

Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**
as it applies in Great Britain

Elemental iron

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B.7. RESIDUE DATA

The active substance, elemental iron, is a stable, non-volatile elementary atomic particle and therefore cannot be degraded. It is also insoluble in water. Iron is the fourth most abundant element and the second most abundant metal to be observed in the earth's crust, accounting for 5.1% (by weight) of the earth's crust. Elemental iron is rarely found in nature, as the ions Fe^{2+} and Fe^{3+} readily combine with oxygen- and sulfur-containing compounds to form oxides, hydroxides, carbonates, and sulfides. Iron compounds are released through weathering of soil and rocks and iron is most commonly found in nature in the form of its oxides. It occurs naturally in terrestrial and aquatic ecosystems.

It is frequently found as oxidic/hydroxidic ore in nature. In agricultural soils, the content of iron is in the range of 0.2 - 5 % corresponding to 2 - 50 g/kg soil. Heavy soils might sometimes contain twice as much iron as sandy soils. The annual removal of iron by growing of wheat and sugar beet is reported to account for 1,500 and 4,500 g/ha. These amounts have to be replaced by fertilization of the soil. In green plants, the content of iron is approximately 30 - 500 mg/kg dry mass.

Elemental iron is a molluscicide which is formulated as a ready-to-use granular bait containing 1.0 % of the active substance. The plant protection product is applied to the soil surface at a rate of 8 kg/ha per treatment which corresponds to 0.08 kg a.s./ha per treatment (maximum total dose: 0.48 kg a.s./ha). Compared to the natural abundance, the amount of powdered iron added by application of the molluscicide is by several orders of magnitude smaller than the natural content commonly found in soils. Additionally, iron is applied in considerable amounts to agricultural soils in chelated fertilizers up to 6 kg Fe/ha in the case of serious iron deficiency.

As iron is a natural constituent of soils serving as essential nutrient in animal and plant physiology, the amount of iron added by application according to the GAP will be negligible compared to the natural content in soil.

Also, elemental iron (carbonyl + electrolytic + hydrogen reduced) compounds are already authorised for the following use:

- in food (Regulation (EC) No 1170/2009)¹, which may be used in the manufacture of food supplements and may be added to food.
- for the manufacturing of dietetic foods (Commission Regulation (EC) No 953/2009)².
- for the manufacturing of processed cereal-based foods and baby foods for infants and young children (Commission Directive 2006/125/EC)³.

B.7.1. STORAGE STABILITY OF RESIDUES

No storage stability study is required as no analysis of residues in plants, plant products and products of animal origin was conducted.

B.7.2. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES

B.7.2.1. Plants

No metabolism and distribution studies in plants after soil application are necessary, because elemental iron is a natural constituent of soil and plants.

¹Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. OJ L 314, 1.12.2009, p. 36.

²Commission Regulation (EC) No 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269, 14.10.2009, p. 9.

³Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 6.12.2006, p. 16.

The fate of elemental iron, as well as of its component ferric ions in soil and plants, is well documented in the published literature. A small selection of published literature regarding the distribution of iron in plants and the soil uptake by plants, are presented below:

Report:	CA 7.2.1/01: Rout G. R., Sahoo S., 2015
Title:	Role of iron in plant growth and metabolism
Document No:	Reviews in Agricultural Science, 3:1-24, 2015.
Guidelines:	Not stated
GLP	No
Summary:	<p>The following abstract has been taken from the review on the role of iron in plant growth and metabolism:</p> <p><i>'Iron is an essential micronutrient for almost all living organisms because of it plays critical role in metabolic processes such as DNA synthesis, respiration, and photosynthesis. Further, many metabolic pathways are activated by iron, and it is a prosthetic group constituent of many enzymes. An imbalance between the solubility of iron in soil and the demand for iron by the plant are the primary causes of iron chlorosis. Although abundant in most well-aerated soils, the biological activity of iron is low because it primarily forms highly insoluble ferric compounds at neutral pH levels. Iron plays a significant role in various physiological and biochemical pathways in plants. It serves as a component of many vital enzymes such as cytochromes of the electron transport chain, and it is thus required for a wide range of biological functions. In plants, iron is involved in the synthesis of chlorophyll, and it is essential for the maintenance of chloroplast structure and function. There are seven transgenic approaches and combinations, which can be used to increase the concentration of iron in rice seeds. The first approach involves enhancing iron accumulation in rice seeds by expressing the ferritin gene under the control of endosperm-specific promoters. The second approach is to increase iron concentrations in rice through overexpression of the nicotianamine synthase gene (NAS). Nicotianamine, which is a chelator of metal cations, such as Fe^{+2} and zinc (Zn^{+2}), is biosynthesized from methionine via S-adenosyl methionine synthase. The third approach is to increase iron concentrations in rice and to enhance iron influx to seeds by expressing the Fe^{+2}- nicotianamine transporter gene OsYSL2. The fourth approach to iron biofortification involves enhancing iron uptake and translocation by introducing genes responsible for biosynthesis of mugineic acid family phytosiderophores (MAs). The fifth approach to enhance iron uptake from soil is the over expression of the OsIRT1 or OsYSL15 iron transporter genes. The sixth approach to enhanced iron uptake and translocation is overexpression of the iron homeostasis-related transcription factor OsIRO2. OsIRO2 is responsible for the regulation of key genes involved in MAs-related iron uptake. The seventh approach to enhanced iron translocation from flag leaves to seeds utilizes the knockdown of the vacuolar iron transporter gene OsVIT1 or OsVIT2. The present review discusses iron toxicity in plants with regard to plant growth and metabolism, metal interaction, iron-acquisition mechanisms, biofortification of iron, plant-iron homeostasis, gene function in crop improvement, and micronutrient interactions.'</i></p> <p>The applicant further expanded upon the abstract with a summary of relevant text taken from the review:</p> <p><i>'Due to its ability to gain and lose electrons, iron works as a cofactor for enzymes involved in a wide variety of oxidation-reduction reactions (i.e, photosynthesis, respiration, hormone synthesis, DNA synthesis, etc.). This function makes iron an essential nutrient, and its deficiency causes iron chlorosis, which seriously constrains normal plant development. Iron uptake by plants is fastest when iron is present in the ferrous form (Fe^{2+}). Iron is primarily absorbed by plants, and it solubilizes Fe^{3+} and then reduces it to Fe^{2+} for absorption or transport into the root. Plants have evolved two separate mechanisms for the acquisition of iron, which can be referred to as Strategy I and Strategy II. Strategy I refers to iron mobilization by dicots and non gramineous monocots in response to iron deficiency stress, and Strategy II refers to that found only with gramineous monocots. Strategy I is used by most plant types, including the model plant Arabidopsis thaliana. Certain plants, namely the grasses, that include most of the world's staple grains, have</i></p>

	<p>evolved a distinct mechanism to acquire iron from the soil, which is known as Strategy II. This strategy is best described as a “chelation” which is similar to that used by many bacteria and fungi and it may have arisen as an adaptation to alkaline soils where acidification of the rhizosphere is difficult to achieve. Strong iron chelators called phytosiderophores (PS) are synthesized by the plant and secreted into the rhizosphere where they bind Fe^{3+}. The Fe (III)-PS complex is then taken up into root cells via transporters that are specific to the complex. Phytosiderophores are chemically quite distinct from bacterial and fungal siderophores, and they belong to a class of compounds called mugineic acids (MAs).</p> <p>Iron is translocated from roots to shoots as a ferric-citrate chelate form, and this is transported to actively growing shoot regions. Iron moves as a citrate complex.</p> <p>Translocation via citrate in the stem exudates of four soybean genotypes was most nearly related to the available iron supply. The transporters that are responsible for loading iron and citrate into the xylem have been recently revealed. Citrate appears to be moved into the xylem, at least in part, via the multidrug and toxic compound extrusion (MATE) family transporter ferric chelate reductase defective 3 (FRD3), which has a mutation that was responsible for the manganese accumulator (<i>man1</i>) phenotype.</p> <p>In leaf tissues, in contrast to the root, this active uptake process is usually not the limiting step in ion uptake. The availability of the micronutrient on the leaf surface is directly related to the water solubility of applied compounds. The precipitation for the crystallization of exogenous elements leads to immobilization, and the higher retention of inorganic iron in epicuticular waxes may be connected with its low solubility. The formation of such an insoluble product would immobilize the element on the leaf surface. However, chelating improves iron solubility in water. The penetration of substances through the cuticle is a diffusive process that is influenced by temperature and concentration gradient. Water and solutes penetrate both stomatous and astomatous cuticles. Cuticles are 10–20 times more permeable to urea than to inorganic ions, and the foliar spray of nitrogen compounds can also enhance iron uptake. Translocation of foliar-applied iron may be enhanced by chelation and by treatment with GA3 or kinetin (6-furfuryl amino purine).’</p>
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Report:	CA 7.2.1/02: Saenchai C., Prom-u-thai C., Lordkaew S., Rouached H., Rerkasem B., 2016
Title:	Distribution of iron and zinc in plant and grain of different rice genotypes grown under aerobic and wetland conditions
Document No:	Journal of Cereal Science, Volume 71, September 2016, pages 108-115
Guidelines:	Not stated
GLP	No
Summary:	<p>The following abstract has been taken from the above study on the distribution of iron and zinc in plant and grain of different rice genotypes grown under aerobic and wetland conditions:</p> <p><i>‘This paper examined the distribution of Fe and Zn in the plant and seed of different rice genotypes in different growing conditions. The Fe and Zn concentrations were determined in different plant tissues during the growth stages of 3 genotypes with high Fe and Zn check genotypes, and in different grain tissues of 15 genotypes grown in aerobic and wetland conditions. Iron and Zn were distributed differently in tissues of the rice plant, with the harvest index (panicle nutrient content as the % of the total above ground nutrients) at 3–4% for Fe and 54–74% for Zn. The concentrations of both Fe and Zn of the endosperm increased with the increasing proportion of the grain nutrient content allocated to the endosperm, but declined when the allocation to the bran fraction increased. The Fe concentrations of the de-husked caryopsis of rice grown in the aerobic soil and the Fe concentration of the de-husked caryopsis of rice grown in the wetland soil were closely related, but not in the endosperm Fe, while the grain Zn concentrations in the aerobic soil were found to correlate with the Zn concentrations in the wetland soil for both the de-husked caryopsis and the endosperm.’</i></p> <p>The applicant further expanded upon the abstract with a summary of relevant text taken from the discussion and conclusions sections of the study:</p> <p><i>‘Iron toxicity and Zn deficiency are the two most common micronutrient disorders in rice</i></p>

