a]Qualitative examination (no differential count).

[b]In addition to the standard sections of the heart, the mitral valve was collected and examined separately.

[c]Cervical spinal cord only.

Slides of bone marrow from control dogs (Animal Nos. 1391 and 1891) and high-dose dogs (Animal Nos. 4391 and 4892) sacrificed at the end of dosing were stained with Prussian blue for detection of ferric iron.

Statistical Analysis

Statistically significant treatment effects on body weight, body weight change from interval to interval, cumulative body weight change from baseline, food consumption, coagulation, clinical chemistry, urinalysis, urine chemistry and organ weights were identified using Bartlett's test for variance homogeneity (Bartlett, 1937), Williams' test (Williams', 1971, 1972), and Dennett's test (Dunnett, 1955, 1964).

Results

Mortality

One male dog (# 4390) that received 2000 mg/kg/day (446 mg Fe/kg/day) of ferric citrate was euthanized on Day 275 (Week 40) due to liver injury attributed to iron toxicity. An increased accumulation of brown pigment in the Kupffer cells/macrophages/hepatocytes, and a significant increase in serum ferritin level (7740 ng/mL) compared to controls (205 ng/mL) was noted in

Week 42. Unsaturated iron binding capacity (UIBC) was <10 µg/mL compared to 144 µg/mL in controls at Week 42. Liver findings were characterized as a marked accumulation of brown pigment in the Kupffer cells, macrophages, and hepatocytes, marked chronic inflammatory foci consisting of brown-pigmented macrophages, moderate bile ductule hyperplasia, and minimal fibrosis.

Increases in serum aspartate aminotransferase (↑4.3 fold), alanine aminotransferase (↑7.7 fold) and alkaline phosphatase (3 fold) and decreases in total protein and albumin (11% and 30%) compared to respective controls correlated with histological and pathological findings at the time of euthanasia.

In addition to this a decrease in reticulocytes (4.5 fold), hemoglobin concentration (41.6%), increases in platelets (55%), mean platelet volume (7%), neutrophils (113%), monocytes (33%) and total white blood cells (60%) compared to controls were also reported. No other mortalities were observed during the study and recovery phase.

Clinical Observation
Incidence of watery/unformed stool and discolored teeth were generally observed in most of the treated animals by the end of the study. Red exudate was also observed under the animal cages. Except for the discolored teeth and dental tartar, the clinical signs generally resolved during the 60-day recovery period. Discoloration of stool was attributed to unabsorbed ferric citrate.

Ophthalmology
There were no ocular findings at the end of study.

Body weight

A significant decrease ($p<0.05$) in mean body weight (↓17%) and body weight gain (↓12%) was observed in males and females treated with the high dosage of ferric citrate from weeks 32 to 42 (Days 222 to 292).

Changes in mean body weight and body weight gains in males and females at low and mid dose levels were comparable to controls. The mean body weight was still decreased in males (14%) and females (11%) in the high dosage group at the end of recovery period.

Food Consumption

An increase in food consumption was noticed in treated animals when compared to controls except at the dosage where food consumption was decreased on days 267-273 (↓5%) and days 274-280 (↓10%).

Hematology

Mean reticulocyte counts in both sexes were significantly decreased at all dosages by the end of dosing period compared to controls (Table 42). Decreases in mean hemoglobin, hematocrit and red blood cell counts were seen in males.

Table 42: Decrease in Mean Reticulocyte Counts in Week 42 Relative to Control (Sponsor's table)

Dose (mg/kg/day)	Males	Females
400	-26%	-22%
1000	-35%[b]	-22%
2000	-45%[b]	-38%

[a]Percent decrease = (mean value of the treated group – mean value of the control group) ÷ mean value of the control group
[b]Absolute values are statistically significantly different from the control values

However, the decreases were generally not dosage- related and not considered as adverse effects of ferric citrate. The decreases in hemoglobin, hematocrit, and RBCs observed in males remained statistically significant at the end of the recovery period. In females, eosinophils were decreased at Week 42 at all doses.

Bone marrow smears were qualitatively evaluated, and there was a finding of mild to marked, dose-related increase in the amount of extracellular and intracellular iron stores at all dosages. Extracellular and intracellular iron pigment was visible as small granules scattered throughout the smear, and as granules in the occasional siderocyte.

Coagulation

The Mean activated partial thromboplastin time (APTT) was increased in males at 1000 and 2000 mg/kg/day and females at 2000 mg/kg/day (Table 43).

Table 43: Change in Mean Activated Partial Thromboplastin Time (APTT) at End of Dosing (Week 42) Compared to Control (Sponsor's table)

	APTT (seconds)	
Dose (mg/kg/day)	Males	Females
400	-1.3	-1.5
1000	+2.9	+0.5
2000	+9.4[a]	+6.1[a]

[a]Absolute values are statistically significantly different from the control values

A prolonged APTT was observed at the end of the recovery period in males and females at mid and high dosage (Table 44).

Table 44: Change in Mean Activated Partial Thromboplastin Time (APTT) at End of Dosing (Week 42) Compared to Control (Sponsor's table)

	APTT (seconds)	
Dose (mg/kg/day)	Males	Females
400	-1.0	-0.8
1000	+4.3[a]	+1.2
2000	+7.5[a]	+3.9[a]

[a]Absolute values are statistically significantly different from the control values

Clinical Chemistry

Serum iron concentrations were significantly increased in males (34%) and females (32%) at high dosage as reflected by increased ferritin and transferrin saturation (TSAT) and decreased

unsaturated iron binding capacity (UIBC) in both genders (Table 45). Iron deposition and overloading was supported by histopathology.

