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Toxicology and safety of Ferrochel and other iron amino acid chelates

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SUMMARY. Iron is estimated to be deficient in the diets of one fifth of the world's population. Iron is commonly provided as a supplemental nutrient in industrialized countries for uses of choice. In other countries of the world, it may be required as an overt addition to the diet to prevent iron deficiency. This may be accomplished through fortification of a common food. As a micronutrient, iron has a relatively narrow range of safety – whether given as a supplement or fortificant, it must be in a high enough dose to be appreciably absorbed, but low enough to avoid toxicity. This concern can be ameliorated by careful choice of the form of iron administered. A source of iron which has proven to be highly bioavailable, yet regulated by dietary need, is iron chelated with amino acids. The structural integrity and longevity of these compounds have been proven by valid chemical and instrumental tests. Proofs of safety of iron amino acid chelate in the dietary administration of iron to swine in both multigenerational and longevity studies are reported. Formal tests of toxicity utilizing ferrous bisglycinate chelate (Ferrochel) carried out in accordance to US-FDA guidelines are also summarized. Ferrochel has been demonstrated to have a No Observable Adverse Effect Level (NOAEL) of at least 500 mg per kg rat body weight, the highest dose tested. This and other results of the detailed toxicity test, as well as other tests of safety and efficacy, have resulted in the US-FDA acknowledging that this product is Generally Recognized As Safe (GRAS) under its approved conditions of use as a source of iron for food enrichment and fortification purposes.
Key words: Iron, Ferrochel, iron amino acid chelate, NOAEL, GRAS.

RESUMEN. Toxicidad e innocuidad de Ferrochel y de otros aminoquelados de hierro. Se estima que el hierro es deficiente en las dietas de una quinta parte de la población del mundo. En paises industrializados el hierro es comúnmente usado como un suplemento dietético; en otros paises la adición de hierro a la dieta se hace necesaria para prevenir la deficiencia de hierro y la anemia ferropriva, lo que se puede lograr fortificando alimentos seleccionados. El hierro presenta límites estrechos de seguridad y cuando se administra ya sea como un suplemento dietético, o como un fortificante, debe darse en una cantidad suficiente para que la cantidad absorbida sea adecuada, y suficientemente baja para prevenir toxicidad. Una fuente de hierro que ha mostrado tener una alta biodisponibilidad y ser regulada por las reservas de hierro del organismo es el hierro quelado con amino ácidos. La innocuidad de este hierro ha sido demostra-da por su administración a generaciones sucesivas de cerdos en los cuales no se ha encontrado ningún efecto nocivo. Pruebas formales de toxicidad del hierro bis-glicinato quelado se han llevado a cabo en ratas siguiendo como guía protocolos del FDA de los E.E.U.U. En estas pruebas se ha encontrado que un nivel de ingesta diaria de 500 mg por kg de peso corporal no producen ningún efecto adverso observable (NOAEL). Con base en estos y otros resultados, el FDA ha reconocido al compuesto como GRAS (Generalmente Reconocido como Innocuo) y ha aprobado su uso para suplementación y fortificación de alimento.
Palabras clave: Hierro, Ferrochel, hierro aminoquelado, NOAEL, GRAS.

INTRODUCTION

Four aspects of iron have long orchestrated the significance of its use in nutrition. The first is that it is absolutely essential to life. It is a required part of hemoglobin, myoglobin, ferredoxins, cytochromes, and several enzymes active in porphyrin synthesis, oxygen regulation and immunity. If iron is absent from its niche within these molecules, they are nonfunctional. The ubiquitous metabolic needs for these molecules make the lives of all aerobic organisms impossible without them. Second, the deliberate intervention of additional bioavailable iron into metabolic systems which are deficient in iron will improve both the functional ability of the metabolites as well as the systems they support, thus reducing iron deficiency and improving the general health of the individual. Third, as a micronutrient, the range of iron sufficiency is relatively narrow. Absorption of iron into the body, transport to its sites of need and the placement of elemental iron into the actual molecules requiring it are subject to narrow ranges of concentration tolerances. Additions of iron which are larger than these tolerances can lead to toxicity expressed as discomfort, pathology or even death.

The fourth aspect of iron nutrition promotes a way to ameliorate the negative effects of the third aspect (toxicity) with the positive consequences of the second aspect (the

possibility of iron remediation). It is important that extradietary iron be supplied to populations who need it, either as short term intervention (supplementation) or long term support (food fortification). The prospect of intervention and the possibility of toxicity need to be favorably balanced, the iron needs to be sufficiently bioavailable to be efficacious and it must be sufficiently free from the toxicities associated with iron to be safe to consume. The fourth aspect, therefore, is the form, or source, of the nutritional iron which dictates its relative bioavailability and safety.