	<p><i>production; a low concentration of these micronutrients in the rice grain makes rice eaters particularly prone to Fe and Zn deficiency.'</i></p> <p><i>'With a harvest index for Fe of only 2-3%, and Fe concentration in the grain tissues being minor fractions of those in the leaves and the stem + leaf sheath, Fe appeared to be kept out of the panicle and the grain. This difference in the distribution of Fe within the rice plant may be related to differential requirement of Fe among the tissues. It has been reported that about 80% of Fe in plants is localized in chloroplasts as it is essential for chlorophyll synthesis, DNA replication, reactive oxygen species detoxification, and electron transport chain in both the mitochondria and the chloroplast. In the photosynthesis system, Fe plays a key role in the capture of excitable energy by opening the PSII reaction center, thereby increasing the quantum yield of PSII electron transport and photochemical quenching coefficient. The actual amount of Fe available for all these biological activities in the rice leaves may be even lower than the determined values as considerable proportion of plant Fe can be biologically inactive, such as when bound with ferritin. Within the panicle, Fe was concentrated mostly in the husk, with four times the concentration of Fe in the de-husked caryopsis. As previously reported, the rice embryo and aleurone had much higher Fe concentration than the endosperm. This is a problem when the nutrient-rich bran fraction (embryo plus aleurone) is removed by polishing to produce white rice, the form preferred by consumers. The polishing process has been reported to cause up to 85% loss of grain Fe. Nevertheless, the Fe in the rice embryo was still only a fraction of the concentration in the leaves and the stem + leaf sheath. The highest Fe concentration in the embryo (115 mg Fe/kg in IR68144) was one quarter of that found in the leaves. Rice, which normally grows in waterlogged soil with much elevated Fe^{2+} concentration in the soil solution, can tolerate up to several hundreds of mg Fe /kg in its leaves and stem. The enrichment of the endosperm of rice with Fe for the benefit of consumers seems to be difficult as evident from this study which demonstrates that the rice plant tends to exclude Fe from the panicle and the grain. Within the rice seed, Fe and Zn are localized together in the scutellum and in most other regions of the embryo, while the rice endosperm is also found with the lowest Fe and Zn concentrations among the grain tissues. Observations suggest that multiple mechanisms may be responsible for the distribution of these micronutrients within the rice plant and the seed. In conclusion, Iron and Zn were distributed differently in tissues of the rice plant, with the harvest index (panicle nutrient content as the % of the total above ground nutrients) at 3–4% for Fe and 54–74% for Zn. The concentrations of both Fe and Zn of the endosperm increased with the increasing proportion of the grain nutrient content allocated to the endosperm but declined when the allocation to the bran fraction increased. The Fe concentrations of the de-husked caryopsis of rice grown in the aerobic soil and the Fe concentration of the de-husked caryopsis of rice grown in the wetland soil were closely related, but not in the endosperm Fe, while the grain Zn concentrations in the aerobic soil were found to correlate with the Zn concentrations in the wetland soil for both the de-husked caryopsis and the endosperm.'</i></p>
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Report:	CA 7.2.1/03: Graham R., Stangoulis C. R., 2003
Title:	Trace Element Uptake and Distribution in Plants
Document No:	The Journal of Nutrition, Volume 133, Issue 5, May 2003, Pages 1502S–1505S
Guidelines:	Not stated
GLP	No
Summary:	<p>The following abstract has been taken from the review on trace element uptake and distribution in plants:</p> <p><i>'There are similarities between mammals and plants in the absorption and transport of trace elements. The chemistry of trace element uptake from food sources in both cases is based on the thermodynamics of adsorption on charged solid surfaces embedded in a solution phase of charged ions and metal-binding ligands together with redox systems in the case of iron and some other elements. Constitutive absorption systems function in nutrient uptake during normal conditions, and inducible "turbo" systems increase the supply of a particular nutrient during deficiency. Iron uptake is the most studied of the micronutrients, and divides the plant kingdom into two groups: dicotyledonous plants have a turbo system that is an upregulated version of the constitutive system, which consists of a membrane-</i></p>

	<p><i>bound reductase and an ATP-driven hydrogen ion extrusion pump; and monocotyledonous plants have a constitutive system similar to that of the dicots, but with an inducible system remarkably different that uses the mugeneic acid class of phytosiderophores (PS). The PS system may in fact be an important port of entry for iron from an iron-rich but exceedingly iron-insoluble lithosphere into the iron-starved biosphere. Absorption of trace metals in these graminaceous plants is normally via divalent ion channels after reduction in the plasma membrane. Once absorbed, iron can be stored in plants as phytoferritin or transported to active sites by transport-specific ligands. The transport of iron and zinc into seeds is dominated by the phloem sap system, which has a high pH that requires chelation of heavy metals. Loading into grains involves three or four genes each that control chelation, membrane transport and deposition as phytate.'</i></p> <p>The applicant further expanded upon the abstract with a summary of relevant text taken from the review:</p> <p><i>'The micronutrients that are known to be required by plants are iron, zinc, copper, manganese, cobalt, nickel, boron, molybdenum and chlorine.</i></p> <p><i>The six micronutrients, iron, zinc, copper, manganese, cobalt, nickel, for higher plants, the transition metals, are generally absorbed as divalent ions via divalent ion channels. These channels either have considerable specificity for each element, or homeostasis is achieved by specific active-excretion mechanisms that are controlled by cytoplasmic concentrations. Because iron and zinc deficiencies are extremely widespread in humans and are also common in some farm animals, this article concentrates on their uptake, transport and loading into grains that constitute the staple foods of most of the human race. What is known about the uptake, transport and loading of the other transition elements is generally analogous to iron and zinc.</i></p> <p><i>Planet Earth is replete in iron that constitutes much of its molten core, and iron is also the fourth most abundant element in the earth's crust. The amount of iron in the soil may be 10,000 times greater than in the vegetation grown in it, yet iron deficiency is common in crop plants. This anomaly is due to the low availability of iron in the presence of oxygen especially at moderate and high soil pH values. The solubility product of some compounds formed in soil that precipitate iron is on the order of 10^{-35}. These forms of iron in the soil are only solubilized by lowering of the pH value, by complexation of ferric iron [Fe(III)] and/or by reduction of Fe(III) to ferrous iron [Fe (II)]. The strategies used by plant roots to access iron exploit each of these chemical options, but the mechanisms vary between species in such a way as to divide the plant kingdom into two groups known as Strategy I and Strategy II plants. The latter group is the Gramineae, and the former includes all dicotyledonous plants together with the nongraminaceous monocotyledonous plants. Both groups have a constitutive system that is adequate to supply plants that are grown in fertile soils having plenty of available forms of iron. The constitutive system consists of a membrane-bound ferric reductase that is linked to a divalent ion transporter or channel and an ATP -driven proton extrusion pump.</i></p> <p><i>Strategy I plants respond to signals of low iron status by upregulating the ferric reductase (by deploying a new 70-kDa protein in the membrane) and the proton-extrusion pump. In addition, many Strategy I plants have a mechanism for excreting iron-binding ligands and soluble reductants, which are commonly phenols.</i></p> <p><i>Insensitivity to bicarbonate is a feature of Strategy II plants, which induce an entirely new mechanism of mobilizing iron under iron stress. Rather than upregulate the constitutive system, Strategy II plants synthesize and release to the soil nonprotein amino acids known as phytosiderophores (PS) or phytometallophores; the latter term recognizes that these amino acids are able to chelate most of the transition metals and not just iron. These form strong soluble chelates with ferric ions in the soil, and because they are soluble and less positively charged, they are free to diffuse toward the root in soil-water films.'</i></p>
Report:	CA 7.2.1/04: Garnett T. P., 2005
Title:	Distribution and Remobilization of Iron and Copper in Wheat
Document No:	Annals of Botany, volume 95, Issue 5, April 2005
Guidelines:	Not stated
GLP	No

Summary:	<p>The following abstract has been taken from the above study on the distribution and remobilization of iron and copper in wheat:</p> <p><i>'The amount of iron (Fe) and copper (Cu) that is loaded into grains of wheat (Triticum aestivum) depends on both the amount of nutrient taken up by the plant post-anthesis and the amount that is remobilized from vegetative organs as they senesce. Previous reports have shown that these two micronutrients behave quite differently in wheat in that Cu is readily remobilized to the grain whilst Fe shows poor remobilization. The object was to quantify the distribution of Fe and Cu in wheat and to show how this distribution changes from anthesis to grain maturity.'</i></p> <p>The applicant further expanded upon the abstract with a summary of relevant text taken from the body of the review:</p> <p><i>'These results show that, in wheat plants, there can be good reproductive remobilization of Fe and Cu to the grain. Apart from the grain, the concentration of Fe in all plant organs dropped over time during grain filling, as expected, due to reproductive remobilization. This was evident in all tissues, but particularly in the peduncle, flag and rachis where there was up to a 10-fold reduction in the Fe concentration. The Fe distribution and remobilization patterns found were very different from those found with field-grown wheat. When comparing the present results with field studies it appears that increased Fe availability in the soil does not increase its content in the grain. This implies that simply increasing Fe levels by increased root uptake capacity or foliar fertilization would not be an effective way of increasing grain levels of Fe.'</i></p>
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RMS comment: The applicant has presented summaries from the available scientific literature to address the metabolism of elemental iron in plants. These summaries consist of the abstract and/or text taken from the literature reviews and study reports. The RMS considers that there is sufficient data already available on the uptake and distribution of iron in plants and that therefore no specific metabolism studies are required to support the approval of elemental iron.

The applicant has proposed that no residue definition is required for elemental iron in plants:

'Considering the role of iron as essential plant nutrients, the immobility of iron as well as its natural occurrence in soil, monitoring of residues originating from soil application of elemental iron is not necessary. Therefore, no residue definition is given.'

It is considered that residue definitions for plants are not required.

B.7.2.2. Poultry

No livestock metabolism studies are necessary, because elemental iron is a natural constituent of soil, plants and animal diet and cannot be degraded therefore the occurrence of further degradation products can be excluded. As pointed out in B.7.2.1 and B.7.3, no relevant residues in plants, in exceedance of natural background, are to be expected after soil application of elemental iron in accordance with the representative use. Consequently, additional uptake of iron by animals by way of feeding crops treated according to the proposed use is of no concern.