Table 45: Normalized serum iron parameters at the end of dosing (Week 42) (Sponsor's table)

	Males		Females	
Dose (mg/kg/day)	**1000**	**2000**	**1000**	**2000**
Serum Iron	-	+34%[b]	-	+32%[b]
Ferritin	1.8X[b]	+13.9X[b]	2.0X[b]	+8.9X[b]
TSAT	-	+47%[b]	-	+35%[b]
UIBC	-	-86%[b]	-	-62%[b]

[a]Percent change was calculated as [(treated value-control value) ÷ control value] x 100. Fold changes from control (X) were calculated as treated value ÷ control value.
[b]Absolute values are statistically significantly different from control.

TSAT = Percent Transferrin Saturation
UIBC = Unsaturated Iron Binding Capacity

The related hepatic parameters were significantly altered due to overloading of iron and injury to liver cells (table 46).

Table 46: Normalized clinical chemistry markers of hepatic function at the end of dosing (Week 42) % Change Relative to Control) (Sponsor's table)

	Males			Females	
Dose (mg/kg/day)	**400**	**1000**	**2000**	**1000**	**2000**
Alanine Aminotransferase	-	+83%[b]	+158%[b]	+32%[b]	+173%[b]
Alkaline Phosphatase	+47%[b]	+57%[b]	+87%[b]	-	+76%[b]
Total Protein	-	-	-7%[b]	-	-9%[b]
Albumin	-	-12%[b]	-18%[b]	-	-15%[b]
Triglycerides	-	-	+34%[b]	-	+41%[b]

[a]Percent change was calculated as [(treated value-control value) ÷ control value] x 100.
[b]Absolute values are statistically significantly different from control.

A slight decrease (↓6-11%) was observed in serum phosphate levels of males and females treated with high dose of test article (2000 mg/kg/day) when compared with respective controls. At high-dosage, changes in serum iron parameters (ferritin, TSAT, UIBC) and related hepatic function parameters , considered to be test article related in both genders, did not return to control levels by the end of 60-day recovery period.

Urinalysis
A significant ($p<0.01$) decrease (47%) in the urinary phosphate/creatinine ratio (Phos/Cre) in males at 2000 mg/kg/day (47%) and in females at 1000 and 2000 mg/kg/day (27% and 42%), was observed at the end of dosing period on week 42, but not by the end of recovery period.

Organ weights
Increased absolute liver weights and liver/body weight and liver/brain weight ratios were observed in males and females at ≥ 1000 mg/kg/day, and were correlated with macroscopic and microscopic findings of enlarged liver.

Absolute thymus weight, thymus/body weight ratio and thymus/brain weight ratio were decreased in males at ≥ 1000 mg/kg/day and in females at 2000 mg/kg/day, but absent any microscopic findings of concern (Table 47).

Table 47: Mean Absolute Organ Weights at the End of Dosing (Week 42)
(% Change Relative to Control) (Sponsor's table)

	Males		Females	
Dose (mg/kg/day)	**1000**	**2000**	**1000**	**2000**
Liver	+16%	+67%[b]	+26%	+47%[b]
Thymus	-47%	-71%[b]	-	-56%
Pituitary	+74%[b]	+48%[b]	-	-
Epididymis	-	-31%[b]	-	-
Prostate	-	-26%	-	-
Testes	-	-31%	-	-
Heart	-	-19%[b]	-	-

[a]Percent change was calculated as [(treated value-control value) ÷ control value] x 100.
[b]Absolute values are statistically significantly different from control.

Neither increased pituitary weight, and pituitary/body and pituitary/brain weight ratios in males at ≥ 1000 mg/kg/day, nor decreased weights of epididymis, prostate and testes in males at 2000 mg/kg/day were associated with any histopathology, and these organ weight changes are not considered to be toxicologically important. Decreased heart weight and heart to brain weight ratio in males at 2000 mg/kg/day was attributed to lower body weights of animals. Changes in liver and heart parameters (Table 48) did not return to normal at the end of recovery period. Such changes were considered to be test article –related.

Table 48: Mean Absolute Organ Weights at the End of Recovery
(% Change Relative to Control) (Sponsor's table)

	Males		Females	
Dose (mg/kg/day)	**1000**	**2000**	**1000**	**2000**
Liver	+46%[b]	+74%[b]	+23%	+87%[b]
Heart	-	-26%[b]	-	-
Thymus	-	+62%	-	-50%

[a]Percent change was calculated as [(treated value-control value) ÷ control value] x 100.
[b]Absolute values are statistically significantly different from control.

Macroscopic and microscopic findings

Dose -related (Table 49-52), brown discoloration , increased size, iron deposits, and bile duct proliferation were found in the liver, gallbladder, digestive tract, and/or lymph nodes in treated animals at mid and high dosage , and was still present at the end of the 30-day recovery period. Dose -related microscopic findings of brown pigmented macrophages and brown pigment were observed in the liver (≥400 mg/kg/day), kidneys (2000 mg/kg/day); digestive tract (≥1000 mg/kg/day); lymph nodes (≥400 mg/kg/day), bone marrow (≥1000 mg/kg/day), and/or spleen (≥400 mg/kg/day) of ferric citrate-treated animals, and reflected the presence of excess iron identified by Prussian blue staining. Histopathological findings were evident in the treated cohort at end of the recovery period (Table 49-52).