Iron nutrition versus iron toxicity

Although the beneficial effects of iron in the body have been recognized from antiquity and its physiological effects have been further elaborated upon since the Renaissance (1,2), the recommended dosage has been widely disparate. Blaud recommended an $FeCO_3$ concoction delivering 193 to 771 mg iron/day as suitable remediation for 'green sickness' or 'chlorosis' (1). Bunge thought all iron supplementation was ineffective and worthless, while Quincke and von Noorden were convinced that no more that 1,5 grains (0,1 mg) of daily iron were sufficient (3). It was only as recent as the 1930's that the quantitative incorporation of iron into hemoglobin was conclusively proven (4,5).

Typical pharmacological recommendations for therapeutic sources of iron are ferrous sulfate heptahydrate, coated anhydrous ferrous sulfate, ferrous gluconate, polysaccharide-iron complex, and finely ground elemental iron. The average dose for treatment of iron deficiency anemia in adults is around 200 mg iron/day, given in three equal doses of around 65 mg iron. Children weighing 15 to 30 kg are presumed to tolerate half of the adult dose, while small children and infants are cited as being able to handle a larger relative proportion (5 mg/kg/day). The main consideration of these recommended doses is that they represent "a practical compromise" between the therapeutic action desired and the toxic effects of the iron (2). Therefore, maximal doses are recommended for the highest absorption of iron ions while the toxic effects are parlayed to their highest tolerance levels. The above recommendations are also made on the presumption that the iron doses are taken on an empty stomach. This is because the absorption rates of iron from its salts are typically limited to 2-10% of the dose in individuals with sufficient iron stores (6), and 10-20% in people suffering iron deficiency anemia (2). These absorbed amounts of iron shrink by an additional 50-67% when taken with meals (7). However, it is acknowledged that when adverse gastrointestinal effects of iron salts are encountered, either the dose should be reduced or the high-dosed iron should be consumed with food (2,8). These adverse effects include nausea, heartburn, abdominal cramping, vomiting, diarrhea and constipation (8). In the event of acute toxicity, the initial feature is still gastrointestinal irritation which confounds the perception that a toxic dose has been taken – the initial signs of toxicity are the same as common side effects of iron supplementation.

Children are more inclined to lethal iron toxicities due to ingesting relatively high doses compared to their weights. Acute toxicities from oral iron preparations include the above side effects of gastrointestinal irritation. Vomiting may be the first sign. These may be followed by gastrointestinal bleeding, lethargy or restlessness, and gray cyanosis. Following these signs, there may be a seeming recovery period of several hours to one to two days. The third phase of toxicity then commences and may include pneumonitis, jaundice, additional signs of liver toxicity and/or convulsions. Additional symptoms are gastrointestinal bleeding and neurological manifestations, including coma. Most deaths occur during this phase. Where individuals survive from three to four days after the acute ingestion of iron, recovery is generally rapid, although long-term leukocytosis may occur. Pyloric constriction and gastric fibrosis may occasionally result. Acute iron toxicity is treated with a combination of induced emesis, gastric intubation with $NaHCO_3$ lavage, and parenteral chelation of the iron with deferoxamine (8,9).

From a therapeutic standpoint, some side effects (toxic symptoms) are expected from the ingestion of ionic sources of iron due to the close proximity of therapeutic and toxic doses of iron salts. Adults taking doses of 200 mg iron/day divided into three equal doses could expect these symptoms to occur in approximately 25% of the cases. If the dose were doubled to 400 mg iron/day, then the incidence of toxic symptoms would increase to approximately 40% (2), although individual tests may indicate greater toxicities. For instance, adolescents and younger children could expect even higher incidences of toxicity at these dose levels or even at lower doses. In a double-blind nutritional study with adolescents having iron deficiency anemia there was a 33,3% incidence of side effects in just 120 mg iron/day from $FeSO_4$ (given in two daily doses of 60 mg iron, each contained in two enteric coated tablets containing 30 mg of iron, each) (10).

Chelation of the iron source for improved bioavailability and safety

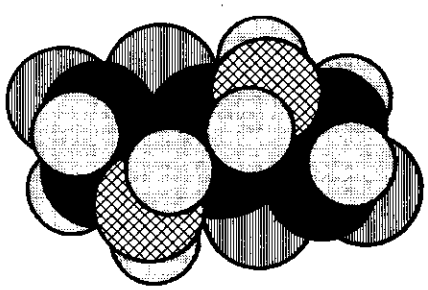
Oral iron sources which are inorganic salts with at least a degree of solubility are typically absorbed from the duodenal segment of the small intestine where the luminal pH is low and the iron is ionized. If the iron is held in a larger molecule, such as in a polysaccharide complex, then the iron needs to be severed from its position in the larger molecule before it can be absorbed by the small intestine. If the iron could be covalently bound to organic ligands which would reduce the charge of the cation and provide some spacial protection on the side of the attachment, then less toxicity due to

gastrointestinal irritation would be expected. The degree of protection inherent in the molecule would vary according to the strength of the ligand bond protecting at least one spacial side of the metal ion and the potential of the remaining charges of the iron cation to irritate the surfaces of the gastrointestinal mucosa or engage in free reactions with metabolites which could contribute to toxic effects in the body.

