CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203565Orig1s000

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	203565
Supporting document/s:	SD 20
Applicant's letter date:	January 30, 2013
CDER stamp date:	January 30, 2013
Product:	Ferric carboxymaltose (Injectafer)
Indication:	Iron deficiency anemia
Applicant:	Luitpold Pharmaceuticals Inc.
Review Division:	Division of Hematology Oncology Toxicology
	(for Division of Hematology Products)
Reviewer:	Brenda J. Gehrke, Ph.D.
Supervisor/Team Leader:	Haleh Saber, Ph.D.
Division Director:	John Leighton, Ph.D., DABT
	Ann Farrell, M.D. (DHP)
Project Manager:	Amy C. Baird

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 203565 are owned by Luitpold Pharmaceuticals Inc. or are data for which Luitpold Pharmaceuticals Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 203565 that Luitpold Pharmaceuticals Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 203565.

Background:
The current submission, Supporting Document 20 for NDA 203565, is a Class 2 Resubmission. NDA 203565 was submitted in September 2011 as a new NDA for Injectafer (ferric carboxymaltose) by Luitpold Pharmaceuticals Inc. for the indication of iron deficiency anemia and a complete response letter was issued in July 2012 due to deficiencies with the manufacturing facility. There were no pharmacology/toxicology concerns with the application, and a review of the impurity and heavy metal acceptance criteria that also contained the proposed labeling recommendations for the pharmacology/toxicology sections (8.1, 12.1, and 13.1) was completed on June 13, 2012. There is no new pharmacology/toxicology information in this resubmission. The proposed acceptance criteria for (b) (4) are the same as those proposed in the 2012 submission and found acceptable; see the previous pharmacology/toxicology review.

Recommendation:
Recommending approval. There are no pharmacology/toxicology issues for NDA 203565 to preclude approval of the drug for the proposed indication.

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/s/

BRENDA J GEHRKE
06/24/2013

HALEH SABER
06/25/2013

MEMORANDUM

ferric carboxymaltose (Injectafer)

Date: June 13, 2012
To: File for NDA 203565
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review of Dr. Chopra (secondary signoff by Dr. Adebayo Laniyonu) and review memorandum and labeling provided by Dr. Gehrke. I agree with Dr Gehrke's conclusion that Injectafer may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
06/14/2012

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	203565
Supporting document/s:	SD 1
Applicant's letter date:	September 30, 2011
CDER stamp date:	October 3, 2011
Product:	Ferric carboxymaltose (Injectafer)
Indication:	Iron deficiency anemia
Applicant:	Luitpold Pharmaceuticals Inc.
Review Division:	Division of Hematology Oncology Toxicology
	(for Division of Hematology Products)
Reviewer:	Brenda J. Gehrke, Ph.D.
Supervisor/Team Leader:	Haleh Saber, Ph.D.
Division Director:	John Leighton, Ph.D.
	Ann Farrell, M.D. (DHP)
Project Manager:	Amy C. Baird

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MEMORANDUM

MEMO DATE: 6/13/2012

TO: To the file for NDA 203565
FROM: Brenda J. Gehrke, Ph.D., Pharmacologist; Division of Hematology Oncology Toxicology, OHOP
THROUGH: Haleh Saber, Ph.D., Supervisory Pharmacologist; Division of Hematology Oncology Toxicology, OHOP

Background

Ferric carboxymaltose injection was originally submitted by Luitpold Pharmaceuticals Inc. under NDA 22054 on June 15, 2006 for the indication of treatment of iron deficiency anemia in heavy uterine bleeding, postpartum, inflammatory bowel disease and hemodialysis patients. A Not Approvable letter was issued on July 9, 2007 with clinical deficiencies. The nonclinical studies for Ferric carboxymaltose were reviewed during this initial submission of NDA 22054. Studies reviewed included pharmacology; safety pharmacology; pharmacokinetics; general toxicology studies including 13-week studies in rats and dogs; genetic toxicology; fertility, embryo-fetal and peri and postnatal development toxicology studies; and local tolerance studies. Carcinogenicity studies were not submitted nor are they needed per ICH S1A due to the short-term intermittent nature of the clinical dosing. There were no pharmacology/toxicology issues at that time, and approval was recommended for ferric carboxymaltose by the pharmacology/toxicology review team (Dr. Yash Chopra and Dr. Adebayo Laniyonu). The NDA was resubmitted on September 12, 2007 and a second Not Approvable letter was issued on March 11, 2008 with clinical deficiencies. Since the indication was changed from the original NDA, NDA 203565 was submitted on September 30, 2011 as a new NDA for Injectafer (ferric carboxymaltose) by Luitpold Pharmaceuticals Inc. for the indication of iron deficiency anemia. No additional pharmacology/toxicology studies are needed for the proposed indication and none were submitted to NDA 203565. The Applicant is cross-referencing the nonclinical data in NDA 22054.

Injectafer (ferric carboxymaltose) is an intravenous formulation of polynuclear iron III hydroxide in complex with 4(R)-(poly-(1→4)-*O*-α-D-glucopyranosyl)-oxy-2(R),3(S),5(R),6-tetrahydroxy-hexanoate. The drug will be administered intravenously as an undiluted slow intravenous push injection or by drip infusion. According to the dosage and administration section of the label, the recommended dosage is 15 mg/kg body weight up to a maximum single dose of 750 mg of iron on two occasions separated by at least 7 days up to a cumulative dose of 1500 mg of iron. Treatment with iron may be repeated if iron deficiency reoccurs. Based on this information, the drug will be administered twice (one week apart), however, repeated dosing is possible and the number of administrations a patient will receive is unknown.

Impurities/heavy metals

The drug substance of ferric carboxymaltose contains high levels of the heavy metals (b) (4). For each heavy metal, the proposed drug substance acceptance criteria, the level of the heavy metal for the maximum iron dose of 750 mg, and the permitted daily exposure (PDE) recommended in the revised USP guidelines (USP 35-NF 30) are shown in the table below.

Heavy metal	Acceptance criteria in DS (µg/g Fe)	Level of heavy metal (µg) per iron dose of 750 mg	USP Parenteral PDE (µg/day)
(b) (4)			

DS: drug substance.

The levels of (b) (4) present in a single 750 mg dose of iron are much higher than the permitted daily exposure values. Patients will receive two doses of ferric carboxymaltose at least 7 days apart. Since repeated dosing of ferric carboxymaltose will be permitted according to the proposed label, the number of administrations a patient will receive is unknown. If multiple administrations of ferric carboxymaltose are administered to a patient, the patient may receive relatively high exposures to (b) (4). Based on this information, the proposed acceptance criteria for (b) (4) are not acceptable. The following comment was sent to the Applicant on May 10, 2012:

> Your proposed acceptance criteria for the heavy metals in the drug substance specification (b) (4) appear to be too high. Based on a maximum iron dose of 750 mg for a single injection of ferric carboxymaltose, these acceptance criteria result in approximately (b) (4) per dose. These levels are higher than the permitted daily exposure (PDE) limits for parenteral administration listed in the USP and EMA guidelines (please see the links below for more information). Lower the acceptance criteria for these heavy metals.
>
> Your proposed acceptance criteria of (b) (4) the drug product is equivalent to (b) (4) content in the drug product, which results in (b) (4) This level is higher than the PDE limit for parenteral administration listed in the EMA guideline document. Please provide justification for the safety of the proposed acceptance criteria for (b) (4) Your justification could include exposure to (b) (4) patients who receive your approved drug Venofer.

USP:
http://www.usp.org/sites/default/files/usp_pdf/EN/hottopics/232_ElementalImpuritiesLimits.pdf
EMA:
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003587.pdf

A response to the comment was received on May 16, 2012. The Applicant emphasized that both the USP and EMA guidelines state that the PDE limits are based on chronic use, and that ferric carboxymaltose is not administered daily and is not intended for chronic use. Based on the periodic short-term use of the drug with a maximum daily iron dose of 750 mg (b) (4) with at least 7 days between doses, the Applicant used the formula (Limit=((b) (4) . The PDE values from the guidelines used in the formula, the new acceptance criteria, and the level of the heavy metal for the maximum iron dose of 750 mg are shown in the table below.

Heavy metal	Acceptance criteria in DS (µg/g Fe)	Level of heavy metal (µg) per iron dose of 750 mg (µg)	USP Parenteral PDE (µg/day)
(b) (4)			

For the new acceptance criteria, the levels of (b) (4) present in a single 750 mg dose of iron are still higher than the USP permitted daily exposure recommendations. It is noted that discussions on safety-based acceptable levels of heavy metals are ongoing as part of the ICH Q3D discussions. However, for the metals listed above, the acceptance criteria provided above are acceptable for the following reasons.

- (b) (4)
- (b) (4)
- (b) (4)

Thus, the revised drug substance acceptance criteria for [redacted] ferric carboxymaltose are acceptable from a pharmacology/toxicology perspective.

Also in the response was the following justification for the safety of the proposed acceptance criteria for [redacted]:

[redacted]

Based on this justification explaining the higher levels of [redacted] present in the approved drug Venofer, the acceptance criteria of [redacted] is acceptable from a pharmacology/toxicology perspective. At this time there are no pharmacology/toxicology issues for NDA 203565 to preclude approval of the drug for the proposed indication.

Comments on Proposed Labeling:

The pharmacologic class under "Indications and Usage" of the HIGHLIGHTS is "iron replacement product", which is consistent with the pharmacologic class for the other intravenous iron products (Feraheme, Ferrlecit, and Venofer).

Ferric caroboxymaltose was administered daily in the embryo-fetal development studies (data presented in section 8.1 of the label) and 3 times per week, on Days 0, 3, and 7 in the fertility and early embryonic development study (data presented in section 13.1 of the label), which differs from the weekly administration in humans. Due to this difference in administration schedules and lack of PK data, dose-to-dose comparisons based on body surface area (mg/m^2) were made to compare the human and animal doses.

Since there are clinical data in nursing women exposed to Injectafer, the maternal health team was consulted for section 8.3 (Nursing Mothers). The maternal health team and the clinical team will be making the labeling recommendations for section 8.3, which has been omitted below.

Journal articles, e.g. Park and Park (2011), suggest that iron is genotoxic. It appears that the genotoxic potential of iron is due to iron overload in the studies published. This observation is not relevant to the current iron product Injectafer,

since Injectafer will be administered to patients with iron deficiency anemia. Administration of Injectafer is not expected to cause iron overload. Additionally, genotoxicity studies with ferric carboxymaltose and other iron products (see labels for Feraheme and Venofer) were compliant with ICHS2 and were negative for genotoxicity. Therefore, information on the potential genotoxicity of iron from overload will not be added to the label.

Reference:

Park, J. and Park, E. (2011). Influence of iron-overload on DNA damage and its repair in human leukocytes *in vitro*. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 718: 56-61.

The proposed draft FDA labeling for ferric carboxymaltose for the pharmacology/toxicology sections is presented below.

Proposed Labeling:

HIGHLIGHTS

----------INDICATIONS AND USAGE----------

Injectafer is an iron replacement product indicated for the treatment of iron deficiency anemia.

----------USE IN SPECIFIC POPULATIONS----------

- Nursing Mothers: (b) (4) exercise (b) (4) when (b) (4) administered to a nursing woman. (8.3)

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

Adequate and well controlled studies in pregnant women have not been conducted. In (b) (4) studies, administration of ferric carboxymaltose to rabbits during the period of organogenesis caused fetal malformations and increased implantation loss at maternally toxic doses; approximately 12% to 23% of the human weekly dose of 750 mg (based on body surface area). Injectafer should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Administration of ferric carboxymaltose to rats as a one-hour intravenous infusion up to 30 mg/kg/day iron on gestation days 6 to 17 did not result in adverse embryofetal findings. This daily dose in rats is approximately 40% of the human weekly dose of 750 mg based on body surface area. In rabbits, ferric carboxymaltose was administered as a one-hour infusion on gestation days 6 to 19 at iron doses of 4.5, 9, 13.5, and 18 mg/kg/day. (b) (4)

(b) (4) Malformations were seen starting at the daily dose of 9 mg/kg (23% of the human weekly dose of 750 mg). Abortion occurred starting at the daily iron dose of 4.5 mg/kg (12% of the human weekly dose based on body surface area). Pre-implantation loss was at the highest dose.

A pre- and post-natal development study was conducted in rats at intravenous doses up to 18 mg/kg/day of iron (approximately 23% of the weekly human dose of 750 mg on a body surface area basis). There were no adverse effects on survival of offspring, their behavior, sexual maturation or reproductive parameters.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Ferric carboxymaltose is a colloidal iron (III) hydroxide in complex with carboxymaltose, a carbohydrate polymer, that releases iron.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility:

Carcinogenicity studies have not been performed with ferric carboxymaltose.

Ferric carboxymaltose was not genotoxic in the following genetic toxicology studies: *in vitro* microbial mutagenesis (Ames) assay, *in vitro* chromosome aberration test in human lymphocytes, *in vitro* mammalian cell mutation assay in mouse lymphoma L5178Y/TK+/- cells, *in vivo* mouse micronucleus test at single intravenous doses up to 500 mg/kg.

In a combined male and female fertility study, ferric carboxymaltose was administered intravenously over one hour to male and female rats at iron doses of up to 30 mg/kg. Animals were dosed 3 times per week (on Days 0, 3, and 7). There was no effect on mating function, fertility or early embryonic development. The dose of 30 mg/kg in animals is approximately 40% of the human dose of 750 mg based on body surface area.

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/s/

BRENDA J GEHRKE
06/13/2012

HALEH SABER
06/13/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 203565 **Applicant: Luitpold Pharmaceuticals Inc.** **Stamp Date: 10/03/2011**

Drug Name: Ferric carboxmaltose **NDA/BLA Type: NDA 505 b1**

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?			There is no pharmacology/toxicology section in this NDA; nonclinical studies were submitted and reviewed under NDA 22054; there is a cross-reference to the non-clinical data in NDA 22054 (paper submission)
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?			Not applicable
3	Is the pharmacology/toxicology section legible so that substantive review can begin?			Not applicable
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)?			Nonclinical studies were reviewed under NDA 22054
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).			Not applicable
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?			Not applicable
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?			Nonclinical studies were reviewed under NDA 22054 and applicant is cross-referencing nonclinical studies in NDA 22054

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?			Nonclinical studies were reviewed under NDA 22054 and applicant is cross-referencing nonclinical studies in NDA 22054
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?	✓		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			No known impurity issues at this time
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

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.

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

Brenda J. Gehrke, Ph.D. 12/1/2011

Reviewing Pharmacologist Date

Haleh Saber, Ph.D. 12/1/2011

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

BRENDA J GEHRKE
12/01/2011

HALEH SABER
12/01/2011

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-054**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**June 15, 2006**
PRODUCT:	**Ferinject (Iron carboxymaltose Injection, VIT-45)**
INTENDED CLINICAL POPULATION:	**Post-partum /IBS/Hemodialysis Anemia**
SPONSOR:	**Luitpold Pharmaceuticals, Inc., Norristown, PA.**
DOCUMENTS REVIEWED:	**Vol. 1:8 to 1:13, 1.15-1.17, 1.28 to 1.53**
REVIEW DIVISION:	**Division of Medical Imaging & Hematology Drug Products (HFD-160)**
PHARM/TOX REVIEWER:	**Yash M. Chopra, M.D., Ph.D.**
PHARM/TOX SUPERVISOR:	**Adebayo Laniyonu, Ph.D.**
ACTING DIVISION DIRECTOR	**R. Dwaine Rieves, M.D.**
PROJECT MANAGER:	**Hyon-Zu, Lee, Pharm.D.**

Date of review submission to Division File System (DFS): June 5, 2007.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. **Recommendation on approvability**: From the preclinical pharmacology and toxicology view point, VIT-45 should be approved.

B. **Recommendation for nonclinical studies**: None.

C. Recommendations on labeling:

The changes in the following sections of the non-clinical portions of the proposed label with edits are shown below: The *underlined italics* represent addition in the original version by the reviewer.

8 USE IN SPECIFIC POPULATIONS

(b) (4)

(b) (4)

II. Summary of nonclinical findings

VIT-45, an intravenous hematinic preparation liberates utilizable iron in the body for intermittent use in iron deficient anemia. The released iron binds with iron binding proteins and accumulates mainly in animal's blood cells with about 76% in red cells,

11% in liver and 2% in spleen and 1% in kidney. Single intravenous dose of 1 g/kg and 0.24 g/kg in rats and dogs, respectively were not lethal but 2 g dose in mice was lethal. The repeat dose 13-week intravenous infusion toxicity study in rats and dogs showed iron deposition in multiple organs including liver, spleen, lymph nodes and kidneys. The dose of 9 mg/kg/week was tolerated well in these species. Similar tissue deposition was also seen in a chronic continuous intravenous infusion 26-week study in dogs, 9 mg/kg/week was identified as a well tolerated dose. VIT-45 was not genotoxic in a battery of tests including, *in vitro* microbial mutagenesis assay, *in vitro* chromosome aberration test in human lymphocytes, *in vitro* mammalian cell mutation assay in mouse lymphoma L5178Y/TK+/- cells, *in vivo* mouse micronucleus test. VIT-45 exerted no adverse effect on the fertility and general reproductive performance in rats. It did not produce a teratogenic defect in pregnant rats but at a maternal toxic dose in rabbits, it caused doomed cranium with hydrocephaly. It did not produce perinatal and postnatal developmental defects in rat pups.

A. Pharmacologic activity

VIT-45, an intravenous hematinic preparation liberates utilizable iron in the body for intermittent use in iron deficient anemia. Sponsor did not submit any new preclinical study to demonstrate the hematinic effects of the compound. VIT-45 is (b) (4) These breakdown products are GRAS listed agents. Malotetriose is synthesized in kidneys from maltose and is hydrolyzed by amylase. Maltotetrose is a metabolite of dextrins and icodextrin used in peritoneal dialysis. It is metabolized into matotetraose, maltotriose, maltose and glucose.

The sponsor submitted a battery of neurological tests, including Irwin tests. VIT-45 did not exert significant effects on body temperature and spontaneous locomotor activity up to 90 mg/kg dose. No significant changes occurred in the respiratory parameters of conscious rats up to 90 mg/kg dose. The renal function test in conscious rats suggested a treatment related transient 33 to and 46 % decrease in urine output in male and female rats at 90 mg/kg dose with a slight decrease in urinary sodium, potassium, and chloride excretion. The total iron binding capacity and plasma iron of the animals were increased in a dose dependent manner. In telemetric dogs, an intravenous dose of up to 90 mg/kg VIT-45 produced a decrease in RR interval and, no significant changes in QT- and QTc-intervals.

B. Nonclinical safety issues relevant to clinical use: None

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-054

Review number: 000

Information to sponsor: Yes ( ) No ( )

Sponsor and/or agent: Luitpold Pharmaceuticals, Norristown, PA.

Manufacturer for drug substance: Luitpold Pharmaceuticals, Norristown, PA.

Reviewer name: Yash M. Chopra, M.D., Ph.D.
Division name: Division of Medical Imaging & Hematology Drugs Products

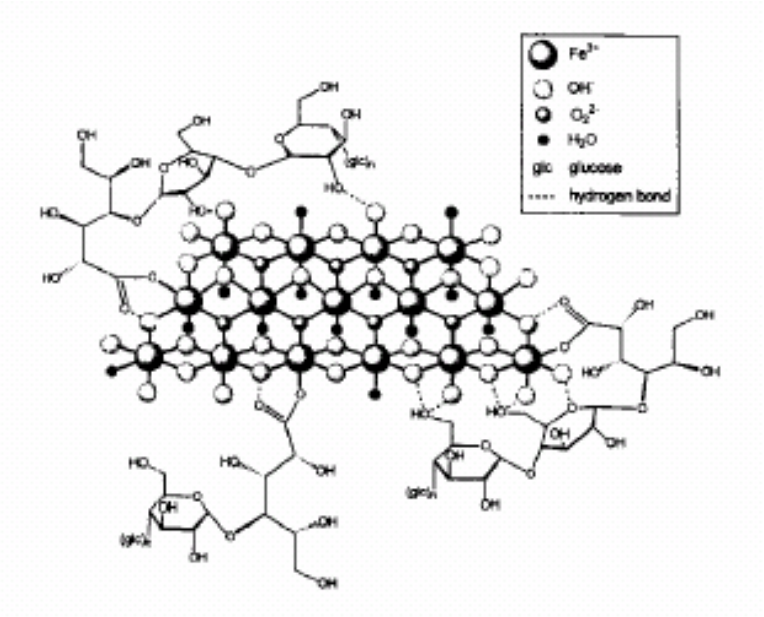
HFD #: 160

Review completion date: June 5, 2007

Drug:

Trade name: Ferinject Injection
Generic name: Iron Carboxmaltose
Code name: VIT-45
Chemical name: The drug substance is a complex of polynuclear iron (III) hydroxide with 4(R)-(poly-(1-->4) –O-⟨-D-glucopyranosyl)-oxy-2(R),3(S),5(R),6-tetrahydroxy-hexanoate.
CAS registry number:
Molecular formula/molecular weight: 150,000 Daltons (Approximately)

Structure:



Relevant INDs/NDAs/DMFs: IND 63,243

Drug class: Hematopoeitic Agent/Hematinic Agent

Intended clinical population: For Iron Overload Patients

Clinical formulation: VIT-45 contains (b) (4) iron hydroxide, sodium chloride and less than (b) (4).

Route of administration: Intravenous Injection

Intended clinical dose: The proposed maximum single dose of Ferinject® (iron carboxymaltose) injection is 1000 mg or 15 mg/kg on day 0 and, maximum repeat doses is 2500 mg/week for the treatment of iron deficiency anemia secondary to Pregnancy/Childbirth (Post-partum Anemia), Heavy Uterine Bleeding (HUB). In chronic hemodialysis patients, the intended clinical dose is 200 mg/dialysis session 2 or 3 times a week.

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

[For (b)(2) applications:

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-054 are owned by Luitpold Pharmaceuticals, Norristown, PA. Any information or data necessary for approval of NDA 22-054, the Luitpold Pharmaceuticals, Norristown, PA. did not own or had a written right to reference constituted/belonged one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously submitted or approved application that Luitpold Pharmaceuticals, Norristown, PA does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-054.

Studies reviewed within this submission:

(I) New Submitted Studies to NDA 22-054:

Type of Study	Study #	Lot	Lab	Page #
Pharmacology	-	-	-	9
Safety Pharmacology	-	-	-	
1.Irwin Dose Range Rats, including body temperature and Locomotor Activity Assessment– Single Intravenous Dose administration	#VFR067/042728	#291100 (4.95% m/v iron)	(b) (4)	10

2.Evaluation of the Effects on Respiration in the Conscious Rat Using Whole Body Bias Flow Plethysmography	#VFR068/042764/#VIT-10-039)		,,	11
3.Effect of VIT-45 on Renal Function in the Conscious Rats	VFR#069/042797/#VIT-10-038, 2005		,,	12
4.Telemeteric Evaluation of cardiovascular (hemodynamic) Effects in Conscious Dogs	# 066/042584	291100	(b) (4)	14
Absorption, Distribution, Metabolism, and Excretion (ADME):				
1. Intravenous Administration of labeled VIT-45 to rats	SR-1075/E01	Not given	--	16
2. Transport of ^{59}Iron Through the Rat Dams Milk after Intravenously Administered VIT-45 to the Offspring:	SR-1077/E01	-	-	17
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6. Placenetal Transfer & Milk secretion studies in Rats after Single IV dose	#VFR 062/033271	#267100M (^{59}Fe VIT-45		23
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8. Pharmacokinetics of a Single Intravenous and Intramuscular Dose Administration of VIT-45 in Rats	#VFR 060/033441 /TEP 9021/1:902/ 4;901/2; 901/4	^{59} CW280303 - ^{9}Fe-VIT-45	-	27
9. Absorption, Distribution and Excretion studies in Dog After Single Intravenous and Intramuscular Doses in Dogs	#VFR059/033758	$^{59}FeCl_3$ (# 267100M-non-labeled; 7.4 MBq		31
10. Determination of the amounts of Degradation Products of the Ligand of VIT-45 using α-Amylase and S9 fraction of Rat Liver Homogenates	#SR-1099-01/E02	#843009M	-	36
TOXICOLOGY:				
Subacute to Subchronic Toxicity				
1. Toxicity Study by Intravenous (Bolus) Administration to CD-Rats Three Times per Week for 13-Weeks	VFR 071/043284	VIT-45 – 291100; Cyclophosphamide – A016418501	(b) (4)	51
2. 26-Week Chronic Intravenous Bolus Injection Toxicity Study in Dogs followed by a 6 Week Recovery Period.	VFR 070/042337	#291100	(b) (4)	63
LABELING	-	-	-	100

The following studies were reviewed earlier under IND 63,243 (Ke Zhang, Ph.D. HFD-180) and their review was available and acceptable. In the present application, this was scanned at the pages as denoted the present review.

Studies reviewed under IND 63,243:

Type of Study	Study #	Lot	Lab
Pharmacology	-	-	-
Absorption, Distribution, Metabolism, and Excretion (ADME):			
I.V. administration of ^{59}Fe labeled VIT-45 in rats	SR-1075	--	--
Transport of iron via maternal mild to offspring	74-1077		
Acute Toxicity:			
Acute i.v. toxicity study in mice	VFR035	0481000	1
Acute i.v. toxicity study in mice	SR-1026/E01	0481000	1
Acute i.v. toxicity study in rats	VFR034	894209B	1
Acute i.v. toxicity study in rats	VFR033	894209B	1
Acute i.v. toxicity study in dogs	VFR032	894209B	1
Subacute to Subchronic Toxicity:			
13-week i.v. toxicity study in rats	VFR042	894209B	1
13-week i.v. toxicity study in dogs	VFR041	894209B	1
Mutagenesis:			
Ames test	VFR029	894209B	1
Mouse lymphoma forward mutation assay in L5178Y+/- cells	VFR030	894209B	1
In vitro chromosomal aberration test in human lymphocyte	VFR028	894209B	1
In vivo mouse micronucleus test	VFR031	894209B	1
Reproductive Toxicity:			
Segment I fertility and general reproductive toxicity study in rats	VFR050	894209B	1
Segment II teratology study in rats	VFR048	894209B	1
Segment II teratology study in rabbits	VFR049	894209B	1
Segment III peri- and post-natal reproductive toxicity study in rats	VFR052	894209B	1
Special Toxicity:			
Intra-arterial tolerance study in rabbits	VFR054		1
Peri-venous tolerance study in rabbits	VFR055		1

Intravenous tolerance study in rabbits	VFR063		1
Dextran Antigenicity to the rabbits	VFR043		1

1 = (b) (4)

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

VIT-45, an intravenous hematinic preparation liberates utilizable iron in the body for intermittent use in iron deficient anemia. The released iron binds with iron binding proteins and accumulates mainly in animal blood cells for more than 762 hr up to 76% in red cells, 11% in liver and 2% in spleen and 1% in kidney. VIT-45 is degraded iron hydroxide and simple oligo-glucose units of matotetraose, maltotriose, maltose and glucose. Malotetriose is synthesized in kidneys from maltose and is hydrolyzed by amylase. Maltotetrose is a metabolite of dextrins and, icodextrin used in peritoneal dialysis and is metabolized in to matotetraose, maltotriose, maltose and glucose.

Iron overload is important outcome of toxic effects of excess iron and has been associated with anemias in patients with refractory and transfusion dependent anemias, thalasemias major, hereditary haematochromatosis and other conditions like porphyria cutanea tarda and chronic liver and renal diseases. The iron induced lipid peroxidation and associated organelle dysfunctions produce liver fibrogenesis. The patients with hemachromatosis (HHC) eventually leads to liver cirrhosis, skin pigmentation, diabetes, heart disease and hypogonadism. VIT-45 a simple short chain starch molecule (b) (4) enzymatic procedures and has been claimed to be effective in iron overload conditions.

2.6.2.2 Primary Pharmacodynamics

Mechanism of action:

VIT-45 a polynuclear iron (Fe^3) complex is intended for a release of iron in patients with iron deficiency anemia. An intravenously or intramuscularly administered VIT-45 was absorbed in a biphasic manner with an initial peak in whole blood for a transient period then peaked to a higher concentration in red blood cells, liver, kidneys and skin. Large amounts were detected in blood cells up to 28 days with only slight decrease. VIT-45 degrades to iron hydroxide and simple oligo-glucose units of maltotetraose and maltoriose, maltose and glucose. The iron released by the compound is transported by an active transport mechanism, intracellular transport and release of iron to transferrin in the portal system. These breakdown products are endogenously liberated substances as malotetriose is synthesized in kidneys from maltose and is hydrolyzed by amylase.

Maltotetrose is a metabolite of dextrins and, icodextrin used in peritoneal dialysis and is metabolized in to matotetraose, maltotriose, maltose and glucose.