Table 49: Ferric Citrate- Related Macroscopic findings in organs of animals (Sponsor's table)

	MALES				FEMALES			
Dose (mg/kg/day)[a]	**0**	**400**	**1000**	**2000**	**0**	**400**	**1000**	**2000**
# animals examined	4	4	4	4[b]	4	4	4	4
LIVER								
Brown Discoloration	-	-	4	4	-	-	3	4
Enlarged	-	-	2	3	-	-	1	2
Firm	-	-	3	4	-	-	1	4
GALL BLADDER								
Abnormal Contents, black material	-	-	-	1	-	-	1	2
LARGE INTESTINE								
Black Discoloration	-	-	-	1	-	-	-	-
ILEO-CECAL JUNCTION								
Black Discoloration	-	-	-	1	-	-	-	-
MESENTERIC LYMPH NODE								
Brown/Red Discoloration	-	-	1	4	-	-	3	4
Enlarged	-	-	-	1	-	-	-	-
MEDIASTINAL LYMPH NODE								
Brown/Red Discoloration	-	2	1	3	-	-	1	1
LYMPH NODE, OTHER[c]								
Brown/Red Discoloration	-	-	4	4	-	-	4	4
Enlarged	-	-	2	3	-	-	4	4

[a]Final dose indicated
[b]Includes unscheduled decedent
[c]Pancreatic, iliac, and/or submandibular lymph nodes

Table 50: Microscopic Findings in the Liver and Kidney of Dogs treated with Ferric Citrate (Sponsor's table)

	MALES				FEMALES			
Dose (mg/kg/day)[a]	**0**	**400**	**1000**	**2000**	**0**	**400**	**1000**	**2000**
# animals examined	4	4	4	4[b]	4	4	4	4
LIVER								
Brown Pigment: Hepatocyte, Kupffer cell, Macrophage								
minimal	-	2	-	-	-	4	-	-
slight	-	1	1	-	-	-	4	-
moderate	-	-	3	1	-	-	-	4
marked	-	-	-	3	-	-	-	-
total	-	**3**	**4**	**4**	-	**4**	**4**	**4**
Chronic Inflammatory Foci, Brown Pigmented								
slight	-	-	3	-	-	-	4	1
moderate	-	-	1	1	-	-	-	3
marked	-	-	-	3	-	-	-	-
total	-	-	**4**	**4**	-	-	**4**	**4**
Hyperplasia, Bile Ductule								
slight	-	-	-	1	-	-	-	3
moderate	-	-	-	1	-	-	-	1
marked	-	-	-	2	-	-	-	-
total	-	-	-	**4**	-	-	-	**4**
Fibrosis								
minimal	-	-	-	1	-	-	-	-
slight	-	-	-	1	-	-	-	-
marked	-	-	-	1	-	-	-	-
total	-	-	-	**3**	-	-	-	-
KIDNEYS								
Brown Pigment, Cortical Tubules								
slight	-	-	-	4	-	-	-	2
total	-	-	-	**4**	-	-	-	**2**

[a]Final dose indicated
[b]Includes unscheduled decedent

Table 51: Microscopic Findings in the Digestive Tract of Dogs treated with Ferric Citrate (Sponsor's table)

	MALES				FEMALES			
Dose (mg/kg/day)[a]	**0**	**400**	**1000**	**2000**	**0**	**400**	**1000**	**2000**
# animals examined	4	4	4	4[b]	4	4	4	4
STOMACH								
Brown Pigment								
minimal	-	-	-	-	-	-	2	2
slight	-	-	-	-	-	-	1	1
total	-	-	-	-	-	-	**3**	**3**
SMALL INTESTINE[c]								
Brown Pigment								
minimal	-	-	1	1	-	-	1	3
slight	-	-	-	1	-	-	-	-
total	-	-	**1**	**2**	-	-	**1**	**3**
Basophilia, Lamina Propria Connective Tissue								
minimal	-	-	-	1	-	-	-	-
total	-	-	-	**1**	-	-	-	-
LARGE INTESTINE/RECTUM/ILEOCOLIC-CECAL JUNCTION[c]								
Brown Pigment								
minimal	-	-	3	2	-	-	4	4
slight	-	-	-	2	--	-	-	-
total	-	-	**3**	**4**	-	-	**4**	**4**
Basophilia, Lamina Propria Connective Tissue								
slight	-	-	-	1	-	-	-	-
total	-	-	-	**1**	-	-	-	-

[a]Final dose indicated
[b]Includes unscheduled decedent
[c]The tabulated incidences and severities reflect the presence of a finding in one or more segments of the tract of a given animal with highest severity shown.

Table 52: Microscopic Findings in Spleen, Bone Marrow and Lymph nodes of Dogs treated with Ferric Citrate (Sponsor's table)

	MALES				FEMALES			
Dose (mg/kg/day)[a]	**0**	**400**	**1000**	**2000**	**0**	**400**	**1000**	**2000**
# animals examined	4	4	4	4[b]	4	4	4	4
SPLEEN								
Increased Brown Pigment, Macrophages								
minimal	-	1	-	1	-	-	-	-
slight	-	-	3	3	-	-	4	2
moderate	-	-	-	-	-	-	-	1
total	-	**1**	**3**	**4**	-	-	**4**	**3**
BONE MARROW[c]								
Increased Brownish-Gray Pigment, Macrophages								
minimal	-	-	4	2	-	-	4	2
slight	-	-	-	2	-	-	-	2
total	-	-	**4**	**4**	-	-	**4**	**4**
LYMPH NODES[d]								
Brown Pigment, Macrophages								
minimal	-	2	-	-	-	3	-	-
slight	-	-	1	-	-	-	2	-
moderate	-	-	3	-	-	-	2	-
marked	-	-	-	2	-	-	-	4
severe	-	-	-	2	-	-	-	0
total	-	**2**	**4**	**4**	-	**3**	**4**	**4**

[a]Final dose indicated
[b]Includes unscheduled decedent
[c]The tabulated incidences and severities reflect the presence of a finding in one or more bone marrow section of a given animal with highest severity shown.
[d]The tabulated incidences and severities reflect the presence of a finding in one or more lymph nodes of a given animal with highest severity shown.