Chelates are formed when the same ligand molecule bonds to the same metal atom through more than one reactive site on the ligand. The ligand thus bends around the metal atom forming a molecular ring structure of atoms. The molecular ring sterically protects the metal atom where the ligand moieties are attached, as well as by the physical barrier of the ligand, itself, between the two moieties. When an additional ligand chelates the same metal atom, the atom is sterically more protected and sequestered within the molecule. Thus, a chelate of elemental iron, preferably having a metal to ligand ratio of 1:2 or greater would provide more sequestration and protection than either a covalently bound organic complex or an organic or inorganic salt. This is illustrated in the space filling structure of an iron bisglycinate chelate shown in Figure 1.

FIGURE 1

Space filling structure of an iron bisglycinate chelate molecule. The iron atom appears in the center with nitrogen, carbon, oxygen and hydrogen atoms of the glycine ligands surrounding the iron atom and bound to it via each nitrogen atom and each carboxyl oxygen atom. The protective capacities of the glycine amino acids with a metal to ligand ratio of 1:2 on each chelated iron atom are illustrated



Provided that the chelating bonds are sufficiently strong to resist cleavage by digestion or through reactive natural foodstuffs (such as, phytic acid, phosphates, tannic acid, and other luminal components which can potentially bind nutritive minerals), the chelates can protect the mineral atoms sufficiently long to be absorbed and utilized nutritionally. It is readily apparent that a 1:1 metal to ligand ratio with glycine or any other amino acid would leave one side of the chelated metal unprotected and vulnerable –both 1) to attack by phytic acid, phosphates, etc., with subsequent loss of nutrient capability and 2) to a greater potential for toxicity due to retaining a reactive site (and, therefore, irritative site) on the metal. The 1:2 metal to ligand ratio restricts unwanted reactions with dietary components, neutralizes the valence of the ferrous iron and protects the gastrointestinal surfaces from being irritated by close contact with the iron atom. Supplemental or fortifying levels of orally administered iron given as a bioavailable chelate would thus provide more protection from iron damage to the body that requires the iron.

There is precedence demonstrating the protection gained from chelates (as well as for improved bioavailability) in the natural iron source from animals known as heme. In this molecule, iron is chelated by a cyclic porphyrin ligand which is a breakdown product of blood hemoglobin and muscle myoglobin. Heme iron is far more bioavailable than inorganic or ionic sources of iron. It has been shown that in a diet containing only 6% of the total iron as heme, 30% of the iron absorbed was acquired from the heme only, at the exclusion of other dietary sources (2). The absorption of iron as heme has been shown to be independent of the presence or composition of concurrently eaten foodstuffs as well as being independent of iron absorption from supplemental organic or inorganic iron salts. These traits are also shared with mineral (metal) amino acid chelates which demonstrate different uptake pathways than either salts or complexes of the same minerals and which do not compete with the uptake of inorganic mineral sources (11-13).

When any mineral atom chelates with reactive ligands, a new molecule is formed which does not have the same properties as either the mineral ion or the free ligands. The new compound better stabilizes both the mineral and the ligands over that of their free forms. The overall properties of the chelated molecule mainly resemble those of the ligand(s), however, since these contribute the most bulk to the new molecule. This may explain the predominant uptake of metal amino acid chelates in the jejunum where amino acids and other products of protein hydrolyzation are typically absorbed (11).

Structural integrity of iron amino acid chelates

The molecular structures of the iron amino acid chelates have been proven through a variety of tests which elucidate chemical structures. Electron paramagnetic resonance (EPR) has been used to determine electron orientations between iron and the chelating amino acids in an animal feed supplement. In addition to proving the presence of chelation, the experiment was designed to determine the structural integrity of the product in premix, mixed loose feed and pelleted (extruded) feed. Both upper and lower g-values demonstrated that the bond orientations of the iron atoms in each case were tetrahedral, which vouched for the integrity of the 1:2, iron:amino acids, molar ratio of the chelated

animal nutrient. Collaborative evidence for the chelation of metal amino acid chelates has been given through a combination of x-ray crystallography and Fourier-transformed infrared spectrometry (FT-IR).

X-ray diffraction was first applied to prove the chelated structure of zinc bisglycinate chelate. This compound was used to verify published infrared absorption peaks for metal amino acid chelates. The appropriate absorption peaks were then applied to the other metal amino acid chelates to determine the structural integrity of these compounds. The molecular structures of these chelates (including iron amino acid chelates manufactured for both animal and human uses) have been proven by these combined instrumental techniques.