2.6.2.3 Secondary pharmacodynamics: No secondary pharmacodynamic effects of its breakdown products were expected. Sponsor speculated that the compound might not exert any secondary pharmacological effects.

2.6.2.4 Safety pharmacology

Neurological effects:

1. **Irwin Dose Range Rats, including body temperature and Locomotor Activity Assessment– Single Intravenous Dose administration**: (Doc #VFR067/042728)

GLP Requirements: The study was conducted in compliance with GLP and QAU of Federal Republic of Germany

Name of the Conducting Laboratory: (b) (4)

Dates of Initiation and Completion: May 4, 2004 and August 25, 2005

Batch #: 291100 (4.95% m/v iron)

Methods: VIT-45 was administered in 0.9% saline solution in 3 groups of rats (4/sex/group) at the intravenous doses of 0, 30 and 90 mg/kg (5 ml/kg) via tail vein at the rate of 1.5 ml/min. The basis of the dose selection was not given in the study. The clinical observations were performed 0-5 min, 15, 30, 60 and 120 after dosing and, on day 2. The rectal temperature and spontaneous motor activity of each of the animals was checked. The Irwin test included the body temperature changes, short term locomotor activity and cage observation of physical changes in animal conditions were noted. The various reflexes; equilibrium and grasping tests and, flexor reflex and foot pinch were noted in treated animals. The tests were analyzed and significance of the difference in the test was evaluated by various statistical methods. Blood was drawn after 7 days of the treatment for iron assay and TIBC assay.

Results:
a. Mortality: None of the animals died during the study.
b. Clinical Observations: There were no changes in the spontaneous motor activity in male and female rats after the treatment with the dose of 30 and 90 mg/kg.
c. Rectal Temperature Changes: The mean rectal temperature before and after 30 min of intravenous 90 mg Fe/kg was 37.8^{o}C and 38.3^{o}C in males and, 37.6^{o}C and 38.6^{o}C in females and there was no significant change in the rectal temperature from the control values.

The spontaneous activity in the VIT-45 treated rats was similar to the control group animals.

d. Plasma Iron and Total Iron Binding capacity: The compound did not affect the total plasma iron and total iron binding capacity of the animals.

On respiratory System:

2. **Evaluation of the Effects on Respiration in the Conscious Rat Using Whole Body Bias Flow Plethysmography:** (Study #VFR068/042764/Luitpod Doc #VIT-10-039)

Methods: The study was conducted under QAU and GLP specifications and in compliance with ICH S7A guidelines for safety pharmacology. Four groups of conscious Wistar rats (8/group) were administered a single intravenous dose of 0, 30 or 90 mg Fe/kg VIT-45 (in 0.9% saline) to determine the effect of the compound on the respiratory parameters (respiratory rate, peak inspiratory and peak expiratory flows, inspiration and expiration times, airways resistance, minute and tidal volume). To an additional group of 8 rats, a solution of 20 mg/kg Morphine sulfate (a positive control) was administered intravenously.

Results: VIT-45 at an intravenous dose of 30 or 90 mg/kg did not produce any significant effect on the respiratory rate, tidal volume and minute volume in male and female rats at 0, 0-30 or 30-60 minutes of administration of the dose. A single IV dose of 20 mg/kg morphine sulfate showed significant depression in respiratory rate, tidal volume and minute volume in both male and female rats. The effect on respiration rate is shown in the following table 1 (vol. 30.323, pp 84).

TABLE 1

Effects of intravenous administration of VIT-45 on respiration rate in the conscious rat

Males

Group	Intravenous treatment	Dose (mg Fe/kg)	Group mean respiration rate (br/min) in the male rat (± sd) at time (minutes) post-dose				
			Pre-dose	0 - 30	60	120	240
1	Vehicle (0.9% w/v saline)	0	313 ±43.3	241 ±37.8	180 ±18.9	161 ±47.4	137 ±31.4
2	VIT-45	30	310 ±42.8	235 ±35.6	176 ±18.3	147 ±42.5	139 ±39.0
3	VIT-45	90	308 ±45.5	221 ±24.6	175 ±22.6	133 ±13.1	133 ±18.3
4	Morphine sulphate	20†	313 ±39.4	105** ±21.6	114** ±19.6	144 ±25.2	216** ±36.3

Females

Group	Intravenous treatment	Dose (mg Fe/kg)	Group mean respiration rate (br/min) in the female rat (± sd) at time (minutes) post-dose				
			Pre-dose	0 - 30	60	120	240
1	Vehicle (0.9% w/v saline)	0	378 ±45.5	233 ±25.8	162 ±48.9	138 ±49.5	160 ±129.6
2	VIT-45	30	383 ±43.6	233 ±30.6	199 ±52.9	171 ±47.4	112 ±27.9
3	VIT-45	90	377 ±48.5	227 ±46.2	185 ±26.5	118 ±25.4	105 ±18.9
4	Morphine sulphate	20†	376 ±49.6	95** ±16.3	107* ±20.5	130 ±36.2	221 ±47.6

sd Standard deviation
† Dose given as salt, not Fe content
br/min Breaths per minute
Statistical significance of difference from vehicle-treated control group: * $p<0.05$, ** $p<0.01$

The total iron binding capacity and plasma iron in animals was increased in a dose related manner as shown in the following sponsor's table 4 (volume 29.323, pp87).

TABLE 4

Effects of intravenous administration of VIT-45 on plasma iron and total iron binding capacity (TIBC) in the conscious rat

Males

Group	Intravenous treatment	Dose (mg Fe/kg)	Group mean results (µmol/L) in the male rat (± sd)	
			Plasma iron	TIBC
1	Vehicle (0.9% w/v saline)	0	69.1 ±10.9	96.0 ±11.1
2	VIT-45	30	118.8** ±17.7	135.8* ±22.9
3	VIT-45	90	286.4** ±18.5	280.9** ±55.7

Females

Group	Intravenous treatment	Dose (mg Fe/kg)	Group mean results (µmol/L) in the female rat (± sd)	
			Plasma iron	TIBC
1	Vehicle (0.9% w/v saline)	0	61.0 ±9.5	91.1 ±13.4
2	VIT-45	30	120.4** ±9.4	132.8 ±36.6
3	VIT-45	90	304.0** ±25.3	252.4** ±61.2

sd Standard deviation

Statistical significance of difference from vehicle-treated control group: * $p<0.05$, ** $p<0.01$

In summary, intravenously administered VIT-45 at 30 or 90 mg/kg produced an increase in the total iron binding capacity and plasma iron of the animals in a dose dependent manner without any significant changes on the respiratory parameters of rats. The 'no effect dose' in the study was the highest dose tested.

Renal Function Study:

3. **Effect of VIT-45 on Renal Function in the Conscious Rats**:
VFR#069/042797/Luitpold Doc #VIT-10-038,2005)

In this GLP study, 3 groups of water only-fasted Wistar rats (8/sex) were treated at 2 different occasions. The males of the study were treated first and then females. The animals were administered intravenous dose of VIT-48 and then were given loading oral dose of water. The animals were randomized and placed in metabolism cages for 24 hr urine collection. The urine samples were recorded at 1, 2, 3, 4, 5 and 24 hr after the treatment. The 5-hr urine samples were analyzed for initial and final urinary flow rates, sodium, potassium and chloride excretion. The results were compared with 5 mg/kg furosemide animals. The males of the test drug groups were compared with males of the positive control group and, VIT-45 treated females were similarly compared with females included in positive treatment group. In a single group of the rats (8/sex), an IV dose of 5 mg/kg furosemide was administered and the increase in the overall initial and final urinary flow rates, sodium, potassium and chloride excretion was studied.

The pH of the furosemide treatment group was not significantly affected. The urine samples were contaminated and not processed further. Sponsor repeated the study with similar doses (VFR 0086/053340). The results of the repeat study showed no deaths and no signs of toxicity but a transient decrease of 46% and 33% in urine output was seen at 90 mg/kg dose in male and female rats up to 5 hr of the dosing. The pH and specific gravity of the urine was not changed. The urinary sodium, potassium and chloride excretion was decreased as seen sponsor's table 1 (vol 29 of 323, pp 223) and scanned below.

TABLE 1

Effects of intravenous administration of VIT-45 on urine output in the rat

Males

Group	Intravenous treatment	Dose (mg Fe/kg)	Group mean cumulative urine output (ml ± sd) at time (h) post-dose					
			1	2	3	4	5	24
1	Vehicle	0	1.6 ±0.91	4.5 ±0.95	5.3 ±0.95	5.9 ±1.04	6.3 ±1.37	14.8 ±5.11
2	VIT-45	30	2.4 ±1.43	4.4 ±1.43	5.5 ±1.26	6.1 ±1.11	7.0 ±1.34	15.5 ±4.11
3	VIT-45	90	0.6 ±0.64	2.6** ±0.98	3.2** ±1.30	3.2** ±1.30	3.4** ±1.44	10.6 ±3.13
4	Frusemide 5 mg/kg	-	6.7** ±0.65	7.6** ±1.05	8.0** ±1.13	8.4** ±1.19	8.5** ±1.11	14.8 ±1.89

Females

Group	Intravenous treatment	Dose (mg Fe/kg)	Group mean cumulative urine output (ml ± sd) at time (h) post-dose					
			1	2	3	4	5	24
1	Vehicle	0	2.0 ±0.90	2.8 ±0.72	3.2 ±0.75	3.7 ±0.83	4.0 ±1.02	12.0 ±2.32
2	VIT-45	30	1.6 ±0.65	2.7 ±0.65	3.0 ±0.41	3.4 ±0.47	3.7 ±0.47	11.1 ±2.39
3	VIT-45	90	1.4 ±0.95	2.3 ±1.03	2.6 ±0.79	2.6** ±0.86	2.7** ±0.92	11.6 ±3.53
4	Frusemide 5 mg/kg	-	5.1** ±0.56	5.4** ±0.64	5.6** ±0.35	5.7** ±0.42	5.8** ±0.37	11.8 ±1.82

sd Standard deviation
Statistical significance of difference from vehicle-treated control group: ** $p<0.01$

The TIBC was decreased in a treatment related manner in both male and female rats and, there was a greater decrease in 30 mg/kg treated animals than the 90 mg/kg treated group (sponsor's table 4, pp 225).

TABLE 4

Effects of intravenous administration of VIT-45 on blood urea, plasma iron and total iron binding capacity (TIBC) in the rat

Males

Group	Intravenous treatment	Dose (mg Fe/kg)	Group mean (± sd) blood urea (mmol/l), Fe (µmol/l) and TIBC (µmol/l)			
			Blood urea		Fe	TIBC
			Pre-dose	24 hours	24 hours	24 hours
1	Vehicle	0	5.2 ±0.39	5.9 ±0.49	64 ±17.27	103 ±2.66
2	VIT-45	30	5.2 ±0.58	5.8 ±0.64	39** ±3.76	102 ±5.88
3	VIT-45	90	4.9 ±0.44	5.2 ±0.94	52** ±8.07	101 ±8.89
4	Frusemide 5 mg/kg	-	5.1 ±0.67	5.6 ±0.74	62 ±16.59	104 ±10.39

Females

Group	Intravenous treatment	Dose (mg Fe/kg)	Group mean (± sd) blood urea (mmol/l), Fe (µmol/l) and TIBC (µmol/l)			
			Blood urea		Fe	TIBC
			Pre-dose	24 hours	24 hours	24 hours
1	Vehicle	0	5.4 ±0.55	5.0 ±0.95	66 ±12.17	96 ±6.30
2	VIT-45	30	5.4 ±1.53	5.4 ±1.07	45* ±5.01	91 ±6.79
3	VIT-45	90	5.4 ±0.39	4.3 ±0.92	59* ±15.36	95 ±8.77
4	Frusemide 5 mg/kg	-	5.1 ±0.75	5.2 ±0.96	65 ±15.44	87** ±3.59

sd Standard deviation
Statistical significance of difference from vehicle-treated control group: * $p<0.05$. ** $p<0.01$

Cardiovascular effects:

4. **Telemeteric Evaluation of Cardiovascular (hemodynamic) Effects in Conscious Dogs**: (Study # 066/042584)

Testing Laboratory: (b) (4)
Dates of Start and Completion of Study: March 22, 2004 and August 25, 2005.
GLP & QAU Requirements: A statement of compliance with GLP and QAU regulations was submitted.
Batch #: 291100

Methods: The study was undertaken to determine the possible cardiovascular toxic effects of intravenously administered VIT-45 in 0.9% saline in dogs (from telemetry colony at (b) (4)). The healthy dogs were implanted with specific surgical telemetry transmitter to evaluate the effects of the intravenously administered compound on blood pressure, heart rate and EKG parameters including QT-prolongation. VIT-45 was

administered in 4 groups of telemetered animals (3/sex) intravenously (being the intended route of administration in human) at the doses of 0, 30, 0 and 90 mg Fe/kg in 4 conscious male dogs implanted with telemetry transducers. The study was conducted in 4 session and each completed/week.

Allocation of the treatment groups/Dosing design

Treatment	Dose(mg Fe/kg)	Test Session	Gr.Number	Target Iron Concen.(mg/ml)	Dose Volume
Control	0	1	1	0	5
Low Dose	30	2		6	
Control	0	3	2	0	
High Dose	90	4		18	

In the first week, was given as control solution and telemetry recordings were obtained. The EKG tracings of each of the animals were recorded at 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 12 hr post dose. The observation period from 0.25 to 4 hr post-dose was light phase and, the 6 to 12 hr post dose was the dark phase. Each of the animals was observed daily, the blood pressure (mean, systolic, diastolic), heart rate, and EKG were recorded Any abnormal EKG was documented and QT converted to QTc (msec). The corrected values from QT-versus RR intervals were used for QTc. The study animals were not sacrificed after the treatment and returned to the telemetry colony of the laboratory. The changes in the behavioral observations, TIBC and iron contents were also calculated.

Results: There was variability of the blood pressure of the animals in the light and dark phases, i.e., 132 to 97 mmHg in the light and, 117 to 96 mmHg in the dark phases in group 1 (control group) dogs. In group 2 animals, mean blood pressure varied from 137 to 101 mmHg in the light phase and, 118 to 99 mmHg in the dark phase. The maximum blood pressure in the light phase was with the sham dosing activities. The heart rate varied from 126 to 81 beats/min in dark phase of group 1 animals and their heart rate was 143 to 71 beats/min in light phase group. The heart rate varied from 113 to 87 in group 1 and, 108 to 88 beats/min in animals of group 2 (90 mg/kg treatment group).

The data in day and dark periods are shown below in the table of sponsor (vol 30 of 323, pp 29). There were no significant changes in the arterial blood pressure of the animals treated with VIT-45. The maximum and minimum heart rates in day were slightly affected but in an insignificant manner.

Treatment and dose	Post-dose time	Maximum and minimum ranges in arterial BP					
		Systolic BP (mmHg)		Diastolic BP (mmHg)		Mean BP (mmHg)	
		max ± s.e.	min ± s.e.	max ± s.e.	min ± s.e.	max ± s.e.	min ± s.e.
Vehicle (Group 1)	Day	175 ± 10.0	141 ± 4.5	116 ± 6.4	89 ± 5.0	136 ± 7.3	87 ± 5.9
	Night	156 ± 7.6	141 ± 6.1	96 ± 6.2	84 ± 4.5	119 ± 7.3	104 ± 4.8
VIT-45 (30 mg Fe/kg)	Day	163 ± 17.5	137 ± 8.5	109 ± 12.5	85 ± 4.6	113 ± 2.0	76 ± 7.4
	Night	150 ± 9.6	135 ± 3.8	96 ± 5.8	83 ± 3.1	112 ± 4.6	99 ± 3.3
Vehicle (Group 2)	Day	150 ± 4.4	130 ± 6.7	102 ± 4.3	85 ± 4.1	116 ± 4.1	101 ± 5.6
	Night	151 ± 5.7	128 ± 3.2	101 ± 4.7	78 ± 5.3	118 ± 4.9	95 ± 5.5
VIT-45 (90 mg Fe/kg)	Day	161 ± 13.2	131 ± 3.6	105 ± 9.0	83 ± 1.6	124 ± 10.5	99 ± 2.1
	Night	148 ± 4.9	131 ± 3.1	96 ± 8.0	82 ± 4.0	114 ± 8.8	98 ± 4.3

The table indicates generally similar maxima and minima regardless of treatment
s.e. Standard error of the mean
0 - 6 h = light phase, 6 - 12 h = dark phase

VIT-45 administration produced insignificant minimal changes in QT-interval and there was no evidence of increase of QTc-interval. There were no behavioral changes in the treated animals. In light and dark phases, there was no significant effect on the mean, systolic and diastolic blood pressure of the animals treated up to 90 mg Fe/kg VIT-45 in dogs. RR interval of the animals treated with 90 mg Fe/kg was reduced in a statistically significant manner after 2 to 4 hr of dosing. No changes in QT and QTc intervals were observed.

A dose related increase in plasma iron content of 42.0 and 108.3 umol/l in animals occured in 30 and 90 mg Fe/kg treatment groups. TIBC of animals treated with low dose was unchanged or similar to control group values but the animals treated with higher dose of 90 mg/kg showed doubling of the concentration of the value in the control (70.5 to 148 umol/l). TIBC was anticipated to be increased in ferrocene method in the presence of VIT-45.

Intravenously administered VIT-45 exerted no significant changes in behavioral and physical observations and, EKG parameters including QT, QTc, RR intervals in dogs were not affected.

2.6.4. PHARMACOKINETICS:

1. **Intravenous administration of ^{59}Fe labeled VIT-45 to rat**
(SR-1075/E01)

Methods: To determine the iron deposit in body, ^{59}Fe labeled VIT-45 was given by intravenous injection at 10 mg to rats fed with iron free diet. Anemia was not evaluated

in this study. The radioactivity in the blood and various organs was determined using a gamma counter over a period of 4 weeks.

Results: The results indicated that the labeled iron was identified in various organs and blood. The results were presented in Table 1 on page 41 in Volume 2.17. This table is attached below.

Table 1: Retrieved ^{59}Fe in relation to the standard dose in red blood cells and serum as well as in organs 14 days (n = 2) and 28 days (n = 2) after intravenous application. The depression factor was applied for all samples except RBC and serum[*].*

Day 14	mean activity[1] (n = 2)	corrected mean activity[2]	corrected mean activity[3] rel. [%]
Liver	**0.050**	*0.054*	10.8
Spleen	**0.008**	*0.009*	1.7
Kidneys	**0.005**	*0.005*	1.0
Tail	**0.362**	*0.387*	---
Faeces	**0.012**	*0.012*	2.5
Urine	**0.003**	*0.004*	0.7
RBC[*]	**0.411**	0.411	82.6
Serum[*]	**0.003**	0.003	0.6
Sum	**0.853**	***0.884***	**100.0**

Day 28	mean activity[1] (n = 2)	corrected mean activity[2]	corrected mean activity[3] rel. [%]
Liver	**0.032**	*0.035*	7.2
Spleen	**0.006**	*0.007*	1.3
Kidneys	**0.006**	*0.006*	1.2
Tail	**0.382**	*0.409*	---
Faeces	**0.007**	*0.007*	1.4
Urine	**0.001**	*0.001*	0.2
RBC[*]	**0.427**	0.427	88.3
Serum[*]	**0.002**	0.002	0.4
Sum	**0.862**	***0.893***	**100.0**

[1] relative values (ratio organ activity /standard dose)
[2] mean activity corrected by the depression factor
[3] after subtracting the activity retained in the tail

The iron concentration was cleared from serum with a half life of 1.5 hours and approximately 76% and 87% of the intravenously injected iron was detected in the red blood cells even after 2 weeks and 4 weeks, respectively.

2. **Intravenous administration of ^{59}Fe labeled VIT-45 to rat dams: transport of iron through mother milk to the offspring**: (SR-1077/E01)

Methods: To determine the transport of iron via milk from dams, ^{59}Fe labeled VIT-45 was given to rat dams right after litter birth by intravenous injection at 10 mg. The radioactivity in the blood, milk, organs, tail, excrements of the dams, and in the offspring was determined over 72 hours using a gamma counter.

Results: The results indicated that the radioactivity retrieved from the milk was very low (up to 0.65% of the injected dose). On day 28, most of the radioactivity remained in the dams and only ~1.2% of the injection dose was retrieved in one offspring. On day 28, approximately 23% of the injected dose was in the blood, 33% in the liver, and 9% in the feces and urine of the dams.

3. **Comparative Pharmacokinetics of Different Parenteral Iron preparations in Rats:** (Document #SR-1005/E01)

The study was conducted to compare the PK of 4 polynuclear ferric hydroxide complex preparations, i.e., iron sucrose, iron polymaltose and 2 iron dextrans in drug-anemic rats. The test was conducted in 16 anemic rats (anemia induced by low-iron diet consisted of Apromin C 1038 pellets - Altromin Spezialfutterwerke GmbH, Lage FRG). The animals were exposed to 12 hr light/dark interval during the study. After the depletion period of 10 days, 4 groups of rats (average wt = 259 g) were administered 10 mg of either iron sucrose, iron polymaltose, iron dextran I or iron dextran II. The body weights were measured on day 1 to 5, 7, 14 and 28 and blood samples for collected for PK parameters estimation. Two/group animals were sacrificed and liver, both kidneys, spleen were separated. The urine and feces samples were collected for radioactivity estimation.

The iron level in erythrocytes was increased in all animals of 4 groups of treated animals during the first 2 weeks post injection. The serum iron in the animals treated with iron sucrose and iron polymaltose was decreased faster than both iron dextran preparations (I and II). The amount of iron in serum was 0.27, 0.29, 3.86, and 2.62 mg in the rats treated with iron sucrose, iron polymaltose and 2 iron dextrans preparations. About 0.7, 0.8, 3.4 and 3.7% of the dose was retrieved in the samples. The iron concentration in the erythrocytes of all the 4 groups of animals was increased and, on day 1, about 21.9, 26.9, 28.6 and 33.3 % of iron was found in erythrocytes in rats treated with iron dextran I, iron dextran II, iron polymaltose and iron sucrose, respectively. The PK data of these 2 iron preparations was shown in table 1 of sponsor (vol, 8.323, pp 100).

Tab. 6: Mean residence time (MRT) and terminal half-life ($t_{1/2}$) of the four iron pharmaceutics iron sucrose, iron polymaltose, Iron dextran (VIT) and iron dextran BP/USP from the serum iron

compound	k_e	MRT	$t_{1/2}$
Iron sucrose	0.60 h^{-1}	1.66 h	1.15 h
Iron polymaltose	0.59 h^{-1}	1.69 h	1.17 h
Iron dextran (VIT)	0.14 h^{-1}	7.17 h	4.97 h
Iron dextran (BP/USP)	0.13 h^{-1}	7.65 h	5.30 h

After 3 weeks of the dosing, 90.8, 93.4, 70.3 and 71.2 % of iron sucrose, iron polymaltose and 2 iron dextrans preparations were incorporated in erythrocytes.

The iron content in tissues after 14 and 28 days of dosing were as shown in the following tables of the submission (vol, 8.323, pp 98 and 99).

Table 5: Retrieved iron in Organs, Blood and Faeces (in mg of a standard dose of 10 mg) **28 days** after injection, as well as the total retrieved iron (n =2 per group).

Iron compound tissue	**Iron Sucrose** mean	**Iron Poly-maltose** mean	**Iron Dextran VIT** mean	**Iron Dextran BP/USP** mean
Kidneys	0,08	0,06	0,07	0,07
Spleen	0,03	0,09	0,33	0,33
Liver	0,32	0,46	0,95	0,34
Faeces	0,28	0,36	0,43	0,47
Urine	0,31	0,00	0,00	0,01
RBC+ Serum	8,94	8,88	6,72	7,11
Sum	9,95	9,85	8,49	8,31

Tab. 3: Retrieved iron (mg) in Red Blood Cells (RBC) at different times after injection of different iron preparations (on days 0 until 14: n =4 per group, days 21 and 28: n =2 per group).

Iron compound	Iron Sucrose		Iron Poly-maltose		Iron Dextran VIT		Iron Dextran BP/USP	
day(s)	mean	SD	mean	SD	mean	SD	mean	SD
0	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
0,25	0,36	0,10	0,26	0,07	0,46	0,13	1,32	0,24
1	1,64	0,39	1,60	0,17	0,96	0,09	1,34	0,25
2	3,33	0,74	2,86	0,42	2,19	0,22	2,69	0,30
3	3,61	0,38	4,24	0,26	3,59	0,63	3,23	0,44
4	3,84	0,62	4,92	0,65	3,48	0,56	3,37	0,35
7	6,07	0,77	5,95	1,22	4,97	0,84	4,25	0,77
14	8,00	1,73	8,45	0,73	6,49	1,06	6,35	0,54
21	9,08	/	9,34	/	7,03	/	7,12	/
28	8,94	/	8,88	/	6,72	/	7,09	/

Table 4: Retrieved iron in Organs, Blood and Faeces (in mg of a standard dose of 10 mg) **14 days** after injection as well as, the total retrieved iron (n =2 per group).

Iron compound tissue	Iron Sucrose mean	Iron Poly-maltose mean	Iron Dextran VIT mean	Iron Dextran BP/USP mean
Kidneys	0,08	0,09	0,07	0,04
Spleen	0,12	0,12	0,35	0,39
Liver	0,51	0,85	0,48	0,27
Faeces	0,22	0,30	0,31	0,34
Urine	0,15	0,00	0,00	0,02
RBC+ Serum	8,02	8,46	6,51	6,36
Sum	10,07	10,35	8,52	7,16

The retrieved iron in the kidneys, the spleen, the liver and the blood after 14 days (Tab. 4) shows that the kidneys contain only 0.9 % or less than the injected dose (iron sucrose 0.8 %, iron polymaltose 0.9 %, Iron dextran (VIT) 0.7 %, Iron dextran (BP/USP) 0.4 %). The spleen is charged higher by Iron dextran (VIT) (3.5 %) and Iron dextran (BP/USP) (3.9 %), than by iron sucrose (1.2 %) or of iron polymaltose (1.2 %). In the liver, iron was found mostly in case of iron sucrose and iron polymaltose.

Most of the administered iron with all the preparations was in animals RBC and serum, the highest amount of iron was in animals treated with iron polymaltose followed by (in decreasing concentrations) iron sucrose, iron dextran (VIT) and iron dextran (B.P/U.S.P.). The iron dextran preparations had greater half lives than other 2 preparations.

4. **Investigation of placental Transfer Permeability for Iron drug VIT-45.**

In an vitro perfusion study using human placenta, the addition of 11 mM ^{59}Fe-VIT-45 (11X the maximum concentration achieved after 200 mg administration in pregnant women) to the maternal circuit produced a 10% decrease in iron concentration within 30 min of the administration on the maternal side with no significant decrease thereafter. The addition of 1.67 mg/ml transferrin to the simulated maternal circuits at one of the 2 phases of the study showed 10% decrease at 10 min following administration on the maternal side. The addition of transferrin did not affect the iron uptake in concentration of 11 mM ^{59}Fe-iron polymaltose (VIT-45) and no radioactivity was detected in fetal circuit. The placental side iron activity was 0.5 to 2% vs 10% in maternal side. The metabolic activity measured as glucose consumption and lactate production were not affected by VIT-45.

Increasing dietary iron at the dose of 35 to 20,000 ug Fe/g in weanling rats, produced a direct increase in non-heme iron and peroxidation and a dose related increase in the heptocytes of the periportal region (zone I). Myocardial degeneration and necrosis with hemosiderin in the interstitial macrophages of myocardial fibers were reported in the rats with heart damage. Spleen lymphoid atrophy and pancreatic atrophy with loss of both endocrine and exocrine pancreatic tissues was also seen in rats with iron overload.

5. **Transport of ^{59}Iron Through the Rat Dams Milk after Intravenously Administered VIT-45 to the Offspring:** (SR-1077/E01)

The study was conducted by administering 10 mg iron/rat as ^{59}Fe VIT-45 in 4 rats dams after birth and the contents of iron absorbed was studied during 4 weeks and the transport of labeled iron was estimated on day 8, 12, 16 and 20 in milk samples. The dams were administered 1 ml oxytocin (synthoconon®). The maximum daily milk in rats is about 14% of the body weight. The offspring were separated from the mothers about 4 hr prior to the collection of the milk samples. The radioactivity was determined in the offspring sacrificed at day 1, 2, 8, 12, 16, 20 and 28. The iron in the blood samples of the dams killed after 6 hr and, at 1, 2, 3 and 4 weeks after the administration of the compound, was determined. The content of iron (radioactivity amounts) liver, spleen, kidneys, stomach, intestines, brains and tails and extremities of the offspring was also estimated.

The milk samples collected on study day 8, 12, 16 and 20 showed significant amount of iron. The amount of radioactivity was about 0.6 to 0.7% of the injected dose of the iron. The amount of iron absorbed gradually increased (see following figure of sponsor, vol 32:323, pp110) and a plateau was seen from day 20 of the administration.