Conclusion
Brown pigmentation reflecting iron overloading, and leading to liver injury was observed in ferric citrate- treated dogs at all dosage levels (400, 1000 and 2000 mg/kg/day) in this 42-Week study. Overt liver damage was well correlated with microscopic and clinical pathological findings, especially at the high dosage. The hepatotoxicity at the mid and high dose was still evident at the end of the recovery period. Liver findings (brown pigmentation that persisted throughout the recovery period, but without any clinical pathology changes) indicative of overt liver damage was observed at 400 mg/kg/day. Accordingly, the NOAEL dosage in this 42-week oral toxicity study in dogs is evidently 400 mg/kg/day (89 mg Fe/kg/day).

7 Genetic Toxicology

Sponsor performed no genetic toxicology studies with ferric citrate.

Gene toxicity data (Table 53) have shown that there is no increase in the number of revertant colonies in *S. typhimurium* strains *(*TA92, TA1535, TA100, TA1537, TA94, and TA98) at a maximum dose 25 mg/plate of ferric citrate in presence of S9 metabolic activation system in Bacterial Reverse Mutation (Ames) test (Ishidate et al 1984).

Polyploidy was observed in 3% of the cells after 48 hours and structural aberration was observed in 1% of the cells after 48 hours with a maximum concentration of 0.5 mg/mL ferric citrate in a chromosomal aberration test using the Chinese hamster lung fibroblasts (Ishidate et al 1984).

In addition, genotoxicity data available from 12 iron-containing compounds (including ferric citrate) are reported in the literature and do not indicate that these ferrous or ferric ion-containing substances demonstrate any genotoxic potential (EFSA and the Joint Food and Agriculture Organization) (FAO)/WHO Expert Committee on Food Additives (JECFA).

Table 53: Gene Toxicity Data for Ferric Citrate (Sponsor's table)

Iron-containing Compound	Study Type	Test System	Test Object	Conc.[a]	Conc. of Iron	Result	Reference
Ferric citrate	Bacterial Reverse Mutation (Ames) Assay	*Salmonella typhimurium*	Strains TA92, TA94, TA98, TA100, TA1535, TA1537	6 conc. up to 25 mg/plate	6 conc. up to 5.25 mg/plate	Negative[b,c]	Ishidate et al 1984
	Chromosomal aberration test	Chinese hamster	Lung fibroblast line	0.5 mg/mL, exposed for 24 and 48 hours	0.105 mg/mL, exposed for 24 and 48 hours	Negative[c]	Ishidate et al 1984

The Bacterial Reverse Mutation (Ames) Test performed with citrate was negative with and without metabolic activation in *S. typhimurium* (in strains TA94, TA97, TA98, TA100, TA104, TA1535, and TA1537), *E. coli,* and *S. cerevisiae*. The results of a yeast gene mutation assay and a chromosomal aberration test of citrate were negative for mutagenicity and clastogenicity potential (Table 54).

Table 54: In Vitro Gene Toxicity Data for Citrate (Sponsor's table)

Study Type	Test System	Test Object	Concentration	Result	Reference
Bacterial Reverse Mutation (Ames) Assay	*Salmonella typhimurium*	TA97, TA98, TA100, TA104	Not stated	Negative[a,b]	OECD SIDS 2001
	Salmonella typhimurium	TA94, TA98, TA100, TA1535, TA1537	Up to 5 mg/plate	Negative[a,b]	Ishidate et al 1984
	Escherichia coli	Not specified	Not specified	Negative[a,b]	OECD SIDS 2001
	Saccharomyces cerevisiae	Not specified	Not specified	Negative[a,b]	
Yeast Gene Mutation Assay	Yeast	Not specified	>3.5 g/kg	Negative[a,b]	
Chromosomal aberration test	Chinese hamster	Fibroblast culture cells	Up to 1 mg citric acid/mL	Negative	

[a] With metabolic activation.
[b] Without metabolic activation.

8 Carcinogenicity

No carcinogenesis studies of ferric citrate were conducted by the Sponsor.

Potential of iron and iron -containing compounds to form reactive oxygen species (ROS) e.g., hydroxyl radical (•OH), and to enhance lipid peroxidation suggest an important role in tumor development. However, life time (96-Week) carcinogenesis studies have shown that oral administration of ferric citrate via drinking water to mice at up to38.5 mg/kg/day and administering iron lactate to rats at up to 186.8 mg/kg was not tumorigenic in the rodent (Inai et al 1994; 2002; Table 55). The ferric citrate was orally administered to $B6C3F_1$ mice (50/sex) at concentrations of 0 (control), 0.06% and 0.12% (16.5% and 18.5% iron) in the drinking water for 96-Weeks. Average survival and mean final body weight in the high-dose males (0.12%) were significantly lower than controls ($p<0.001$ and $p<0.05$, respectively). Gross autopsy and histological evaluation of mice revealed no significant differences between controls and treated animals for non-neoplastic lesions (Table 55). Ferric chloride added to drinking water at concentrations providing up to 114 mg/Kg/day was not tumorigenic (table 55).The dosages tested in the mice and the rats significantly reduced body weight compared to mean weight in relevant control group.