The fate of elemental iron in animals, is well documented in the published literature. A small selection of published literature regarding the distribution of iron in animal, is presented below:

Report:	CA 7.2.2/01: Ohira Y., Hegenauer J., Saltman P., Edgerton V. R., 1981
Title:	Distribution and Metabolism of Iron in Muscles of Iron-Deficient Rats
Document No:	Biological trace element research, Vol. 4, Issue 1, 45-56 (1982)
Guidelines:	Not stated
GLP	No
Summary:	<p>The following abstract has been taken from the above study on the distribution and metabolism of iron in muscles of iron-deficient rats:</p> <p><i>'Iron-deficiency anemia leads directly to both reduced hemoglobin levels and work performance in humans and experimental animals. In an attempt to observe a direct link between work performance and insufficient iron at the cellular level, we produced severe iron deficiency in female weanling Sprague-Dawley rats following five weeks on a low-iron diet. Deficient rats were compared with normal animals to observe major changes in</i></p>

	<p>hematological parameters, body weight, and growth of certain organs and tissues. The overall growth of iron-deficient animals was approximately 50% of normal. The ratio of organ weight: body weight increased in heart, liver, spleen, kidney, brain, and soleus muscle in response to iron deficiency. Further, mitochondria from heart and red muscle retained their iron more effectively under the stress of iron deficiency than mitochondria from liver and spleen. Metabolism of iron in normal and depleted tissue was measured using tracer amounts of ^{59}Fe administered orally. As expected, there was greater uptake of tracer iron by iron-deficient animals. The major organ of iron accumulation was the spleen, but significant amounts of isotope were also localized in heart and brain. In all muscle tissue examined the ^{59}Fe preferentially entered the mitochondria. Enhanced mitochondrial uptake of iron prior to any detectable change in the hemoglobin level in experimental animals may be indicative of nonhemoglobin related biochemical changes and/or decrements in work capacity.'</p>
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Report:	CA 7.2.2/02: Rouault T. A., 2003
Title:	How Mammals Acquire and Distribute Iron Needed for Oxygen-Based Metabolism
Document No:	PLoS Biol 1(3): e79; https://doi.org/10.1371/journal.pbio.0000079
Guidelines:	Not stated
GLP	No
Summary:	<p>The applicant provided a summary of the above literature review with a summary of relevant text taken directly from the document:</p> <p><i>'Virtually all cells and organisms require iron to perform basic cellular processes. In respiration, iron proteins capture energy released from oxidation of food by synthesizing high-energy compounds, such as NADH, that are used to fuel cellular metabolism. Iron also enables hemoglobin in red blood cells to bind and transport oxygen to tissues throughout the body. Since iron is indispensable for respiration and oxygen transport, it is not surprising that cellular acquisition of iron has not been left to chance. It can be a challenge for organisms to obtain sufficient iron because much environmental iron is very insoluble. Thus, elaborate systems for uptake of iron and distribution within organisms are found throughout the kingdoms of life. Iron deficiency has been a common affliction throughout human history, and it is now estimated to affect 1 billion people worldwide. In mammals, dietary iron is absorbed from digestion of food in the duodenal portion of the gut. Specific iron transporters are present in epithelial cells that line the duodenum, and these transporters are much more abundant in iron-deficient animals. Mammals—including humans—have the ability to communicate their iron needs to the duodenum. Once iron has crossed the barrier cells of the duodenum, it is released into blood and is circulated to tissues throughout the body. However, it is not practical to circulate free elemental iron because iron can bind indiscriminately to many proteins. Therefore, iron is transported in an unreactive form by a specific carrier protein, transferrin. Most of the iron that gains access to the circulating blood binds tightly to serum transferrin, an abundant protein that binds one (monoferric) or two ferric iron atoms (diferric or holotransferrin) with high affinity. When ferric iron is bound to transferrin, it is nonreactive, meaning that it does not engage in single-electron transfers and it does not threaten other proteins and blood vessel walls with its reactivity. Also, because transferrin is a large protein, it remains in the circulation instead of being lost in urine when it passes through the kidney. Thus, cells and tissues that need to replenish their iron stores can do so by taking up transferrin that contains bound iron. Cells accomplish this task by synthesizing transferrin receptors, proteins that are present as pairs (dimers) on the cell surface. Membranes of iron-starved cells contain many more transferrin receptors than those of iron-replete cells, indicating that cells can appraise their own iron needs and increase transferrin receptor synthesis when they are iron-starved.'</i></p>

Report:	CA 7.2.2/03: Papanastasiou DA, Vayenas DV, Vassilopoulos A., Repanti M., 2000
Title:	Concentration of iron and distribution of iron and transferrin after experimental iron overload in rat tissues in vivo: study of the liver, the spleen, the central nervous system and other organs.
Document No:	Pathol Res Pract. 2000, 196(1): 47-54

Guidelines:	Not stated
GLP	No
Summary:	<p>The following abstract has been taken from the above study on the concentration and distribution of iron and transferrin after experimental iron overload in rat tissues <i>in vivo</i>:</p> <p><i>'The purpose of this study was to estimate the iron concentration in the liver, spleen and brain of control rats and rats overloaded with iron and to determine the distribution of iron and of transferrin (TF). Iron was administered to Wistar rats by food supplemented with 3% carbonyl iron for 3 months, or intraperitoneally, or intravenously as iron polymaltose for 4 months (total administered dose: 300 or 350 mg/rat, respectively). Iron concentration was estimated by atomic absorption spectrophotometry and iron- and TF-distribution histochemically and immunohistochemically, respectively. In control rats the organ with the highest iron content was the spleen, followed by the liver and brain. After iron loading, the increase of iron in the liver was greater than that of the spleen; iron concentration in the brain did not change significantly. Distribution of iron in the liver was in Kupffer cells throughout the lobule and in hepatocytes at its periphery. No difference in the number of positive cells or staining intensity for TF was observed between control rats and iron overloaded animals in the liver or central nervous system (CNS); the spleen was negative for TF. Distribution of TF in the liver showed a centrilobular localisation in hepatocytes. TF reaction in the brain occurred in oligodendrocytes, vessel walls, choroid plexus epithelial cells and some neurons. In conclusion, experimental iron overload in rats leads to iron uptake mainly by reticuloendothelial (RE) cells and hepatocytes, indicating that hepatocytes are of particular importance for iron metabolism. Iron uptake by the brain was not significant, probably because the brain is protected against iron overload. Iron overload did not influence location and quantity of TF in the liver and CNS, whereas the visualisation of iron and TF did not coincide. This indicates that TF may have other functions beyond iron transport.'</i></p>

RMS comment: The applicant has presented summaries from the available scientific literature to address the metabolism of elemental iron in animals. These summaries consist of the abstract and further text taken from literature reviews and study reports. The RMS considers that there is sufficient data already available on the uptake and distribution of iron in animals and that therefore no specific metabolism studies are required to support the approval of elemental iron.

No residue definition for residues in animal matrices has been given, considering the role of iron as an essential nutrient in animals.

B.7.2.3. Lactating ruminants

No livestock metabolism studies are necessary, because elemental iron is a natural constituent of soil, plants and animal diet and cannot be degraded therefore the occurrence of further degradation products can be excluded. As pointed out in B.7.2.1 and B.7.3, no relevant residues in plants, in exceedance of natural background, are to be expected after soil application of elemental iron in accordance with the representative use. Consequently, additional uptake of iron by animals by way of feeding crops treated according to the proposed use is of no concern.

The fate of elemental iron in animals, is well documented in the published literature. See discussion under B.7.2.2.

B.7.2.4. Pigs

No livestock metabolism studies are necessary, because elemental iron is a natural constituent of soil, plants and animal diet and cannot be degraded therefore the occurrence of further degradation products can be excluded. As pointed out in B.7.2.1 and B.7.3, no relevant residues in plants, in exceedance of natural background, are to be expected after soil application of elemental iron in accordance with the representative use. Consequently, additional uptake of iron by animals by way of feeding crops treated according to the proposed use is of no concern.

The fate of elemental iron in animals, is well documented in the published literature. See discussion under B.7.2.2.

B.7.2.5. Fish

No fish metabolism studies are necessary, because elemental iron is a natural constituent of soil, plants and animal diet and cannot be degraded therefore the occurrence of further degradation products can be excluded. As pointed out in B.7.2.1 and B.7.3, no relevant residues in plants, in exceedance of natural background, are to be expected after soil application of elemental iron in accordance with the representative use. Consequently, additional uptake of iron by fish by way of feeding crops treated according to the proposed use is of no concern. The fate of elemental iron in fish, is well documented in the published literature. A small selection of published literature regarding the distribution of iron in fish, is presented below:

Report:	CA 7.2.5/01: Zhao L., Xia Z., Wang F., 2014
Title:	Zebrafish in the sea of mineral (iron, zinc, and copper) metabolism
Document No:	Front Pharmacol. 2014; 5: 33.
Guidelines:	Not stated
GLP	No
Summary:	<p>The following abstract has been taken from the review on trace element homeostasis studies using the zebra fish model, with a focus on iron, zinc, copper, selenium, manganese, and iodine:</p> <p>‘Iron, copper, zinc, and eight other minerals are classified as essential trace elements because they present in minute in vivo quantities and are essential for life. Because either excess or insufficient levels of trace elements can be detrimental to life (causing human diseases such as iron-deficiency anemia, hemochromatosis, Menkes syndrome and Wilson's disease), the endogenous levels of trace minerals must be tightly regulated. Many studies have demonstrated the existence of systems that maintain trace element homeostasis, and these systems are highly conserved in multiple species ranging from yeast to mice. As a model for studying trace mineral metabolism, the zebrafish is indispensable to researchers. Several large-scale mutagenesis screens have been performed in zebrafish, and these screens led to the identification of a series of metal transporters and the generation of several mutagenesis lines, providing an in-depth functional analysis at the system level. Moreover, because of their developmental advantages, zebrafish have also been used in mineral metabolism-related chemical screens and toxicology studies.’</p> <p>The applicant provided a summary of the above literature review with the following text taken directly from the document (Overview of Iron Metabolism):</p> <p><i>‘Iron is present in nearly all living organisms. As an essential component of heme and iron-sulfur cluster-containing proteins, iron plays a central role in many biological activities, including oxygen transport, cellular respiration, and DNA synthesis. Of all the trace elements, the iron homeostasis system is one of the best characterized, primarily because of iron's role in erythropoiesis and its causative relationships with iron-deficiency anemia and hematochromatosis. To date, many major proteins involved in the uptake, transport, storage and release of iron have been identified.</i></p> <p><i>Under normal conditions, dietary iron is absorbed by enterocytes through Divalent Metal Transporter 1 (DMT1); from there, it is exported to the circulation through Ferroportin 1 (Fpn1). In the blood, iron is transported in the form of Transferrin (Tf)-Fe³⁺, which is taken up by endocytosis into cells with surface Transferrin receptors (TfRs). Iron in the endosomes is then released to the cytoplasm and delivered to the mitochondria, where it is used to make iron-sulfur (Fe-S) clusters, to synthesize heme, or to be stored as Ferritin. Most of the iron used for producing hemoglobin in erythrocytes is obtained from the recycling iron pool released from senescent red blood cells that are phagocytized by macrophages. Aside from transport and storage proteins, Hephadin - a peptide hormone released by the liver - plays an important role in regulating iron levels by binding to Fpn1 and promoting its internalization. Other factors such as oxidoreductases [e.g., Duodenal Cytochrome b (Dytb), Ceruloplasmin (Cp), Hephaestin (Heph), and STEAP3] and modulatory proteins (e.g., Hemochromatosis (HFE), Hemojuvelin (HJV), Iron Regulatory Protein (IRP) 1/2, and Transmembrane Serine Protease 6 (TMPRSS6)] also play an active role in iron metabolism.</i></p>