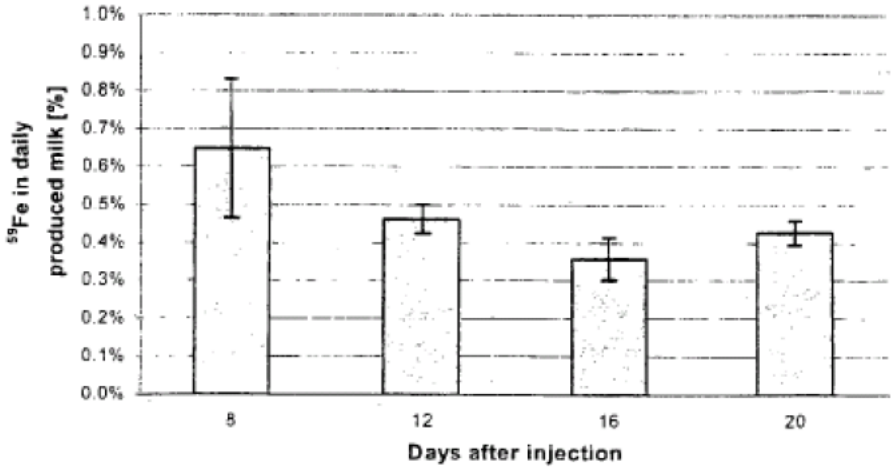

Fig. 1: Calculated average percentage of injected dose in milk produced in 24 hours, on day 8, 12, 16 and 20 after injection.

About 22.3% of the iron was absorbed in red blood cells and decrease to 19.6% after 6 hr of the dose.

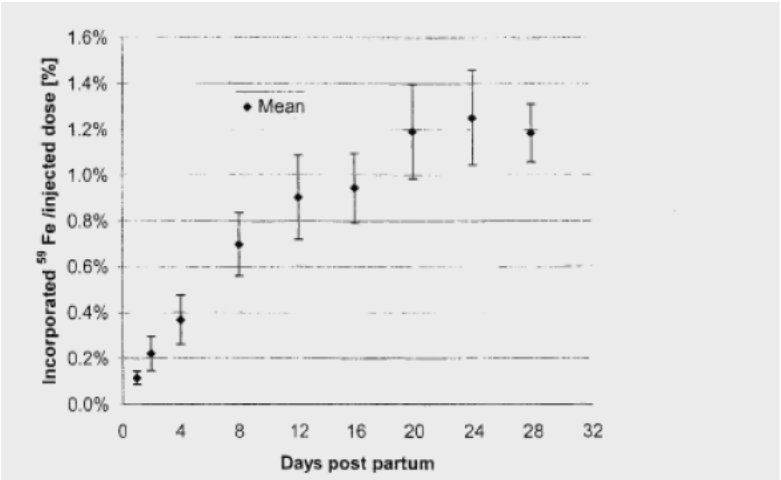

Fig. 2: Average percentage of injected dose retrieved in offspring (n = 5-9) after injection of rat dams.

The distribution of ^{59}Fe VIT-45 was (in descending order: liver, blood, fecal matter, carcass, tail, spleen, intestines, kidneys and stomach. The iron in the VIT-45 was also absorbed/transferred to offspring.

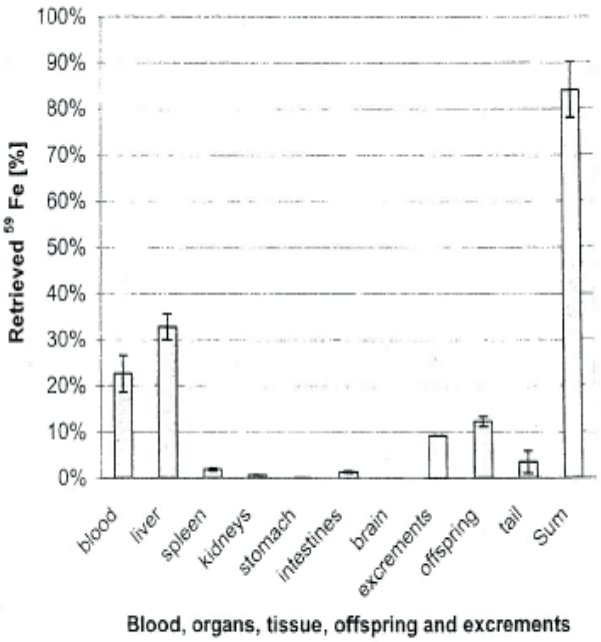

Fig. 5: Average percentage of injected dose retrieved in whole blood, selected organs, excrements as well as in offspring and in tail 28 days after the injection of the rat dams.

Intravenously administered Vit-45 in rat dams was absorbed/transferred in rat fetus.

6. **Placental Transfer and Milk Secretion Studies in the Rat after Single Intravenous Dose.** (Study #VFR 062/033271)

Methods: The study was conducted to determine the distribution and excretion of 5 mg/rat ^{59}Fe VIT-45 (batch #267100M) in pregnant rats, fetuses and secretion in milk of lactating dams. In phase A of the study, 10 pregnant animals were given intravenous (caudal vein) dose of 5 mg Fe-VIT-45/rat on day 12 of gestation and the 2 animals were killed at 0.5, 6, 24, 72 and 168 hr post dose. The blood was collected and was used to determine the whole blood and plasma distribution of the compound. The maternal and fetal tissues were separated and the radioactivity in the tissues was determined by LSC.

In Phase B, the animals were treated with 1-ml oxytocin (synthoconon®) 3 hr prior to the collection of milk to stimulate the milk secretion and their litters removed. On day 7 following parturition, 15 animals were treated with a single intravenous dose of 5 mg Fe-VIT-45/rat and animals. The blood samples from 3 time mated dams/time interval were collected at 1, 6, 24, 120 and 240 hr post dose. The milk samples collected by gently expressing milk by hand in tubes were used to determine the amount of the compound.

The content of iron (radioactivity amounts) in the brain, liver, spleen, kidneys, uterus, ovaries, lungs, amniotic fluid and mammary glands was estimated. The PK data of the compound in plasma and milk were obtained.

Results:

In phase 1, the plasma concentration of the radioactivity was seen from 1 hr post dose to 240 hr of the study (maximum time period of the study). The radioactivity was seen in milk from the first sample at 1 hr post dose to 120 hr post dose sample. The radioactivity was not detected in sample collected on 240 hr post dose.

In phase 2 (stimulated by oxytocin), the ratio of milk:plasma radioactivity was increased from 0.02 at 1 hr to 1.39 (highest peak) ug eq. Fe/g at 24 hr of the administration. It was detected in a significant amount at 120 hr milk-samples (figure 2 from sponsor's submission). The radioactivity injected to mother was transferred to the fetus thus indicating that the compound could pass the placental barrier and it was secreted via mother milk. Thus, the compound should be administered in females with caution during pregnancy and lactation period.

Phase 1 Tissue Distribution:
The plasma concentration of the radioactivity was high in comparison to the concentration in blood cells initially and then decreased. In 24 hr post dose sample, the plasma concentration was in a steady state until 168 hr. Cmax's of the whole blood and blood cells increased, i.e., 1.086, 43.53 and 66.15 ug eq Fe/g after 0.5, 24 and 168 hr. The amount of radioactivity was about 0.6 to 0.7% of the injected dose of the iron. The amount of iron absorbed gradually increased (see following figure of sponsor, vol 32:323, pp110) and a plateau was seen after day 20 of the administration.

About 22.3% of the iron was absorbed in red blood cells and decrease to 19.6% after 6 hr of the dose. The tissue distribution of the amount of radioactivity tagged with ^{59}Fe VIT-45 was (in descending order is liver, blood, fecal matter, carcass, tail, spleen, intestines, kidneys and stomach (vol 32.323, figure 3, pp 46).

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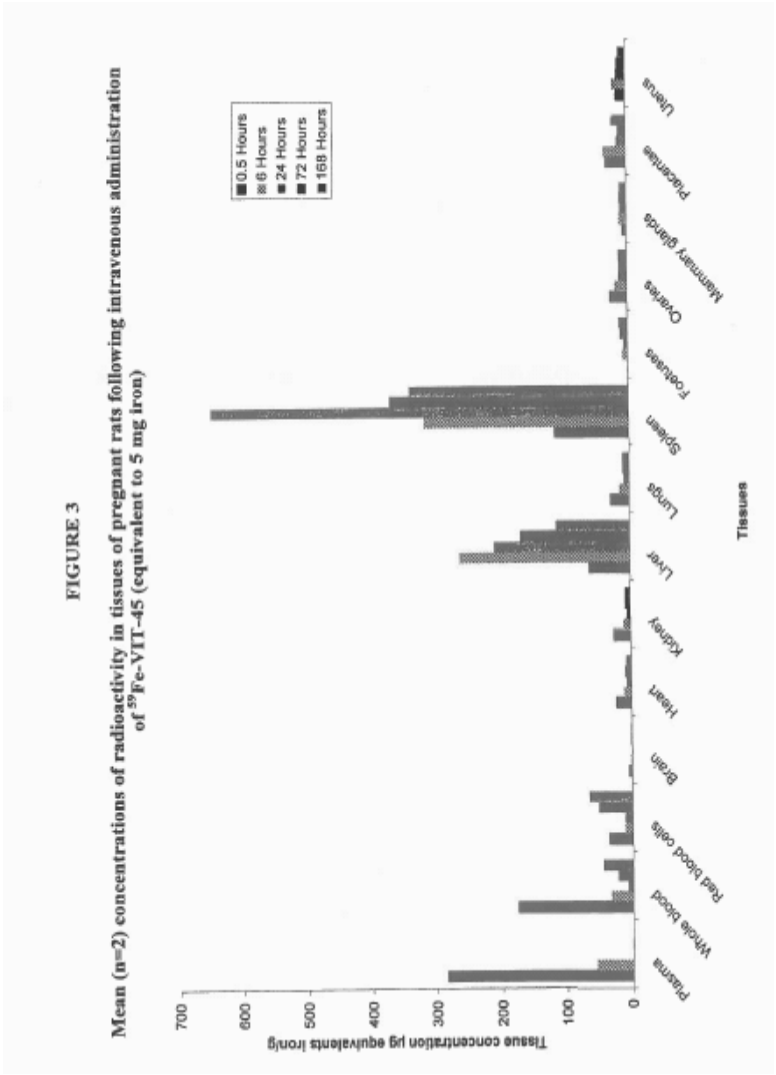
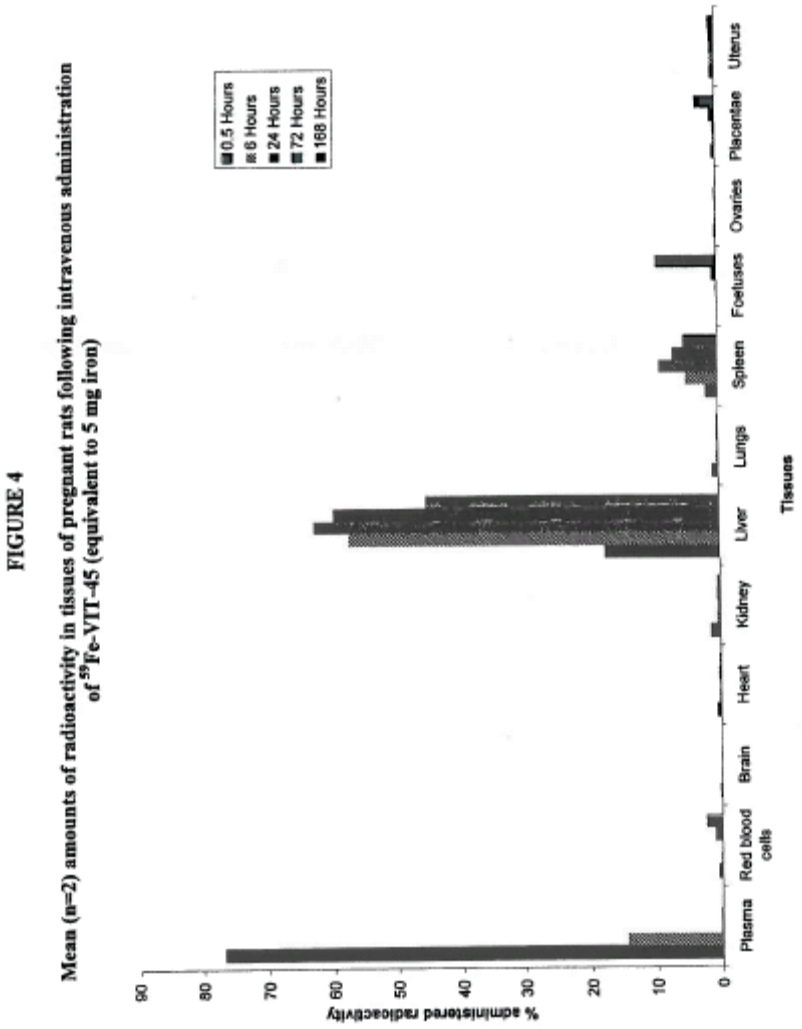
FIGURE 3

Mean (n=2) concentrations of radioactivity in tissues of pregnant rats following intravenous administration of ^{59}Fe-VIT-45 (equivalent to 5 mg iron)



The highest amount of the compound was in spleen followed by plasma, liver and whole blood etc as shown below in figure 3.



In phase 2, the compound was in the maximum amounts in plasma followed by liver, spleen, fetus, placenta, red blood cells and kidneys.

Thus, iron of VIT-45 was absorbed/transferred to the rat offspring and was identified in rat tissues, i.e., liver, blood, fecal matter, carcass, tail, spleen, intestines, kidneys, and stomach.

7. **<u>In vitro perfusion study using human placenta</u>**: The addition of 11 mM ^{59}Fe-VIT-45 (11X the highest concentration achieved after a dose of 200 mg in pregnant women) in to the maternal circuit caused 10% decrease in iron concentration within 30 min of the administration on the maternal side. There was only an insignificant amount of iron level decrease in the placenta. The addition of 1.67 mg/ml transferrin to the simulated maternal circuits showed 10% decrease in the initial 10 min of its administration on the

maternal side. The addition of transferrin did not affect the iron uptake of 11 mM ^{59}Fe-VIT-45 and no radioactivity was detected in fetal circuit. The placental side iron activity was 0.5 to 2% vs. 10% in maternal side. The metabolic activity measured as glucose consumption and lactate production were not affected by VIT-45.

The increasing dietary iron at the dose of 35 to 20,000 ug Fe/g in weanling rats, produced a direct increase in non-heme iron, peroxidation activity and a dose related increase in the heptocytes of the periportal region (zone I). The myocardial degeneration and necrosis with hemosiderin in the interstitial macrophages of myocardial fibers were reported in the rats with heart damage. The spleen lymphoid atrophy and pancreatic atrophy with loss of both endocrine and exocrine pancreatic tissues were also seen in rats with iron overload.

8. **Pharmacokinetics of a Single Intravenous and Intramuscular Dose Administration of VIT-45 in Rats**: (Study #VFR 060/033441/TEP 9021/1:902/4;901/2; 901/4)

Methods:
This 4 phase study (Phase A to D) was done in 4 phases in 102 rats using ^{59}Fe-VIT-45 (batch # CW280303) and non-radioactive VIT-45 (batch # 267100M).

Phase A: Six sub groups of 3 rats/sex were treated with a single intravenous dose to assess the pharmacokinetic and distribution of the compound. Two blood samples were collected from each subgroup as described below: i) 5 minutes, 4 and 72 hr post dose; ii) 0.25, 6 and 120 hr; iii) 0.5, 8 and 168 hr; iv) 1, 16 and 336 hr; v) 2, 24 and 504 hr and vi) 3, 48 and 672 hr after dosing the animals. The animals were sacrificed and radioactivity distribution was estimated in the tissues and organs separated from the animals sacrificed at 168, 336 and 672 hr.

Phase B: Three sub groups of 21 rats/sex were treated with a single intramuscular dose and 2 blood samples collected by cardiac puncture from each subgroup at intervals as described below: i) 5 minutes, 4 and 48 hr post dose; ii) 1, 8 and 96 hr and iii) 2, 16 and 120 hr after dosing to estimate PK of the compound.

Phase C: A single intramuscular dose of ^{59}Fe-VIT-45 was administered to a group of 21 rats/sex. The blood samples were collected by cardiac puncture from each subgroup of 3/sex at 6, 24, 72, 168, 336, 504 and 672 hr post dose and radioactivity was determined the quantitative distribution by LSC methodology.

Phase D: A single intramuscular dose of ^{59}Fe-VIT-45 was administered in 3/sex animals and the urine and feces were collected. The urine samples were collected at 0-6 and 6-24 hr, and at 24 hr interval up to 168 hr post dose and fecal matter were collected at 24 hr interval up to 168 hr post dose and radioactivity in plasma, cells and whole blood was determined by LSC methodology. The PK of the compound was also estimated
The animals were sacrificed and radioactivity was estimated in the tissues and organs separated from the animals sacrificed at 168, 336 and 672 hr.

Results:

There were no changes in the clinical signs of the animals and none of the animals died. The amount of the radioactivity was 98.8% of the projected dose.

The intramuscular injection of 5 mg VIT 45 produced the mean peak concentration of 60.34 and 61.66% of the injected radioactivity in the muscles up to 24 hr after which it reduced to 41.03 and 42.6% at 72 hr observation period in male and female rats. The radioactivity was accumulated in the muscles up to last observation study period of 672 hr. The administered radioactivity was decreased in the tissues but increased amounts were found in whole blood and blood cells. At the peak, 12.26 and 12.27% radioactivity was still detected at 672 hr. The percent dose seen in other tissues is shown in the table below (sponsor's table 15 a for males and b for female, vol 31.323, pp 65 and 66).

TABLE 13

Mean total amounts of radioactivity in organs or tissues of male and female rats following the administration of single intravenous doses of ^{59}Fe-VIT-45 (equivalent to 5 mg of iron)

Results are expressed as total % dose

a) Males

Organ/Tissue	168 hours		336 hours		504 hours		672 hours	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Plasma*	29.81	3.98	43.28	3.61	57.07	2.46	75.82	11.45
Whole blood*	29.81	3.97	43.77	3.60	57.08	2.43	75.86	11.48
Blood cells†	0.1103	0.0294	0.1937	0.0085	0.1811	0.0628	0.1281	0.0541
Brain	0.4957	0.0197	0.4738	0.1794	0.5175	0.1587	0.9652	0.4328
Heart	0.6099	0.1893	0.8143	0.8915	0.8415	0.0674	0.9589	0.2177
Liver	38.52	4.37	28.82	1.42	22.82	0.08	20.37	2.65
Lungs	0.6045	0.3167	0.7534	0.4865	0.5819	0.1322	1.054	0.611
Spleen	4.173	1.353	2.405	0.524	1.517	0.397	1.638	0.514
Adrenal glands	0.0377	0.0082	0.0429	0.0149	0.0485	0.0117	0.0418	0.0017
Pituitary gland	-	-	-	-	-	-	-	-
Thymus	0.1033	0.0522	0.1110	0.0443	0.1453	0.0556	0.1593	0.0764
Epididymis	0.0512	0.0082	0.0636	0.0144	0.0643	0.0090	0.0727	0.0042
Testes	0.4734	0.0319	0.4345	0.0304	0.5389	0.1444	0.5157	0.0860
Stomach wall	0.0830	0.0122	0.1304	0.0073	0.1395	0.0237	0.1146	0.0164
Small intestine wall	0.7164	0.0582	0.4865	0.1357	0.5043	0.0365	0.3920	0.0249
Large intestine wall	0.2172	0.0895	0.2652	0.1070	0.2653	0.0865	0.1788	0.0606

b) Females

Organ/Tissue	168 hours		336 hours		504 hours		672 hours	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Plasma*	19.97	1.29	33.84	3.28	39.83	0.58	55.20	8.47
Whole blood*	19.96	1.29	33.85	3.28	39.84	0.60	55.19	8.48
Blood cells†	0.0806	0.0158	0.0898	0.0096	0.1216	0.0184	0.1499	0.0262
Brain	0.2514	0.0427	0.3537	0.1307	0.2615	0.0814	0.2803	0.0813
Heart	0.4796	0.0177	0.6329	0.0532	0.7189	0.0694	0.8595	0.1189
Liver	43.82	2.66	37.98	1.86	34.47	0.73	29.19	1.29
Lungs	0.4539	0.0813	0.5120	0.0512	0.5447	0.1086	0.6634	0.0525
Spleen	4.062	0.409	3.695	0.126	4.544	0.531	7.529	0.452
Adrenal glands	0.0470	0.0017	0.0614	0.0051	0.0559	0.0288	0.0712	0.0098
Pituitary gland	-	-	-	-	-	-	-	-
Thymus	0.1534	0.0806	0.1389	0.0554	0.1619	0.0233	0.1482	0.0416
Ovaries	0.0815	0.0173	0.0798	0.0160	0.0774	0.0167	0.0844	0.0055
Stomach wall	0.0753	0.0091	0.1402	0.0413	0.1137	0.0122	0.1345	0.0185
Small intestine wall	0.8637	0.3063	0.4141	0.0489	0.5892	0.0727	0.3857	0.0581
Large intestine wall	0.2017	0.0932	0.2198	0.1023	0.2116	0.0671	0.1925	0.0286

sd Standard deviation
- Not calculated (not detected in at least two out of the three samples)
* % dose for these tissues calculated from estimated tissue weights
† % dose for blood cells calculated from estimated tissue weight and PCV measurements

After intramuscular dose, the maximum plasma concentrations were 12.12 and 14.6 ug. eq/g in male and female rats, respectively with in 1 hr of the administration. The maximum concentration of radioactivity was 24.7 and 27.5 ug.eq/g blood in male and female rats. Radioactivity concentration in whole blood declined from 60 and 91 ug.eq.h/g in male and female rats and then increased. In males, the radioactivity of whole blood first declined from 7.1 ug.eq/g to 2.2 ug eq Fe/g at 0.5 hr. It was again increased from 8 hr which peaked at the 672 hr of observation period. In females, the radioactivity of whole blood was 8.6 ug. eq/g at 1.0 hr to 1.95 ug eq Fe/g at 8 hr and it again increased like in males, to peak at the 672 hr observation period. The PK data of intramuscular dose of the radioactivity is shown in the following table. The terminal half

life in whole blood could not be estimated but plasma half life in male was 1/3rd of female.

TISSUE DISTRIBUTION:

I.M.

Intramuscularly administered VIT-45 attained the maximum plasma concentrations of 429.5 and 501.2 ug.eq/g in male and female rats, respectively after 5 min of its administration. The maximum concentration of radioactivity in whole blood was 260.8 and 280.2 ug.eq/g blood in male and female rats. The radioactivity concentration in whole blood declined first and then again it increased to maximum till 672 hr of the study time.

PK data of intravenously administered dose of the radioactivity is shown in the following table (Sponsor Table 5, vol 31.323, pp 55) . The terminal half life in whole blood could not be estimated but plasma half life in female was 25.1 hr but in males, the half life was not estimated because of the limited data.

VFR 060/033441

TABLE 5

Pharmacokinetic parameters of radioactivity after a single intravenous administration of ^{59}Fe-VIT-45 to rats at a dose level equivalent to 5 mg iron

Plasma

Sex	C_0 (µg eq/g)	C_{max} (µg eq/g)	T_{max} (hours)	AUC_{672} (µg eq.h/g)	V_0 (g)	k (hours^{-1})	$t_{½}$ (hours)
Male	429.5	417.3[a]	0.083[a]	1560	11.4	b	b
Female	501.2	481.4[a]	0.083[a]	1881	9.8	0.0276	25.1

Whole Blood

Sex	C_0 (µg eq/g)	C_{max} (µg eq/g)	T_{max} (hours)	AUC_{672} (µg eq.h/g)	V_0 (g)	k (hours^{-1})	$t_{½}$ (hours)
Male	260.8	242.9[a]	0.083[a]	54839	18.8	b	b
Female	280.2	269.9[a]	0.083[a]	50052	17.5	b	b

Red Blood Cells

Sex	C_{max} (µg eq/g)	T_{max} (hours)	AUC_{672} (µg eq.h/g)
Male	342.3[a]	672[a]	135840
Female	316.2[a]	672[a]	127057

eq	Equivalents
a	Value taken from experimental data
b	Terminal rate constant could not be estimated
C_0	Concentration at time 0 hours
C_{max}	Maximum observed concentration of radioactivity
T_{max}	Time of occurrence of maximum observed concentration of radioactivity
AUC_{672}	Areas under the radioactivity concentration-time curves up to 672 hours post-dose
k	terminal rate constant
t½	terminal half-life
Vo	apparent volume of distribution at time zero

Intravenously administered compound was distributed in tissues and blood extensively. At 168 and 336 hr following the administration of the drug, the whole blood had the highest amounts of 39.8 and 29.9% in males and, 29.8 and 39.6% in females of the administered amounts. The rat tissue concentrations were highest at 168 hr and were decreased gradually up to 672 hr excepting in whole blood, blood cells and liver (slight decrease from 168 hr period). The half life was 25.1 hr in females and was not estimated for males because terminal rate was not estimated in males.

9. Absorption, Distribution and Excretion studies in Dog After Single Intravenous and Intramuscular Doses in Dogs: (Study #VFR 059/033758)

Methods: Eleven dogs (12 months old and weighing 8.7 to 11.3 kg), were divided in 2 groups, i.e. 4 dogs in 1 group and 7 male dogs in group 2. Group 1 animals were administered a single intravenous (via cephalic vein) dose of 50 mg iron and animals in group 2 were given deep intramuscular injection (thigh muscle). The mononuclear iron

solution was prepared by dissolving (b) (4) sucrose in labeled $^{59}FeCl_3$ (batch # 267100M- non-labeled; 7.4 MBq in HCl, volume = 1.0 ml, pH = 12.72) and the solution was added to filtered VIT-45 and the final pH adjusted to 5.66. The blood samples from each animal given intravenous dose were collected at 5, 10 and 15 min and, 1, 2, 3, 4, 6, 24, 48 and up to 672 hr post dose. One of the dogs was killed at 168 and 172 hr post dose and 2 were sacrificed 672 hr post dose. The tissues as enumerated in the result section were separated. Among the animals given an intramuscular dose, only the blood samples were collected at 0.5, 1, 2, 3, 4, 6, 24, 48, 72, 120, 168, 240 336, 504 and 672 hr after treatment and the dogs were sacrificed at 168, 336 and 672 hr. The excreta from animals given IM dose was collected at 24 hr interval from the animals. The radioactivity was measured by COBRA II gamma scintillating counter and, the plasma concentration of the compound was determined by ELISA method. The PK of the compound was estimated in the study.

Results: No adverse effects were reported at the dose of 50 mg VIT-45.
Plasma concentration (IV): The peak plasma concentration ($AUC_{0\text{-}t\ hr}$) of 72.89 ug iron.h/g was attained immediately after injection with $t_{1/2}$ of 3.1 hr. The peak concentration in whole blood (plasma + blood cells) was 5123 ug iron.h/g and half life of 3.2 hr.

(IM): The concentration in whole blood and plasma after an IM dose of 50 mg iron was 3642 and 13.62 ($AUC_{0\text{-}t\ hr}$) of 72.89 ug iron.h/g was attained after 0.5 hr after injection with $t_{1/2}$ of 5.2 hr. The bioavailability of radioactivity in plasma following intramuscular dose of 50 mg iron was about 0.33 (calculated to be 70% in whole blood). An intravenous dose of VIT-45 equivalent to 50 mg iron in male dogs produced a bioavailability of radioactivity of 0.33 (based on plasma mean AUC). The mean ratio of whole blood to plasma for radioactivity was 123:1 and 270:1 for intravenous and intramuscular administered doses.

The intramuscular injection of 50 mg iron (5 mg/kg, assumption 10 kg dog weight) VIT 45 achieved a mean peak concentration of radioactivity equivalent to 2.002 ug Fe/g initially and it fell to 0.228 ug Fe/g after 24 hr of the dosing. The concentration of iron was again increased, and attained a peak of 16.47 ug Fe/g at 672 hr of the dosing. The peak concentration of 3.7 ug Fe/g was seen in plasma after 0.5 hr of the dosing (at 1st sampling) and then dropped to 1.08 ug/g 6 hr post dosing and it decreased below the limit of estimation at this time to the termination time of 672 hr. The t1/2 of the IM administered dose of the compound in blood and plasma was 4.3 and 5.2 hr, respectively.