Table 55: 96 and 104 week-eek Oral Tumorigenicity Study of Ferric Citrate in B6C3F1 mice and Ferric chloride and iron Lactate in F344 rats (Sponsor's table)

Study Type and Duration	Test Article	Species (Strain)	Route of Administration	Average Iron Dose (mg/kg/day)	Reference
96-Week Tumorigenicity	Ferric citrate	Mouse ($B6C3F_1$)	PO/drinking water	Males: 0, 17.5, 38.5 Females: 0, 12.3, 28.0	Inai et al 1994
104-Week Carcinogenicity	Ferric chloride	Rat (F344)	PO/drinking water	Males: 0, 57.7, 108.7 Females: 0, 63.9, 114.2	Sato et al 1992
104-Week Carcinogenicity	Iron lactate	Rat (F344)	PO/dietary	Males: 0, 83.2, 168.5 Females: 0, 91.8, 186.8	Imai et al 2002

PO=oral.

Initial Assessment Report evaluated the carcinogenicity of [redacted] and reported that [redacted] is not a suspected carcinogen. In a non-GLP lifetime carcinogenicity study with groups of 20 male rats receiving up 3% or 5% (approx. 1.2 and 2 g/kg/day, respectively) [redacted] in the feed for 2 years, no evidence of carcinogenicity was reported. No increase in deoxyribonucleic acid synthesis in the bladder epithelium was found in rats administered 1.7% sodium citrate (approx. 0.74 g/kg/day) for 8 weeks. [redacted] was thus determined to have no tumorogenic potential.

9 Reproductive and Developmental Toxicology

No reproductive and developmental toxicology studies were conducted. , However, Sponsor has agreed to carry out the following juvenile animal studies as per a pediatric research plan (PSP) to address the potential for toxicity of ferric citrate in children (≥ 6 month):

- A one-week tolerability study in 7-day old rats
- A 21-day pilot study in 7-day old rats, and
- A 56-day juvenile toxicity study with a 4-week recovery period in 7-day old rats.

A significant weight loss and irreversible damage were seen in testis of adult rats when iron compounds were injected locally in the testis as a single dose of ferrous sulfate and ferric chloride at a dosage and dosing volume of 0.08 mmol/kg/2mL distilled water (Kamboj and Kar, 1964). A significant weight loss, signs of sterility and loss of spermatozoa were observed in rats after seven days of administration ferric chloride in this study. An 8-generation study in Wistar rats has shown no signs of impaired reproductive performance when iron oxide was orally administered to provide 25 mg /iron/day. In a similar study (Fisch et al, 1975), an intramuscular administration of dextran-iron formulation at a dosage providing 20 mg/kg iron to the 6-week old female Sprague-Dawley rats, weekly from 7 to 13 weeks prior to mating at 14 weeks of age and following the same dosing and mating protocol to the next 4 generations did not alter reproduction performance parameters when compared to that of controls. No abnormality was observed in rat fetuses in this study. Data from developmental studies conducted in chick embryos, CD1-mice and Wistar-rats with iron containing compounds (Ferrous sulfate; Ferric sulfate; Ferrous gluconate; Pyrophosphate) revealed no teratogenic activity in developing embryos (Table 56).

Table 56: Teratological Studies of Iron Containing Compounds (Sponsor's table)

Study Type	Test Article	Species	Route of Administration	Average Iron Dose	Reference
Chick Embryo Assay	Ferric pyrophosphate	Single-Comb White Leghorn Chicken	Direct injection into fertile eggs	2.4/egg	Verrett et al 1980
	Ferrous sulfate			0.325 mg/egg	
	Ferrous gluconate			0.925 mg/egg	
Teratology	Ferric sodium pyrophosphate	CD-1 Mouse	PO / gavage	0, 3.5, 16.3, 75.9, 353.5 mg Fe/kg/day	Bailey and Morgareidge 1975
Teratology	Ferric sodium pyrophosphate	Wistar Rat	PO / gavage	0, 3.5, 16. 3, 75.9, 353.5 mg Fe/kg/day	Bailey and Morgareidge 1975
Teratology	Ferrous sulfate	CD-1 Mouse	PO / gavage	0, 0.6, 2.7, 12.8, 59.2 mg Fe/kg/day	Bailey and Morgareidge 1974
Teratology	Ferrous sulfate	Wistar Rat	PO / gavage	0, 0.7, 3.4, 16, 74 mg Fe/kg/day	Bailey and Morgareidge 1974

PO=oral.

10 Special Toxicology Studies

No special toxicology studies of ferric citrate were conducted by the Sponsor.

Data from published reports are cited below showing the effects of iron -containing compounds on various organ systems.

Cardiovascular system
In view of complications leading to death in humans due to hemochromatosis and congestive heart failure as a result of iron overloading, carbonyl iron (~3000 mg Fe/Kg/day) was administered to male weanling Sprague-Dawley rats for 12 week time period. An increase (30%) in non-heme cardiac iron concentration and heart to body weight ratio (180% increase), and cardiomyopathy with inflammatory cells and degeneration or necrosis of my fibers were observed at dosages providing 525 and 3000 mg of Fe/Kg/day. However, cardiomyopathy also occurs spontaneously at a high frequency in rats (83% i.e., 10/12 control rats in one study: Whittaker et al, 1997).