	<i>Zebrafish absorb waterborne iron via the gastrointestinal tracts and the gills. The fish branchial iron uptake has high- and low-affinity components, with K_m of 5.9 nmol/l Fe and V_{max} of 2.1 pmol/g·h at low Fe concentration (<40 nmol/l), and a linear manner increase of the uptake rate at higher Fe concentration (40–200 nmol/l). Zebrafish branchial iron transport can be inhibited by high level of Cd, but not by other divalent metals such as Zn, Cu, and Mn. Moreover, low iron diet fed zebrafish exhibited a significant increase in tissue Cd accumulation, suggesting an interaction between Fe and Cd assimilation in fish.'</i>
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Report:	CA 7.2.5/02: Carriquiriborde P., Handy R. D., Davies S. J., 2003
Title:	Physiological modulation of iron metabolism in rainbow trout (<i>Oncorhynchus mykiss</i>) fed low and high iron diets
Document No:	The Journal of Experimental Biology 207, 75-86
Guidelines:	Not stated
GLP	No
Summary:	<p>The following abstract has been taken from the study on the physiological modulation of iron metabolism in rainbow trout (<i>Oncorhynchus mykiss</i>) fed low and high iron diets:</p> <p><i>'Iron (Fe) is an essential element, but Fe metabolism is poorly described in fish and the role of ferriredutase and transferrin in iron regulation by teleosts is unknown. The aim of the present study was to provide an overview of the strategy for Fe handling in rainbow trout, <i>Oncorhynchus mykiss</i>. Fish were fed Fe-deficient, normal and high-Fe diets (33, 175, 1975 mg Fe /kg food, respectively) for 8 weeks. Diets were chosen so that no changes in growth, food conversion ratio, haematology, or significant oxidative stress (TBARS) were observed. Elevation of dietary Fe caused Fe accumulation particularly in the stomach, intestine, liver and blood. The increase in total serum Fe from 10 to 49 $\mu\text{mol/L}$ over 8 weeks was associated with elevated total Fe binding capacity and decreased unsaturated Fe binding capacity, so that in fish fed a high-Fe diet transferrin saturation increased from 15% at the start of the experiment to 37%. Fish on the high-Fe diet increased Fe accumulation in the liver, which was correlated with elevation of hepatic ferriredutase activity and serum transferrin saturation. Conversely, fish on the low-Fe diet did not show tissue Fe depletion compared with normal diet controls and did not change Fe binding to serum transferrin. Instead, these fish doubled intestinal ferriredutase activity which may have contributed to the maintenance of tissue Fe status. The absence of clear treatment-dependent changes in branchial Fe accumulation and ferriredutase activity indicated that the gills do not have a major role in Fe metabolism. Some transient changes in Cu, Zn and Mn status of tissues occurred.'</i></p> <p>The applicant provided a summary of the above study with a summary of relevant text taken directly from the document:</p> <p><i>'Fish acquire iron predominantly from the diet, and with negligible iron uptake at the gills compared with the gut, teleost fish have a dietary iron requirement of ~30–200 mg/kg dry mass (d.m.) of food.</i></p> <p><i>Iron forms insoluble ferric (hydro)oxides at neutral pH and molecular evidence suggests that the small fraction of Fe^{3+} presumably present in the gut lumen will be reduced to Fe^{2+} prior to import into the gut enterocytes of fish. In mammals, ferriredutase activity in the brush border of the intestinal mucosa facilitates the reduction of Fe^{3+} to Fe^{2+} prior to Fe^{2+} import on divalent metal ion transporter 1 (DMT 1). Although intestinal ferric reductase activity has not been measured in rainbow trout, in the European flounder at least, Fe^{2+} is absorbed three times faster than Fe^{3+}. DMT 1 genes are also expressed in fish intestine, for example, rainbow trout and zebrafish Intracellular Fe is stored as Fe^{3+} by ferritin, a 450-kDa protein with a spherical cavity capable of carrying 4500 iron atoms. Ferritins are an ancient group of proteins conserved in bacteria, plants and man, and have also been found in fish. The precise mechanism of how imported Fe^{2+} is re-oxidised to Fe^{3+} by cytoplasmic ferritin, or how the Fe^{3+} is subsequently reduced to Fe^{2+} for export from the cell to the blood, remains controversial in mammals and unknown in fish. In mammals basolateral export of Fe^{2+} from the cell to the blood is against the electrochemical gradient, and probably mediated by iron regulated transporter (IREG 1, also called MTP 1 or ferroportin), and recent evidence from the zebrafish genome suggests IREG 1 genes are</i></p>

	<p>present in fish. In mammals, exported Fe^{2+} is oxidised on the extracellular surface of the serosal membrane by a membrane bound copper oxidase (a ceruloplasmin homologue, hephaestin), and the resulting Fe^{3+} binds rapidly to extracellular transferrin to facilitate bulk iron transport in the blood. Fish have long been known to have transferrin for bulk iron transport in the blood, and in the hagfish (<i>Myxine glutinosa</i>) at least, the transferrin has a similar structure to that in humans.</p> <p>Elevation of dietary Fe above normal caused Fe accumulation in the fish, but the Fe-deficient diet generally did not cause tissue Fe depletion. Fish fed the high-Fe diet showed statistically significant increases in Fe concentration in the intestine, stomach, liver and serum, but not muscle or gill compared with fish on either normal or low-Fe diets (diet effect ANOVA, $P < 0.05$). The biggest increases occurred in the intestine (86-fold increase), liver (6.5-fold increase) and stomachs (3.9-fold increase) of fish fed the high-Fe diet, compared with the initial fish.'</p>
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RMS comment: The applicant has presented summaries from the available scientific literature to address the metabolism of elemental iron in fish. These summaries consist of the abstract and further text taken from literature reviews and study reports. The RMS considers that there is sufficient data already available on the uptake and distribution of iron in fish and given the expected additional uptake of iron by fish by way of feeding crops treated according to the proposed use, no specific metabolism studies are required to support the approval of elemental iron.

No residue definition for residues in fish has been given.

B.7.3. MAGNITUDE OF RESIDUE TRIALS IN PLANTS

Elemental iron is a molluscicide which is formulated as a **ready-to-use** granular bait containing 1.0 % of the active substance. The plant protection product is applied to the soil surface at a rate of 8 kg/ha per treatment which corresponds to 0.08 kg a.s./ha per treatment (maximum total dose: 0.48 kg a.s./ha).

Compared to the natural abundance, the amount of elemental iron added by application of the molluscicide is by several orders of magnitude smaller than the natural content commonly found in soils. Additionally, iron is applied in considerable amounts to agricultural soils in chelated fertilizers.

As indicated in B.7.2.1, relevant residues of elementary iron in food of plant and animal origin, in exceedance of natural background, are not expected to occur. Therefore, supervised residue trials are not necessary.

The essential role of iron, in soil and plants, is well documented in the published literature. A small selection of published literatures regarding the natural background in soil and plant, the iron deficiency and soil treatments and the sufficiency nutrient concentrations required for production of several crops, is presented below:

Report:	CA 7.3/01: Vitosh M.L., Warncke D.D., Lucas R.E., 1994
Title:	Secondary and Micronutrients for Vegetables and Field Crops
Document No:	Michigan State University Extension, Departement of Crop and Soil Sciences, E-486, Revised August 1994
Guidelines:	Not stated
GLP	No
Summary:	<p>The applicant provided a summary of the above study with a summary of relevant text taken directly from the document:</p> <p><i>'Iron is a constituent of many organic compounds in plants. It is essential for synthesizing chlorophyll, which gives plants their green colour. Iron deficiency can be induced by high levels of manganese. High iron levels can also cause manganese deficiency.'</i></p> <p><u>Iron Deficiency Symptoms:</u></p> <p><i>Iron deficiency symptoms are marked and show up first in terminal leaves as a light yellowing. The symptoms are very similar to those of manganese deficiency. A lack of iron in field and vegetable crops is not common in soils with pH below 7.0.</i></p> <p><u>Correcting Iron Deficiency:</u></p> <p><i>Soil treatments usually require applications of iron chelates at a rate equivalent to 1/2 to 1 pound of iron per acre. Often it is difficult to correct iron deficiency with soil applications when soils are alkaline. Soil applications are effective if soils are acid or neutral in reaction. Under alkaline soil conditions, foliage sprays are recommended. Sometimes the best cure for Fe deficiency is to grow varieties that are not sensitive to Fe deficiency. For instance, some soybean varieties are more sensitive to Fe deficiency than others.</i></p>

	<p><u>Iron Toxicity:</u> <i>Injury due to high soil iron concentrations is not common under neutral or high pH soil conditions. Toxic situations occur primarily on acid soils (< pH 5.0) and where excess soluble iron salts have been applied as foliar sprays or soil amendments. The first symptoms of iron toxicity are necrotic spots on the leaves.'</i></p>
Report:	CA 7.3/02: Chen Y., Barak P., 1982
Title:	Iron nutrition of plants in calcareous soils
Document No:	Advances in Agronomy, 35, 217 – 240
Guidelines:	Not stated
GLP	No
Summary:	<p>The applicant provided a summary of the above review with a summary of relevant text taken from the document:</p> <p><i>Iron is the fourth most abundant element in the earth's lithosphere, following oxygen, silicon, and aluminum. Most of the iron in the earth's crust is in the form of ferromagnesium silicates. Weathering of such minerals in soil is usually accomplished by combined hydrolysis and oxidation due to reaction with water and air. Most of the iron released by weathering is precipitated as oxides or hydroxides; only a small part of the iron is incorporated into secondary silicate minerals or complexed by soil organic matter.</i></p> <p><i>The solubility of Fe(III) oxides decreases in the order: Fe(OH)₃ (amorphous) > Fe(OH)₃ (soil) > γ-Fe₂O₃ (maghemite) > γ-FeOOH (lepidocrocite) > α-Fe₂O₃ (hematite) > α-FeOOH (goethite). Goethite, because of its great stability, is found in almost every soil and climate and is the most abundant soil iron oxide.</i></p> <p><i>Hematite, the second most abundant soil iron oxide, is absent from recent soils in humid temperate climates. Lepidocrocite is found exclusively in noncalcareous hydromorphic soils, having had a Fe(II) hydroxy compound precursor. Maghemite is common in highly weathered soils formed from basic igneous rocks in tropical and subtropical climates. Ferrihydrite (previously called "amorphous ferric hydroxide") has been identified in numerous soil environments.</i></p> <p><i>Relatively little is known of solid phase organoiron complexes in soils since investigation by necessity involves some type of extraction which alters the natural state of the complexes by making them soluble. The most common extractant of organoiron complexes is sodium pyrophosphate. In a study of 33 Ando soils, the iron in organoiron complexes ranged from 0.05 to 2.91% of soil weight and even exceeded the weight of iron in soil iron oxides in a number of soils.</i></p> <p><i>Iron concentrations in saturated extracts of 68 soils representing 30 soil series in California ranged from 0.01 to 0.8 ppm, with a mean and median of 0.05 and 0.03 ppm, respectively. Such high iron concentrations are almost certainly due to either colloidal iron hydroxide or soluble organoiron complexes. Colloidal iron hydroxide has been found to pass 100Å membrane filters, so that evidence of soluble organoiron complexes must be indirect.</i></p> <p><i>This chapter reviews iron nutrition of plants in calcareous soils. Many agricultural crops worldwide, especially in semiarid climates, suffer from iron deficiencies.</i></p> <p><i>Deficiencies are usually recognized by chlorotic, or yellowed, intervein areas in new leaves and are typically found among sensitive crops grown in calcareous soils (calcareous soils cover over 30% of the earth's land surface). Iron deficiency in extreme cases may lead to complete crop failure.</i></p> <p><i>Two principal methods of treating iron deficiencies are accepted:</i></p> <ul style="list-style-type: none"> - <i>Spraying foliage with inorganic salts has been shown to be of benefit, but often gives spotty results because of, limited penetration of iron into leaves. Also, repeated treatments are required during the course of canopy development.</i> - <i>Soil treatment with synthetic chelates, principally FeEDDHA (ethylenediaminedi-o-hydroxyphenylacetic acid), has been found to be an unqualified success, but for the drawback of their high costs.</i> <p><i>Iron deficiency in plants causes chlorosis of leaf tissue because of inadequate chlorophyll synthesis. In a healthy plant, 60% of all leaf iron is concentrated in chloroplasts. The exact role of iron in chlorophyll synthesis is not certain but there is evidence of the involvement of ferrous iron in the condensation of succinic acid and glycine to form γ-aminolevulinic acid. Magnesium is incorporated into the molecule to form chlorophyll, possibly with the catalytic</i></p>