The half lives of the compound after these 2 routes were similar and the bioavailability of the compound was 70% when whole blood data were considered and the compound resided in blood cells than in plasma. The PK data of radioactivity of plasma and whole blood are shown below:

Table for the Mean Pharmacokinetic Parameters in the Dogs

Treatment Gr.	Cmax (ug Fe/kg)	Tmax (hr)	T ½ (h)	$AUC_{(0\text{-}th)}$ (ugEq h/g)	λ_z /hr
I.M.					
Plasma	3.70 ± 1.322	0.5	4.3	13.63 ± 7.01	0.118 ± 0.06
Whole Blood	12.5 ± 5.28	504	5.2	3642 ± 2919	0.133 ± 0.031
I.V.					
Plasma	76.9 ± 5.1	-	3.1	61.5 ± 6.0	0.2264± 0.08
Whole Blood	44.4 ± 4.7	-	3.2	5123 ± 4532	0.215 ± 0.055

Tissue Distribution:

I.V.: The peak concentration of the compound-tagged radioactivity was in liver at 168 and 336 hr post dose which declined from 672 hr of the dosing (from 103.0 to 37.0 ug Fe/g in liver and, from 8.9 to 1.8 ug Fe/g in lymph nodes). But the concentration in blood cells (from 15 to about 40 ug Fe/g) and whole blood (6.17 to approximately 20 ug Fe/g) increased during this period suggesting the preferable affinity of the compound to blood cells. The radioactivity tagged with the compound was in higher amounts in all the tissues of the animal killed at 672 hr than in 168 hr killed animal.

TABLE 9

Concentrations of radioactivity in organs and tissues of male dogs following administration of single intravenous doses of ^{59}Fe-VIT-45 (equivalent to 50 mg of iron)

Results are expressed as µg equivalents iron/g

Sample	1M	2M	3M	4M
	168 h	336 h	672 h	672 h
Plasma	ND	ND	ND	ND
Whole-blood	6.169	11.35	24.41	16.32
Blood cells	SS	21.94	43.18	36.72
Brain	ND	ND	0.284	0.295
Heart	0.577	0.978	1.711	1.525
Kidney	1.517	1.326	1.872	1.472
Liver	103	75.23	37.61	37.32
Lungs	2.000	2.669	6.472	4.180
Spleen	27.65	28.28	37.90	42.88
Adrenal glands	3.382	2.020	2.381	2.878
Lymph nodes	8.897	7.269	1.868	1.749
Pituitary gland	ND	ND	ND	ND
Thymus	0.550	0.517	0.848	0.836
Epididymis	0.268	0.426	0.418	0.414
Testes	0.392	0.383	0.521	0.685
Muscle	ND	0.338	0.496	0.398
Stomach	ND	ND	ND	ND
Small intestine	ND	ND	ND	ND
Large intestine	ND	ND	ND	ND

ND Not detected
SS Sample spoiled prior to analysis

I.M.:

The peak of the radioactivity at the site of injection, muscle, liver and lymph nodes was noted in animal killed 6 hr post dose after which the radioactivity declined and increased again in blood cells and liver. The greatest amounts of radioactivity in liver, blood cells and lymph nodes was at 72 hr after dosing and remained high in animals killed at 672 hr. The animal had higher amounts than animals killed at 24 hr time period. The

concentration in blood cells increased slowly and was the highest at 672 hr period than at any observation period suggesting the preferable affinity of the compound to blood cells. The radioactivity tagged was in higher percent amounts in the tissues of the animal killed at 672 hr than in animals killed at 24 hr observation post dose. The data is shown below in sponsor's table 11 and scanned here.

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TABLE 11

Concentrations of radioactivity in organs and tissues of male dogs following the administration of single intramuscular doses of ^{59}Fe-VIT-45 (equivalent to 50 mg iron)

Results are expressed as µg equivalents iron/g

Sample	5M	6M	7M	8M	9M	10M	11M
	6 h	24 h	72 h	168 h	336 h	672 h	672 h
Dose site (muscle)[1]	142.900	71.570	57.760	12.040	23.010	35.660	16.650
Dose site (other)[2]	316.900	81.640	166.200	114.800	31.980	35.720	129.400
Plasma	1.095	ND	ND	ND	ND	ND	ND
Whole-blood	SS	ND	2.285	5.322	11.780	16.630	16.300
Blood cells	ND	ND	ND	6.606	9.250	26.820	34.080
Brain	ND	ND	ND	ND	ND	ND	ND
Heart	ND	ND	ND	ND	ND	1.127	1.015
Kidney	ND	ND	0.700	0.970	1.283	2.205	1.571
Liver	75.770	92.310	81.840	74.910	84.060	49.060	47.650
Lungs	ND	ND	0.926	1.886	3.087	4.890	7.657
Spleen	1.027	5.867	8.392	19.000	29.700	18.580	36.070
Adrenal glands	ND	ND	ND	1.524	2.235	2.204	2.713
Lymph nodes (local)[3]	538.900	590.100	1557.000	911.200	76.070	474.000	511.200
Lymph nodes (remote)[4]	ND	ND	ND	6.005	ND	ND	4.908
Pituitary gland	ND	ND	ND	ND	ND	ND	ND
Thymus	ND	ND	ND	ND	ND	0.955	1.002
Epididymis	ND	ND	ND	ND	ND	ND	ND
Testes	ND	ND	ND	ND	ND	ND	ND
Muscle	ND	ND	ND	ND	ND	ND	ND
Stomach	ND	ND	ND	ND	ND	ND	ND
Small intestine	ND	ND	ND	ND	ND	ND	ND
Large intestine	ND	ND	ND	ND	ND	ND	ND

SS Sample spoiled prior to analysis
ND Not detected
1 Section of muscle including site of dose administration
2 Section of connective tissue (fat, muscle, skin) surrounding site of dose administration
3 Sampled from the hind leg used for dose administration
4 Sampled from the hind leg not used for dose administration

The amount of radioactivity (expressed as total of %dose) was highest in the muscle tissues from the time of administration, followed by liver and spleen etc. It was also seen in lymph nodes as shown below in sponsor's table 11 and scanned here

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TABLE 12

Total amounts of radioactivity in organs of male dogs following the administration of single intramuscular doses of ^{59}Fe-VIT-45 (equivalent to 50 mg of iron)

Results are expressed as total % dose

Sample	5M	6M	7M	8M	9M	10M	11M
	6 h	24 h	72 h	168 h	336 h	672 h	672 h
Dose site (muscle)[1]	15.92	7.644	4.984	1.076	2.575	3.228	1.015
Dose site (other)[2]	13.47	6.376	17.71	9.846	2.095	2.941	8.087
*Plasma (5%)	1.302	ND	ND	ND	ND	ND	ND
*Blood cells (4%)	ND	ND	ND	5.841	7.262	27.33	28.37
Brain	ND	ND	ND	ND	ND	ND	ND
Heart	ND	ND	ND	ND	ND	0.2247	0.1545
Kidney	ND	ND	0.0822	0.1346	0.1225	0.2388	0.1432
Liver	58.35	70.62	54.12	52.63	54.06	36.19	31.70
Lungs	ND	ND	0.1424	0.3023	0.4802	0.7672	0.9905
Spleen	0.1312	1.185	1.479	7.722	7.639	3.516	11.31
Adrenal glands	ND	ND	ND	0.0050	0.0047	0.0060	0.0067
*Lymph nodes (local)[3]	0.1361	0.6219	1.448	2.149	0.0803	0.3823	0.7094
*Lymph nodes (remote)[4] (1%)	ND	ND	ND	1.327	ND	ND	1.021
Pituitary gland	ND	ND	ND	ND	ND	ND	ND
Thymus	ND	ND	ND	ND	ND	0.0224	0.0247
Epididymis	ND	ND	ND	ND	ND	ND	ND
Testes	ND	ND	ND	ND	ND	ND	ND
*Muscle (39%)	ND	ND	ND	ND	ND	ND	ND
Stomach	ND	ND	ND	ND	ND	ND	ND
Small intestine	ND	ND	ND	ND	ND	ND	ND
Large intestine	ND	ND	ND	ND	ND	ND	ND

ND Not detected
1 Section of muscle including site of dose administration
2 Section of connective tissue (fat, muscle, skin) surrounding site of dose administration
3 Sampled from the hind leg used for dose administration
4 Sampled from the hind leg not used for dose administration
* Calculated assuming that tissue represents the proportion of total bodyweight indicated in parentheses

I.M.:

The compound-tagged radioactivity was concentrated at site of injection immediately after injection (17134 $\pm$ 1217 cpm) which was declined to 3722 $\pm$ 1492 cpm at 24 hr post dose. The radioactivity was declined slowly from the site of injection as the concentrations were 5019, 3019 and 2186 ug Fe/g at 48, 168 and 672 hr post dose, respectively. The compound was accumulated in surrounding tissues and lymph nodes, liver, plasma and spleen after 6 hr of the dosing and these were 75.77, 1.095 and 538.9 ug Fe/g in liver, plasma and spleen. After 24 and 72 hr and at the time of sacrifice, the compound was still be seen in surrounding tissues and lymph nodes, liver and low

concentrations were in kidneys, liver, lung and spleen. The compound was concentrated in muscle, kidneys and lungs for a period of 672 hr indicating a slow removal of the compound from the body tissues.

Excretion and Metabolism: The IM administered compound was excreted slowly and after 672 hr of the dosing, only a minor amount of the compound was present in urine and no quantifiable amount of the activity was excreted in dog feces. The compound was retained in the body up to 672 hr post dose.

50% of the administered compound was present in liver, 5 to 10% in spleen and, 30% in blood cells (at 672 hr post dose period). The PK profile of the IM and IV administered doses were similar.

10. **Determination of the Amount of Degradation Products of the Ligand of VIT-45 using α-Amylase and S9 fraction of the rat Liver Homogenates.** (Documents #SR-1099-01/E02)

This in vitro test was conducted to show that the ligand malto-dextrin molecule of the compound was incompletely hydrolyzed by α-amylase cleavage (at α-1, 4 linked bonds) and α-1, 6-linked bond remained unaffected. The ligand was shown to be degraded to dextrins products maltotetrose, maltotriose, maltose and glucose. In the rat liver homogenates, 0.67 ml VIT-45 (equivalent to 5% Fe or 1 g Fe) in 20 ml VIT-45 FP solution with α-amylase resulted in a maximum amount of 1.2% of glucose, maltotriose and maltotetrose and maltose. Thus the compound was degraded at 1, 4 bondage. But in the presence of 1, 6-α-glucosidases and after 15 hr incubation, the recovery of the simple sugars like glucose and maltose was increased as shown below in sponsor's graph.

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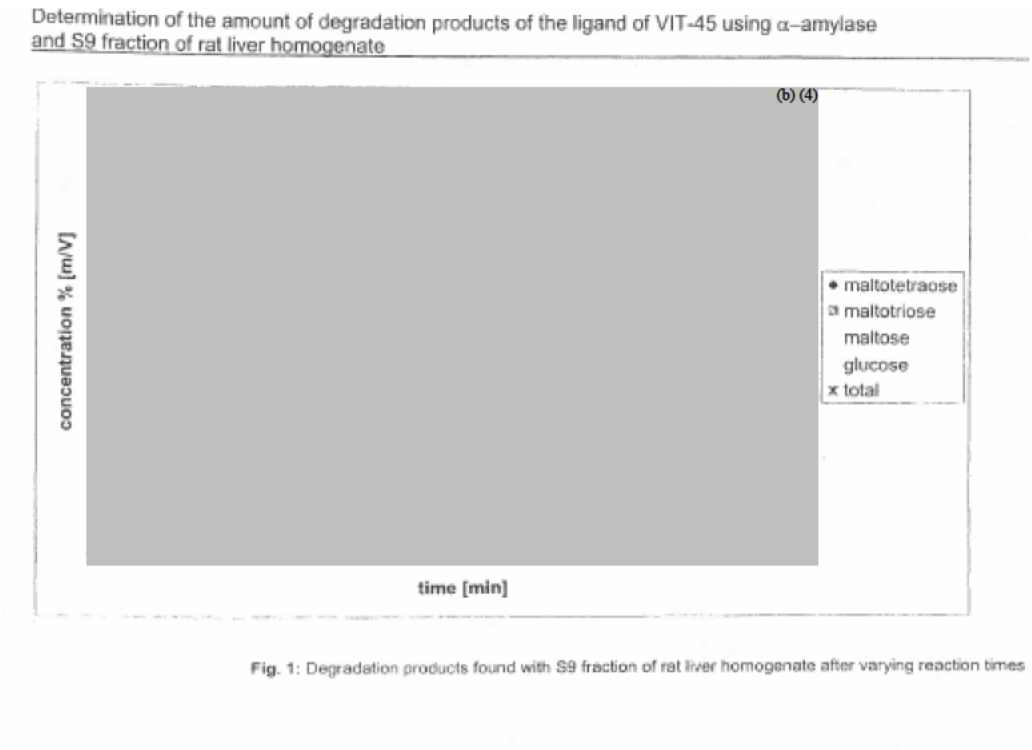

Fig. 1: Degradation products found with S9 fraction of rat liver homogenate after varying reaction times

Thus the compound was degraded into simple sugars and their contents was increased in the presence of 1, 6 glucosidases.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary:

The acute tolerance studies in rats, mice and monkey showed the minimal lethal single intravenous dose in mice was 2000 mg/kg for both males and females. Signs of toxicity were piloerection, lethargy, reduced body temperature, hunched posture prior to death. A dose of 1000 mg/kg was non-lethal dose. VIT-45 was not lethal in rats up to 240 mg/kg although piloerection, swollen limbs, dark extremities, swollen extremities and enlarged spleen were seen. In dogs, a single dose of up to 240 mg/kg of iron dextrin complex was not lethal. In 13-week intravenous toxicity study in rats and dogs, intravenous infusion of 0, 9, 30, and 90 mg iron/kg/week doses for 13 weeks were administered. None of the animals died of treatment related causes. Dose dependent, but non-time dependent iron deposition in multiple organs including the macrophages were seen in both species. Induced humoral immune response to T-cell dependent SRBC antigen and increased liver

enzyme activities was seen in rat study and, liver, spleen, lymph nodes, and kidney were identified as the target organs of toxicity. 9 mg/kg/day and 30 mg/kg/day were highest tolerable doses in 13 week rat and dog toxicity studies. In 26-week toxicity study in dogs, intravenous bolus doses of 0, 3, 9 and 30 mg iron/kg/week VIT-45 was given in 3 equal divided doses. Multi-organs iron deposition, renal medullary mineralization and glomerular mesangial cells and, pigmentation in the Kupffer's cells and sinusoidal/phagocytic cells in liver and mesenteric lymph node were suggestive of liver, kidney and lymph nodes as target organs of toxicity.

2.6.6.2 Single-dose toxicity:

Study Title: **Acute intravenous toxicity study in mice**
Study No: VFR 035/003710/AC
Conducting Laboratory and Location: (b) (4)

Date of study initiation: June 28, 2000
Report date: September 22, 2000
GLP compliance: This study was conducted in accordance with Good Laboratory Practice Regulations of the UK and OECD.
QA-Report Yes (x) No ()

- **Methods**: A single dose of VIT-45 was given to mice (5/sex/group) by intravenous injection at 1000 and 2000 mg/kg. There were no control groups.

Dosing:

- **species/strain**: Albino mice of ICR origin
- **#/sex/group or time point**: 5/sex/group
- **age**: ~5-7 weeks old
- **weight**: male/female: 20-31 g.
- **satellite groups used for toxicokinetics or recovery**: None
- **dosage groups in administered units**: Intravenous injection: 1000 and 2000 mg/kg.
- **route, form, volume, and infusion rate**: 20 ml/kg.

Drug, lot#, radiolabel, and % purity: 0481000.
Formulation/vehicle: 0.9% sodium chloride.
Observations and times:

- **Clinical sings**: Clinical signs of toxicity were observed daily for 14 days.
- **Body weights**: Body weights were determined before dosing on day 1, and on days 8 and 15.
- **Gross pathology**: Animals were necropsied at the end of study.

Results: The following deaths occurred: 3 males and 2 females at 2000 mg/kg within 3 days of dosing. Clinical signs of toxicity prior to death were piloerection, lethargy, reduced body temperature, and hunched posture. No treatment related changes were noted in the remaining mice of the 2000 mg/kg/day group and in all animals of the 1000 mg/kg group. Enlarged spleen was noted in some treated animals.

Key Study Findings: In this study, the minimal lethal intravenous dose was 2000 mg/kg for both males and females. Clinical signs of toxicity prior to death were piloerection, lethargy, reduced body temperature, hunched posture. The dose of 1000 mg/kg was non-lethal dose.

Another acute intravenous toxicity study (study #SR-1026/E01) in mice (NMRI(SPF) mice) was conducted at 250 mg/kg. There were no deaths. No treatment related toxicity was noted. Iron deposit in various organs was found.

Study Title: **Acute intravenous toxicity study in rats**
Study No: VFR 033/000038
Conducting Laboratory and Location: (b) (4) .
Date of study initiation: June 2, 1999
Report date: February 15, 2002
GLP compliance: This study was conducted in accordance with Good Laboratory Practice Regulations of UK and OECD.
QA-Report Yes (x) No ()

- **Methods**: A single dose of iron dextrin complex was given to rats (3 rats/sex/group) by intravenous infusion over 1 hour at 60, 120, and 240 mg/kg. There were no control groups.

Dosing:

- **species/strain**: Crl:CD BR rats
- **#/sex/group or time point**: 3/sex/group
- **age**: ~33-37 days old
- **weight**: male/female: 113-120 g for males and 102-113 g for females.
- **satellite groups used for toxicokinetics or recovery**: None
- **dosage groups in administered units**: Intravenous infusion at 60, 120, and 240 mg/kg.
- **route, form, volume, and infusion rate**: over 1 hour, dose volume: 8 ml/kg.

Drug, lot#, radiolabel, and % purity: 894209B.
Formulation/vehicle: 0.9% sodium chloride.
Observations and times:

- **Clinical sings**: Clinical signs of toxicity were observed daily for 7 days.
- **Body weights**: Body weights were determined before dosing and twice weekly after treatment.
- **Food consumption**: weekly
- **Hematology**: On the day following treatment.
- **Clinical chemistry**: on the day following treatment.
- **Gross pathology**: Animals were necropsied at the end of study (day 7).

Results: There were no deaths and no treatment related changes in clinical signs of toxicity, body weight, food consumption. Brown discoloration (clotted old blood) of the pancreas was noted in all high dose animals.

Key Study Findings: In this study, none of animals died and minimal lethal intravenous dose was not identified. Brown discoloration indicating the iron deposit in the pancreas was noted in 240 mg/kg treated animals.

Study Title: **Acute intravenous toxicity study in rats**

Study No: VFR 034/003711/AC
Conducting Laboratory and Location: (b) (4)

Date of study initiation: July 4, 2000
Report date: September 22, 2000
GLP compliance: This study was conducted in accordance with Good Laboratory Practice Regulations of UK and OECD.
QA-Report Yes (x) No ()

- **Methods**: A single dose of iron dextrin complex was given to 3 rats/sex by intravenous infusion over 1 hour at 1000 mg/kg. There were no control groups.

Dosing:

- **species/strain**: CD rats of Sprague-Dawley origin
- **#/sex/group or time point**: 5/sex/group
- **age**: ~5-7 weeks old
- **weight**: male/female: 113-135 g.
- **satellite groups used for toxicokinetics or recovery**: None
- **dosage groups in administered units**: Intravenous injection at 1000 mg/kg.
- **route, form, volume, and infusion rate**: 2 ml/minutes intravenous injection.

Drug, lot#, radiolabel, and % purity: 0481000.
Formulation/vehicle: 0.9% sodium chloride.
Observations and times:

- **Clinical sings**: Clinical signs of toxicity were observed daily for 15 days.
- **Body weights**: Body weights were determined before dosing on day 1, and on days 8 and 15.
- **Food consumption**: weekly
- **Gross pathology**: Animals were necropsied at the end of study (day 15).

Results: There were no deaths in this study. Treatment related changes in clinical signs of toxicity included piloerection, swollen limbs, dark extremities, and swollen extremities. Enlarged spleen was noted in all males and 4 females.

Key Study Findings: In this study, the minimal lethal intravenous dose was not identified. Treatment related changes in clinical signs of toxicity included piloerection, swollen limbs, dark extremities, and swollen extremities. Enlarged spleen was noted in all males and 4 females.

Study Title: **Acute intravenous toxicity study in dogs**
Study No: VFR 032/000037

Conducting Laboratory and Location: (b) (4)

Date of study initiation: June 4, 1999
Report date: July 9, 2002
GLP compliance: This study was conducted in accordance with Good Laboratory Practice Regulations of UK and OECD.
QA-Report Yes (x) No ()

- **Methods**: A single dose of iron dextrin complex was given to Beagle dogs (1/sex/group) by intravenous infusion over 1 hour at 60, 120, and 240 mg/kg. There were no control groups.

Dosing:

- **species/strain**: Beagle dogs
- **#/sex/group or time point**: 1/sex/group
- **age**: ~22-24 weeks old
- **weight**: male/female: 6-6.8 kg.
- **satellite groups used for toxicokinetics or recovery**: None
- **dosage groups in administered units**: Intravenous injection at 60, 120, and 240 mg/kg.
- **route, form, volume, and infusion rate**: intravenous infusion over 1 hour.

Drug, lot#, radiolabel, and % purity: 894209B.
Formulation/vehicle: 0.9% sodium chloride.
Observations and times:

- **Clinical sings**: Clinical signs of toxicity were observed daily for 7 days.
- **Body weights**: Body weights were determined before dosing on day 1 and then twice weekly.
- **Food consumption**: daily.
- **ECG and blood pressure**: before dosing, 0.5 and 24 hours after dosing.
- **Hematology and clinical chemistry**: before dosing, and then on the day following dosing.
- **Gross pathology**: Animals were necropsied at the end of study (day 7).

Results: There were no deaths in this study. There were no treatment related changes in clinical signs of toxicity, food consumption, ECG, and blood pressure. Dark coloration of the lymph nodes were noted at the mid and high dose groups.

Key Study Findings: In this study, the minimal lethal intravenous. dose was not identified. Dark coloration of the lymph nodes were noted at the mid and high dose groups, indicating iron deposit in the lymph nodes.

2.6.6.3 Repeat-dose toxicity

RATS:

Study Title: **13-Week intravenous infusion Toxicity Study in CD Rats**
Study No: VFT 042/993363
Conducting Laboratory and Location: (b) (4) .

Date of study initiation: June 2, 1999
Report date: February 15, 2002
GLP compliance: This study was conducted in accordance with Good Laboratory Practice Regulations of the United States Food and Drug Administration (21 CFR Part 58).
QA-Report Yes (x) No ()

- Methods: VIT-45 (Iron Dextrin Complex) was given to rats (10 rats/sex/group) by intravenous infusion over 1 hour at 0, 9, 30, and 90 mg/kg once a week for 13 weeks.

Dosing:

- species/strain: Crl:CD BR rats
- #/sex/group or time point: 10/sex/group
- age: ~7 weeks old
- weight: male: 206-256 g, female: 154-190 g.
- satellite groups used for toxicokinetics or recovery:
- dosage groups in administered units: Rats received test drug at 0, 9, 30, and 90 mg/kg.
- route, form, volume, and infusion rate: intravenous infusion, dosing volume: 6 ml/kg.

Drug, lot#, radiolabel, and % purity: 894209B
Formulation/vehicle: 0.9% sterile sodium chloride

Observations and times:

- Clinical signs: Clinical signs of toxicity were observed daily.
- Body weights: Body weights were determined weekly.
- Food consumption: Food consumption was determined weekly.
- Hematology, clinical chemistry, and urinalysis: During weeks 6 and 13.
- Ophthalmologic Examination: Before treatment, during week 12.
- Gross pathology: Animals were necropsied at termination.
- Organ weighed: Organs were weighed at termination.
- Histopathology: Following organs or tissues were examined histopathologically from each animal in all groups: adrenals, aorta, brain, cecum, colon, cervix, duodenum, epididymis, eyes, esophagus, gonads, Harderian glands, heart, ileum, jejunum, kidneys, larynx, lachrymal glands, liver, lungs, lymph node, mammary gland, optic nerve, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle,

skin, spinal cord, spleen, sternum, stomach, thymus, thyroid, tongue, trachea, urinary bladder, uterus, vagina, and all gross lesions.

Methods: To assess the repeated dose toxicity of VIT-45 in rats, VIT-45 was given to rats (10 rats/sex/group) by intravenous infusion over 1 hour at 0, 9, 30, and 90 mg/kg once a week for 13 weeks. In the current study, clinical signs of toxicity were observed daily. Body weights and food consumption were determined weekly. Hematology, clinical chemistry, and urinalysis were determined during weeks 6 and 13**.** Ophthalmological examination was conducted before treatment, during weeks 12. All animals were necropsied at termination and organ weights were determined. Histopathological examination was conducted in all animals from all groups.

Results:

- Clinical Signs: There were no clear treatment related changes.
- Mortality: There were no deaths in this study.
- Body Weights: The mean initial and final body weights in the control group were 222 and 447 g for males and 174 and 256 g for females, respectively. The terminal body weight gain was decreased by ~19% and 38% in the mid and high dose males as compared to the control. The terminal body weight gain was decreased by 10% in the high dose females as compared to the control.

- Food Consumption: The average food consumption in the control group was 26.8 g/rat/day in males or 18.8 g/rat/day in females. The food consumption was comparable in the treatment groups to the control.
- Ophthalmoscopy: There were no treatment related changes.
- Hematology: Slight increase in mean corpuscular hemoglobin, mean hemoglobin concentration, and mean corpuscular volume (<5%) was seen in high dose male during week 6. Slight increase in mean corpuscular hemoglobin (7-7.7%), mean hemoglobin concentration (2.3-2.6%, and mean corpuscular volume (4.7%) was also noted in high dose male during week 13. Red blood cell counts were decreased in the low (4%), mid (5%), and high (8.7%) dose males as compared to control. These changes were not seen in treated females.

- Clinical Chemistry**:** Mean plasma iron level was increased in the mid and high males during weeks 6 (106% and 135%) and 13 (139% and 200%). Mean plasma iron level was increased in the mid and high females during weeks 6 (38% and 43%) and 13 (42% and 44%). Significant increases in alkaline phosphatase (78%), alanine aminotransferase (628%), and aspartate aminotransferase (439%) were found in the high dose males during week 13. Significant increases in alkaline phosphatase (110%), alanine aminotransferase (50%), and aspartate aminotransferase (49%) were found in the high dose females during week 13. Slight increase in alkaline phosphatase was also noted in the high dose males and females during week 6. Total bilirubin (100%) and urea (29%) were significantly increased in the high dose males during week 6. Total bilirubin (50%) and urea (27%) were significantly increased in the high dose females during week 13.

- Urinalysis: Decreased urinary outputs of electrolytes (Na, K, and Cl) were noted in the high dose males during weeks 6 and 13 (31-51%).

- Organ Weights: Increased liver weight was seen at high dose (20% in males and 16% in females) as compared to the control. The spleen weight was increased by 63-67% in high dose males and females as compared to the control.
- Gross Pathology: Enlarged spleen was noted in the mid and high dose group.
- Histopathology: Deposition of iron-containing pigment was noted in tissues or organs including the liver, spleen, kidney, and lymph nodes in a dose dependent manner. These were not associated with any histopathological changes.

Key study findings: In the 13-week intravenous toxicity study in rats, VIT-45 was given to rats by intravenous infusion over 1 hour at 0, 9, 30, and 90 mg iron/kg/week for 13 weeks. Terminal body weight gain was decreased by ~19% and 38% in the mid and high dose males and by ~10% in high dose females as compared to the control. The liver enzyme activities were significantly increased in the high dose group. Slight decrease in red blood cell counts was in treated males (~4-9%). Iron deposition was noted in multiple organs including the liver, spleen, lymph nodes, and kidney in a dose dependent manner.

Study Title: Toxicity Study by Intravenous (Bolus) Administration to CD-Rats Three Times per Week for 13-Weeks

Study No: VFR 071/043284
Conducting Laboratory and Location: (b) (4)

Date of study initiation: March 25, 2004
Report Issue date: October 28, 2005
GLP compliance: This study was conducted in accordance with Good Laboratory Practice Regulations of the United States Food and Drug Administration (21 CFR Part 58).
QA-Report: Yes (x) No ()

Methods & Materials:

-Dosing: VIT-45 (Iron Dextrin Complex) was administered 3 times a week to 5 groups of rats (10 rats/sex/group) by intravenous bolus injection of 0, 1, 3, 10 and 30 mg/kg 3 times a week (days 1, 3 and 5 of each week) for 13 weeks. Total weekly doses were 0, 3, 9, 30 and 90 mg Fe/kg VIT-45.
- species/strain: Crl:CD BR rats
- #/sex/group or time point: 10/sex/group
- age: ~7 weeks old
- weight: male: 206-256 g, female: 154-190 g.