The safety of iron amino acid chelates including Ferrochel

The safety of iron amino acid chelates has been demonstrated through a variety of animal tests and human clinical studies. Three kinds of chelated iron products have been used in these tests. For clarity, these are herein identified as animal grade iron amino acid chelate for the animal feed grade product made from feed grade ingredients and human grade iron amino acid chelate which utilizes USP ingredients. The third form is Ferrochel, which is also made from USP ingredients and predominately maintains the chelated iron in the ferrous state. Ferrochel may also be referred to as ferrous bisglycinate chelate. These products have been validated as amino acid chelates by FT-IR and other appropriate instrumental methods.

Studies utilizing animal grade iron amino acid chelate. Long-term multigenerational feeding of iron amino acid chelate in sows

Two breeding sow operations from Ontario Province in Canada were chosen as treatment and control farms to conduct a toxicological assessment of animal grade iron amino acid chelate (also referred to as Iron Metalosate) as well as other metal amino acid chelates (14,15). Pigs on the treatment farm had received this dietary iron amino acid chelate for several years, resulting in five generations of pigs which had received dietary minerals in the form of metal amino acid chelates (Metalosates). The pigs on both farms were raised under similar confinement housing systems and the farms were within 20 km of each other. All animals received inoculations against erysipelas, leptospirosis, *Parvo* virus and *Escherichia coli*. The treatment animals additionally received Ivomec® for deworming and lice control. The breeds of the sows on both farms were similar, being mixtures of both York and York-Landrace stock. The nutritional constituents of the feeds were similar for both treatment and control sows with the exception that the treated sows received iron amino acid chelate as a supplemental source of iron, as well as other metal amino acid chelates.

Four feed rations were formulated for the pigs at the treatment farm. The first two, Weaner and Grower, were fed sequentially to all production offspring. Production pigs were marketed upon achieving 105 kg weights. Once the sows which became production sows were grown, they mostly received Dry Sow ration. Lactation ration was given to sows starting three weeks prior to parturition and continued for 25 days after birth, at which time the litters were weaned and began receiving the Weaner ration. While all four of the rations contained iron amino acid chelate, the greatest amount was formulated into the Lactation ration with the equivalent amount of 183,86 mg iron per kg of finished feed. Additionally, nursing sows were allowed to consume 7,257 kg of the Lactation ration per day (which supplied 1334,27 mg iron per day) to nourish their litters, while sows on the Dry Sow ration were allowed just 2,722 kg of feed per day (contributing 272,2 mg iron per day). Due to a change in the Lactation ration, prior to assessing the contributions of iron amino acid chelate to the health of the sows, all of the sows in the test group, except one, received 756,54 mg iron per day from the Lactation ration during their last farrowing. All of the sows also received the iron which was inherent in their food. In comparing the Dry Sow ration with the Lactation rations, it can be seen that the total consumption of iron as iron amino acid chelate was largely dependent on the number of farrowings (litter births) which the sows achieved in their lifetimes.

Six breeding sows were chosen for assessment from each of the farm herds. The two sets of six sows each were delivered to a local abattoir (slaughter house) on the same morning. Three of the six treatment animals represented the fourth filial generation of breeding sows to receive iron amino acid chelate. One of the treatment sows was of the third filial generation and the other two were of the second filial generation. The total amounts of iron from iron amino acid chelate consumed by the sows on the basis of their age and number of farrowings (parity) were less in the fourth generation animals since these tended to be the youngest sows. One of these animals had lived long enough to have six farrowings, however, so her total consumption was greater that the other two. Sows from the fourth filial generation thus consumed 131, 193 and 556 g of iron. The single individual of the third filial generation consumed 734 g, while the two individuals of the second filial generation consumed 580 and 687 g.

A local veterinarian, who had been certified with the Canadian Veterinary Medical Association, was enlisted to conduct both premortem and postmortem examinations of the sows. The veterinarian was kept blind as to which pigs had received iron amino acid chelate (treatment sows) and which had not (control sows). He was not appraised of the sow identifications until he had submitted his written report to Albion Laboratories Inc.

Following euthanasia of the sows by cerebral electrocution

and exsanguination, internal organs and skeletal tissues were excised for histopathological examination. The tissues excised included: brain, duodenum, jejunum, ileum, large intestine, muscle, heart, liver, spleen, bone marrow, mesenteric lymph node, kidney and ovary. The certified veterinarian excised all of the tissues from all of the animals and made all of the collections from the same parts of the organs utilized in order to standardize the assessments of both the treatment and control animals. He also made internal examinations as to the health of the animals. The tissues were trimmed into small blocks (approximately,1 cm X 1 cm X 2,5 cm) for ease of infiltration of the tissue fixative and for subsequent microtoming for examination slides. Following excision, the tissue blocks were submerged in 10% formalin solution in small capped bottles and shipped the next day by overnight courier to Albion Laboratories. Following receipt at Albion, the fluid in the bottles was changed out for 10% buffered formalin and shipped to a certified veterinary histopathologist in the United States. The veterinary report on premortem and postmortem examinations was also reviewed by the histopathologist although he was kept blind as to the identifications of treatment and control sow groups in both the on-site veterinarian's report and his own tissue samples until he had issued his draft report. At that time, he was made aware of the sow identifications so he could finalize his report as to group and sow identifications.