	<i>action of iron.</i>
Report:	CA 7.3/03: Vose P. B., 1982
Title:	Iron nutrition in plants: a world overview
Document No:	Journal of Plant Nutrition, 5, 233
Guidelines:	Not stated
GLP	No
Summary:	<p>The following abstract has been taken from the review on iron nutrition in plants:</p> <p><i>'Fe-deficiency chlorosis has been recognized since 1844 (Gris) and was the first plant nutrient deficiency to be investigated. It is a reflection of the continuing importance of iron nutrition problems in major crops worldwide that anybody interested in any aspect of plant nutrition cannot but be aware of them, whether as Fe-deficiency or Fe-toxicity.</i></p> <p><i>Iron, as the element, is seldom deficient as it comprises 4.2% of the earth's crust, the fourth most abundant element, and commonly occurs as 2–6% Fe₂O₃ in temperate soils, increasing to as much as 60% Fe₂O₃ for certain tropical Ferralsols (Bould, 1963). Therefore, iron deficiency and toxicity are functions of the whole soil system and its effect on the availability of iron to the plant, and an understanding of this is essential to an appreciation of the problems.'</i></p> <p>The applicant provided a summary of the review with a summary of relevant text taken from the document:</p> <p><i>'Iron is contained in the iron-bearing primary minerals of the soil mainly in the ferrous, Fe²⁺, state. Following weathering the iron is largely converted to the ferric, Fe³⁺, state and forms various hydrated oxides. Reducing (poor aeration) conditions can lead to reversion to Fe²⁺ state. The availability of Fe³⁺ in soils is governed largely by pH, being highly available at pH 6.0 and below, while at pH 7.0 availability is already markedly decreased and above this pH it declines dramatically, through precipitation of Fe-compounds, becoming limiting to plant growth in many cases. It has been shown that above pH 4.0 the Fe³⁺ activity in solution decreases a thousand-fold for each unit increase of pH. Decomposing organic matter in calcareous soils gives high bicarbonate production. This may increase available phosphate, which reduces the Fe available to the plant.</i></p> <p><i>Although Fe-deficiency is mainly associated with calcareous soils of high pH ("lime-induced chlorosis"), Fe-deficiency can also occur associated with Mn-toxicity at low pH on sandy soils.</i></p> <p><i>Iron toxicity is not as universally common as Fe-deficiency. On acid soils, where iron is most available, Fe²⁺ can become toxic to plants usually in association with unfavourable factors such as poor drainage, highly reducing conditions and high sulphide, such as occur in some rice soils.</i></p> <p><i>Plants can absorb iron in divalent form, but in normal aerated soils Fe³⁺ is the form present, Fe²⁺ being associated with abnormally reduced conditions. Fe³⁺ is apparently made available for absorption as Fe²⁺ by reduction at the root surface.</i></p> <p><i>The Fe-content of plants varies considerably, the range for "normal" content being from about 60-300 ppm, "deficient" plants may have 10-30 ppm, while "excess" conditions can give levels of 400-1000 ppm.'</i></p>
Report:	CA 7.3/04: Pasian C. C., 2001
Title:	Micronutrient disorders
Document No:	Ohio State University Fact Sheet HYG-1252-98
Guidelines:	Not stated
GLP	No
Summary:	<p>The applicant provided a summary of the above article with a summary of relevant text taken from the document:</p> <p><i>'A micronutrient disorder may be a deficiency (when the micronutrient is in deficit) or a toxicity (when the micronutrient is in excess). Micronutrient management is complex and difficult.</i></p>

Small variations from the optimum level required for plant growth can be damaging. By the same token, level slightly above those required for good growth can be toxic. It is very important for growers to have a clear understanding about micronutrient management. This article is a brief overview of the principles that control the availability of nutrients in soilless mixes and how to correct imbalances.

Nutrient availability:

Sometimes, the micronutrient present in a growing mix is not available to the plant (the plant cannot take it up). Micronutrient availability is influenced by media pH: except for molybdenum, the availability of micronutrients decreases with increasing media pH and vice versa. Water alkalinity is an important factor modifying media pH and hence micronutrient availability. It is important to maintain the pH for soilless media between 5.5 and 6.3.

The General critical foliar ranges for floral crops:

Nutrient	Minimum ppm	Maximum ppm
Iron (Fe)	50	?
Manganese (Mn)	30	500
Zinc (Zn)	20	100-200
Copper (Cu)	5	20-100
Boron (Bo)	25	100-300
Molybdenum (Mo)	0.5	15

Correct diagnosis:

Iron availability is reduced by high levels of manganese and nitrate-nitrogen fertilizer.

How to correct the problem:

Deficiencies can be corrected by adding the micronutrient that is deficit or by correcting the factor that makes it unavailable (e.g. high pH). This second course of action is very common among growers who have high alkalinity irrigation water.

Micronutrients can be I) added over time in small amounts with the irrigation water, II) applied once with a concentrated solution during a normal watering, III) applied as single foliar spray.'

Report:	CA 7.3/05: Nand Kumar Fageria, Baligar V.C., Wright R.J., 1990
Title:	Iron nutrition of plants: an overview on the chemistry and physiology of its deficiency and toxicity
Document No:	Pesq. agropec. bras., Brasília, 25(4):553-570, abr. 1990
Guidelines:	Not stated
GLP	No
Summary:	<p>The applicant provided a summary of the above review with a summary of relevant text taken from the document:</p> <p><i>'Iron deficiency and toxicity are important yield limiting factors in crop production around the world.</i></p> <p><i>Iron stress (deficiency or toxicity) in crop plants often represents a serious constraint for stabilizing and/or increasing crop yields. Any factor that decreases the availability of Fe in a soil or competes in the plant absorption process contributes to Fe deficiency.</i></p> <p><i>Iron deficiency occurs in a variety of soils. Affected soils usually have a pH higher than 6. Iron deficient soils are often sandy, although deficiencies have been found on fine-textured soils, mucks, and peats. Iron deficiency is potentially a problem on most calcareous soils. It is estimated that as much as about 5.2 million hectares of the world land surface is calcareous and might therefore be susceptible to Fe-deficiency problems. The most severely affected Fe-deficient areas tend to have less than 50 cm annual rainfall on major soil association of xerosols, arenosols, solonch, rendzinas and chernozems. Iron toxicity is not as common as Fe-deficiency. On acid soils, where Fe is most available, Fe²⁺ can become toxic to plants. Iron toxicity is most commonly found in rice soils where unfavorable factors such as poor drainage, highly reducing conditions, and high sulphide content occur.</i></p>

Soil analysis is the most widely used test of nutritional status. It consists of chemical and physical measurements made on a soil. The best method so far reported for Fe extraction seems to be the DTPA (diethylene triamine pentaacetic acid) test, which involves 1:2 soil/solution extraction with 0.005 M DTPA, 0.01 M CaCl₂, and 0.1 M triethanolamine, adjusted to pH 7.3.

Plant analysis is the determination of the concentration of an element or extractable fraction of an element in a sample from a particular part or portion of a crop sampled at a certain time or stage of morphological development.

Plant analysis is not as commonly used as analysis to evaluate soil fertility. The main reason for this is the high cost involved in plant tissue analysis and lack of calibration data for many plants and for many growing conditions.

For interpretation of plant analysis results, it is essential to have pre-established critical or sufficiency nutrient levels for each crop and agroclimatic region.

To determine critical or sufficiency level, a calibration curve is constructed relating nutrient concentration in a specific plant part to growth. Growth is usually expressed as a percent of the treatment giving maximum growth. If there is plant response to the applied nutrient, the calibration curve generally is represented by four zones. The first zone is known as the deficiency zone in which plant growth increases sharply as more nutrient is absorbed, but there is little change in the concentration of the nutrient in the plant part analyzed. The second zone is the transition zone, in which both nutrient concentration and growth increase as more nutrient is absorbed. The third zone, called adequate or sufficiency zone, is the region of the curve where each addition of the nutrient raises nutrient concentration without a corresponding increase in growth. If the applied nutrient is in excess, a fourth zone known as the toxic zone is developed. In this zone there is an increase in nutrient concentration, but yield is decreased. The critical concentration lies within the transition zone and is usually associated with a 10% reduction in growth. The sufficiency or adequate values of Fe reported for some important crops are presented in the table below:

Iron Sufficiency Level for Different Crops

Crop	Plant Part Analysed	Stage of Growth	Sufficiency range (mg/kg)	Reference
Barley	Whole tops	Heading	50-150	Ward et al. 1973
Common Bean	Fully developed trifoliolate	Flowering	100-450	Wilcox & Fageria 1976
Corn	Ear leaf	At silk	50-200	Jones Junior & Eck 1973
Cotton	Mature leaves	Early bloom	30-300	Sabbe et al. 1972
Peanut	Upper Stem & Leaves	Flowering	50-300	Small & Ohlrogge 1973
Rice	Whole Top	Tillering	70-300	Fageria 1984
Sorghum	3 rd Leaf	At bloom below head	65-100	Lockman 1972
Sugarbeet	Blade	Not given	60-140	Nagarajah & Ulrich 1966
Sugarcane	Blade	Not given	20-600	Schmehl & Humbert 1964
Soybean	Fully developed trifoliolate	Prior to pod set	51-350	Small & Ohlrogge 1973
Wheat	Whole Tops	Heading	50-150	Ward et al. 1973