The present study was undertaken to assess the toxicity of VIT-45 administered intravenously by bolus injection three times per week for 13 weeks. The treatment (3 times per week dosing) was done to simulate the condition of clinical application of the

drug and to observe if the dosing of the compound caused any additional toxicity in animals. The study was done in 2 groups of animals, i.e., main toxicology groups and, satellite toxicology groups for immunotoxicity portion of the study.

Main Toxicology Group
- dosage administered in 5 groups (10/sex) in units: Rats received bolus injection of the test drug at 0, 1, 3, 10 and 30 mg/kg 3 times a week, i.e. on days 1, 3 and 5 of each week at the total doses of 0, 3, 9, 30, and 90 mg/kg.

Immunotoxicity Phase (for study portion):
- dosage administered in 4 groups (10/sex) in units: Rats received bolus injection of the test drug at 0, 3, 10 and 30 mg Fe/kg 3 times a week (days 1, 3 and 5 of each week). One additional group (5/sex) of rats was administered 50 mg/kg cyclophosphamide (volume = 10 ml/kg) 2 days prior to termination.
- route, form, volume: intravenous bolus injection, dosing volume, 5 ml/kg.

Drug, lot#, radiolabel, and % purity: VIT-45 – 291100; Cyclophosphamide – A016418501

Formulation/vehicle: supplied as 5% w/v iron solution in 0.9% saline, ready for use; Cyclophosphamide in 0.9% saline – 97% pure

Observations and times:

- Clinical signs: Clinical signs of toxicity were observed
Daily immediately before and after dosing in cage. The animals were observed after the completion of treatment in each group, between 1-2 hr after the completion of treatment
- Body weights: Body weights were determined one week before the treatment and then weekly during the study.
- Food consumption: Food consumption was determined weekly.
- Hematology, clinical chemistry, and urinalysis: During
weeks 4 and 13 (before treatment) from overnight fasting animals. For immunotoxicity phase animals, samples were obtained from fasting animals.
- Ophthalmologic Examination: Before treatment, during week
6 and 13.

Immunotoxicology**:**

SRBC specific antibodies screening cells were determined by using a modified original Jerne plaque forming cells (PFC) assay. The animals were challenged with a single IV injection of $2X10^8$ SRBC cells in 0.9% saline 4 days prior to termination. One of the group of the animals (positive control - cyclophosphamide) was treated at the dose of 50 mg/kg 2 days prior to the termination. On assay day, $1X10^6$ cells and $2X10^6$ cells splenocytes from the each of the animals were mixed with guinea pig serum specific antigen and this plaguing mixture was added to tubes containing a mixture of 0.5% agar and 20% SRBC. Antigen specific antibody bound to SRBC produced complement-

mediated lysis and formed clear plaques and each plaque was a replica of single specific lymphocyte secreting SRBC specific antibody and these were evaluated microscopically. These were indicated as expressed as PFC/10^6 spleen cells and PFC/spleen.

- Gross pathology: At termination the animals were necropsied and full macroscopic examination of organs and orifices was done.
- Organ weighed: the tissues and organs of the animals of main and immunotoxicology parts of the study were cleaned and weighed at termination.
- Histopathology: Following organs or tissues of the toxicology phase animals were examined histopathologically from each animal included in all treatment groups: adrenals, aorta, brain, cecum, colon, duodenum, epididymis, eyes, esophagus, gonads, Harderian glands, heart, ileum, jejunum, kidneys, larynx, lachrymal glands, liver, lungs, lymph node, mammary gland, optic nerve, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, thymus, thyroid, and parathyroid, tongue, trachea, urinary bladder and cervix, uterus, vagina, and all gross lesions.
The following organs or tissues of the immunotoxicology phase animals were examined histopathologically (each animal in all groups: adrenals, femur, kidneys, liver, lymph nodes, injection site, spleen, sternum and thymus.

Results:
- Clinical Signs: The increased incidences of hair loss on head were 3, 8, 5, 3 and 7 males and, 4, 7, 4, 3 and 9 females of the main toxicity study. Brown staining on the head was seen in 0, 0, 1, 0 and 1 male and, 1, 4, 5, 7 and 6 females out of 10 animals/sex. The hair loss from the ventral side was seen in 0, 0, 1, 0 and 1 males and, 1, 4, 5, 7 and 6 females of 10/sex animals of 0, 3, 9, 30 and 90 mg/kg/week treatment groups of the toxicology group.
- Mortality: There were no unscheduled deaths in this study.
- Body Weights: The mean initial and final body weights in the control group were 226.3 and 509.4 g for males and, 172.6 and 273.2 g for females, respectively. The terminal body weight of the animals was 283.2, 261.3, 246.1 and 225.2 g in males and, 100.6, 102.3, 97.4, 86.3 and 67.6 g in females. The reduction in body weight gain among males was 7.7, 13.1, 20.6 and, 47.4% in males, and 0, 2.6, 13.7 and 32.4% in females included in 3, 9, 30 and 90 mg/kg/week treatment groups.
- Food Consumption: The average food consumption in the control group was 26.9 g/rat/day in males or 17.5 g/rat/day in females. The food consumption was decreased by 9.7 and 29.7% in males of 30 and 90 mg/kg/week treatment groups. The food consumption of animals in these treatment groups was reduced by 5.2 and 14.9%, respectively.

Ophthalmoscopy: There were no treatment related changes.

- Hematology: An increase in mean corpuscular hemoglobin ($p<0.01$) and mean hemoglobin volume ($p<0.01$) was noted in 30 and 90 mg/kg/week treatment group males and females during week 13. The platelet counts, PT and APTT among males and females of the treatment groups (toxicology part of the study) were not affected on week

13 excepting a slight increase of statistical significance was observed in females of 30 and 90 mg/kg/week treatment groups. An increase in the total number of WBCs and neutrophils in males and females of 90 mg/kg/week treatment group was observed.

- Clinical Chemistry**:** Mean plasma iron level (umol/l) was increased at week 13 and the increase was 82.3 and 217.6% in males and, 55.2 and 89.7% in females in 30 and 90 mg/kg/week treatment groups. Significant increases in total iron binding capacity (umol/l) in males to 138 umol/l in males and, not significant increase to 126 umol/l in females of 90 mg/kg/week group was seen. Increases in serum alkaline phosphatase (36.8% in males and 93.5% in females), alanine aminotransferase (581.8% in males and, 285.7% in females), and aspartate aminotransferase (388.6% in males and, 300% in females) were noted in 90 mg/kg/week males during week 13. The increase in alkaline phosphatase during week 4 and slight increase in total protein in week 13 among males of the high dose males could be due to the significant ($p<0.01$) decrease excretion in urine.

Tissue Iron Content: A treatment related increase in the iron content was observed in liver, kidney and spleen as shown in the following table (sponsor's submission, vol 36 of 323, table 11, pp 118). The exposure ratio in the liver was maximum, i.e., 24.2 and 20.4 times of the animals of 3 mg/kg/week treatment group.

Table 12

Exposure ratio of tissues analysed for iron content

GROUP	:	1	2	3	4	5
COMPOUND	:	Control		VIT-45		
DOSAGE (mg Fe/kg/week)	:	0	3	9	30	90

Dose	Dose Ratio	Exposure ratio		
		Liver	Kidney	Spleen
Males				
3	1	1	1	1
9	3	2.75	1.00	1.84
30	10	9.90	1.47	2.63
90	30	24.23	2.58	4.25
Females				
3	1	1	1	1
9	3	2.68	1.19	1.80
30	10	8.45	1.79	2.07
90	30	20.45	2.98	3.20

On week 13, the total iron binding capacity (TIBC) was higher in males of 90 mg/kg/ week VIT-45 treatment group and TIBC was not affected in animals of the 9 and 30 mg/kg/week groups.

Table 11 - continued

Tissue Iron content – group mean values (mg Fe/kg of tissue sample) for animals on the Toxicity Phase

GROUP		1	2	3	4	5
COMPOUND		Control		VIT-45		
DOSAGE (mg Fe/kg/week)		0	3	9	30	90
GROUP		LIVER	KIDNEYS	SPLEEN		
1F	Mean	344.0	168.0	1930.0		
	Sd	44.8	14.76	727.0		
		**		**		
2F	Mean	573.0	170.0	3990.0		
	Sd	57.6	20.00	706.4		
		**	**	**		
3F	Mean	1536.0	203.0	7164.0		
	Sd	180.0	30.93	1677.1		
		**	**	**		
4F	Mean	4841.0	304.0	8276.0		
	Sd	376.9	36.27	1327.0		
		**	**	**		
5F	Mean	11720.0	507.0	12760.0		
	Sd	846.3	74.69	1639.2		

**P<0.01

The total serum protein, alpha-1, alpha-2 and beta globulins were increased in males of 30 and 90 mg/kg/week groups. Slightly higher beta globulin amount was seen in females of 90 mg/kg/week group. The mean creatinine amounts were low in females of 9, 30 and 90 mg/kg/week treatment groups.

Immunotoxicology: Like in main study group animals, higher mean iron was noted in males and females of 90 mg/kg/day group and, females of 30 mg/kg/day group in animals of immunotoxicity group. Slightly higher protein content in males and not in females of 90 mg/kg/day treatment group were similar changes to the findings of treatment phase animals. No immunotoxicity was observed in lymphoid tissues or bone marrow of the animals of this group.

- Urinalysis: Decreased (13-51%) urinary outputs and excretion of electrolytes (Na, K, and Cl) were noted in the high dose males during weeks 6 and 13.

- Organ Weights: The absolute weight of pituitary, adrenal, seminal vesicle and, prostate weight was decreased statistically. The absolute weight of kidney was increased by 1.2 times and, liver by 1.63 times in males of 90 mg/kg/week treatment group. The spleen weights in treatment groups were 1.2, 1.3, 1.4 and 1.8 times the control group males. The adjusted weights of spleen and liver were also increased in females belonging to 30 and 90 mg/kg/week treatment groups. The absolute weights of liver were 10.35, 12.2 and 17.3 g and, spleen weights were 0.51, 0.75 and 0.95 g in females belonging to control, 30 and 90 mg/kg/week treatment groups.

- Gross Pathology: The liver and adrenal of the animals in 30 and 90 mg/kg/week treatment groups were dark colored and, the salivary glands had brown discoloration due to iron pigmentation. The spleen of the animals treated with 30 and 90 mg/kg/week were enlarged. Pancreatic lymph node had brown discoloration in 4 and 10 males and, 0 and 10 females of 30 and 90 mg/kg/week treatment groups.

- Histopathology: Deposition of iron-containing pigment (brown pigment) was noted in tissues or organs including the liver, spleen, kidney, and lymph nodes (after Perl's stain)

in a dose dependent manner. In liver, the sinusoidal/phagocytic cells laden with iron pigment was treatment related as the intensity of pigmentation was dose dependent, that is moderate and marked in 30 and 90 mg/kg/week treatment group males and females. The perivascular fibrosis at the site of injection was in higher incidences in 30 and 90 mg/kg/day treatment groups than in control group animals. The iron pigment in sinusoidal/phagocytic cells, Kupffer's cells and periportal Kupffer's cells was treatment related and is shown in the following table (Sponsor's submission vol 36.323, table for Toxicity Phase, pp 46 -51):

-

Toxicity phase - continued

(Perls' stain)		Males					Females				
Group		**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Dosage level (mg Fe/kg/week)		**0**	**3**	**9**	**30**	**90**	**0**	**3**	**9**	**30**	**90**
Iron pigment deposition in vascular endothelium	Total	0	0	0	1	9c	0	0	0	1	9c
	Minimal	0	0	0	1	9	0	0	0	1	9
Macrophages containing iron pigment in lumen of blood vessels	Total	0	0	0	0	8c	0	0	0	0	6a
	Minimal	0	0	0	0	8	0	0	0	0	6
Iron pigment within hepatocyte cytoplasm – periportal	Total	0	0	0	10c	10c	0	0	0	10c	10c
	Minimal	0	0	0	7	0	0	0	0	10	0
	Slight	0	0	0	3	0	0	0	0	0	0
	Moderate	0	0	0	0	10	0	0	0	0	10
Iron pigment within hepatocyte cytoplasm – centrilobular	Total	0	0	5a	10c	10c	0	1	10c	10c	10c
	Minimal	0	0	5	0	0	0	1	10	0	0
	Slight	0	0	0	10	0	0	0	0	10	0
	Moderate	0	0	0	0	10	0	0	0	0	10
Number of livers examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

a – $p < 0.05$, c – $p < 0.001$ with Fisher's Exact Test (on total incidence only)

(Perls' stain)		Males					Females				
Group		**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Dosage level (mg Fe/kg/week)		**0**	**3**	**9**	**30**	**90**	**0**	**3**	**9**	**30**	**90**
Clumps of sinusoidal/phagocytic cells containing iron pigment	Total	0	0	0	10c	10c	0	0	1	10c	10c
	Minimal	0	0	0	0	0	0	0	1	0	0
	Moderate	0	0	0	10	0	0	0	0	10	0
	Marked	0	0	0	0	10	0	0	0	0	10
Generalised iron pigment within Kupffer cells	Total	0	2	5a	7b	10c	0	8c	10c	10c	10c
	Minimal	0	2	1	0	0	0	8	0	0	0
	Slight	0	0	4	0	0	0	0	10	0	0
	Moderate	0	0	0	7	0	0	0	0	10	0
	Marked	0	0	0	0	10	0	0	0	0	10
Iron pigment within Kupffer cells – predominantly periportal	Total	0	8c	5a	3	0	0	2	0	0	0
	Minimal	0	8	0	0	0	0	2	0	0	0
	Slight	0	0	5	0	0	0	0	0	0	0
	Moderate	0	0	0	3	0	0	0	0	0	0

Iron pigment deposition in vascular endothelium	Total	0	0	0	1	9c	0	0	0	1	9c
	Minimal	0	0	0	1	9	0	0	0	1	9
Macrophages containing iron pigment in lumen of blood vessels	Total	0	0	0	0	8c	0	0	0	0	6a
	Minimal	0	0	0	0	8	0	0	0	0	6
Iron pigment within hepatocyte cytoplasm – periportal	Total	0	0	0	10c	10c	0	0	0	10c	10c
	Minimal	0	0	0	7	0	0	0	0	10	0
	Slight	0	0	0	3	0	0	0	0	0	0
	Moderate	0	0	0	0	10	0	0	0	0	10
Iron pigment within hepatocyte cytoplasm – centrilobular	Total	0	0	5a	10c	10c	0	1	10c	10c	10c
	Minimal	0	0	5	0	0	0	1	10	0	0
	Slight	0	0	0	10	0	0	0	0	10	0
	Moderate	0	0	0	0	10	0	0	0	0	10
Iron pigment in macrophages – predominantly in red pulp	Total	10	10	10	10	10	10	10	10	10	10
	Minimal	8	0	0	0	0	7	0	0	0	0
	Slight	2	10	0	0	0	3	8	3	0	0
	Moderate	0	0	10	9	0	0	2	7	10	3
	Marked	0	0	0	1	10	0	0	0	0	7
Iron pigment in capsule	Total	0	0	0	10c	10c	0	0	0	10c	10c
	Minimal	0	0	0	10	6	0	0	0	10	6
	Slight	0	0	0	0	4	0	0	0	0	4
Number of spleens examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Iron pigment within glomerular mesangial cells	Total	0	0	0	10c	10c	0	0	1	10c	10c
	Minimal	0	0	0	10	7	0	0	1	10	5
	Slight	0	0	0	0	3	0	0	0	0	5
Iron pigment deposition in macrophages	Total	0	0	0	10c	10c	0	0	0	10c	10c
	Minimal	0	0	0	10	8	0	0	0	10	5
	Slight	0	0	0	0	2	0	0	0	0	5
Iron pigment deposition in cortical tubular epithelium	Total	5	3	4	9	10a	5	2	3	10a	9
	Minimal	5	2	4	9	10	5	2	3	10	7
	Slight	0	1	0	0	0	0	0	0	0	2
Number of kidneys examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Deposition of brown pigment in macrophages	Total	0	0	0	9c	10c	2	1	4	10c	10c
	Minimal	0	0	0	9	0	2	1	4	6	0
	Slight	0	0	0	0	5	0	0	0	4	5
	Moderate	0	0	0	0	5	0	0	0	0	5
Generalised brown pigment within zona glomerulosa cells	Total	0	0	0	0	10c	0	0	0	1	10c
	Minimal	0	0	0	0	5	0	0	0	1	9
	Slight	0	0	0	0	5	0	0	0	0	1
Number of adrenals examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Mandibular											
Deposition of brown pigment in macrophages	Total	0	1	8c	10c	10c	0	1	10c	10c	10c
	Minimal	0	1	6	0	0	0	1	6	0	0
	Slight	0	0	2	4	0	0	0	4	0	0
	Moderate	0	0	0	5	4	0	0	0	9	1
	Marked	0	0	0	1	6	0	0	0	1	9
Number of mandibular lymph nodes examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Mesenteric											
Deposition of brown pigment in macrophages	Total	0	10c	10c	10c	10c	0	10c	10c	10c	10c
	Minimal	0	4	0	0	0	0	3	0	0	0
	Slight	0	6	0	0	0	0	7	0	0	0
	Moderate	0	0	10	0	0	0	0	10	1	0
	Marked	0	0	0	10	10	0	0	0	9	10
Number of mesenteric lymph nodes examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Lumbar											
Deposition of brown pigment in macrophages	Total	0	7b	10c	10c	10c	1	9b	10c	10c	10c
	Minimal	0	7	0	0	0	1	8	1	0	0
	Slight	0	0	5	0	0	0	1	6	0	0
	Moderate	0	0	5	3	0	0	0	3	0	1
	Marked	0	0	0	7	10	0	0	0	10	9
Number of lumbar lymph nodes examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Perivascular fibrosis	Total	3	2	1	3	9a	5	6	4	9	9
	Minimal	2	2	0	1	0	3	2	1	4	1
	Slight	1	0	1	2	5	2	4	2	5	4
	Moderate	0	0	0	0	4	0	0	1	0	4
Intimal proliferation	Total	2	0	0	0	4	6	4	4	4	9
	Minimal	1	0	0	0	2	1	1	1	1	2
	Slight	1	0	0	0	2	3	2	3	2	5
	Moderate	0	0	0	0	0	2	1	0	1	2
Thrombus/thrombi		1	0	0	0	2	0	1	1	1	2
Number of parenteral injection sites examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Perivascular brown pigmented macrophages	Total	0	0	0	1	3	0	0	0	0	4
	Minimal	0	0	0	1	2	0	0	0	0	1
	Slight	0	0	0	0	1	0	0	0	0	3
Perivascular inflammatory cell infiltration	Total	4	3	1	5	9	5	6	6	6	9
	Minimal	4	1	1	4	6	1	1	1	3	5
	Slight	0	2	0	1	3	4	5	5	3	3
	Marked	0	0	0	0	0	0	0	0	0	1
Number of parenteral injection sites examined		**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Key study findings: In this 13-week rat toxicity study, 5 groups of animals were given intravenous bolus injection of 0, 3, 9, 30, and 90 mg iron/kg/week to compare the findings of the previous 13-week IV infusion toxicity study. A dose related iron accumulation in the plasma, reduction of body weight gain in the mid and high dose males and females and, increased liver enzyme activities in both sexes were noted. The accumulation of iron in macrophages of liver, spleen, kidneys, adrenals, lymph nodes etc., were dose related and time dependent (> in 13 weeks than 4 weeks) indicating a humoral immune response to T-cell dependent SRBC antigen. Based on this, the liver,

spleen, lymph nodes, and kidney these were identified as the target organs of toxicity and a dose 9 mg/kg/day produced minimal treatment related iron deposition.

DOG:

Study Title: **13-Week Intravenous Infusion Toxicity Study in Dogs**
Study No: VFR 041/993362
Conducting Laboratory and Location: (b) (4)

Date of study initiation: June 18, 1999
Report date: December 20, 2002
GLP compliance: This study was conducted in accordance with Good Laboratory Practice Regulations of the United States Food and Drug Administration (21 CFR Part 58).
QA-Report Yes (x) No ()

- **Methods**: VIT-45 was given to dogs (4/sex/group) by intravenous infusion over 1 hour at 0, 9, 30, and 90 mg/kg once a week for 13 weeks.

Dosing:

- **species/strain**: Beagle dogs.
- **#/sex/group or time point**: 4/sex/group.
- **age**: 21-26 weeks old.
- **weight**: males/females: 7.5-11.7 kg.
- **satellite groups used for toxicokinetics or recovery**:
- **dosage groups in administered units**: Dog received test drug at 0, 9, 30, and 90 mg/kg.
- **route, form, volume, and infusion rate**: intravenous infusion.

Drug, lot#, radiolabel, and % purity: 894209B.
Formulation/vehicle: 0.9% sodium chloride.
Observations and times:

- Clinical signs: Clinical signs of toxicity were observed daily.
- Body weights: Body weights were determined weekly.
- Food consumption: Food consumption was determined weekly.
- Hematology, clinical chemistry, and urinalysis: During weeks 6 and 13.
- Ophthalmologic Examination: Before treatment, and during week 12.
- ECG and blood pressure: before treatment, on day 1, and week 13.
- Gross pathology: Animals were necropsied at termination.
- Organ weighed: Organs were weighed at termination.
- Histopathology: Following organs or tissues were examined histopathologically from each animal in all groups: adrenals, aorta, brain, cecum, colon, cervix, duodenum, epididymis, eyes, esophagus, gonads, Harderian glands, heart, ileum, jejunum, kidneys, larynx, lachrymal glands, liver, lungs, lymph node, mammary gland, optic nerve, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, thymus, thyroid, tongue, trachea, urinary bladder, uterus, vagina, and all gross lesions.

Methods: To assess the repeated dose toxicity of VIT-45 in dogs, VIT-45 was given to dogs by intravenous infusion over 1 hour at 0, 9, 30, and 90 mg/kg once a week for 13 weeks. In the current study, clinical signs of toxicity were observed daily. Body weights were determined twice weekly. Food consumption was determined daily. Hematology, clinical chemistry, and urinalysis were conducted before treatment and during weeks 6 and 13. Ophthamological examination was conducted before treatment and during week 12. EKGs and blood pressure were determined before treatment, on day 1, and during week 13. All animals were necropsied at termination and organ weights were determined. Histopathological examination was conducted in all animals from all groups.

Results:

- Clinical Signs: Yellow discoloration of the eyes and gums were noted in the high dose group. Liquid feces were noted all treatment groups with a higher incidence in the mid and high dose groups.
- Mortality: There were no deaths.
- Body Weights: The initial and final body weights in the control group were 10.2 and 12.9 kg for males or 7.8 and 10.8 kg for females. There were no treatment related changes.
- Food Consumption: The average food consumption in the control group was 396 g/dog/day for males and 376 g/dog/day for females. There were no treatment related changes.
- Hematology: Decreases in red blood cell count, hemoglobin level, and hematocrit were noted in the high dose males during week 6 (~9-10%) and week 13 (21-23%). Smaller changes of these parameters were also noted in the mid dose males during week 6 (~6%) and 13 (~16%).
- Clinical Chemistry: Mean plasma iron level was increased in the mid and high males during weeks 6 (high dose 107%) and 13 (32% and 129%). Mean plasma iron level was increased in the mid and high females during weeks 6 (high dose 83%) and 13 (18% and 79%).
- Urinalysis: There were no obvious treatment related changes.
- Ophthalmologic Examination: There were no treatment related changes.
- ECG and blood pressure: There were no treatment related changes.
- Organ Weights: The mean liver weight was increased dose dependent manner by 35-50% in the mid dose group and by 94-110% in the high dose group.
- Gross Pathology: Enlarged liver was noted in the mid and high dose group animals. Dark discoloration of lymph nodes was found in the mid and high dose groups animals.
- Histopathology: Iron deposition was noted in tissues or organs including the liver, spleen, and kidney in all treatment groups. The iron deposition was not associated with any histopathological changes. One high dose male had focal hepatocyte necrosis.

	Male				Female			
Dosage level (mg Fe/kg/week)	**0**	**9**	**30**	**90**	**0**	**9**	**30**	**90**
Generalised brown pigment in Kupffer cells								
Total	0	4	4	4	0	4	4	4
Slight	0	0	0	0	0	2	0	0
Moderate	0	4	0	0	0	2	0	0
Marked	0	0	4	0	0	0	4	0
Severe	0	0	0	4	0	0	0	4
Clumps of sinusoidal/phagocytic cells containing brown pigment								
Total	0	4	4	4	0	4	4	4
Moderate	0	4	0	0	0	4	0	0
Marked	0	0	4	0	0	0	4	0
Severe	0	0	0	4	0	0	0	4
Extramedullary haemopoiesis								
Total	0	4	4	4	0	4	4	4
Slight	0	2	3	0	0	0	0	0
Moderate	0	2	1	0	0	4	2	0
Marked	0	0	0	3	0	0	2	4
Severe	0	0	0	1	0	0	0	0
Hepatocyte necrosis								
Total	0	0	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Increased perivascular fibrosis/associated cellularity								
Total	0	0	2	4	0	0	4	4
Slight	0	0	2	0	0	0	0	0
Moderate	0	0	0	0	0	0	4	0
Marked	0	0	0	2	0	0	0	4
Severe	0	0	0	2	0	0	0	0
Perls' stained								
Generalised iron pigment with Kupffer cells								
Total	0	4	4	4	0	4	4	4
Moderate	0	0	0	0	0	4	0	0
Marked	0	4	0	0	0	0	0	0
Severe	0	0	4	4	0	0	4	4
Clumps of sinusoidal/phagocytic cells containing iron pigment								
Total	0	4	4	4	0	4	4	4
Moderate	0	4	0	0	0	1	0	0
Marked	0	0	0	0	0	3	1	0
Severe	0	0	4	4	0	0	3	4
Fine granules of iron pigment within hepatocytes								
Total	0	0	4	4	0	0	4	4
Minimal	0	0	3	0	0	0	0	0
Slight	0	0	1	0	0	0	4	0
Moderate	0	0	0	0	0	0	0	0
Marked	0	0	0	4	0	0	0	4
Number of livers examined	4	4	4	4	4	4	4	4

	Male				Female			
Dosage level (mg Fe/kg/week)	**0**	**9**	**30**	**90**	**0**	**9**	**30**	**90**
Deposition of brown pigment in macrophages, mainly red pulp								
Total	0	0	4	4	0	1	4	4
Minimal	0	0	3	0	0	0	2	0
Slight	0	0	1	0	0	1	1	0
Moderate	0	0	0	1	0	0	1	1
Marked	0	0	0	3	0	0	0	3
Extramedullary haemopoiesis								
Total	0	0	4	4	0	1	1	4
Minimal	0	0	3	0	0	1	1	0
Slight	0	0	1	0	0	0	0	0
Moderate	0	0	0	1	0	0	0	1
Marked	0	0	0	3	0	0	0	3
Perls' stained								
Iron pigment in macrophages-mainly red pulp								
Total	4	4	4	4	4	4	4	4
Minimal	3	1	0	0	4	2	0	0
Slight	1	3	0	0	0	1	1	0
Moderate	0	0	4	0	0	1	3	1
Marked	0	0	0	4	0	0	0	3
Number of spleens examined	4	4	4	4	4	4	4	4

	Male				Female			
Dosage level (mg Fe/kg/week)	**0**	**9**	**30**	**90**	**0**	**9**	**30**	**90**
Brown pigment within glomerular mesangial cells								
Total	0	0	0	4	0	0	0	4
Slight	0	0	0	4	0	0	0	2
Moderate	0	0	0	0	0	0	0	2
Interstitial cells containing brown pigment								
Total	0	0	0	2	0	0	0	0
Minimal	0	0	0	2	0	0	0	0
Perls' stained								
Iron pigment within glomerular mesangial cells								
Total	0	0	4	4	0	0	4	4
Minimal	0	0	4	0	0	0	4	0
Moderate	0	0	0	4	0	0	0	4
Iron pigment within interstitial cells								
Total	0	0	0	4	0	0	0	4
Slight	0	0	0	4	0	0	0	4
Aggregations of iron containing macrophages-inner medulla								
Total	0	0	0	4	0	0	0	4
Minimal	0	0	0	1	0	0	0	1
Slight	0	0	0	3	0	0	0	3
Number of kidneys examined	4	4	4	4	4	4	4	4

Brown pigment deposition was reported in many other tissues:

Key study findings: In the 13-week intravenous toxicity study in dogs, VIT-45 was given to dogs by intravenous infusion over 1 hour at 0, 9, 30, and 90 mg iron/kg/week for 13 weeks. Iron deposition was noted in multiple organs including the liver, spleen, and kidney mainly in the two high dose groups. One high dose male had hepatocyte necrosis.