Gastrointestinal Tract
The GI tract is one of the most common targets for iron deposits, presence of pigmented macrophages and iron toxicity after oral ingestion of iron-containing compounds, especially iron salts. Moderate necrosis was observed in the mucosa of the rat stomach and the villi of the duodenum 24 hours after administration of ferrous ion at (200 mg/kg dose. Findings of mucosal/submucosal thickening (including increased size and/or numbers of glands, increased goblet cells per gland, increased number and/or crowding of enterocytes per gland), inflammatory cell infiltrates, and basophilia, were observed in the small and large intestines of rats receiving ferric citrate at ≥1400 mg/kg/day (Benoni et al 1993).

Hepatic/Biliary System
A 9.5-fold (at 1500 mg Fe/kg/day) to 30-fold (at 3000 mg Fe/kg/day) increase in liver non-heme iron concentration, 2.3-fold (at 3000 mg Fe/kg/day) increase in liver to body weight ratios, and 2-fold (at 1500 and 3000 mg Fe/kg/day) increase in liver conjugated diene levels were observed in male weanling Sprague-Dawley rats when administered carbonyl iron at dosages providing up to 1500 and 3000 mg Fe/kg/day (Whittaker and Chanderbhan 2001).

Hepatocellular hypertrophy, characterized by enlarged hepatocytes, and located predominantly in the periportal region of the liver lobule, was observed at dosages of 1500 and 3000 mg Fe/kg/day, the incidence to the latter exposure being 6/10 animals.

Renal/Urinary System
Data from published literature documents an occurrence of kidney degeneration (<750 mg Fe/kg/day) and nephropathy (1500 mg Fe/kg/day) in rats exposed to carbonyl iron for 12 weeks. Incidence and severity of brown pigment likely hemosiderin or lipofuscin in the kidney was increased at dosages of iron ≥446/605 mg Fe/kg/day in rats dosed with ferric citrate for 32-weeks.

Endocrine System
Brown pigment was observed in the pancreas of 1 male, edema of the pancreas in 2 males and 1 female and yellow discoloration of the pancreas in 1 male and 1 female at a dosage of ferric citrate providing 605 mg Fe/kg/day in a 16-week study in dogs sponsored by Keryx. Pancreatic atrophy was observed in male Sprague-Dawley rats receiving 3500 to 20000 µg/g diet (about 525 to 3000 mg Fe/kg/day) for 12 weeks.

Immune System
Dosage dependent increases in incidence of brown-pigmented macrophages were observed in the spleen of all dogs treated with ferric citrate for 16 weeks (Keryx). Histopathologic evidence of iron deposition was observed in the spleen of male and female rats at dosages ≥500 mg/kg/day (108 mg Fe/kg/day) in 32-weeks repeat dose study. Severe lymphocyte depletion was observed in F344 rats fed a diet providing carbonyl iron at a dosage of 1500 mg Fe/kg/day.

11 Integrated Summary and Safety Evaluation

Brief Background / Introduction
End-stage renal disease (ESRD) develops when chronic kidney disease (CKD) has worsened to the point at which kidney function is less than 10% of normal and no longer able to sustain life. Patients in ESRD need dialysis or a kidney transplant for life support. A decrease in phosphorus excretion in the urine in ESRD can lead to hyperphosphatemia, extra- skeletal calcification and hyperparathyroidism (Iida et al 2013). Moreover, a high intake of dietary phosphate has been shown to decrease renal function, and low phosphate intake preventing the progression of CKD to ESRD (Iida et al 2013).

It has been shown that ferric citrate can reduce phosphate absorption, lower serum phosphate, calcium, and intact parathyroid hormone; and decrease parathyroid hyperplasia, ectopic calcification in the aorta, and bone abnormalities in an experimental CKD model in rats (Iida et al 2013). To lower the serum phosphate level, Sponsor has proposed an oral administration of KRX-0502, a ferric citrate coordination complex, in ESRD patients on dialysis.

Ferric citrate binds phosphate in the GI tract, precipitating phosphate as ferric phosphate that is excreted in the stool. The sequestration leads to lower serum phosphate levels. The free ferric ion is either excreted or transported into the enterocyte via the divalent metal transporter (DMT1) to increase the iron stores as ferritin or exported into the blood by ferroportin.

Ferric citrate as a food supplement is generally regarded as safe (GRAS) in 21 CFR 184.1298. The maximum human recommended dose (MHRD) of 12 g/day (200 mg/kg/day for a 60 kg patient,) provides 2.52 g ferric iron (42 mg ferric iron/kg) and 7.2 g citrate/day (120 mg citrate/kg) and stops or reverses the occurrence of hyperphosphatemia. Sponsor requested to classify this drug product as a phosphate-binder indicated for lowering the serum phosphate concentration in ESRD patients on dialysis, while maintaining the iron stores.

To maintain a normal physiological function, the recommended daily intake of iron as approved from the American Medical Association Committee on Iron Deficiency is shown in Table 57.

Table 57: Recommended Daily Intake Values for Iron (Sponsor's table)

Population	mg/day	Source
Adult men (>18 years of age) Adult women (50+ years of age)	10	[National Research Council 1989]
Adult women (>18-50 years of age)	15	
Adult men (moderately active, 65 kg body weight)	5-9[a]	WHO recommendations (as summarized in 571. Iron [WHO 1983])
Adult women (moderately active, 55 kg)	14-28[a]	

[a] Lower value applies when over 25% of calories come from animal foods and higher value when animal foods represent less than 10% of calories.
WHO=World Health Organization.