Soils and plants are the most important factors affecting Fe availability to plants. The availability of Fe is particularly sensitive to changes in the soil environment. Part of the sensitivity to these changes is related directly to the performance of the root system in

	<p><i>exploring the soil volume for this nonmobile element, and part is related to the pool or bonding of the element in the soil. In the case of an insoluble nutrient like Fe, the transport process from soil to plant roots is generally the rate limiting step in nutrient uptake. At present, foliar application is the only feasible means for overcoming Fe-deficiency in most crop plants. Inorganic sources of Fe are ineffective for soil application when applied at low or moderate rates and higher doses are uneconomical. However, foliar application of nutrients is not the right solution in modern agriculture where higher productivity is the goal. In addition, foliar application is restricted by weather conditions and cost. This means more field research is needed to solve Fe-deficiency or toxicity problems. One feasible and economical approach may be selection of crop genotypes which are more efficient or tolerant under low and high Fe concentrations, respectively. To achieve this objective, it is necessary to have a cooperative effort among soil scientists, plant physiologists and plant breeders. A lot of work has been done in identifying genotypes with high efficiency at resistance to toxicity, but these results have not been taken to farmers. '</i></p>
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Natural Iron Background in Soil and Plant

As reported in the environmental fate section (Volume 3 B.8 (AS) – Section B.8.1.), iron is frequently found as oxidic/hydroxidic ore in nature. In agricultural soils, the content of iron is in the range of 0.2 - 5 % corresponding to 2 - 50 g/kg soil. Heavy soils might sometimes contain twice as much iron as sandy soils. The annual removal of iron by growing of wheat and sugar beet is reported to account for 1,500 and 4,500 g/ha. These amounts have to be replaced by fertilization of the soil.

From the United States Department of Agriculture, USDA Food Composition Database (<https://ndb.nal.usda.gov/ndb/nutrients/index>), natural backgrounds in plant is presenter in the table below:

Matrix	Min. Iron Content		Max. Iron Content	
	mg/100g	mg/kg	mg/100g	mg/kg
Cereal grains and Pasta	0.14	1.4	18.54	185.4
Fruits and Fruit juices	0.05	0.5	6.8	68
Legumes and legume Product	0.29	2.9	20	200
Nut and Seed Products	0.11	1.1	19.2	192
Fats and oils	0.01	0.1	2	20
Dairy and egg foods	0.01	0.1	12.82	128.2
Beef product	0.17	1.7	44.55	445.5

Therefore, application of elemental iron as a plant protection product will thus not result in relevant residues in crops, compared to natural background values.

Sufficiency Nutrient Concentrations

Iron is essential for crop growth and food production, even though only small amounts are required compared to some other nutrients. Plants require iron for a large number of metabolic processes. Table 7.6-01 and Table 7.6-02 show the acceptable or sufficiency nutrient concentrations required for production of several crops. The values reported for vegetables are general and should be used only as guidelines. Nutrient values below the sufficiency concentration may indicate a deficiency. Values above the sufficiency concentration may be excessive and possibly toxic.

Table 7.6-01 Acceptable or sufficiency nutrient concentrations required for production of several crops (see CA 7.6/01)

Nutrient sufficiency ranges for corn, soybeans, alfalfa, wheat sugar beets, potatoes and vegetables							
Element	Corn (Ear leaf sample in initial silk)	Soybeans (Upper fully developed leaf sampled prior to initial flowering)	Alfalfa (Top 6 inches sampled prior to initial flowering)	Wheat (Upper leaves sampled prior to initial bloom)	Sugar Beets (Center fully developed leaf sampled in midseason)	Vegetables (Top fully developed leaves)	Potatoes (Petioles from most recently matured leaf sampled in midseason)
Percent (%)							
Nitrogen	2.76-3.50	4.26-5.50	3.76-5.50	2.59-3.00	3.01-4.50	2.50-4.00	2.50-4.00
Phosphorous	0.25-0.50	0.26-0.50	0.26-0.70	0.21-0.50	0.26-0.50	0.25-0.80	0.18-0.22
Potassium	1.71-2.50	1.71-2.50	2.01-3.50	1.51-3.00	2.01-6.00	2.00-9.00	6.00-9.00
Calcium	0.21-1.00	0.36-2.00	1.76-3.00	0.21-1.00	0.36-1.20	0.35-2.00	0.36-0.50
Magnesium	0.16-0.60	0.26-1.00	0.31-1.00	0.16-1.00	0.36-1.00	0.25-1.00	0.17-0.22
Sulfur	0.16-0.50	0.21-0.40	0.31-0.50	0.20-0.40	0.21-0.50	0.16-0.50	0.21-0.50
Parts per million (ppm)							
Manganese	20-150	21-100	31-100	16-200	21-150	30-200	30-200
Iron	21-250	51-350	31-250	11-300	51-200	50-250	30-300
Boron	4-25	21-55	31-80	6-40	26-80	30-60	15-40
Copper	6-20	10-30	11-30	6-50	11-40	8-20	7-30
Zinc	20-70	21-50	21-70	21-70	19-60	30-100	30-100
Molybdenum	0.1-2.0	1.0-5.0	1.0-5.0	0.03-5.0	0.15-5.0	0.5-5.0	0.5-4.0

Table 7.6-02 Iron sufficiency level for different crops (see CA 7.6/05)

Crop	Plant Part Analysed	Stage of Growth	Sufficiency range (mg/kg)	Reference
Barley	Whole tops	Heading	50-150	Ward et al. 1973
Common Bean	Wholly developed trifoliate	Flowering	100-450	Wilcox & Fageria 1976
Corn	Ear leaf	At silk	50-200	Jones Junior & Eck 1973
Cotton	Mature leaves	Early bloom	30-300	Sabbe et al. 1972
Peanut	Upper Stem & Leaves	Flowering	50-300	Small & Ohlrogge 1973
Rice	Whole Top	Tillering	70-300	Fageria 1984
Sorghum	3 rd Leaf	At bloom below head	65-100	Lockman 1972
Sugarbeet	Blade	Not given	60-140	Nagarajah & Ulrich 1966
Sugarcane	Blade	Not given	20-600	Schmehl & Humbert 1964
Soybean	Fully developed trifoliate	Prior to pod set	51-350	Small & Ohlrogge 1973
Wheat	Whole Tops	Heading	50-150	Ward et al. 1973

Overall, the sufficiency range is 11-600 mg/kg for plants. The plant protection product is applied to the soil surface at a rate of 8 kg/ha per treatment which corresponds to 0.08 kg a.s./ha per treatment (maximum total

dose: 0.48 kg a.s./ha). The application of elemental iron as a plant protection product will thus not result in relevant residues in crops, compared to natural background values.

Correcting Iron Deficiency

Where crop deficiencies occur, the problem usually is associated with low availability of iron rather than with the amount of iron present in the soil.

Availability of iron in the soil is affected by pH. Availability is low in soils of high pH (>7.0) and especially when free calcium carbonate is present. Worldwide, iron deficiency is common in calcareous and semi-arid soils. It has been estimated that some 40% of world soils are prone to iron deficiency (see CA 7.6/02 and CA 7.6/03). Within Europe, deficiency is found mainly in the Mediterranean area. In the UK, iron deficiency is found in fruit and nursery plants but very rarely, if at all, in field crops.

Soil and plant tissue analysis are not always regarded as reliable indicators of deficiency. However, 50 mg Fe/kg dry-matter has been proposed as a minimum satisfactory leaf concentration for ornamentals (see CA 7.6/04) and strawberries (<http://strawberry.ifas.ufl.edu/fertilizer.htm>). The value below which deficiencies may occur has been given as 4.5 ppm or as 6 ppm for field soils and 5 ppm for glasshouse soils (see CA 7.6/02).

Soil treatments usually require applications of iron chelates (soluble complex of iron, sodium and a chelating agent such as ethylenediaminetetraacetate (EDTA), EDDHA, or others, used to make the iron soluble in water and, for the purposes of agriculture, accessible to plants) at a rate equivalent to 0.56-1.12 kg a.s./ha (see CA 6.6/01). However, typical application rate for chelated iron is 100 g Fe/ha as a foliar spray and up to 3 kg Fe/ha as a soil application.

Again, application of elemental iron as a plant protection product (with supported soil application rate of 0.0048 kg a.s./ha, corresponding to 1 mg/kg in soil) will thus not result in relevant residues in crops, compared to considerable amounts used as chelated fertilizers.

Iron Toxicity

Iron toxicity is not as common as Fe-deficiency. Soil and plant tissue analysis are not always regarded as reliable indicators of deficiency. However, concentrations of 300 – 2000 mg Fe/kg dry-matter have been associated with toxicity. The plant protection product is applied to the soil surface at a rate of 8 kg/ha per treatment which corresponds to 0.08 kg a.s./ha per treatment (maximum total dose: 0.48 kg a.s./ha). Application of elemental iron as a plant protection product will thus not result in relevant residues in crops, that will affect elemental iron toxicity.

RMS comment: The information provided by the applicant is considered sufficient to support the case that supervised residue trials are not necessary for the approval of elemental iron. Iron is already present in soil in much higher levels than would be introduced by the application of elemental iron. Iron is commonly added to soil as part of commercial chelated fertilisers, given its important role in plant health. Residues of elemental iron in food of plant and animal origin, in exceedance of natural background, are not expected to occur based on the proposed representative uses.

However, it is noted that the proposed GAP does not specify a pre-harvest interval/latest timing of application. This could be interpreted as a zero-day PHI which is not considered to be appropriate for edible crops. Therefore, for edible crops a 1-day PHI should be stipulated.

B.7.4. FEEDING STUDIES

No relevant residues of elemental iron, in exceedance of natural background, are to be expected in plants and feeds after soil application of the elementary iron. Consequently, additional uptake of iron by animals by way of feeding is of no concern and livestock metabolism studies are not necessary. Therefore, a waiver on livestock feeding studies is justified as well.

B.7.4.1. Poultry

Poultry feeding studies are not required (See point B.7.2.2.).

B.7.4.2. Ruminants

Ruminant feeding studies are not required (See point B.7.2.3.).

B.7.4.3. Pigs

Pig feeding studies are not required (See point B.7.2.4.).

B.7.4.4. Fish

Pig feeding studies are not required (See point B.7.2.5.).

B.7.5. EFFECTS OF PROCESSING

No relevant residues of elemental iron are to be expected in crops after application of elemental iron according to the proposed representative use (see point B.7.3.). Consequently, no processing study is required.

B.7.5.1. Nature of the residue

Not required. See discussion under point B 7.5 above.

B.7.5.2. Distribution of the residue in peel and pulp

Not required. See discussion under point B 7.5 above.

B.7.5.3. Magnitude of residues in processed commodities

Not required. See discussion under point B 7.5 above.