2. **Study Title**: **26-Week Chronic Intravenous Bolus Injection Toxicity Study in Dogs followed by a 6 Week Recovery Period.**

Study No: VFR 070/042337
Conducting Laboratory and Location: (b) (4)

Date of study initiation: March 08, 2004
Report Issue date: October 28, 2005
GLP compliance: This study was conducted in compliance with Good Laboratory Practice Regulations of the United States Food and Drug Administration (21 CFR Part 58).
QA-Report Yes (x) No ()

Methods: The study was conducted in 3 phases of (a) Interim phase (3/sex/group), (b) main study group (4/sex/group) and (c) Recovery Phase consisting of two groups (2/sex/group) of 0 (control) and 30 mg/kg/week groups. The dogs in this study were treated with intravenous bolus injection 3 times a week (day 1, 3 and 5 of each week) for 13 weeks.

Dosing:
- **species/strain**: Beagle dogs.
- **#/sex/group or time point**: Interim Phase: 3/sex/group,
  Main Study Phase: 4/sex/group,
  Recovery Phase: 2 groups 2/sex/group
- **age**: 24-29 weeks old.
- **weight**: males/females: 9.7 to 13.4 kg (males); 7.1 to 11.9 kg (Females).
- **satellite groups used for toxicokinetics or recovery**: 2 groups of 2/sex in 0 and high dose groups

- **dosage groups in administered units**: Dog received test drug at 0, 3, 9, and 30 mg/kg/week and each dose was given in 3 equal divided doses.
- **route, form, volume, and infusion rate**: intravenous bolus dose.
Drug, lot#, radiolabel, and % purity: 291100.
Formulation/vehicle: Ampoules in 0.9% sodium chloride.
Observations and times:
- Clinical signs: Clinical signs of toxicity were observed daily in the first week and twice weekly 2-4, weekly up to week 14 for changes in the appearance and physical activities.
- Body weights: Body weights were determined weekly once prior to the treatment and during the study.

- Food consumption: Food consumption was determined by the remaining and spilled food recorded daily and computed to obtain weekly consumption up to study week 26 and recovery period.
- Hematology, clinical chemistry, and urinalysis: During weeks 13 and 26 before dosing and week 6 of recovery, 0.5 ml of blood samples was collected from each of the animals.
- Tissue Iron Content: The iron content in liver, kidney and spleen was determined on the tissues of the animals treated for 13 and 26 weeks of treatment by coupled plasma optical emission spectrometry.

- Ophthalmologic Examination: Before treatment and during study week 12 and 25 and during recovery week 6 (for recovery animals).
- ECG and blood pressure: Recorded on day 1 and, on week 13 and 26 at predose, at 2 and 24 hr after the dosing.
- Gross pathology: Animals were necropsied at the termination of the treatment and recovery group animals were killed at the end of week 26.
- Organ weighed: Organs were weighed at termination.
- Histopathology: Following organs or tissues from each of the animals of all the groups were separated and for histopathological examination: adrenals, aorta, brain, cecum, colon, cervix, duodenum, epididymis, eyes, esophagus, gonads, Harderian glands, heart, ileum, jejunum, kidneys, larynx, lachrymal glands, liver, lungs, lymph node, mammary gland, optic nerve, pancreas, pituitary, prostate, rectum, salivary glands (mandibular, parotid and sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin spinal cord, spleen, sternum, stomach, thymus, thyroid, tongue, trachea, urinary bladder, uterus and vagina.

Anti-drug Antibodies determination: One ml of blood samples was collected before treatment on day 1, week 13 and 26 and during week 6 of recovery from each of the animals for sera preparation for antibody determination. The test results were not included in the present submission and the sera preserved for future testing.

Results:

- Clinical Signs: Sponsor stated that there were no clinical changes in the clinical signs of any of the treatment group animals. No table or data were submitted to substantiate the statement.

- Mortality: There were no deaths among the treatment group animals.
- Body Weights: The initial and final body weights in the control group males were 12.1 and 14.6 kg and, females 9.9 and 12.3 kg. The percent reduction in body weight gain was 16.0, 9.9 and 24.1% in males and, 8.0, 12.0 and 20% in females of the study. During the recovery period, the body weights gains were similar, that is 0.2 and 0.3 kg in male and, 0.3 and 0.2 kg among females of control and, 30 mg/kg/week treatment groups.
- Food Consumption: The average food consumption in the dogs included in the treatment groups was similar to the control group animals during the study. The control group food consumption was 280.2 and 241.1 g/dog/day for males and females, respectively. There were no treatment related food consumption changes.

- Hematology: Among high dose treated dogs, the decreases in red blood cell count (17.7 in males and 9.9% in females), hemoglobin level (15.2 and 14.1%), and hematocrit (15.0 and 5.8%) were noted in the high dose treated animals. APTT was only slightly increased (1.2 times in males and 1.2 times in females). These changes were smaller and were only of statistical significance.
- Clinical Chemistry: On week 26, a slight dose related reduction (only of mathematical importance) in serum creatinine and cholesterol was observed in male dogs of high dose treatment group. Serum creatinine levels were 75, 71, 65 and 62 umol/L in males and, 66, 69, 66 and 60 umol/L in females of 0, 3, 9 and 30 mg/kg/week treatment groups. Serum cholesterol change was more intense in high dose males than females (M:F = 16.3/38.2%) . Mean plasma iron levels were increased in the mid and high females during weeks 6 (high dose 83%) and 13 (18% and 79%). The total serum protein, alpha-1, alpha-2 and beta globulins were increased in males of 30 and 90 mg/kg/week groups. Slightly higher beta globulin amount was seen in females of 90 mg/kg/week group. The mean creatinine amount was low in only study females of 9, 30 and 90 mg/kg/week treatment groups but was not significantly affected in males.

Mean tissue iron level was increased in the treatment related manner as shown in the following table of sponsor (Table 11 of Tissue Iron levels, vol 43:323, p 12).

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TABLE 11

Tissue iron content - group mean values (mg Fe/kg of tissue sample) - interim animals

GROUP		1	2	3	4
COMPOUND		Control	-----VIT-45------		
DOSAGE(mg Fe/kg/week):		0	3	9	30
GROUP		KIDNEY	LIVER	SPLEEN	
1M	N	3	3	3	
	MEAN	47.0	190.0	583.3	
	SD	2.6	55.7	90.7	
2M	N	3	3	3	
	MEAN	52.7	1260.7**	645.0	
	SD	10.7	189.6	39.7	
3M	N	3	3	3	
	MEAN	81.3*	3380.0**	896.7**	
	SD	16.9	243.3	81.5	
4M	N	3	3	3	
	MEAN	150.0**	8850.0**	2180.0**	
	SD	20.0	26.5	520.3	

* p<0.05, ** p<0.01

Iron content increase was similar in males and females on week 13 and 26 as shown below in sponsor's table, and scanned here.

Summary of Tissue Iron Levels

Tissue	Fold increase over Control of Iron concentration during Week 13		Week 26		Week 6 Recovery	
	M	F	M	F	M	F
3 mg Fe / kg / Week						
Kidney	1.1	1.3	1.5	1.5	-	-
Liver	6.6	5.0	7.6	6.2	-	-
Spleen	1.1	1.1	1.5	1.1	-	-
9 mg Fe / kg / Week						
Kidney	1.7	1.5	2.1	2.1	-	-
Liver	17.8	12.4	19.6	17.8	-	-
Spleen	1.5	1.4	3.0	3.0	-	-
30 mg Fe / kg / Week						
Kidney	3.2	3.5	5.0	4.6	6.0	5.5
Liver	46.6	30.6	50.9	41.5	64.9	34.1
Spleen	3.7	4.8	6.8	6.9	8.1	4.4

Tissue Iron Contents: After the intravenous bolus injections of 0, 1, 3 and 10 mg Fe/kg VIT-45 three times a week in dogs, a treatment related increase in the iron concentration was observed in study week 13 and 26. The exposure ratio of the compound in the liver of the treatment groups animals in comparison to the control group was 6.63, 17.8 and 46.6 times in males and, 5.0, 12.4 and 30.6 times in females included in 3, 9 and 30 mg/kg/day treatment groups. The iron contents of tissues like kidneys, liver and spleen are shown above in summary table.

On week 13 and 26, the total iron binding capacity (TIBC) was high in males treated with 90 mg/kg/week VIT-45 and TIBC was not affected in animals treated with the doses of 9 and 30 mg/kg/week.

- Urinalysis: There were no significant treatment related changes.
- Ophthalmologic Examination: There were no treatment related changes.
- ECG and blood pressure: On week 26, no treatment related changes in the blood pressure and heart rate parameters of the males and females of the study were noted. On week 13, the heart rate was decreased in treatment related manner in male and female dogs. These were 141, 101, 91 and 108 b/min in males and, 132, 12, 141 and 112 b/min in females. On week 13, the QT-interval was slightly affected ($p<0.05$) in males and, not among females. The systolic blood pressure was increased from 128 to 154 b/min at 2 hr post dose observation period in males but not at 24 hr post dose the diastolic pressure was also slightly affected (from 86 to 102 mmHg).

- Organ Weights: On week 13, absolute weight of liver was increased significantly in male and females of 30 mg/kg/day treatment group and it was 38.1 and 64.6%, respectively. On week 26, the increase was more intense and was seen in all of the treatment groups of animals, the adjusted body weight were increased to 24.8, 42.7 and 87.7% in males and, 12.2, 25.8 and 85.0% in the females of low, mid and high dose treatment groups. The absolute weight of prostate in males of high dose group was observed to be increased, by 143% of the control. The testes weights were not affected.

- Gross Pathology: Enlarged liver was noted in all 4/4 males and females of 30 mg/kg/week treatment group and brown coloration of mandibular lymph nodes of all male and females was seen. The clotted blood/iron deposition (brown coloration) was seen in various lymph nodes of male and females of mid and high dose group. The subcutaneous hemorrhage, thickening and brown coloration was noted at the site of injection (cephalic vein - left/right).

-Histopathology: A material positive to Perl's stain was present in glomerular mesangial cells of 3 of 3 males and females of 30 mg/kg/day treatment group. Medullary mineralization was limited to 1/3 males of 9 and 30 mg/kg/day treatment groups. None of the females showed this change. Pigmentation in the Kupffer's cells was noted in 0, 3, 3, and 3 males and, 0, 3, 3, and 3 females out of 3 animals/sex in the study. These cells were Perl's positive. The Perl's positive clumps of sinusoidal/phagocytic cells in liver were also present in 0, 3, 3, and 3 males and, 0, 3, 3, and 3 females out 3/sex animals of the study. The pigmented macrophages in sinuses of mesenteric node were in 3 and 3 males and, 2 and 3 females out of 9 and 30 mg/kg/day treatment groups. None of the control and low dose group animals showed this abnormality. The pigmented macrophages in the spleen was present in 0, 1, 2 and 3 males and, 1, 0, .2 and 3 females out of 3/sex animals of 0, 3, 9 and 30 mg/kg/day treatment groups. The pigmented macrophages were present in sternum and bone marrow of 3 males and 3 females out of 3/sex of 30 mg/kg/day treatment group. Generally, the histiocytes in sinuses of lymph nodes of the animals contained pigmentations (as shown in the table below.):

		Male				Female			
Group		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dosage (mg Fe/kg/week)		**0**	**3**	**9**	**30**	**0**	**3**	**9**	**30**
Pigmented Kupffer cells	Total	0	4	4	4	0	4	4	4
	Minimal	0	1	0	0	0	0	0	0
	Slight	0	3	0	0	0	4	0	0
	Moderate	0	0	4	0	0	0	4	2
	Marked	0	0	0	4	0	0	0	2
Pigment in sinusoidal/phagocytic cells	Total	0	4	4	4	0	4	4	4
	Minimal	0	1	0	0	0	1	0	0
	Slight	0	3	0	0	0	3	0	0
	Moderate	0	0	4	0	0	0	4	2
	Marked	0	0	0	4	0	0	0	2
Extramedullary haemopoiesis	Total	3	1	3	4	2	1	2	4
	Minimal	3	1	3	1	2	1	2	0
	Slight	0	0	0	3	0	0	0	4
Perls' stain Iron in Kupffer cells	Total	1	4	4	4	2	4	4	4
	Minimal	1	0	0	0	2	0	0	0
	Slight	0	4	0	0	0	4	0	0
	Moderate	0	0	4	0	0	0	4	1
	Marked	0	0	0	4	0	0	0	3
Perls' stain Iron in sinusoidal/phagocytic cells	Total	0	4	4	4	0	4	4	4
	Minimal	0	0	0	0	0	1	0	0
	Slight	0	4	0	0	0	3	0	0
	Moderate	0	0	4	0	0	0	4	1
	Marked	0	0	0	4	0	0	0	3
Perls' stain Iron in hepatocytes	Total	0	0	2	4	0	0	0	4
	Minimal	0	0	2	4	0	0	0	4
Spleen									
Pigmented macrophages	Total	1	4	4	4	4	4	4	4
	Minimal	1	4	1	0	4	4	0	0
	Slight	0	0	2	2	0	0	3	0
	Moderate	0	0	1	2	0	0	1	4
Perls' stain Iron in macrophages	Total	3	4	4	4	4	4	4	4
	Minimal	3	4	0	0	4	4	0	0
	Slight	0	0	3	2	0	0	3	0
	Moderate	0	0	1	2	0	0	1	4

Kidneys:

Pigment in glomerular mesangial cells	Total	0	0	0	3	0	0	0	1
	Minimal	0	0	0	3	0	0	0	1
Perls' stain Iron in glomerular mesangial cells	Total	0	0	0	4	0	0	0	4
	Minimal	0	0	0	4	0	0	0	4
Perls' stain Iron in interstitium	Total	0	0	0	3	0	0	0	2
	Minimal	0	0	0	3	0	0	0	2
Parenteral injection sites									
Left cephalic Perivascular pigmented macrophages	Total	0	3	4	4	0	3	4	4
	Minimal	0	3	2	0	0	2	2	1
	Slight	0	0	2	4	0	1	2	3
Right cephalic Perivascular pigmented macrophages	Total	0	3	3	4	1	4	4	4
	Minimal	0	2	2	0	1	3	2	0
	Slight	0	1	1	4	0	1	2	3
	Moderate	0	0	0	0	0	0	0	1

Key study findings: In the 26-week intravenous toxicity study in dogs, VIT-45 was given to dogs by intravenous bolus doses of 0, 3, 9 and 30 mg iron/kg/week in 3 equal divided doses. Iron deposition was noted in multiple organs, renal medullary mineralization and glomerular mesangial cells, pigmentation in the Kupffer's cells, sinusoidal/phagocytic cells in liver and sinuses of mesenteric node. These were suggestive for the liver, kidney and lymph nodes as target organs of toxicity.

2.6.6.4 Genetic toxicology

VIT-45 was negative in the Ames test, in vitro chromosomal aberration test, mouse lymphoma forward mutation assay, and mouse micronucleus test.

Study Title: Ames test
Study report No: VFR 029/985223
Testing Laboratory: (b) (4)
Date of study initiation: December 9, 1998
Date of study report: February 3, 1999
GLP Compliance: This study was conducted in accordance with Good Laboratory Practice Regulations of the UK and OECD.
QA-report: Yes (x) No ()
Drug Batch No.: 894209B
Study Endpoint: To determine the potential mutagenic effects of Fe Dextrinate (VIT-45).

Methods: To examine the potential mutagenic effects of VIT-45, the reverse mutation assay (Ames test) was conducted using pre-incubation method in four strains of Salmonella typhimurium (TA 98, TA100, TA1535 and TA1537) and one strain of E coli WP2 trp uvr in the presence and absence of metabolic activation. The following concentrations were tested: 5, 15, 50, 150, 500, 1500, and 5000 µg/plate.

- **Strain/species/cell line**: Four strains of Salmonella typhimurium (TA98, TA100, TA1535 and TA1537).
- **Dose selection criteria**:
- **Basis of dose selection**: The high concentration of 5000 µg/plate was used.
- **Metabolic activation system**: Metabolic activation, S-9 mix, was from rat liver.
- **Control**:
- **Negative control**: dimethyl sulfoxide.

Positive control: N-ethyl-N-nitro-N-nitrosoguanidine, 9-aminoacridine, 2-nitrofluorane, 2-aminoanthracene, and benzo[a]pyrene, were tested.

- **Exposure conditions**: The reverse mutation assay (Ames test) was conducted using the pre-incubation method.
- **Dose used in defining study**: The following concentrations were tested: 5, 15, 50, 150, 500, 1500, and 5000 µg/plate with and without S-9.
- **Analysis**:
- **Cytotoxic endpoints**: The condition of the bacterial backgound lawn was evaluated for evidence of cytotoxicity.
- **Genetic toxicity endpoints/results**: Number of revertant colonies.
- **Statistical methods**: Number of revertant colonies were averaged for each concentration.

Criteria for positive results: The results should be considered positive if the test substance induced a two fold increase in the mean revertant colonies as compared to the control and this increase should be a dose response to increasing concentrations of the test article.

Results:

- **Study validation**: The positive controls significantly increased the colonies compared to the solvent controls.

- **Study outcome**: VIT-45 did not significantly increase the colonies as compared to the solvent control in the presence and absence of S-9 mix.

Summary: The results suggest that VIT-45 was not mutagenic in this test system.

Study Title: Forward mutagenicity test in the L5178Y$^{+/-}$ mouse lymphoma cells at TK locus

Study report No: VFR 030/992265

Testing Laboratory: (b) (4)

Date of study initiation: December 17, 1998

Date of study report: April 1, 1999

GLP Compliance: This study was conducted in accordance with Good Laboratory Practice Regulations of the UK and OECD.

QA-report: Yes (x) No ()

Drug Batch No.: 894209B.

Study Endpoint: To determine the potential mutagenic effects of VIT-45.

Methods: To examine the potential mutagenic effects of VIT-45, the L5178Y TK+/- mouse lymphoma cell assay was conducted in the presence and absence of metabolic activation, S-9 mix from rat liver. The following concentrations of VIT-45 were used: 78, 156, 312, 625, 1250, 2500, and 5000 µg/ml with and without S9. The TK+/- cell suspension was incubated with test drug for 4 hours at ~37^{o} C and cells were then washed free of drug. The expression period was 2 days. Plates were incubated for 10-11 days at ~37^{o} C. The number of TK+/- mutant colonies were then determined.

- **Strain/species/cell line**: Mouse lymphoma L5178Y TK+/- cell line.

- **Metabolic activation system**: Metabolic activation, S-9 mix, was from rat liver.
- **Control**:
  - **Vehicle**: DMSO
- **Positive control**: Methyl methanesulfonate, and 20-methylcholanthrene were tested.
- **Exposure conditions**: The TK+/- cell suspension was incubated with test drug for 4 hours at ~37^{o} C and cells were then washed free of drug. The expression period was 2 days. Plates were incubated for 12 days at ~37^{o} C.
- **Dose used in defining study**: The following concentrations were tested at 78, 156, 312, 625, 1250, 2500, and 5000 µg/ml µg/ml with and without S9.
- **Analysis**:
- **Counting method**: The number of TK+/- mutant colonies were then determined using automatic colony counter (size of colonies was not differentiated).
- **Cytotoxic endpoints**: A cell count was determined to measure the reduction in cell growth relative to the concurrent vehicle control cell cultures.
- **Genetic toxicity endpoints/results**: The number of TK+/- mutant colonies.
- **Statistical methods**: Numbers of colonies were averaged for each concentration.
- **Criteria for positive results**: The result is considered positive if a significant dose dependent increase in mutation frequency is observed and the result is reproducible.

Results:

- **Study validation**: The positive controls significantly increase the colonies compared to the solvent controls.

- **Study outcome**: Treatment with VIT-45 significantly increased the mutant frequency in the cultures at concentrations of 1250 and 2500 µg/ml in the absence of S9. This was not reproducible. The results were presented in Table 7 on page 25 in Volume 2.25. This table is attached below.

Table 7: Test 2 in the absence of S-9 mix, mutant frequency

Concentration of Fe Dextrinate (µg Fe/ml)	Empty Wells	Total wells	P(0)	Mean P(0)	Cloning Efficiency	Mutant Frequency	Mean mutant frequency
0	149	192	0.776		0.000127	0.000105	
0	150	192	0.781	0.741	0.000123	0.000118	0.000111
0	134	192	0.698		0.000180	0.000127	
0	136	192	0.708		0.000172	0.000093	
312.5	109	192	0.568	0.680	0.000283	0.000317	0.000207
312.5	152	192	0.792		0.000117	0.000097	
625.0	172	192	0.896	0.813	0.000055	0.000148	0.000152
625.0	140	192	0.729		0.000158	0.000156	
1250.0	119	192	0.620	0.656	0.000239	0.000268	0.000240
1250.0	133	192	0.693		0.000184	0.000212	**
2500.0	123	192	0.641	0.651	0.000223	0.000431	0.000434
2500.0	127	192	0.661		0.000207	0.000436	**
MMS - positive control							
5	28	192	0.146	0.169	0.000963	0.002157	0.002028
5	37	192	0.193		0.000823	0.001900	**

** $p < 0.01$

Summary: The results indicated that VIT-45 was not genotoxic in this test system.

Study Title: **In vitro chromosomal aberration test in Human lymphocytes**
Study No: VFR 028/992094
Testing Laboratory: (b) (4)

Date of study initiation: December 17, 1998
Date of study report: March 1, 1999
GLP Compliance: This study was conducted in accordance with Good Laboratory Practice Regulations of the UK and OECD.
QA-report: Yes (x) No ()
Drug Batch No.: 894209B
Study Endpoint: To determine the potential clastogenic effects of VIT-45.

Methods: To examine the potential induction of chromosomal aberrations by VIT-45, the *in vitro* chromosomal aberration test was conducted using human lymphocytes in the presence and absence of metabolic activation, S-9 mix from rat liver. VIT-45 was tested at 39, 78, 156, 312, 625, 1250, 2500, and 5000 µg/ml. Cells were exposed to the test drug for 4 hours and sampled 73 hours after exposure. Cells were arrested in metaphase using colcemid ~3 hours before harvest.

- **Strain/species/cell line**: Human peripheral lymphocytes.
- **Basis of dose selection**: The highest concentrations (5000 µg/ml) were used.
- **Metabolic activation system**: Metabolic activation, S-9 mix, was from rat liver.
- **Control**:
- **Negative control**: Water.
- **Positive control**: Chlorambucil and cyclophosphamide were tested.
- **Exposure conditions**: Cells were exposed to the test drug for 4 hours and sampled 73 hours after exposure.

Dose used in defining study: VIT-45 was tested at 39, 78, 156, 312, 625, 1250, 2500, and 5000 µg/ml with and without S9.

- **Analysis**:
- **Counting method**: Slides were prepared and stained for analysis of chromosomal aberration.
- **Cytotoxic endpoints**: Percentage of cell survival was used to measure the cytotoxicity.
- **Genetic toxicity endpoints/results**: percentage of cells with chromosomal aberration.
- **Statistical methods**: Percent of aberrant cells is analyzed by one-tail binomial test and compared pair wise to the control.
- **Criteria for positive results**: The result is considered positive if a significant increase in the number of cells with chromosomal aberrations is observed at one or more concentrations.

Results:

- Study validation: The positive controls significantly increased the frequency of the chromosomal aberration.

- Study outcome: VIT-45 did not significantly increase the frequency of the chromosomal aberration.

Summary: The results indicated that VIT-45 was not clastogenic in this test system.

Study Title: **In vivo mouse micronucleus test**

Study report No: VFR 031/992700

Testing Laboratory: (b) (4)
Date of study initiation: December 17, 1998
Date of study report: April 8, 1999
GLP Compliance: The study was conducted in accordance with GLP Regulations of the UK and OECD.
QA-report: Yes (x) No ()
Drug Batch No.: 894209B.
Study Endpoint: Frequency of cells with micronucleated reticulocytes.

Methods: To examine the potential mutagenic effects of VIT-45, the micronucleus test was conducted using mouse bone marrow cells. A single intravenous dose of VIT-45 was given to mice at 125, 250, and 500 mg/kg. In the current study, bone marrow was collected at termination. Vehicle and positive controls (mitomycin C) were also tested. The frequency of micronucleated reticulocyte was determined.

- **Strain/species/cell line**: CD-1 mice.
- **Metabolic activation system**: None.
- **Control**:
  - **Vehicle**: 0.9% sodium chloride.
- **Positive control**: Mitomycin C.
- **Exposure conditions**: Mice were sacrificed 24 hours after dosing and bone marrow was collected.
  - **Dose used in defining study**: 125, 250, and 500 mg/kg
  - **Analysis**:
- **Counting method**: Slides were prepared and examined for presence of micronucleated polychromatic erythrocytes.
- **Cytotoxic endpoints**: Proportion of reticulocytes to total erythrocytes was determined as an indicator of bone marrow toxicity.
- **Genetic toxicity endpoints/results**: Frequency of micronucleated reticulocytes.
- **Statistical methods**: Frequency of micronucleated reticulocytes was analyzed.
- **Criteria for positive results**: The result is considered positive if a significant increase in the micronucleated reticulocytes is observed dose-dependently.

Results:

- **Study validation**: The positive controls significantly increased the frequency of micronucleated reticulocytes.
    - **Study outcome**: VIT-45 did not significantly increase the frequency of micronucleated reticulocytes as compared to the control.

Summary: VIT-45 did not induce any toxicity at concentrations up to 500 mg/kg. VIT-45 was not mutagenic under this testing condition.

2.6.6.5 CARCINOGENICITY: Studies were not submitted by sponsor.

2.6.6.6 REPRODUCTIVE AND DEVELOPMENT TOXICOLOGY:

In the Segment I fertility and reproductive performance study in rats, VIT-45 was given by intravenous infusion over 1 hour to male and female rats at 0, 3, 9, and 30 mg/kg (18 to 180 mg/mm^2) doses three times a week. VIT-45 produced no effects on the fertility and general reproductive performance of the rats at the dose of 30 mg/kg which was identified as the highest tolerable dose. In the Segment II teratology study in rats, VIT-45 was given by intravenous infusion over 1 hour to rats at doses of 0, 3, 9, and 30 mg/kg (18 to 180 mg/mm^2) and VIT-45 was not teratogenic in rats. In the developmental Segment II teratology study in rabbits, VIT-45 was lethal in dams of 4.5, 13.5, and 18 mg/kg/day treatment groups and markedly domed shaped cranium, marked internal hydrocephaly or suspected hydrocephaly were seen in fetuses of the 13.5 and 18 mg/kg groups. The lowest dose of 4.5 mg/kg was maternally toxic with dam deaths, however there were no adverse fetal effects observed. These major malformations were found at maternally toxic doses. In the Segment III pre- and pos-natal reproductive toxicity study in rats, VIT-45 up to 18 mg/kg/day did not produce any adverse effects on parturition, lactation, physical and reflexological development of the offspring.

Study Title: Study of effects on the fertility and early embryonic development to implantation in CD rats by intravenous infusion administration
Study no.: VFR050/004685

Volume # 2.22, and page #:1
Conducting laboratory and location: (b) (4)

Date of study initiation: April 7, 2000
GLP compliance: Statements of compliance with GLP regulations and the quality assurance unit were included.
QA reports: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: 894209B.
Formulation/vehicle: 0.9% sodium chloride.

Methods:

Species/strain: Crl:CD(SD)BR rats

Doses employed: 3, 9, and 30 mg/kg
Route of administration: intravenous infusion.
Number/sex/group: 22/sex/group.
Study design:

To study the potential effects of VIT-45 on the fertility and reproductive performance in rats, VIT-45 was given by intravenous infusion over 1 hour to male rats for 4 weeks prior to mating until termination (the drug was given three times a week) and to female rats for 2 weeks prior to mating and throughout mating (the drug was given three times a week), on days 0, 3, and 7 after mating at 0, 3, 9, and 30 mg/kg. The females were sacrificed 14 days after mating for examination of their uterine contents.

Results:

Mortality: There were no deaths in this study.
Clinical signs: There were no clear treatment related changes.
Body weight:

The initial and final body weights in the control group were 320 and 424 g in males and 241 and 332 g in females (final body weight for female was determined on gestation day 14). The terminal body weight gain was decreased by ~42% in the high dose males as compared to the control. The body weight gain was not clearly affected by the treatment in females.
Food consumption: The average food consumption in the control group was 30-31 g/rat/day in males and 25-32 g/rat/day in females. The food consumption was slightly lower in the high dose males (26-30 g/rat/day) as compared to the control (30-31 g/rat/day).

Reproductive performance:
Estrous cycle, mating performance, and fertility were not affected. The number of corpora lutea, implantations, and resorption were not affected. Pre- and post-implantation loss and litter size were not affected. The sex ratio was not affected.

The sperm motility was not affected in study males.

Necropsy of the parental females revealed orange or brown discoloration of various organs including adrenal glands, liver, pancreas, and lymph nodes. The incidence of this finding appears dose related.

Key study findings: The compound from 3 to up to 30 mg/kg/day (18 to 1800 mg/mm^2) reduced body weight gain and food consumption in males. Treatment with VIT-45 did not affect the fertility and general reproductive performance of the rat.