This study was conducted in order to assess two conditions: 1) the long term effects of continuous feeding of iron amino acid chelate on single individuals, and 2) possible cumulative effects of this iron amino acid chelate on multiple generations of production sows. The certified veterinary histopathologist's conclusion was that, "No histopathologic tissue alterations were observed that could be attributed to dietary administration of Iron Metalosate® to sows." He also noted that, "When the histopathologic findings in pigs of Group I [Control] were compared to those of Group II [Treatment], there were no biologically significant differences between the groups. It was concluded that no histopathologic tissue alterations were observed that could be attributed to Iron Metalosate® given as a mineral premix to sows " (16).

Long-term feeding of iron amino acid chelate in sows achieving six or greater parity

This investigation was a companion study to the long-term multigenerational study described above. As noted in the previous study, three of the test animals were of the fourth filial generation of production sows which had received iron amino acid chelate throughout their lives. Two of these animals had only been alive for a third to a half of the time of other test animals in the study. It was considered important to organize an additional investigation to verify that production sows which were uniformly older, at least by production standards, and which had received this iron amino acid chelate throughout their production lives, would be likewise free from pathology (17). Parity is an additional manner of expressing the number of farrowings which a sow has achieved over her productive lifetime. Six parities denotes a sow that has lived a longer than average production life.

Two farms in Ontario Province of Canada were chosen for this study. The control farm was the same one used for the multigenerational study, but the treatment farm was different. Both farms were still within 20 km of one another, however. Pigs at both farms had been inoculated for erysipelas, leptospirosis, *Parvo* virus and *Escherichia coli*. The control animals additionally received Ivomec® for internal and external parasites. Of the four sows selected for assessment from the control farm, three were of the Landrace breed and one was a York-Landrace cross. Eight sows were taken for assessment from the treatment farm. All of these were of the York-Landrace cross. The nutritional constituents of the feeds were similar for both treatment and control sows with the exception that the treated sows received iron amino acid chelate as a supplemental source of iron, as well as other minerals in the metal amino acid chelate form.

Four feed rations were formulated for the pigs at the treatment farm. These included Pig Starter, Grower, Gestation (dry) and Lactation rations. Iron amino acid chelate was supplied in all but the Grower ration. Production pigs were fed Pig Starter ration from weaning at 12-14 kg until they were 18-20 kg. This ration contained 110 mg iron per kg of finished feed from the iron amino acid chelate source. Pigs in this weight range consumed an average of 0,888 kg of finished feed per day. Grower ration was given to the production pigs from 18-20 kg until marketed at 100 kg, or above. Sows which were kept on the farm for litter production were then placed on either Gestation (dry) ration or the Lactation ration depending on the stage of their pregnancies. Two weeks prior to parturition, pregnant sows received an additional product called Litter Booster which also contained iron amino acid chelate. This was supplemented at the rate of 64,5 mg iron per kg finished feed. The Litter Booster addition was continued in each the sow's feed until her litter was weaned at a mean of 26 days after birth. Lactation ration was given to sows starting one week prior to parturition and continuing until the litters were weaned and began receiving Pig Starter ration. The greatest amount of iron from iron amino acid chelate was formulated into Gestation (dry) ration at the inclusion rate of 225 mg iron per kg of finished feed. The same iron chelate was formulated into Lactation ration at 189,5 mg iron per kg of finished feed. Sows on Gestation (dry) Sow ration were allowed just 2,04 kg of feed per day, while nursing sows were allowed to consume 7,71 kg of Lactation ration per day to nourish their litters. With the

consumption of more Lactation ration being allowed on a per day basis, more of the chelated iron was consumed via Lactation diet during the days of its administration. Since Litter Booster commenced two weeks prior to parturition and since 2,04 kg of the Gestation (Dry) ration was being given to the gravid sows, they received 591 mg iron per day from two weeks to one week prior to parturition. At the commencement of one week prior to parturition, the gravid sows were put on Lactation diet with Litter Booster and were allowed to consume 7,71 kg feed per day, until a mean of 26 days postparturition. During this time, the adult sows received 1958 mg iron per day from the amino acid chelated source. All pigs also received iron which was intrinsic in their feed. At times other than two weeks prior to parturition and during lactation, the sows received Gestation (Dry) ration and were limited to 2,04 kg of feed per day. During this time the sows received 459 mg iron per day.