B.7.6. RESIDUES IN SUCCEEDING OR ROTATIONAL CROPS**B.7.6.1. Metabolism in rotational crops**

Uneaten bait does decompose, leaving the active ingredient, elemental iron. The elemental iron does not dissolve as would be conventionally expected with this type of process but reacts with oxygen in the air, to form insoluble hydrated iron oxide (rust), the major natural constituent of iron in the soil environment, not soluble iron.

Application of elemental iron as a plant protection product will thus not result in relevant residues in succeeding or rotational crops, compared to natural background values. Iron also occurs naturally in soil. It can be utilized by plants as a source of ferric ions via uptake through roots, but this process is very slow. Consequently, the amount of iron taken up by plants is negligibly small. For this reason, iron is frequently added to agricultural soils as fertilisers (Tisdale and Nelson, 1975, Soil Fertility and Fertilizers).

The uptake of elemental iron, as well as of its components ferric ions, from the soil by the plant is well documented in the published literature.

Iron Uptake

Iron arrives in the vicinity of the root as various chemical compounds or organic complexes, rarely as elemental Fe. Iron is taken up by plants as the ion Fe^{2+} but is transported to the root surfaces as organo-mineral complexes. Iron in the soil solution can be moved to the plant root as a component in the bulk soil pore solution moving toward the root as water is taken into the plant to replace that lost by transpiration or used in growth processes. Iron also can move to the root by diffusion from a region of high concentration to a lower one (at the root surface as Fe is taken up by the plant). Roots also can intercept Fe compounds in the soil as the roots grow and expand into additional soil volume. Root density and extension are very important factors in the plant's ability to obtain Fe.

Iron uptake by the plant is not as simple as with other essential elements. Iron is taken up by plant roots in greatest amounts in the zone of the root between cell elongation and maturation, about 1 to 4 cm behind the root tip. Uptake of Fe by the plant is an active process, that is, energy is expended by the plant to take in Fe. Iron

uptake is dependent on the plant's ability to reduce Fe^{3+} to Fe^{2+} and remove it from the complex or chelating compound. Research evidence shows this reduction occurs at the cell surface and that electrons from within the cell are used. The same 1- to 4-cm area behind the root tip where most Fe is absorbed also is the area of the root where most protons and reductants are released. The chelated Fe in the soil solution moves to the root by mass flow or by diffusion. At the root, Fe is reduced and removed from the chelating molecule and moved across the cell membrane. Iron uptake can be interfered with by other cations in the soil solution such as manganese (Mn) and calcium (Ca).

Report:	CA 7.6/01: Kobayashi T., Nishizawa N. K., 2003
Title:	Iron Uptake, Translocation, and Regulation in higher plants
Document No:	Annu. Rev. Plant Biol. 2012. 63:131–52
Guidelines:	Not stated
GLP	No
Summary:	<p>The applicant provided a summary of the above review with a summary of relevant text taken from the document:</p> <p>Iron uptake</p> <p><i>Iron is essential for the survival and proliferation of all plants. Despite its abundance in the soil, Fe is only slightly soluble under aerobic conditions, especially in high-pH and calcareous soils. The Fe acquisition mechanisms in various plant species have been placed into two categories: Strategy I in nongraminaceous plants and Strategy II in graminaceous plants. The two main processes in the Strategy I response, which is utilized by all higher plants except those in the Gramineae family, are the reduction of ferric chelates at the root surface and the absorption of the generated ferrous ions across the root plasma membrane. The Strategy II response relies on biosynthesis and secretion of mugineic acids (MAs), which are specific to graminaceous plants. Nine types of MAs have been identified to date, all of which are synthesized through a conserved pathway from S-adenosyl-L-methionine. This pathway includes three sequential enzymatic reactions mediated by nicotianamine synthase (NAS), nicotianamine aminotransferase (NAAT), and deoxymugineic acid synthase (DMAS), generating 2'-deoxymugineic acid (DMA), the precursor of all other MAs.</i></p> <p>Figure 1</p> <p>Fe acquisition strategies in higher plants: Strategy I in nongraminaceous plants (<i>left</i>) and Strategy II in graminaceous plants (<i>right</i>). Ovals represent the transporters and enzymes that play central roles in these strategies, all of which are induced in response to Fe deficiency. Abbreviations: DMAS, deoxymugineic acid synthase; FRO, ferric-chelate reductase oxidase; HA, H^+-ATPase; IRT, iron-regulated transporter; MAs, mugineic acid family phytosiderophores; NA, nicotianamine; NAAT, nicotianamine aminotransferase; NAS, nicotianamine synthase; PEZ, PHENOLICS EFFLUX ZERO; SAM, S-adenosyl-L-methionine; TOM1, transporter of mugineic acid family phytosiderophores 1; YS1/YSL, YELLOW STRIPE 1/YELLOW STRIPE 1-like.</p> <p>Iron translocation</p> <p><i>Because of the poor solubility and high reactivity of Fe, its translocation inside the plant body must be associated with suitable chelating molecules and proper control of redox states between</i></p>

the ferrous and ferric forms. Fe translocation in plants involves various steps, including radial transport across the root tissues, which must include symplastic transport to pass through the Casparian strip; xylem loading, transport, and unloading; xylem-to-phloem transfer; phloem loading, transport, and unloading; symplastic movement toward the site of demand; and retranslocation from source or senescing tissue.

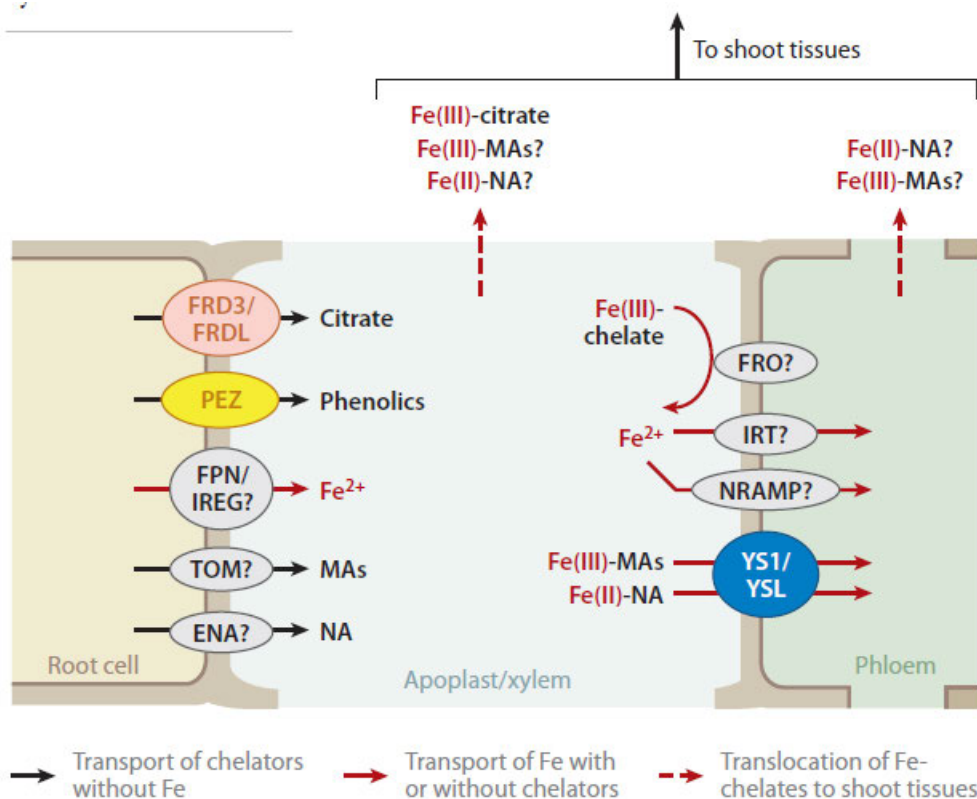


Figure 2

Molecules involved in xylem and phloem Fe loading. Only a few components have been proven to be responsible for a specific step in transport; the involvement of others (indicated by question marks) has been suggested by physiological, genetic, or biochemical studies. Abbreviations: ENA, efflux transporter of nicotianamine; FPN/IREG, ferroportin/iron regulated; FRD3/FRDL, FERRIC REDUCTASE DEFECTIVE 3/FERRIC REDUCTASE DEFECTIVE 3-like; FRO, ferric-chelate reductase oxidase; IRT, iron-regulated transporter; MAs, mugineic acid family phytosiderophores; NA, nicotianamine; NRAMP, natural resistance-associated macrophage protein; PEZ, PHENOLICS EFFLUX ZERO; TOM1, transporter of mugineic acid family phytosiderophores 1; YS1/YSL, YELLOW STRIPE 1/YELLOW STRIPE 1-like.

Higher plants have developed two distinct strategies to acquire iron, which is only slightly soluble, from the rhizosphere: the reduction strategy of nongraminaceous plants and the chelation strategy of graminaceous plants. Key molecular components—including transporters, enzymes, and chelators—have been clarified for both strategies, and many of these components are now thought to also function inside the plant to facilitate internal iron transport. Transporters for intracellular iron trafficking are also being clarified. A majority of genes encoding these components are transcriptionally regulated in response to iron availability. Recent research has uncovered central transcription factors, cis-acting elements, and molecular mechanisms regulating these genes. Manipulation of these molecular components has produced

transgenic crops with enhanced tolerance to iron deficiency or with increased iron content in the edible parts.

Regulation of iron responses:

Gene regulation is a crucial step for coping with fluctuating environments. Plants induce or repress various genes related to Fe homeostasis in response to Fe deficiency or excess. Induction of Fe acquisition-related genes under low Fe availability is especially pronounced in both nongraminaceous and graminaceous plants, and the central regulators of these genes have been clarified in this decade.

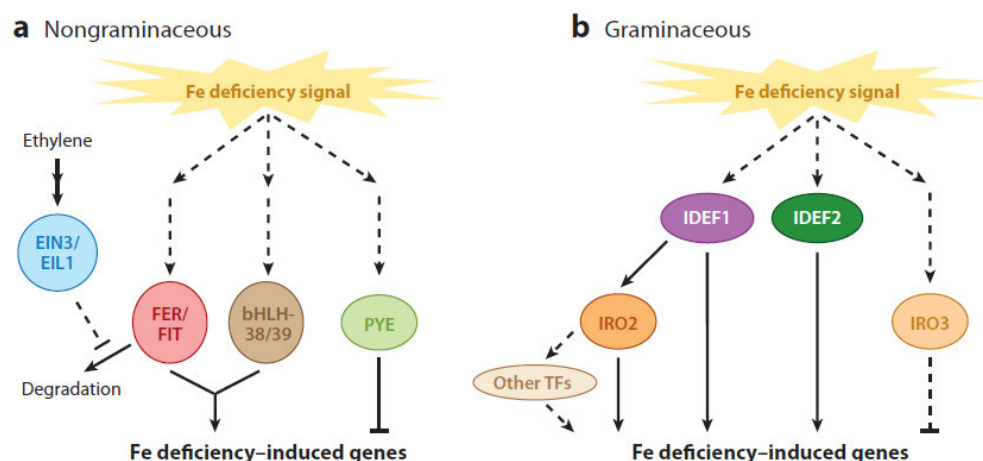


Figure 3

Regulation of Fe deficiency responses in (a) nongraminaceous and (b) graminaceous plants. Ovals indicate central transcription factors (TFs) involved in the regulation of Fe deficiency-induced genes. Dashed lines indicate putative or unverified pathways. Several other signaling molecules, including nitric oxide (NO), affect expression or activity of some of the depicted key factors through unknown mechanisms. On the other hand, the response to Fe overload is mediated through distinct pathways which are partly linked to the circadian clock and NO. Abbreviations: bHLH, basic helix-loop-helix; EIN3/EIL1, ETHYLENE INSENSITIVE 3/ETHYLENE INSENSITIVE 3-LIKE; FER/FIT, T3238FER/FER-like iron deficiency-induced transcription factor; IDEF, iron deficiency-responsive element-binding factor; IRO, iron-related transcription factor; PYE, POPEYE.