Study Title: Study of effects on embryo-fetal toxicity in rats by intravenous infusion administration

Study no.: VFR048/002163
Volume # 2.22, and page #257
Conducting laboratory and location: (b) (4)

Date of study initiation: January 20, 2000
GLP compliance: Statements of compliance with GLP regulations and the quality assurance unit were included.
QA reports: yes (x) no ( )
Drug, lot# 894209B
Formulation/vehicle: 0.9% sodium chloride.

Methods:

Species/strain: Crl:CD(SD)BR pregnant rats
Doses employed: 0, 3, 9, and 30 mg/kg
Route of administration: intravenous infusion.
Number/sex/group: 22/group.

Study design:

To study the potential for the developmental defects by VIT-45 in rats, VIT-45 was given by intravenous infusion over 1 hour to rats at doses of 0, 3, 9, and 30 mg/kg once daily from gestation days 6 to 17. All animals were sacrificed on gestation day 20.

Parameters and endpoints evaluated: Clinical signs of toxicity and mortality were observed daily. Body weight and food consumption were determined. At termination, all pregnant females were examined for the followings: number of corpora lutea, gravid uterus weight, number and position of live fetuses, dead fetuses, resorptions, external appearance of fetuses, fetus weight, placental weight and number of ribs. Each fetus was then sacrificed for external, skeletal and visceral examinations.

Results:

Mortality: There were no deaths.

Clinical signs: There were no treatment related clinical signs of toxicity.

Body weight**:** The mean body weights on gestation days 6 and 20 in the control females were 269 and 378 g, respectively. The body weight gain during the treatment period of gestation days 6-17 was lower (18-26%) in the high dose group as compared to the control.

Food consumption: The average food consumption in the control groups was 25-31 g/rat/day. The food consumption during the treatment period of gestation days 6-17 was slightly lower (21-29 g/rat/day) in the high dose group.

Necropsy: Orange/brown discoloration in various organs was noted in treated dams in all groups as a result of iron infusion. Swollen or enlarged liver was found in the mid and high dose groups.

Litter Data: There were no significant treatment related changes in pre- and post implantation loss, litter size, and sex ratio.

Malformation: There were no treatment related changes on the minor and major malformations. The results were summarized in Tables 7, 8, and 9 on pages 286-288 in Volume 2.22. These tables are attached below.

TABLE 7

Fetal examinations – major abnormalities – group incidences

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex ----------		
Dosage (mgFe/kg/day)	:	0	3	9	30

	Fetuses				Litters			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (Iron Dextrin Complex mgFe/kg/day)	**0**	**3**	**9**	**30**	**0**	**3**	**9**	**30**
Number examined	283	299	303	316	22	22	22	22
Number affected	2	2	2	2	2	1	2	2
Microphthalmia	-	1	-	-	-	1	-	-
Microphthalmia with retinal fold	-	-	-	1	-	-	-	1
Multiple thoracic irregularities, scoliosis	1	-	-	-	1	-	-	-
Thoracolumbar vertebral irregularities	-	-	1	-	-	-	1	-
Thickened/kinked/irregular ossification ribs	-	-	-	1	-	-	-	1
Small diaphragmatic hernia	1	-	-	-	1	-	-	-
Lumbar scoliosis, spina bifida, absent kidney, ureter, ovary, uterine horn, additional forelimb, forelimb flexure	-	-	1	-	-	-	1	-
Interrupted vertebral column sacrocaudal region, imperforate anus	-	1	-	-	-	1	-	-

TABLE 8

Fetal examinations – minor skeletal abnormalities/variants – group incidences

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex ----------		
Dosage (mgFe/kg/day)	:	0	3	9	30

		Fetuses				Litters			
Group		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (Iron Dextrin Complex mgFe/kg/day)		**0**	**3**	**9**	**30**	**0**	**3**	**9**	**30**
Number examined		142	150	149	158	22	22	22	22
Cranial	sutural bone	1	2	4	-	1	2	3	-
	interparietal fissure	-	1	-	-	-	1	-	-
	bipartite ossification supra-occipital	-	-	-	1	-	-	-	1
Vertebral element abnormality									
	thoracic	3	1	2	1	3	1	2	1
Ribs	thickened/kinked	-	1	-	8	-	1	-	5
	partially fused	-	1	-	-	-	1	-	-
Sternebrae	offset	-	-	2	-	-	-	2	-
	misshapen	-	-	-	1	-	-	-	1
	bifurcated xyphisternum	-	1	-	-	-	1	-	-
Total affected by one or more of the above		4	7	8	9	4	6	7	6
Rib and vertebral configuration									
Cervical rib		1	-	2	3	1	-	2	2
Short/rudimentary 13th rib		-	1	-	1	-	1	-	1
Number with 13/14 or 14/14 ribs		16	19	6	14	10	9	6	11
Offset alignment pelvic girdle		-	1	-	2	-	1	-	2
Incomplete ossification									
Cranial centres		4	8	4	10	4	7	3	7
Vertebrae	cervical	2	1	1	1	2	1	1	1
	thoracic	4	3	7	5	3	3	6	4
	sacrocaudal	9	4	8	1	5	3	3	1
Sternebrae	5th and/or 6th	59	80	78	81	18	20	20	22
	other	4	2	-	3	4	2	-	2
	total	59	81	78	81	18	20	20	22
Pelvic bones		5	2	3	1	3	1	2	1
Metacarpals/metatarsals		3	-	-	2	3	-	-	2
Generalised incomplete ossification		-	-	-	1	-	-	-	1
Precocious ossification									
Cervical vertebral centra (>3 ossified)		13	13	2	11	6	5	1	5

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

TABLE 8 - continued

Fetal examinations – minor skeletal abnormalities/variants – group incidences

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex -------		
Dosage (mgFe/kg/day)	:	0	3	9	30

	Fetuses				Litters			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (Iron Dextrin Complex mgFe/kg/day)	**0**	**3**	**9**	**30**	**0**	**3**	**9**	**30**
Number examined	142	150	149	158	22	22	22	22
Additional observations at necropsy								
Renal cavitation	9	1	2	4	5	1	2	1
Hydroureter	9	1	2	4	3	1	2	2
Shiny skin	1	-	-	-	1	-	-	-
Dark adrenals	-	3	-	-	-	2	-	-
Left umbilical artery	-	1	-	-	-	1	-	-

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

TABLE 9

Fetal examinations – minor visceral abnormalities – group incidences

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex --------		
Dosage (mgFe/kg/day)	:	0	3	9	30

		Fetuses				Litters			
Group		**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Dose (Iron Dextrin Complex mgFe/kg/day)		**0**	**3**	**9**	**30**	**0**	**3**	**9**	**30**
Number examined		139	147	152	156	22	22	22	22
Number affected		25	28	37	29	12	17	17	17
Brain	dilated cerebral aqueduct	-	-	1	-	-	-	1	-
Eye(s)	variation in size	2	1	-	1	1	1	-	1
Thyroid	rudimentary	1	-	-	-	1	-	-	-
Thymus	partially undescended	-	-	1	-	-	-	1	-
Innominate artery	absent/rudimentary	-	1	1	1	-	1	1	1
Heart	small ventricular septal defect	-	-	1	-	-	-	1	-
Diaphragm	thin with protruding liver	4	7	3	4	3	4	3	2
Liver	additional lobe	-	1	1	-	-	1	1	-
	protrusion	-	1	1	-	-	1	1	-
Adrenal	dark	-	1	-	-	-	1	-	-
Kidney(s)	rudimentary/absent papilla	3	2	-	1	2	2	-	1
	misshapen	-	1	-	-	-	1	-	-
Ureter(s)	dilated	11	1	1	1	7	1	1	1
Umbilical artery	left sided	2	-	1	-	2	-	1	-
Testis(es)	displaced	2	2	6	5	2	2	5	5
Tail	short	-	1	-	-	-	1	-	-
Haemorrhages									
Brain/spinal cord		1	1	3	-	1	1	2	-
Subcutaneous		1	8	13	6	1	6	6	4
Other	dorsal fat pad	-	2	-	1	-	2	-	1
	intra-abdominal	-	3	6	6	-	3	5	5
	within liver	3	-	7	6	3	-	4	6
	within kidney	-	1	-	-	-	1	-	-

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded.

Sponsor did not provide historical control data for this strain of rat in the testing laboratory.

Key study findings: Treatment with a dose of 3 to 30 mg/kg/day (18 to 1800 mg/mm^2) VIT-45 reduced body weight gain and food consumption in the high dose group. VIT-45 was not teratogenic in this study.

Study Title: Study of the effects on embryo-fetal toxicity in the pregnant rabbit by intravenous infusion administration

Study no.: VFR049/004349
Volume # 2.23, and page # 1:
Conducting laboratory and location: (b) (4)

Date of study initiation: August 5, 2000
GLP compliance: Statements of compliance with GLP regulations and the quality assurance unit were included.
QA reports: yes (x) no ( )
Drug, lot #894209B.
Formulation/vehicle: 0.9% sodium chloride.

Methods:

Species/strain: Females (3.45-5.55 kg, 19-27 weeks old)
New Zealand White rabbits
Doses employed: 4.5, 9, 13.5, and 18 mg/kg/day.
Route of administration: intravenous infusion.

Study design: To study the potential teratogenic effects of VIT-45 in pregnant rabbits, VIT-45 was given by intravenous infusion to rabbits (22/group) at 0, 4.5, 9, 13.5, and 18 mg/kg/day from gestation days 6 to 19.

Number/sex/group: 22/group (10 rabbits in the group of 18 mg/kg/day).
Parameters and endpoints evaluated: Clinical signs of toxicity and mortality were observed daily. Body weight and food consumption were determined daily. At termination, all pregnant females were examined for the followings: number of corpora lutea, gravid uterus weight, number and position of live fetuses, dead fetuses, resorptions, external appearance of fetuses, fetus weight, placental weight, number of ribs. Each fetus was then sacrificed for skeletal and visceral examinations.

Results:

Mortality: Two dams each in groups of 4.5, 13.5, and 18 mg/kg/day were either found dead or sacrificed. In addition, one dam each in groups of 4.5, 9, and 18 mg/kg/day and two in the group of 13.5 mg/kg/day aborted and these dams were also sacrificed prior to the scheduled termination. Prior to death or sacrifice, the dams in the 13.5 and 18 mg/kg groups had lost weight and consumed little food. Most of these dams had liver tinged orange with accentuated lobular pattern. However, these changes were not found at the two low doses of 4.5 and 9 mg/kg. No death or abortion was found in the control.

Clinical signs: Orange/brown eyelids were noted in the treated rabbits at 18 mg/kg/day. Brown/dark pinna was noted in the treated rabbits at 9 and 13.5 mg/kg/day.

Body weight: The mean body weights on gestation days 0 and 19 in the control females were 4.6 and 4.8 kg, respectively. There were no clear treatment related changes on the body weight gain in the survived animals during treatment period.

Food consumption: The average food consumption in the control groups was 169-209 g/animal/day. Food consumption was lower in the high dose group during gestation days 17-19 (105-115 g/rabbit/day) as compared to the control during same period (182-187 g/rabbit/day).

Necropsy: Orange/brown discoloration in various organs were noted in treated dams at 9, 13.5, and 18 mg/kg/day as a result of iron infusion. This was not seen at 4.5 mg/kg/day.

Litter Data:

The pre-implantation loss was higher in the group of 18 mg/kg/day (33%) as compared to the control (18%). There were no significant treatment related changes in other parameters. The results were summarized in Tables 7 and 8 on pages 34 and 35 in Volume 2.23. These tables are attached below.

TABLE 7

Litter data - group values

Group	:	1	2	3	5	4
Compound	:	Control	------- Iron Dextrin Complex -------			
Dosage (mgFe/kg/day)	:	0	4.5	9	13.5	18

Group		Corpora Lutea	Implantations	Resorptions			Live young			Sex ratio (% M)	Implantation loss (%)	
				Early	Late	Total	Male	Female	Total		Pre-	Post-
1	Mean	12.5	10.1	0.9	1.0	1.9	4.1	4.1	8.2	52.4	18.1	17.4
	SD	3.4	2.9	0.9	1.0	1.3	1.7	2.2	2.3			
	n	22	22	22	22	22	22	22	22	22	22	22
2	Mean	13.1	11.5	0.5	1.4	1.9	4.9	4.7	9.6	51.9	13.1	15.0
	SD	1.9	2.8	0.7	1.2	1.3	1.7	1.8	2.5			
	n	16	16	16	16	16	16	16	16	16	16	16
3	Mean	11.6	9.6	0.6	0.7	1.3	3.9	4.4	8.3	46.8	16.3	14.0
	SD	2.0	3.1	0.8	0.9	1.1	2.0	2.1	3.5			
	n	19	19	19	19	19	19	19	19	19	19	19
5	Mean	12.1	10.6	0.7	0.8	1.4	4.1	5.1	9.2	44.9	12.2	13.7
	SD	2.0	2.7	0.8	1.1	1.5	2.2	2.4	2.7			
	n	16	16	16	16	16	16	16	16	16	16	16
4	Mean	12.1	8.4	0.1	1.4	1.6	3.1	3.7	6.9	42.3	32.7	17.0
	SD	1.9	3.4	0.4	1.2	1.3	2.8	2.2	3.1			
	n	7	7	7	7	7	7	7	7	7	7	7

Malformations:

Three fetuses in 18 mg/kg group and two fetuses in 13.5 mg/kg group had marked domed cranium. The two fetuses at 13.5 mg/kg with marked domed cranium also had marked internal hydrocephaly or suspected hydrocephaly. These malformations were classified as major malformation. One fetus in 9 mg/kg group had moderately domed cranium which was classified was minor skeletal malformation. However, the marked or moderately domed cranium is considered treatment related since the incidence and severity were dose related. There was no instance of domed cranium reported in the 4.5 mg/kg group or in the background control data provided by the sponsor. The results were presented on Tables 9-12 on pages 36-40 in Volume 2.23. These tables are attached below.

TABLE 9

Fetal examinations - major abnormalities - group incidences

	Fetuses				Litters			
Group	**1**	**2**	**3**	**5**	**1**	**2**	**3**	**5**
Dose (Iron Dextrin Complex, mgFe/kg/day)	**0**	**4.5**	**9**	**13.5**	**0**	**4.5**	**9**	**13.5**
Number examined	181#	154	158	147	22	16	19	16
Number affected	2	1	2	3	2	1	2	3
Marked internal hydrocephaly/suspected, marked domed cranium	-	-	-	2	-	-	-	2
Partially fused bilateral frontal, small left orbital socket	1	-	-	-	1	-	-	-
Fused bilateral parietal to interparietal, partially fused bilateral frontal, dilated pulmonary trunk, narrow ascending aorta, retroesophageal aortic arch, large/small atria, misshapen ventricle	-	1	-	-	-	1	-	-
Palatine irregularity, protruding tongue, incompletely ossified cranial bones and vertebral elements, irregularly ossified, kinked and medially thickened ribs, irregularly ossified long bones	-	-	-	1	-	-	-	1
Dilated ascending aorta and aortic arch, transposition of ascending aorta and dorsally displaced pulmonary trunk, displaced ductus arteriosus, ventricular septal defect	-	-	1	-	-	-	1	-
Lumbar scoliosis	-	-	1	-	-	-	1	-
Lumbosacral vertebral irregularities	1	-	-	-	1	-	-	-

Includes 1 early birth

TABLE 10

Fetal examinations - minor skeletal abnormalities/variants - group incidences

		Fetuses				Litters			
Group		**1**	**2**	**3**	**5**	**1**	**2**	**3**	**5**
Dose (Iron Dextrin Complex mgFe/kg/day)		**0**	**4.5**	**9**	**13.5**	**0**	**4.5**	**9**	**13.5**
Number examined		179#	153	156	144	22	16	19	16
Number intact		118	102	105	94	22	16	19	16
Cranial	fissures / extra sutures	-	-	3	-	-	-	3	-
	domed cranium	-	-	1	-	-	-	1	-
	unossified area	-	-	1	2	-	-	1	2
	sutural bone	-	2	2	1	-	2	2	1
	small interparietal	1	-	-	-	1	-	-	-
	fused centres	-	1	-	-	-	1	-	-
	bent cornua of hyoid	4	1	1	2	3	1	1	2
Vertebral elements	abnormality								
	cervical	3	-	-	-	2	-	-	-
	thoracic	-	-	1	1	-	-	1	1
	lumbar	-	-	-	1	-	-	-	1
	caudal	1	-	-	1	1	-	-	1
	additional centre	1	-	-	1	1	-	-	1
	scoliosis, minimal	-	-	-	1	-	-	-	1
Ribs	branched/fused	-	-	1	-	-	-	1	-
	medially thickened	1	-	-	-	1	-	-	-
	offset/bent/misshapen	-	-	-	1	-	-	-	1
Sternebrae	additional centre(s)	4	4	11	7	4	3	5	4
	bridge of ossification/fused	-	-	4	-	-	-	3	-
	offset/bipartite	1	2	-	-	1	2	-	-
	bifurcated	1	-	1	-	1	-	1	-
	wide/misshapen	-	-	2	-	-	-	2	-
Costal cartilage	offset	1	1	-	-	1	1	-	-
Scapulae	elongated acromion	1	-	-	1	1	-	-	1
Limb	small claw	1	-	-	-	1	-	-	-
Total affected by one or more of the above		**14**	**9**	**24**	**13**	**10**	**6**	**12**	**8**
Rib and vertebral configuration									
Cervical rib		-	-	1	5	-	-	1	2
Short 12th rib		-	-	1	-	-	-	1	-
Number with 12/13 or 13/13 ribs		124	102	100	109	21	16	18	16
18 thoracolumbar vertebrae		-	-	1	-	-	-	1	-
20 thoracolumbar vertebrae		47	33	33	56	15	12	12	13
Offset alignment pelvic girdle		5	12	14	7	5	9	10	7

Includes 1 early birth

Note: Individual fetuses/litters may occur in more than one category. Fetuses with major abnormalities excluded

TABLE 11

Fetal examinations - minor visceral abnormalities - group incidences

		Fetuses				Litters			
Group		**1**	**2**	**3**	**5**	**1**	**2**	**3**	**5**
Dose (Iron Dextrin Complex, mgFe/kg/day)		**0**	**4.5**	**9**	**13.5**	**0**	**4.5**	**9**	**13.5**
Number heads examined at detailed visceral		61	51	51	50	22	16	17	16
Head	dilated orbital sinus	2	-	-	-	2	-		
	folded retina	-	3	1	-	-	2	1	-
Subdural haemorrhage cerebellum		1	-	-	1	1	-	-	1
Number of heads affected		**3**	**3**	**1**	**1**	**3**	**2**	**1**	**1**
Number examined at necropsy		179#	153	156	144	22	16	19	16
Eyes	lenticular opacity	-	1	-	-	-	1	-	-
Lungs	atelectatic	1	-	1	1	1	-	1	1
Abdomen	fluid in	2	-	-	3	1	-	-	1
Gall bladder	small	-	-	-	1	-	-	-	1
	haemorrhage on	3	5	5	3	3	4	5	2
	adjacent cyst	1	-	-	-	1	-	-	-
Stomach	gas in	1	1	-	-	1	1	-	-
	content dark	-	-	2	1	-	-	1	1
Limbs and tail	haemorrhagic	1	-	-	-	1	-	-	-
Number of fetuses affected		**8**	**6**	**8**	**9**	**5**	**5**	**6**	**5**

Includes 1 early birth

TABLE 12

Fetal examinations - placental abnormalities - group incidences

		Fetuses				Litters			
Group		1	2	3	5	1	2	3	5
Dose (Iron Dextrin Complex, mgFe/kg/day)		0	4.5	9	13.5	0	4.5	9	13.5
Number examined at necropsy		181#	154	158	147	22	16	19	16
Amniotic sac	milky fluid in	1	-	-	-	1	-	-	-
	tinged orange	-	-	-	55	-	-	-	6
Placenta	milky fluid around	1	1	-	-	1	1	-	-
	maternal portion tinged orange	-	-	-	95	-	-	-	10
	pale and swollen	-	-	-	9	-	-	-	1
Number of fetuses affected		1	1	-	122	1	1	-	13

Includes 1 early birth

Key study findings: VIT-45 at doses of 4.5, 9, 13.5 and 18 mg/kg induced abortion and maternal toxicity. Treatment with VIT-45 produced markedly domed cranium and hydrocephaly at 18 mg/kg group and 13.5 mg/kg treatment groups and these malformations were the major malformations. One fetus in 9 mg/kg group had moderately domed cranium which was classified as minor skeletal malformation. All doses were maternally toxic so a NOEL for maternal toxicity was not defined. The NOEL for fetal effects was 4.5 mg/kg/wk.

Study Title: Study of the effects on the Peri- and Post-natal development in CD rat by intravenous infusion administration.

Study no.: VFR052/013368
Volume # 2.24, and page # 1:
Conducting laboratory and location: (b) (4)

Date of study initiation: September 19, 2000
GLP compliance: Statements of compliance with GLP regulations and the quality assurance unit were included.
QA reports: yes (x) no ( )
Drug, lot #894209B
Formulation/vehicle: 0.9% sodium chloride.

Methods:

Species/strain: Females: Crl:CD(SD)IGS BR rats (240-300 g, 11-13 weeks old).
Doses employed: 0, 3, 9, and 18 mg/kg/day.
Route of administration: intravenous infusion over 1 hour.
Study design: To study the potential effects of VIT-45 on the prenatal and postnatal reproductive functions in rats, VIT-45 was given by intravenous infusion over 1 hour to rats from gestation days 6-19, and on days 1, 4, 7, 10, and 14 of lactation. The tested doses were 0, 3, 9, and 18 mg/kg/day.
Number/sex/group: 22/group.
Parameters and endpoints evaluated: In this study, the treated rats were observed for clinical signs of toxicity and body weight daily. Food consumption was recorded during gestation. All pregnant rats were allowed to litter and rear their offspring for assessment of the offspring growth, survival and development. Dams were then sacrificed on or shortly after post partum day 25. The litters were also sacrificed and examined macroscopically.

Results:

Dams:

Mortality: There were no deaths in this study.

Clinical signs: There were no treatment related changes.

Body weight: The mean body weights on gestation day 6 and (gestation days 6-10) was lower in the high dose group as compared to the control as the individual weight gain was 8 g in treated rats and it was 14 g in untreated animals. The terminal body weight changes in the treatment groups were comparable to the control.

Food consumption: The average food consumption was 29-84 g/rat/day in the control group. There were no treatment related changes.

Necropsy: Orange discoloration in various organs was noted in treated dams in all treatment groups as a result of iron infusion.

Litter Data:

There were no treatment related changes in the gestation length, litter size, number of implants, viability index, live birth index, and post-implantation survival index. The results were summarized in Tables 7-9 on pages 50-52 in Volume 2.24. These tables are attached below.

TABLE 7

Gestation length and gestation index – group values (F0)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)	:	0	3	9	18

Group	Number of pregnant animals		Gestation length (days)			Number of live litters born	Gestation index (%)
			22	22.5	23		
1	22	n	18	2	2	22	100
		(%)	(82)	(9)	(9)		
2	22 A	n	12	5	4	21	95
		(%)	(57)	(24)	(19)		
3	22 B	n	16	5	0	20	91
		(%)	(76)	(24)			
4	22	n	15	4	3	22	100
		(%)	(68)	(18)	(14)		

n Number of animals in category

A Percentage distribution of gestation lengths calculated from 21 animals - one pregnant female killed for reasons of animal welfare on Day 22 after mating

B Percentage distribution of gestation lengths calculated from 21 animals - one pregnant female failed to litter

TABLE 8

Macropathology – group incidence of principal findings (F0)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)	:	0	3	9	18

Macropathology findings	Females affected			
Group:	1	2	3	4
Dosage (mgFe/kg/day)	0	3	9	18
Number of females examined	22	22	22	22
Liver: tinged orange	0	0	2	10
Spleen: tinged orange	0	0	0	8
Lymph nodes: tinged orange	0	5	12	12
Adrenal glands: tinged orange	0	0	2	11
Ovaries: tinged orange	0	0	0	1
Uterus: implantation scars tinged orange	0	0	1	2
Uterus: tinged orange	0	0	0	6
Mammary tissue: tinged orange	0	0	1	0

TABLE 9

Litter size – group mean values (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)	:	0	3	9	18

Group		Implants	Total litter size	Live litter size on Day of age								
				Before cull		After cull						
			Day 1	1	4	4	7	10	14	18	21	28
1	Mean	16.1	15.1	15.0	14.8	10.0	9.9	9.8	9.8	9.8	9.8	9.8
	SD	1.5	1.8	1.7	1.8	0.0	0.5	0.5	0.5	0.5	0.5	0.5
	n	22	22	22	22	22	22	22	22	22	22	22
2	Mean	16.3	15.5	15.3	15.0	10.0	10.0	9.9	9.8	9.8	9.8	9.7
	SD	1.9	2.0	1.9	2.1	0.0	0.2	0.4	0.4	0.4	0.4	0.6
	n	21	21	21	21	21	21	21	21	21	21	20
3	Mean	15.6	15.1	15.0	14.0	9.6	9.6	9.6	9.6	9.6	9.6	9.6
	SD	2.0	2.1	2.1	3.7	1.8	1.8	1.8	1.8	1.8	1.8	1.8
	n	20	20	20	20	20	20	20	20	20	20	20
4	Mean	15.5	14.8	14.8	14.4	10.0	10.0	10.0	9.9	9.9	9.9	9.9
	SD	1.6	1.6	1.6	1.7	0.0	0.2	0.2	0.3	0.3	0.3	0.3
	n	22	22	22	22	22	22	22	22	22	22	22

SD Standard deviation
n Number of litters

F1 generation: The physical signs of the offspring were not affected. There were no treatment related changes in viability, body weight, physical and reflexological development, and sex ratio. The results were summarized in Tables 10-11, 16, 25-31 on pages 53-54, 59, 68-76 in Volume 2.24. These tables are attached below.