The achievement of six parities for the animals used in this study was the minimum requirement. Of the four control sows used in the study, three had achieved six parities while the fourth one had reached ten parities. Among the eight treatment sows, three had achieved six parities, two had passed seven parities, one had achieved eight parities and two were at nine parities. Lifetime ingestion of iron as iron amino acid chelate reflected the age of the sows as apparent from the respective parity numbers (farrowings) which they had achieved. The three treatment sows with six parities ingested 768, 776 and 785 g iron as iron amino acid chelate throughout their lives. The two sows with seven parities received 893 and 907 g of iron in this form while the sow with eight parities reached 1073 g iron. Lastly, the two treatment sows which achieved nine parities ingested 1074 and 1103 g iron from the iron amino acid chelate source over the courses of their lives. All of these lifetime ingested amounts of iron were more than the highest amount of iron ingested by any of the six treatment sows assessed in the long-term multigenerational study reported above.

A local certified veterinarian was enlisted to conduct both premortem and postmortem examinations of the sows. The veterinarian was kept blind as to which pigs had received iron amino acid chelate (treatment sows) and which had not (control sows). He was not appraised of the sow identifications until he had finalized his written report to Albion Laboratories Inc.

Following euthanasia of the sows, their internal organs and skeletal tissues were excised by the certified veterinarian for histopathological examination. The veterinarian made all of the collections from the same parts of the organs utilized in order to standardize the assessments of both the treatment and control animals. He also made internal examinations as to the health of the animals. The tissues were trimmed into small blocks (approximately, 0,5 cm X 1 cm X 2,5 cm) for ease of infiltration of the tissue fixative and microtoming for examination slides. The tissues excised included brain, duodenum, jejunum, ileum, large intestine, muscle, heart, liver, spleen, bone marrow, mesenteric lymph node, kidney and ovary. Following excision, the tissue blocks were submerged in 10% buffered formalin solution in small capped bottles and these were shipped the next day by courier to Albion Laboratories. Following receipt at Albion, the fixed tissues were shipped to the same certified veterinary histopathologist in the United States who had assessed the slides made from tissues of pigs used in the long-term multigenerational study. In order to keep the veterinary histopathologist blind as to the identifications of the sow tissues, they were sent to him as three sets of four animals each (comprising one set for control animals and two for the treatment animals). The veterinary report on premortem and postmortem examinations was also reviewed by the histopathologist although he was kept blind as to the identifications of the three groups of sow tissues which he had received. After he had issued his draft report, he was made aware of the sow identifications so he could finalize his report as to group and sow identifications.

This study was conducted to assure that production sows receiving supplemental iron as iron amino acid chelate did not show any cumulative pathological effects due to the iron source. The certified veterinary histopathologist's conclusion was that, "No histopathologic tissue alterations were observed that could be attributed to dietary administration of the test article [Iron Amino Acid Chelate]."

Studies utilizing Ferrochel

LD_{50} determination of Ferrochel in rats

The LD_{50} for Ferrochel (human grade ferrous bisglycinate chelate) was determined using good laboratory practices (GLP) as specified for non-clinical studies by the US-FDA. Five male and five female rats were used for each of four dosing levels. The dosing levels of Ferrochel were calculated to yield 150, 300, 600 and 1200 mg iron/kg body weight. These doses were administered to the rats via oral gavage (gastric lavage) on the first day of the study. All of the animals were observed for toxic effects at 1, 2,5 and 4 hours on the day of dosing and twice a day for the next 14 days. All surviving animals were sacrificed at the end of the 14 day observation period, necropsied, and given a complete examination for gross pathology (18).

The oral LD_{50} for iron in Ferrochel was calculated to be the same for both male and female rats. It was 560 mg iron (as Ferrochel) per kg body weight of rat.

90-Day subchronic toxicity study of Ferrochel in rats administered via diet according to US-FDA guidelines

An acute dose LD_{50} toxicity test supplies relative

information as to susceptibility of animal physiological systems to relatively large single doses of the test article. A further assessment of the cumulative effects of receiving potentially toxic or subtoxic doses can be gained by supplying daily (chronic) doses of the test article over a period of time. The standard 90-day (3-month) subchronic toxicity test in rats has been designed to provide this information. The term, subchronic, implies that the assessment is being made over a finite period of time in an effort to answer specific physiological questions about the toxicity of the test article rather than supplying the article to the rats chronically for one to two years. When young rats are used for the test, 90 days can bracket the period of their highest growth rate. In addition to a control group of rats which do not receive the article being tested, the actual daily doses of the test article are set by best efforts to bracket a benign dose, a median dose and a compromised or toxic dose.