Due to risk of anemia in CKD patients and increased blood loss (during frequent laboratory tests and post-dialysis bleeding), iron is often administered intravenously as single doses of 60 to 510 mg of elemental iron to meet the higher requirement of iron in ESRD patients on dialysis. In view of these considerations, a maximum dose of 12 g ferric citrate/day (b) (4) for the treatment of hyperphosphatemia in ESRD patients on dialysis has been proposed by the Sponsor.

Pharmacology
Ferric citrate binds with dietary phosphate and forms an insoluble ferric-phosphate complex in the gastrointestinal (GI) tract to prevent the systemic absorption of phosphate and thereby reduce serum phosphate level. The insoluble ferric phosphate complex is excreted. Free Fe^{+3} is either excreted or transported into the enterocyte via the divalent metal transporter (DMT1) where it can either be stored as ferritin or be exported into the blood by ferroportin. Citrate is metabolized to carbon dioxide in the citric acid cycle and expelled by the lungs or is converted to bicarbonate as an aspect of acid-base balance.

Ferric citrate hydrate (JTT-751), similar in composition to KRX-0502, reduces the intestinal absorption of phosphate in rats in a dose dependent manner (Iida et al 2013). Ferric chloride when administered to rats at dosages binding about 84% of the phosphate in the diet, causes rickets (osteomalacia in adults), within 3 weeks unless the diet is supplemented with phosphate. Dietary iron salts are fed to rats rabbits, guinea pigs, and chicks in amounts able to bind 50% induces large decreases in serum phosphate and bone ash, and develop rickets within 3 weeks, in those species as well.

Sponsor has not performed any safety pharmacological studies based on the fact that iron is not absorbed even after 28 days of oral dosing to rats (3500 mg/kg/day) and to dogs (1000 mg/kg/day). Impairment in long term memory and oxidative brain damage has been observed in adult rats treated postnatally with oral iron (10 mg Fe/kg), suggesting greater absorption and/or susceptibility in juveniles. It has been shown that level of brain serotonin and dopamine are decreased and meningeal hemorrhage, congestion, edema, and degenerated cortical neurons are observed in Wistar rats fed with ferrous sulfate (~20 mg/kg/day) for 10 weeks. Dietary administration of iron (15, 35, 150, or 300 mg Fe/kg diet) to female Sprague Dawley rats for 4 months increased heart low molecular weight iron and malonaldehyde leading to heart injury in

this model of ischemia/reperfusion injury. No studies of respiratory effects, if any, of oral iron formulations are documented in the literature.

Pharmacokinetics
No standard pharmacokinetic studies (C_{max}, AUC or $t_{½}$) were determined in the toxicology studies sponsored by Keryx. Iron parameters (serum or plasma iron, ferritin, TIBC, and TSAT) that reflect the total iron status of the animal are values cited in the literature. Prior to absorption, ferric iron (Fe^{+3}) is converted to ferrous iron (Fe^{+2}) and transported into enterocytes via the DMT1 and stored as ferritin or exported into the blood by ferroportin (Papanikolaou and Pantopoulos 2005).

Species differences have been reported e.g., absorption of iron from the diet in rats and dogs is approximately 2 to 5 –fold greater than in humans when adjusted for body surface area. Absorbed iron is stored and usually not excreted. A very small amount of total body iron (~ 0.1% bound to transferrin) is present in serum while ~60% to 70% of iron is present in the erythrocytes within hemoglobin and 7.5% to 15% is found in myoglobin.

Toxicology
Acute toxicity studies of ferric citrate were not carried out by Keryx. However, toxicity studies of ferric iron and citrate have been published by other investigators. Results of acute studies have shown a decreased activity, weakness, decreased muscular control, prostration, urination, bowel obstruction, symptomatic gastroenteritis (diarrhea, vomiting, dehydration, hemoconcentration, and electrolyte imbalance), dyspnea, convulsions, coma, respiratory failure, and cardiac arrest in animals exposed to lethal dosages of ferric iron.

There are 7 repeated -dose toxicological studies conducted by the Sponsor including 3 studies in Sprague-Dawley rats (28 and 90-day; 32-week), and 4 studies in beagle dogs (28 and 33-day; 16 and 42-week). There were no treatment related deaths in rats and dogs in any of these studies. In 32 Week oral rat studies, ferric citrate was added to Certified Rodent Diet to promote iron content from basal level (0.19 mg/g) to 1.6% to 3.8% iron content. In 90 Day rat study the basal iron content was 0.74 mg/g, and ferric citrate promoted iron levels to 7% to 15%. In all 4 dog studies, ferric citrate was added to canned food to promote its iron content from 0.13 mg/g to 0.5% to 1.4%.

The 90-Day repeat- dose oral toxicity study in rats revealed dosage- dependent iron deposits presenting as black material in the cecum, colon, spleen, and liver of males and females treated at all dose levels, and identifying the GI tract as the primary target for ferric citrate toxicity. Increases in serum phosphate were observed in all studies (at dosages ≥2000 mg/kg/day administered for 28 days, and ≥500 mg/kg/day administered for 90 days). Decreased urinary phosphate excretion was noticed in all dosage groups (≥2000 mg/kg/day administered for 28 days and ≥500 mg/kg/day administered for 13 weeks or more) and viewed as an expected physiological response to maintain serum phosphate level. Pigmented macrophages were present in the stomach, intestine, and colon, and iron granules were present in epithelial cells.