TABLE 10

Offspring survival indices – group mean values (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)	:	0	3	9	18

Group		Post-implantation survival index %	Live birth index %	Viability index (%)	Lactation index (%)					
					7	10	14	18	21	28
1	Mean	93.8	99.5	98.5	98.6	98.2	98.2	98.2	98.2	98.2
	n	22	22	22	22	22	22	22	22	22
2	Mean	95.0	98.9	97.5	99.5	98.6	97.6	97.6	97.6	97.0
	n	21	21	21	21	21	21	21	21	20
3	Mean	96.7	99.1	93.0	100.0	99.5	99.5	99.5	99.5	99.5
	n	20	20	20	20	20	20	20	20	20
4	Mean	95.1	100.0	97.2	99.5	99.5	99.1	99.1	99.1	99.1
	n	22	22	22	22	22	22	22	22	22

n Number of litters

TABLE 11

Sex ratio – group mean values (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------	
Dosage (mgFe/kg/day)	:	0	3	9	18

Group		Total Day			Live (before cull)						Live (after cull)					
		1			1			4			4			21		
		M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
1	Mean	7.9	7.2	52.0	7.9	7.2	52.0	7.8	7.0	52.6	5.0	5.0	49.5	4.9	5.0	49.6
	SD	1.7	1.5	9.6	1.7	1.4	9.4	1.7	1.5	9.7	0.5	0.5	4.9	0.6	0.7	5.8
	n	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
2	Mean	8.2	7.3	53.4	8.1	7.2	53.6	8.0	7.0	53.8	5.2	4.8	51.9	5.0	4.7	51.7
	SD	1.8	2.6	12.7	1.8	2.3	12.1	1.7	2.3	11.5	0.4	0.4	4.0	0.5	0.5	4.4
	n	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
3	Mean	7.3	7.8	48.8	7.3	7.7	49.0	7.0	7.1	50.5	4.7	5.0	48.5	4.7	4.9	48.8
	SD	2.3	2.7	15.2	2.3	2.7	15.1	2.7	3.2	16.2	1.4	1.5	11.4	1.4	1.5	11.5
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
4	Mean	7.3	7.5	49.3	7.3	7.5	49.3	7.0	7.3	49.2	5.0	5.0	49.5	4.9	5.0	49.5
	SD	1.5	1.6	9.2	1.5	1.6	9.2	1.6	1.8	10.4	0.4	0.4	3.8	0.4	0.4	4.1
	n	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22

SD Standard deviation
n Number of litters

TABLE 16

Pre-weaning development – group mean values for offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)	:	0	3	9	18

Pre-weaning assessment	Group			
	1	2	3	4
Surface righting (age of attainment)				
Mean (Day of age)	4.9	5.0	5.4	5.4
SD	0.7	1.1	0.9	1.1
n	22	21	20	22
Air righting (age of attainment)				
Mean (Day of age)	15.2	15.3	15.5	15.6
SD	0.5	0.5	0.6	0.6
n	22	21	20	22
Pupil reflex				
Percentage successful	100.0	98.9	100.0	99.1
n	22	21	20	22
Startle response				
Percentage successful	100.0	100.0	100.0	100.0
n	22	21	20	22

SD Standard deviation
n Number of litters
Surface righting: no statistical significance ($p>0.05$)

TABLE 25

Sexual development – group mean age and bodyweight at attainment for males and females (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)#	:	0	3	9	18

Group		Vaginal opening (Day of age)	Bodyweight (g) at vaginal opening	Preputial separation (Day of age)		Bodyweight (g) at	
				onset	completion	onset	completion
1	Mean	34.0	115	36.7	44.1	153	221
	SD	1.3	12	1.4	1.4	16	19
	n	20	20	20	19	20	19
2	Mean	34.4	113	36.5	44.9	146	222
	SD	1.5	12	1.2	2.3	15	18
	n	20	20	20	20	20	20
3	Mean	34.5	110	36.8	45.0	141	212
	SD	2.1	12	1.2	3.2	14	16
	n	20	20	20	20	20	20
4	Mean	34.2	110	37.3	45.2	152	225
	SD	2.0	12	1.6	2.6	12	20
	n	20	20	20	20	20	20

\# Treatment refers to F0 females – F1 generation untreated
SD Standard deviation
n Number of animals

TABLE 26

Motor activity – group mean scores for offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)#	:	0	3	9	18

Group /sex	Number of animals	Beam level	Time (minutes)										
			6	12	18	24	30	36	42	48	54	60	Total
1M	20	High	19.4	11.7	7.7	6.3	2.7	1.3	2.4	3.2	3.1	2.8	60.4
		SD	13.9	9.4	6.8	9.5	5.1	3.0	3.2	6.2	7.3	4.7	40.2
		Low	79.8	44.1	39.4	29.8	13.7	12.6	13.4	15.7	14.4	16.3	279.1
		SD	23.9	19.6	27.5	24.6	20.0	20.3	19.1	26.8	21.0	21.5	141.9
2M	20	High	21.5	11.4	8.3	6.3	5.2	6.9	5.8	4.3	3.8	5.3	78.6
		SD	10.3	6.4	8.1	5.8	6.4	9.8	10.2	6.9	5.8	7.4	54.5
		Low	77.2	45.3	38.0	32.2	25.1	29.9	24.0	25.0	27.2	22.5	346.2
		SD	28.8	25.0	24.6	23.2	26.8	30.4	29.1	30.7	26.5	28.4	189.8
3M	20	High	18.7	7.3	7.3	3.2	4.3	3.1	3.4	1.2	1.3	1.4	51.0
		SD	13.7	6.9	7.2	4.0	5.7	4.2	5.5	2.3	2.6	3.6	29.8
		Low	86.9	40.3	39.8	25.5	26.5	27.0	23.0	15.6	14.8	16.7	315.8
		SD	36.4	22.9	33.4	26.8	27.6	34.5	36.5	27.0	23.1	31.0	224.6
4M	20	High	23.9	15.3	6.2	6.1	2.8	2.1	2.5	1.6	2.1	0.7	63.1
		SD	15.1	9.6	7.5	7.6	4.4	2.9	5.1	2.7	4.8	2.2	28.2
		Low	86.4	49.0	35.0	29.1	26.1	21.5	14.0	14.3	11.2	7.6	293.9
		SD	23.3	21.8	26.5	27.1	22.8	23.0	21.7	22.4	22.8	20.4	144.6

\# Treatment refers to F0 females – F1 generation untreated

TABLE 26 - continued

Motor activity – group mean scores for offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)#	:	0	3	9	18

Group /sex	Number of animals	Beam level	Time (minutes)										
			6	12	18	24	30	36	42	48	54	60	Total
1F	20	High	19.1	11.4	8.9	6.2	3.3	4.7	3.5	4.1	4.2	5.8	71.0
		SD	15.3	8.7	6.8	8.8	4.5	6.4	5.1	4.9	7.9	13.3	49.8
		Low	79.3	44.1	38.9	31.7	24.4	20.2	16.8	22.3	20.0	20.8	318.2
		SD	29.8	15.3	24.3	31.4	25.6	26.6	17.9	23.7	24.2	25.3	164.3
2F	20	High	18.1	8.4	4.0	4.1	4.4	3.7	3.5	2.0	2.0	1.6	51.6
		SD	16.8	6.9	4.6	5.6	7.7	8.6	6.7	3.7	5.0	3.6	41.8
		Low	82.4	43.5	27.4	19.8	24.3	21.7	21.3	13.3	13.7	10.2	277.4
		SD	30.2	31.7	20.5	23.2	31.9	25.1	29.0	17.4	22.8	16.0	141.7
3F	20	High	12.8	8.4	7.5	3.5	2.5	3.8	2.2	2.3	1.0	2.2	46.0
		SD	8.4	6.7	7.0	4.5	3.9	5.0	3.1	3.7	2.4	4.5	23.7
		Low	72.6	39.1	29.1	27.3	23.8	24.6	23.9	18.4	8.7	15.3	282.6
		SD	24.0	23.2	25.4	33.5	24.0	21.8	28.6	23.5	15.0	22.7	171.6
4F	20	High	18.3	10.1	8.2	4.5	5.4	4.7	2.5	3.9	2.8	4.3	64.4
		SD	10.8	6.2	7.4	6.4	6.5	8.6	4.7	7.5	5.2	8.1	40.7
		Low	89.6	42.1	40.4	27.2	23.6	20.4	19.1	19.6	11.2	18.0	311.1
		SD	32.6	21.2	28.4	24.4	18.6	27.3	26.8	24.3	16.2	20.8	154.2

Treatment refers to F0 females – F1 generation untreated

TABLE 27

Morris water maze performance – group mean values for offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)#	:	0	3	9	18

Group	Number of animals		Day 1			Day 2			Day 3			Day 4		
			T	F	S	T	F	S	T	F	S	T	F	S
1M	20	Mean	58.5	1.3	16.9	37.8	0.5	11.4	22.8	0.2	7.9	18.9	0.1	6.4
		SD	21.1	1.0	4.7	17.2	0.5	4.2	10.2	0.4	3.3	12.6	0.3	3.0
		%		80.0			50.0			15.0			10.0	
2M	20	Mean	59.2	1.4	16.8	32.9	0.2	10.4	29.8	0.3	9.0	26.9	0.3	9.5 a
		SD	19.1	0.8	4.6	13.2	0.4	3.2	18.6	0.6	4.3	16.4	0.6	4.6
		%		90.0			20.0			15.0			20.0	
3M	20	Mean	58.6	1.3	17.8	42.3	0.6	13.5	34.9	0.2	11.4	17.4	0.1	6.4
		SD	15.8	0.8	4.9	20.2	0.7	5.3	21.0	0.7	5.2	8.8	0.3	2.6
		%		85.0			50.0			10.0			10.0	
4M	20	Mean	61.3	1.5	17.4	33.1	0.5	10.7	25.1	0.3	8.1	14.8	0.1	5.5
		SD	14.4	0.8	3.4	17	0.6	4.7	17.1	0.6	4.4	9.9	0.2	3.2
		%		90.0			40.0			20.0			5.0	

\# Treatment refers to F0 females – F1 generation untreated
T Trial time (seconds: mean of 3 trials)
F Number of failed trials (90 seconds)
S Sector entries (mean of 3 trials)
% Percentage of animals with at least 1 failed trial (90 seconds)
a Significant when compared with Group 1: a - $p<0.05$

TABLE 27 - continued

Morris water maze performance – group mean values for offspring (F1)

Group : 1 2 3 4
Compound : Control ---------- Iron Dextrin Complex----------
Dosage (mgFe/kg/day)# : 0 3 9 18

Group	Number of animals		Day 1			Day 2			Day 3			Day 4		
			T	F	S	T	F	S	T	F	S	T	F	S
1F	20	Mean	61.7	1.4	16.9	33.9	0.3	10.2	33.1	0.3	9.8	21.8	0.1	7.0
		SD	16.3	0.8	5.8	14.8	0.6	3.6	20.6	0.6	5.4	12.1	0.3	3.1
		%		95.0			25.0			25.0			10.0	
2F	20	Mean	58.5	1.2	17.2	36.5	0.5	11.5	27.4	0.2	9.2	23.0	0.1	7.4
		SD	19.3	0.9	4.2	17.0	0.7	4.3	17.6	0.4	5.1	16.4	0.3	4.2
		%		80.0			35.0			20.0			10.0	
3F	20	Mean	60.1	1.3	17.1	38.0	0.4	12.2	27.4	0.2	8.7	18.5	0.0	6.1
		SD	16.9	0.9	4.6	16.3	0.5	4.0	13.5	0.4	3.9	10.3	0.0	2.5
		%		80.0			40.0			15.0			0.0	
4F	20	Mean	61.6	1.3	17.2	36.4	0.3	11.9	31.1	0.3	10.0	20.1	0.2	6.9
		SD	16.0	0.9	4.3	17.8	0.5	4.4	18.6	0.6	4.1	18.6	0.7	4.1
		%		80.0			30.0			25.0			5.0	

\# Treatment refers to F0 females – F1 generation untreated
T Trial time (seconds: mean of 3 trials)
F Number of failed trials (90 seconds)
S Sector entries (mean of 3 trials)
% Percentage of animals with at least 1 failed trial (90 seconds)

TABLE 28

Accelerating rotarod – group mean times for offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)#	:	0	3	9	18

Group	Number of animals		Maximum time achieved*	
			Males	Females
1	20a	Mean	209	208
		SD	33	26
2	20	Mean	202	215
		SD	32	36
3	20	Mean	202	212
		SD	35	32
4	20	Mean	206	210
		SD	33	36

\# Treatment refers to F0 females – F1 generation untreated
SD Standard deviation
* During three trials (300 seconds maximum).
a 19 females only - one animal excluded from group mean calculation.

TABLE 29

Pre-coital interval – group values (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)#	:	0	3	9	18

Group	Number of animals		Pre-coital interval (days)				
			1-4	5-8	9-12	13-16	17-21
1	20	n	19	1	0	0	0
		(%)	(95)	(5)			
2	20	n	20	0	0	0	0
		(%)	(100)				
3	20	n	19	1	0	0	0
		(%)	(95)	(5)			
4	20	n	20	0	0	0	0
		(%)	(100)				

\# Treatment refers to F0 females – F1 generation untreated
n Number of animals in category

TABLE 30

Mating performance and fertility – group values (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)#	:	0	3	9	18

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1M	20	20	20	100	100	100
2M	20	20	19	100	95	95
3M	20	20	19	100	95	95
4M	20	20	20	100	100	100
1F	20	20	20	100	100	100
2F	20	20	19	100	95	95
3F	20	20	19	100	95	95
4F	20	20	20	100	100	100

\# Treatment refers to F0 females – F1 generation untreated

TABLE 31

Gestation length and gestation index – group values (F1)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)#	:	0	3	9	18

Group	Number of pregnant animals		Gestation length (days)				Number of live litters born	Gestation index (%)
			21.5	22	22.5	23		
1	20	n	0	15	3	2	20	100
		(%)		(75)	(15)	(10)		
2	19	n	0	9	7	3	19	100
		(%)		(47)	(37)	(16)		
3	19	n	0	11	6	2	19	100
		(%)		(58)	(32)	(11)		
4	20	n	1	8	8	3	20	100
		(%)	(5)	(40)	(40)	(15)		

\# Treatment refers to F0 females – F1 generation untreated
n Numbers of animals in category

F2-generation:

No adverse effects were noted in the F2 generation of animals.
The results were summarized in Tables 32-34 on pages 77-79 in Volume 2.24. These tables are attached below.

TABLE 32

Litter size – group mean values (F2)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)#	:	0	3	9	18

Group		Implants	Total litter size Day 1	Live litter size on Day of age				
				Before cull		After cull		
				1	4	4	7	10
1	Mean	15.6	14.4	14.4	13.8	10.0	9.6	9.4
	SD	1.4	1.6	1.7	1.9	0.0	1.4	1.7
	n	19	19	19	19	19	19	19
2	Mean	15.3	14.5	14.5	14.1	9.9	9.6	9.5
	SD	2.0	2.3	2.3	2.3	0.5	1.1	1.2
	n	19	19	19	19	19	19	19
3	Mean	15.0	14.2	13.9	13.3	9.9	9.7	9.6
	SD	2.2	2.2	2.3	2.5	0.3	0.6	0.8
	n	19	19	19	19	19	19	19
4	Mean	14.9	13.8	13.7	13.5	10.0	10.0	10.0
	SD	1.3	1.7	1.7	1.7	0.0	0.2	0.2
	n	20	20	20	20	20	20	20

\# Treatment refers to F0 females – F1 generation untreated
SD Standard deviation
n Number of litters
No statistical significance ($p>0.05$) – implants and litter size on Day 1 analysed

TABLE 33

Offspring survival indices – group mean values (F2)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex---------		
Dosage (mgFe/kg/day)#	:	0	3	9	18

Group		Post-implantation survival index (%)	Live birth index (%)	Viability index (%)	Lactation index (%)	
					7	10
1	Mean	91.9	99.6	96.5	95.8	94.2
	n	19	19	19	19	19
2	Mean	94.6	100.0	97.3	96.8	95.8
	n	19	19	19	19	19
3	Mean	94.9	98.0	95.6	97.8	96.7
	n	19	19	19	19	19
4	Mean	92.8	99.3	98.6	99.5	99.5
	n	20	20	20	20	20

\# Treatment refers to F0 females – F1 generation untreated
n Number of litters

TABLE 34

Sex ratio – group mean values (F2)

Group	:	1	2	3	4
Compound	:	Control	---------- Iron Dextrin Complex----------		
Dosage (mgFe/kg/day)#	:	0	3	9	18

Group		Total Day			Live (before cull)						Live (after cull)					
		1			1			4			4			10		
		M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
1	Mean	7.7	6.7	53.8	7.7	6.6	54.0	7.5	6.4	53.8	4.9	5.1	49.5	4.7	4.7	49.4
	SD	2.0	2.2	13.4	2.0	2.1	13.1	2.1	2.0	13.3	0.7	0.7	7.1	1.1	1.1	9.0
	n	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
2	Mean	6.8	7.7	47.1	6.8	7.7	47.1	6.6	7.5	46.6	4.9	4.9	50.1	4.7	4.8	49.7
	SD	2.0	1.9	11.3	2.0	1.9	11.3	2.1	2.0	12.2	0.6	0.8	6.9	0.6	0.9	5.5
	n	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
3	Mean	7.3	6.9	51.8	7.2	6.8	51.9	6.7	6.6	51.6	5.2	4.7	52.2	5.0	4.6	52.6
	SD	2.2	2.4	15.0	2.2	2.6	15.8	2.0	2.7	15.4	1.1	1.1	11.1	1.1	1.3	12.1
	n	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
4	Mean	7.2	6.6	52.4	7.1	6.6	52.1	7.1	6.5	52.5	5.1	4.9	51.0	5.1	4.9	50.7
	SD	1.9	2.1	12.3	1.8	2.1	12.1	1.8	2.0	12.0	0.4	0.4	4.5	0.5	0.4	4.7
	n	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

\# Treatment refers to F0 females – F1 generation untreated
SD Standard deviation
n Number of litters

Key study findings: Treatment with VIT-45 at doses up to 18 mg/kg/day did not produce any adverse effects on parturition, lactation, physical and reflexological development of the offspring.

SPECIAL TOXICOLOGY STUDIES:

2.6.6.7 Local tolerance

Intra-arterial tolerance study in rabbits
(VFR 054/032138)

Methods: To determine the local irritation potential of VIT-45, 0.5 ml VIT-45 was given to 4 rabbits by a single intra-arterial injection into auricular artery of the right ear over 30 seconds. Same animals received 0.5 ml of sterile physiological saline in left ears. The animals were observed for 4 days and then sacrificed. The injection sites were evaluated histopathologically.

Results: All animals survived during the study. No clinical signs of toxicity were observed. The results indicated that there were no dermal reactions at the injection sites.

Paravenous tolerance study in rabbits
(VFR 055/032139)

Methods: To determine the local irritation potential of VIT-45, 0.2 ml VIT-45 was given to 4 rabbits by a single paravenous injection alongside the lateral ear vein of the right ear over 30 seconds. Same animals received 0.2 ml of sterile physiological saline in left ears. The animals were observed for 4 days and then sacrificed. The injection sites were evaluated histopathologically.

Results: All animals survived. No clinical signs of toxicity were observed. The results indicated that there were no dermal reactions at the injection sites.

Intravenous tolerance study in rabbits
(VFR 063/032140)

Methods: To determine the local irritation potential of VIT-45, 0.2 ml VIT-45 was given to 4 rabbits by a single intravenous injection into the lateral ear vein of the right ear over 30 seconds. Same animals received 0.2 ml of sterile physiological saline in left ears. The animals were observed for 4 days and then sacrificed. The injection sites were evaluated for microscopic changes.

Results: All animals survived. No clinical signs of toxicity were observed. The results indicated that there were no dermal reactions at the injection sites.

Dextran antigenicity to the rabbit
(VFR 043/003504)

Methods: Passive cutaneous anaphylaxis test was conducted in rabbits. Conjugated dextran with bovine serum albumin was given to rabbits at 0.5 mg/kg once during week 1 and once during week 5. The serum from the rabbits was then injected into shaven back of guinea pigs. These guinea pigs were then re-challenged with intravenous administration of dextran at 50 µg/animal, iron dextrinate at 3 ml/kg, and venofer at 3 ml/kg immediately followed by IV dose of 2% Evan blue. The injection sites on the back of guinea pigs were then examined for a sign of bluing which is used as an indicator of positive response and scored as followings:
0 = no bluing, + = slight bluing, ++ = moderate bluing, and +++ = marked bluing.

Results: The results indicated that Dextran produced positive PCA responses as indicated by marked bluing. However, re-challenge with iron dextrinate or venofer did not produce specific PCA reactions.

2.6.6.8. LABELING:

The proposed labeling generally conforms to the Section 4 of CFR (201.50) to (201.57). Based on the present review, the following changes in the proposed label have been suggested. The proposed suggested modifications to the label have been made by striking out the existing version by the proposed changes.

The changes in the following sections of the non-clinical portions of the proposed label with edits are shown below: (~~strike out~~) indicate the deletion of the original version and the *italic underline* represents addition.

8 USE IN SPECIFIC POPULATIONS

(b) (4)

(b) (4)

OVERALL CONCLUSIONS AND RECOMMENDATIONS:

VIT-45, a polynuclear iron (III)-hydroxide complex, is under clinical development for the parenteral iron replacement therapy in iron deficiency anemia in pregnancy and anemia of chronic diseases. Following IV injection, VIT-45 releases utilizable systemic iron in the liver, spleen, and red blood cells. The sponsor conducted a battery of safety pharmacology tests on central nervous system, cardiovascular system, respiratory system and renal system and it did not show significant effects on these systems of animals other than the expected pharmacologic effects of iron overload. The released iron binds with iron binding proteins and accumulates mainly in animals' blood cells with about 76% in red cells, 11% in liver, 2% in spleen and 1% in kidney. Single intravenous dose of 1 g and 0.24 g in rats and dogs, respectively were not lethal but 2 g dose in mice was lethal.

The studies submitted included preclinical safety pharmacology studies, pharmacokinetic studies, acute intravenous toxicity studies in mice, rats and dogs, 13-week intravenous toxicity studies in rats and dogs, genetic toxicity studies including Ames test, *in vitro* chromosomal aberration test, mouse lymphoma forward mutation assay, and *in vivo* mouse micronucleus test, reproductive toxicity studies including Segment I (rats), II (in rats and rabbits), and III (rats), and special toxicity studies.

The results of pharmacokinetic studies indicated that two weeks after intravenous administration of ^{59}Fe-labeled VIT-45 at 10 mg in rats fed with iron free diet, ~76% of the administered iron was recovered in red blood cell, 11% in the liver, 2% in the spleen, and 1% in kidney. The serum half life was ~1.5 hours in rats. In another pharmacokinetic study, 28 days after intravenous administration of ^{59}Fe-labeled VIT-45 at 10 mg in lactating rats, ~28% of the administered iron was recovered in the red blood cell, 33% in the liver, 1.9% in the spleen, 0.6% in the kidney, and 0.36-0.65% in the milk. The results of this study also indicated that ~0.1-1% of the administered iron was recovered in the offspring via milk. Most of the iron was in the cells and had a long residence time.

In mice, the minimal lethal intravenous dose was 2000 mg/kg. Piloerection, decreased body temperature, and hunched posture were the signs of toxicity prior to death. In rats, the minimal lethal intravenous dose was not identified. The clinical signs of toxicity after single intravenous dose of VIT-45 at 1000 mg/kg were similar to those in mice; with swollen limbs, dark and swollen extremities as additional signs. In dogs, the intravenous doses of 120 and 240 mg/kg were not lethal and the signs of toxicity were dark coloration of the lymph nodes.

The 13-week intravenous toxicity study in rats was conducted at 0, 9, 30, and 90 mg iron/kg VIT-45 by intravenous infusion over 1 hour once a week. The percent reduction in body weight gain was ~19% and 38% in the mid and high dose males and by ~10% in high dose females with increases in alkaline phosphatase (78%), alanine aminotransferase (628%), and aspartate aminotransferase (439%) seen in the high dose males during week 13. Among females, significant increase in alkaline phosphatase

(110%), alanine aminotransferase (50%), and aspartate aminotransferase (49%) was noted in the high dose. The increase in alkaline phosphatase in the high dose males and females was noted from week 6. Total bilirubin (100%) and urea (29%) were significantly increased in the high dose males during week 6. Total bilirubin (50%) and urea (27%) were significantly increased in the high dose females during week 13. Iron deposition was noted in multiple organs including the liver, spleen, lymph nodes, and kidney mainly in the two high dose groups.

In 13-week intravenous toxicity study in CD-rats, VIT-45 was given to 5 groups of rats (10 rats/sex/group) by intravenous bolus injection of 0, 1, 3, 10 and 30 mg/kg 3 times a week (total doses were 0, 3, 9, 30 and 90 mg Fe/kg/week) to compare the findings of an earlier 13-week iv infusion toxicity study and also to simulate the human clinical dose. As in the earlier study, a dose related iron accumulation in the blood cells, liver, spleen and lymph nodes were found in the mid and high dose males and females and, these were associated with increased liver enzyme activities in both sexes. The iron accumulation in macrophages of different organs was dose related and time dependent (> in 13 weeks than 4 weeks) indicating a humoral immune response to T-cell dependent SRBC antigen. Based on the iron deposition noted in multiple organs, the liver, spleen, lymph nodes, and kidney these were identified as the target organs of toxicity.

The 13-week toxicity study in dogs was conducted at intravenous infusion (over 1 hr) doses of 0, 9, 30, and 90 mg iron/kg/week for 13 weeks. Iron deposition was noted in multiple organs including the liver, spleen and kidney mainly in the two high dose groups. One high dose male had necrosis of hepatocytes.

To observe the effect of the compound on chronic 26 weeks use and to determine the reversal of toxicity after 6 weeks, the study was conducted at the doses of 0, 3, 10 and 30 mg Fe/kg/week (day 1, 3 and 5 of each week). The study weekly dose regimen was to simulate the clinical dose in patients. In the recovery phase two groups (2/sex/group) of animals were given 0 (control) and 30 mg/kg/week for 26 weeks. Iron deposition was noted in multiple organs, renal medullary mineralization and glomerular mesangial cells and pigmentation in the Kupffer's cells in liver was present in males and females. The Perl's positive clumps of sinusoidal/phagocytic cells in liver and sinuses of mesenteric node indicated the liver, spleen, and kidney and lymph nodes as target organs of toxicity.

VIT-45 was not mutagenic in the Ames test and mouse lymphoma forward mutation assay and, was non-clastogenic in a vitro chromosomal aberration and mouse micronucleus test.

In the Segment I fertility and reproductive performance study in rats, VIT-45 was given by intravenous infusion to male rats for 4 weeks prior to mating until termination, for a total of 6 weeks (the drug was given three times a week) and to female rats for 2 weeks prior to mating and throughout mating and during gestation and lactation, (the drug was given three times a week), on days 0, 3, and 7 after mating at 0, 3, 9, and 30 mg/kg (18 to 180 mg/mm^2) three times a week. A reduction of body weight gain associated with the reduced food intake was observed in males of the 30 mg/kg group.

Treatment with VIT-45 up to 30 mg/kg/day dose did not affect the fertility and general reproductive performance of the rat.

In the Segment II teratology study in rats, VIT-45 was given by intravenous infusion over 1 hour to rats at doses of 0, 3, 9, and 30 mg/kg (18 to 180 mg/mm^2) once daily from gestation days 6 to 17. Treatment with VIT-45 reduced body weight gain and food consumption in the high dose group. VIT-45 was not teratogenic in this study.

In the Segment II teratology study in rabbits, VIT-45 was given by intravenous infusion to rabbits (22/group) at 0, 4.5, 9, 13.5, and 18 mg/kg/day (36 to 108 mg/mm^2) from gestation days 6 to 19. VIT-45 induced abortion at all doses. Deaths of 2 dams in each of 4.5, 13.5, and 18 mg/kg/day treatment groups were seen. The dams in the 13.5 and 18 mg/kg groups had weight loss and consumed little food before death. Most of these dams had liver tinged orange with accentuated lobular pattern and these changes were not found at the two low doses of 4.5 and 9 mg/kg. There were no clear treatment related changes on the body weight gain in the survived animals during treatment period. Treatment with VIT-45 produced markedly domed cranium in 3 fetuses in 18 mg/kg group and 2 fetuses in 13.5 mg/kg group (maternal toxic doses). The two fetuses at 13.5 mg/kg with marked domed cranium also had marked internal hydrocephaly or suspected hydrocephaly and not in 4.5 mg/kg/day group. The NOEL for maternal toxicity was not identified. However, the NOEL for embryo-fetal toxicity was 4.5 mg/kg/day in the study.

In the Segment III pre- and pos-natal reproductive toxicity study in rats, VIT-45 was given by intravenous infusion over 1 hour to rats from gestation days 6-19, and on days 1, 4, 7, 10, and 14 of lactation. The tested doses were 0, 3, 9, and 18 mg/kg/day (18 to 108 mg/mm^2). Treatment with VIT-45 at doses up to 18 mg/kg/day did not produce any adverse effects on parturition, lactation, physical and reflexological development of the offspring.

The preclinical safety pharmacology studies indicated that VIT-45 did not exert an adverse effect on the CNS, cardiovascular system of conscious rat and dogs and, it did not produce a significant change in QTc intervals in telemetric dogs. Parenterally administered single dose of the compound was absorbed in blood cells for a prolonged period of 10 days and it had a long residence time of 28 days in dogs and rats. The compound was evaluated in 13-week intravenous repeat dose toxicity studies in rats and dogs. In these studies, VIT-45 was given to rats or dogs by intravenous infusion over 1 hour up to the doses of 0, 9, 30, and 90 mg iron/kg once a week for 13 weeks. The major toxicity in the animals was related to iron deposition in organs including the liver, spleen, lymph nodes, and kidney at doses of 30 and 90 mg/kg/week and therefore, the high dose of 90 mg/kg/week was considered as tolerated dose in rats and dogs. The 13-week toxicity study in rats and dogs were conducted to simulate the clinical dose administration. The studies revealed that the VIT-45 produced similar dose related iron deposition in the liver, spleen, lymph nodes, and kidney as seen in earlier 13-week toxicity studies in rats and dog. Similar target organs of major toxicity were seen. The iron deposition in organs including the liver, spleen, lymph nodes, and kidney were noted

in 26-week bolus injection study in dogs. The identified target organs of toxicity of VIT-45 were the liver, spleen, and kidney and lymph nodes. VIT-45 administration did not show mutagenic effects in the Ames test, in vitro chromosomal aberration test, mouse lymphoma forward mutation assay, and mouse micronucleus test and also showed no reproductive toxicity in rats and rabbits.

From preclinical view point, VIT-45 should be approved provided the sponsor amends the proposed label as suggested in the review.

RECOMMENDATIONS:

1. The proposed application of VIT-45 for the proposed indications of treatment of iron deficiency patients secondary to pregnancy (child birth), heavy uterine bleeding, inflammatory bowel disease and hemodialysis should be approved.

2. Sponsor should amend the proposed label by inserting the suggested changes in the present review.

Signatures (optional):

Reviewer Signature _____
(Yash M. Chopra, M.D., Ph.D.)

Supervisor Signature_____ Concurrence Yes ___ No ___
(Adebayo A. Laniyonu, Ph.D.)

APPENDIX/ATTACHMENTS NONE

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

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6/5/2007 12:22:47 PM
PHARMACOLOGIST

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