A 90-day subchronic toxicity test was carried out for Ferrochel (19). The rat breed used was Sprague Dawley CD® strain. In a preliminary study on three rats of each sex at 0, 300 and 500 mg Ferrochel/kg body weight, one female was found with signs of internal irritation at the 500 mg/kg dose. No deaths or other signs of toxicity were apparent. It was determined that the highest dose should be set at 500 mg Ferrochel/kg rat body weight in anticipation that some toxicity would be generated at this level over the course of the 90-day study allowing an assessment of the toxicity of Ferrochel. For the 90-day subchronic study, 20 male and 20 female rats per dosing level received either 0, 100, 250 or 500 mg Ferrochel/kg rat body weight mixed in their diets, making a total of 160 rats involved in the study. Iron levels from Ferrochel were confirmed by atomic absorption spectrometry on alternate weeks throughout the study. Structural integrity of the Ferrochel in the feed was confirmed periodically by Fourier-transformed infrared spectrometry (FT-IR).

No deaths occurred in any group. Both growth rates and feed consumption for the treatment animals matched the control animals for all dosage groups. Premortem and postmortem examinations failed to reveal any pathology that could be attributed to the iron dosages. Forty-eight tissues from each of ten males and ten females randomly selected from the 500 mg Ferrochel/kg dose level and the controls were excised, fixed, mounted on microscope slides and examined. None of the tissues revealed signs of iron pathology. Additionally, blood chemistries and clinical chemistries were also free from changes due to the increased iron in the rat diets. The No Observable Adverse Effect Level (NOAEL) was determined to be at least 500 mg Ferrochel/kg body weight for both male and female rats. Further details of this 90-day subchronic toxicity study and its conclusions have been published elsewhere (20).

Physiological control of absorption of Ferrochel

When an iron source shows high bioavailability, a commonly expressed concern is for toxic overload of iron. At issue is the question as to whether the highly bioavailable iron source is regulated in a similar fashion to heme iron or inorganic salt sources of iron. Subjectively, this has not been a concern for the iron amino acid chelates. Early LD_{50} estimates for several of the metal amino acid chelates routinely indicated that these sources were safer than inorganic mineral sources (21). Similar inferences have been gained in additional toxicological tests, such as, the multigeneration pig study (14-16) and the six-parity pig study (17), summarized above. A very strong implication of physiological regulation of these iron amino acid chelates is the 90-day subchronic toxicity study (19,20) assessing the impact of continual high doses of dietary Ferrochel. The finding of no toxicological evidence, no hematological or biochemical aberrations and virtually no impact other than normalcy speaks very strongly for the regulation of tissue uptake of iron taken as Ferrochel.

More direct measures of the regulation of iron uptake when in the amino acid chelated form were made by Pineda(22) on data that had been reported by Mervyn (23). The data were from a crossover study of 6 male and 6 female non-anemic individuals in the Mount Sinai School of Medicine in New York who received 18 mg iron daily from $FeSO_4$ for a week, followed by a washout period, then a replicate week-long dose of 18 mg iron/day from human grade iron amino acid chelate. Fecal iron was measured as the reciprocal of absorbed iron. In all of the individuals, more iron was absorbed from the amino acid chelated source. The mean improvement in absorption was 59%. Since the data were obtained from the same individuals, Pineda arranged the pairs of absorbed amounts of iron from the two sources from the least to the greatest. Then he calculated linear regressions for both sets. Presuming different inherent needs for iron among the subjects of the test, the data could be expected to plot the iron needs of a small sampling of a population. This resulted in two regression lines which differed in position (due to the higher absorption from the iron amino acid chelate source), but which were very similar in slope. Pineda additionally plotted the correlation of iron absorbed from the iron amino acid chelate against the respective data for iron absorption from $FeSO_4$, with the paired data arranged from least to highest. The correlation plot had an r^2 of 0,9411. These paired data indicate a linear correlation between the amounts of iron absorbed from the two iron sources and also imply that both iron sources are similarly regulated.

Additional data supporting the regulation of iron absorption from the amino acid chelated source have been provided by Olivares *et al* (24). These researchers noted that

unmodified cow's milk would inhibit the absorption of any non-heme source of iron, due to the high concentration of iron absorption inhibitors, including casein, calcium, whey protein and phosphates. In comparing the iron absorbed from Ferrochel (termed: ferrous bis-glycine chelate in this paper) versus $FeSO_4$, they found that, while absorption of iron as Ferrochel was decreased by the cow's milk, iron from this source was still absorbed from 2 to 2,5 times higher than iron from $FeSO_4$. This suggests that the Ferrochel iron source was less influenced by the inhibitors than was ferrous sulfate. They additionally found an inverse relationship between serum ferritin content and iron absorption as Ferrochel. This was correlated to the absorption of iron from ferrous ascorbate, thus further suggesting the regulation of Ferrochel iron absorption.