Microscopic findings in the cecal mucosa (thickening, basophilia, and increased mixed inflammatory cell infiltrate) and colonic epithelium (goblet cell hyperplasia, increased size of

colonic glands) were observed in rats at mid dosage (≥ 1400 mg/kg/day; (302 mg Fe/kg/day). The colon was severely affected in male rats and colonic goblet cell hyperplasia and/or hypertrophy was observed even at the lowest dosage of ≥500 mg/kg/day (108 mg Fe/kg/day). Females in the low dosage group (500 mg/kg/day) showed complete recovery of increased lymphocytic infiltration, while such was still present in the mid- and high-dosage groups. Partial recovery was found in sinus ectasia/cysts in females, but not in males. Partial recovery was also noticed in pigmented macrophage aggregation in mediastinal lymph nodes, but not in mesenteric lymph nodes. The NOAEL in rat studies was considered to be 500 mg/kg/day by this reviewer rather than 2800 mg/kg/day as judged by the Sponsor.

In 16-Week repeat dose study of oral ferric citrate in beagle dogs, a significant increase (~9 & 15 fold) in serum ferritin was seen in females and males at high dosage (2800 mg/kg/day) level when compared with controls as evident from an increase in serum iron level,(113% greater than control value in male ; 67% greater in females). Increased liver weight, bile duct hyperplasia, GI tract and liver injury in high dosage treated animals were seen primarily because of iron overload, and associated with histopathological and clinical findings. Dose- dependent findings were evident in dogs at all dosage levels (400, 1000 or 2000 mg/kg/day) in a42-Week repeat dose study.

An unscheduled death of a beagle dog was seen in high dosage (2000 mg/kg/day) at week 40 of treatment due to liver injury attributed to iron overload and correlated with histopathologic findings and clinical pathology. Moderate histopathological changes were observed in liver at mid dosage level (1000 mg/kg/day) while at the low dosage (400 mg/kg/day) changes were limited to brown pigmentation still present at the end of 60-Day recovery period. The NOAEL dose was established at 400 mg/kg/day (89 mg Fe/kg/day) in 42-week oral toxicity study in dogs.

Ferric citrate, showing neither mutagenic potential in the bacterial reverse mutation assay (Ames test), nor clastogenic activity in the chromosomal aberration assay in Chinese hamster fibroblast cells, is not considered to be a genotoxic following oral administration.

Data from published life- time carcinogenesis/mutagenesis studies have demonstrated that ferric citrate and other iron salts are not carcinogenic in mice and rats when administered intramuscularly or subcutaneously.

Data from reproductive and developmental toxicological studies did not show any teratogenicity or adverse effects of iron salts on reproductive performance. .

The ratio of the highest tolerated iron dosage (1600 mg ferric sodium pyrophosphate/mg, or 353.5 mg ferric iron/kg) in rats to the highest proposed dose in human (12 g/day, or 200 mg ferric citrate/kg in a 60 kg human, or 42 mg ferric iron/kg) is 8.4 on a mg/kg basis, or 1.36 on a body surface area basis. The highest oral iron dosage in mice (1600 mg ferric sodium pyrophosphate/kg, or 353.5 mg ferric iron/kg/day) is also 8.4 –fold higher on an mg/kg basis, or 0.68 on a body surface area basis than the maximum proposed dose in humans. Based on the NOAEL dosage in the most sensitive species-the dog (400 mg/kg/day), the calculated HED (400x0.54=216 mg/kg/day) would carry a small safety margin over the proposed human therapeutic dose of 200 mg/kg in a 60 KG patient (216/200=1.08). However, the projected safety

margin, based on more overt toxicology, and persistent histopathology e.g., inflammatory liver foci occurring in the dog at 1000-2000 mg/Kg/day, is 5-10 times on a mg/Kg basis. For local toxicity, and liver toxicity as a result of intestinal-hepatic portal delivery a safety margin based on mg/Kg rather that body surface area may be more appropriate. Results of published animal studies have shown the effects of iron compounds on, GI, cardiovascular, hepatic/biliary, renal, endocrine and immune system due to iron overloading and deposition. GI tract is one of the most common targets for iron toxicity as evident from the overt presence of residual pigmented macrophages.

Conclusion

Data obtained both from published non-proprietary studies and animal studies sponsored by Keryx have shown the effects of orally administered ferric citrate on liver, GI tract, renal, cardiovascular, and endocrine and immune systems at high dosage levels, including lethal hepatotoxicity in the dog, the most sensitive species . The proposed maximum human therapeutic dose (12g/day, or 200 mg ferric citrate/kg in a 60 kg human, or 42 mg ferric iron/kg) is 8.4 fold lower than the maximum iron dosage used in mice and rats when calculated on mg/kg basis. In view of an extensive human experience (Studies: PBB00101; 201; 202; 304; 305) and results published in the nonclinical studies, the present application (NDA 205874) is approvable from the Pharm/Tox perspective.

It is noted that sponsor's request that their product be designated as an (b) (4) has been denied (b) (4)

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RAMA S DWIVEDI
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ALBERT F DEFELICE
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PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 205,874 **Applicant:** Keryx Biopharmaceuticals Inc. **Stamp Date:** 08/07/2013

Drug Name: KRX-0502 (Ferric Citrate) **NDA/BLA Type:**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?			Yes. Submission is primarily based on published literature.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			N/A
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?			Yes.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?			See comment # 4

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			N/A
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			None yet identified.
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

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

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

None

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None

Rama Dwivedi	August 14, 2013
Reviewing Pharmacologist	Date
Thomas Papoian	August 14, 2013
Team Leader/Supervisor	Date

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/s/

RAMA S DWIVEDI
08/14/2013

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08/15/2013
Concur.