Data from a study by Iost *et al.* describing the successful repletion of hemoglobin levels in anemic young children with low fortificant doses of Ferrochel also indicate regulation of iron uptake against iron stores (25). One hundred and eighty-five young children (134 being 1-year old at the commencement of the study) were given 3 mg iron as Ferrochel in 1 L of cow's milk per day. Hemoglobin amounts were measured initially and at 133 ± 13 and 222 ± 2 days into the study. Mean and standard deviations for each sampling were 9,3 ± 1,5, 10,5 ± 1,6 and 11,2 ± 1,5 g hemoglobin/dL, respectively, demonstrating the repletion of iron deficiency anemia in the children over the course of the study. The data were additionally divided by degree of anemia, ≤ 9,4 g hemoglobin/dL whole blood being the most severe with 9,5 - 11,0 g hemoglobin/dL being less severe. Children having hemoglobin levels ≥ 11,1 g/dL were considered normal. Over the course of the study, the greatest changes were noted in the most severely anemic group. However, among children with normal hemoglobin values, there were no significant differences in hemoglobin amounts at any of the measurement times ($P > 0,10$). While ferritin assays were not a part of this study, the hemoglobin concentrations among the normal children implied that less amounts of iron were absorbed where there were less needs for iron.

The absorption of iron as Ferrochel has additionally been assessed in whole maize meal porridge by Bovell-Benjamin, Viteri and Allen (13). Whole maize is high in phytates which normally bind iron fortificants and may greatly limit their absorption. In an experiment involving 10 non-anemic men who consumed porridge containing $^{59}FeSO_4$ on the first day of the study and porridge containing ^{55}Fe-ferrous bisglycinate chelate on the second day, blood analyses done on the sixteenth day revealed that iron from the bisglycinate chelated source was absorbed greater than was iron from the $FeSO_4$ source. The two sources of iron were additionally consumed together in whole maize porridge by the same 10 men following the above blood sampling and final blood samples were taken 14 days later. The second blood sampling demonstrated that there was no exchange of the differently labeled iron isotopes between sources in the whole maize porridge when consumed at the same time. The lack of label exchange in the second experiment demonstrated that there was no breakdown of the ferrous bisglycinate in the intestinal pool before entering the mucosal cell. The $FeSO_4$ source was expected to breakdown in the intestine, resulting in free cationic iron being presented to the intestinal mucosal cells for absorption. The absence of label exchanging also implies that the Ferrochel iron was entering the mucosal cell intact as the bisglycinate chelate. The mean of the differences in iron absorption between the separate and mixed blood samplings was 4,7 times greater from the ferrous bisglycinate chelate than from ferrous sulfate. Additionally, scatter plots of the natural logarithm (ln) of percent iron absorption versus the ln of serum ferritin concentration for both the ferrous bisglycinate and $FeSO_4$ sources of iron yielded regression lines with similar correlation coefficients and slopes, although the ferrous bisglycinate data potted higher in absorption. This demonstrated that the absorptions of both iron as Ferrochel and iron as $FeSO_4$ were effectively regulated by the iron reserves of the body.

Safety of ferrous bisglycinate chelate determined

In 1997, after its review of many scientific papers on the efficacy and safety of Ferrochel (ferrous bisglycinate chelate), a panel of scientific experts nationally recognized in the USA and representing physiological, toxicological and medicinal disciplines concluded that Ferrochel was affirmed as "Generally Recognized As Safe" (GRAS) as both a food fortificant and dietary supplement. In 1999, following the self-affirmation of Ferrochel as GRAS and the subsequent review of the findings of the expert panel, the Office of Premarket Approval of the Center for Food Safety and Applied Nutrition of the US-FDA acknowledged the GRAS self-affirmation of Ferrochel (ferrous bisglycinate chelate) and advised that it had no questions or concerns regarding the GRAS status of this product under its proposed conditions of use for food enrichment and fortification purposes.

CONCLUSIONS

The importance of iron to the well being and healthy functionality of the body cannot be underestimated. Among the pathologies which may result from the deficiency of any physiologically required mineral, of paramount importance and of paramount occurrence are the morbidities associated with iron deficiency anemia. A fifth of the world's entire population is estimated to present this anemia. This is the major mineral deficiency to address and solve.

Inherent in iron are the seeds of its own noncompliance to efforts to reduce the effects of compromised absorption. In the inorganic form, which has been the form of choice for supplementation and fortification attempts in diets where it has been low or lacking, iron consistently demonstrates restricted bioavailability with ever greater toxic symptoms if the supplementary sources are increased in an attempt to increase its absorption. Modifications on the inorganic forms of iron, such as, the addition of ascorbic acid to keep the iron in the ferrous state prior to absorption, gain some improvements in absorption, but apply additional costs to the intervention.

Ferrochel may be the best form for supplementation and fortification of iron into human diets. It presents iron in the ferrous state, resists cleavage once ingested, is absorbed intact in its protective state, repletes iron levels at relatively small doses, has been proven remarkably nontoxic, even at relatively high daily doses, demonstrates the characteristics of being physiologically regulated, and increases iron absorption over that obtainable from ferrous sulfate, the current standard for iron intervention.

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