In another approach to understanding Fe deficiency responses, cell type-specific microarray analyses in Fe-deficient *Arabidopsis* roots were carried out. They reported large variability in the genes responsive to Fe deficiency between cell layers: Genes involved in metal transport and chelation were induced mainly in the epidermis, whereas those involved in signaling and stress were enriched among the stele-induced genes. The key elements and factors of the Fe deficiency response in graminaceous plants have been clarified through another strategy. Stepwise promoter analysis of the barley *IDS2* gene in transgenic tobacco led to identification of the cis-acting iron deficiency-responsive element 1 (*IDE1*) and *IDE2*, which are the first identified elements related to micronutrient deficiency in plants. *IDE1* and *IDE2* synergistically induce Fe deficiency-responsive expression in tobacco roots as well as in rice roots and leaves.

In response to Fe overload, plants induce a subset of Fe homeostasis-related genes, including those encoding ferritin, a ubiquitous protein for Fe storage. Through a precise promoter analysis and gel-retardation assay, Petit et al. identified an iron-dependent regulatory sequence (*IDRS*), a cis-acting element that depresses the expression of the maize and *Arabidopsis* ferritin genes (*ZmFer1* and *AtFer1*, respectively) under Fe overload. Nitric oxide (NO) is necessary to mediate transcriptional regulation by *IDRS* and thus regulates the two opposite responses to Fe availability: Fe deficiency and Fe overload. So in conclusion, the regulation of the Fe deficiency response is mediated by a combination of transcriptional and posttranscriptional control, the former being more pronounced among Fe acquisition-related genes. A network of transcription factors has been clarified, which is only partially conserved among nongraminaceous and graminaceous plants. The response to Fe overload is mediated through different pathways than the response to Fe deficiency, but some signaling molecules, such as NO, link both responses.

Report:	CA 7.6/02: Banin A., Navrot J., 2008
Title:	Pattern of iron distribution in the soil-plant system and its possible relation to iron-chlorosis
Document No:	Journal, Communications in Soil Science and Plant Analysis, volume 3, 1972 – Issue 3
Guidelines:	Not stated
GLP	No
Summary:	<p>The following abstract has been taken from the article on the pattern of iron distribution in the soil-plant system:</p> <p><i>'The frequent concentration ranges of various nutrient elements in soils and in plants are compared. Iron is different from almost all other nutrient elements in the fact that its optimal concentration range in plants is much lower than its frequent concentration range in soils. It is suggested that this observation is related to a chemical-physiological mechanism of control on the uptake of iron by plants which in turn may explain the situations in which iron deficiency conditions in plants arise.</i></p> <p><i>Iron deficiency in plants and the typical discoloration ("chlorosis") related to it, are very widespread in agricultural plants. the causes and the mechanism of the deficiency are not yet clear and not agreed upon.'</i></p> <p>The applicant provided a summary of the above review with a summary of relevant text taken from the document:</p> <p><i>'It is seen that the nutrient elements can be divided into three groups according to the relation between the optimal concentration ranges in the plant and those found in soils:</i></p> <p><i>(1) The concentration ranges in plants fall inside or overlap the range in soils. Most of the elements belong to this group: P, K, Ca, Mg, S, Zn, Cu, B, Mo.</i></p> <p><i>(2) The concentration ranges in plants are much higher than in soils. Nitrogen definitely belongs to this group, and possibly phosphorous and sulfur.</i></p> <p><i>(3) The optimal concentration in plants is much lower than the concentration range in soils. Definitely iron, and possibly manganese, belong to this group.</i></p> <p><i>Thus, it is seen that iron and manganese are in a unique position among the nutritional elements in as much as the plant may actually have to "screen" itself against their uptake. The mechanisms by which ions are transported from the soil to the plant are by root interception, mass flow of soil solution, and ion diffusion.</i></p> <p><i>Moreover, iron usually appears in soils as amorphous oxides covering particles or as adsorbed ions on surfaces. This may lead to a high probability of contact with roots followed by iron uptake if iron is absorbed in mechanisms similar to the other elements. Thus, a controlling mechanism developed by the plants to adapt to the prevailing soil conditions is plausible. It seems to us that a possible mechanism available to plants to control the uptake of these two elements and to absorb only the quantities needed for normal metabolism is related to this chemical property.</i></p> <p><i>Furthermore, it seems that the uptake of such elements is dependent on their reduction. It has been proven that plants prefer ferrous iron (II) to ferric iron (III) in their uptake. So is the situation with Mn, in which the absorbed species is the divalent. It, therefore, suggests that there is a general coupling between the uptake of iron (and manganese) and the respiration activity of roots, and that by this coupling, the amounts may be adjusted to suit the plant needs.'</i></p>

RMS comment: Iron is naturally abundant in soils, but the process of iron uptake by plants is slow. This is established in the available scientific literature provided by the applicant. The amount of iron introduced by application of elemental iron is negligible compared to the levels already found in soil. The information provided by the applicant is considered sufficient to support the case that the nature and magnitude of residues in succeeding crops does not need to be addressed with specific trials data.

B.7.6.2. Magnitude of residues in rotational crops

As mentioned before (see points B.7.2.1. and B.7.3.), no relevant residues of elemental iron are to be expected in plants after soil application of the elemental iron. Studies on metabolism in rotational crops are not necessary. Therefore, a waiver on magnitude of residues in rotational crops is justified as well.

B.7.7. OTHER STUDIES**Iron in Drinking Water**

Report:	CA 7.7/01: Anon., 2008
Title:	Iron in Drinking-water
Document No:	WHO/SDE/WSH/03.04/08
Guidelines:	Not stated
GLP	No
Summary:	<p>The applicant provided a summary of the above review with a summary of relevant text taken from the document:</p> <p><i>'Anaerobic groundwaters may contain iron (II) at concentrations up to several milligrams per litre (usually be 0.5–10 mg/litre, but concentrations up to 50 mg/litre can sometimes be found) without discoloration or turbidity in the water when directly pumped from a well. The median iron concentration in rivers has been reported to be 0.7 mg/litre. Concentrations of iron in drinking-water are normally less than 0.3 mg/litre but may be higher in countries where various iron salts are used as coagulating agents in water-treatment plants and where cast iron, steel, and galvanized iron pipes are used for water distribution. Taste is not usually noticeable at iron concentrations below 0.3 mg/litre, although turbidity and colour may develop in piped systems at levels above 0.05–0.1 mg/litre. Laundry and sanitary ware will stain at iron concentrations above 0.3 mg/litre. Iron in water can be determined by atomic absorption spectrometry (detection limit 1 µg/litre) or by colorimetric methods (detection limit 5 µg/litre).</i></p> <p><i>Iron is an essential element in human nutrition. Estimates of the minimum daily requirement for iron depend on age, sex, physiological status, and iron bioavailability and range from about 10 to 50 mg/day.</i></p> <p><i>As a precaution against storage of excessive iron in the body, JECFA established a provisional maximum tolerable daily intake (PMTDI) in 1983 of 0.8 mg/kg of body weight (14), which applies to iron from all sources except for iron oxides used as colouring agents, and iron supplements taken during pregnancy and lactation or for specific clinical requirements. Allocation of 10% of this PMTDI to drinking-water gives a value of about 2 mg/litre, which does not present a hazard to health. The taste and appearance of drinking water will usually be affected below this level, although iron concentrations of 1–3 mg/litre can be acceptable for people drinking anaerobic well-water.</i></p> <p><i>No health-based guideline value for iron is proposed.'</i></p>

B.7.7.1. Effect on the residue level in pollen and bee products

As mentioned before (see point B.7.3), soil application of the compound will not result in relevant residues in plants, pollen or bee products. No additional study is required.

B.7.8. REFERENCES RELIED ON

Literature search

A literature review has been carried out for the active substance, elemental iron. The review has been conducted in accordance with Article 8(5) of Regulation (EC) No. 1107/2009 and are based on the EFSA guidance document as published in EFSA Journal 2011; 9(2):2092.

The literature review report aims to collate information on the active substance, iron powder, that has been published in the scientific peer-reviewed open literature between January 2006 and December 2017. Additional compounds and alternative physical forms of iron (for example, ionic forms, iron salts, iron compounds and iron complexes) were considered by Adama to not be within the scope of the literature. The literature review submitted in relation to the active substance is considered in this dossier with respect to residues.

In November 2016, a search strategy based on a single concept was performed by bibra in the Toxline and TRACE databases for information on the active substance (iron powder). In November 2017, a similar search was performed, via single platform

Databases searched

The table below summarises the databases searched by the notifier as part of the literature search:

Summary of databases searched

Total number of databases searched	11
List of databases used in the literature review and date of last database update	Agricola (18/11/2017), Analytical Abstracts (11/12/2017), BIOSIS Toxicology (11/12/2017), CAB ABSTRACTS (11/12/ 2017), Embase, Environment Abstracts (15/12/2017), Medline (15/12/2017), ToxFile (15/12/2017), Toxicology Abstracts (18/11/ 2017), Toxline (13/12/2017), TRACE (19/12/2017)
Search period	01/01/2006 – 18/12/2017

With respect to residues, during the initial (rapid) filter stage, it was taken into account that iron is ubiquitous in the environment and, as such, is widely present in the earth's biotmatter and natural resources (such as water bodies). Therefore only studies considering the (excess) levels in plant matter/the environment (i.e. residues) that may have generated as a result of iron (powder) application would be considered as potentially relevant. In fact, no such studies were identified.

Search criteria: The databases were searched using the active substance:

- Iron synonyms, CAS number, codes and abbreviations, molecular structure, molecular formula, molar mass and other names/codes were used :
7439-86-6, Iron, Ancor B, Ancor en 80/150, Armco Iron, Atomel 28, Atomel 300M200, Atomel 500M, Atomel 95, Atomiron 44MR, Atomiron 5M, Atomiron AFP 25, Atomiron AFP 5, ATW 230, ATW 432, carbonyl iron, DSP 1000, DSP 1288, DSP 135, DSP 135C, DSP 138, EF 1000, EF 250, EFV 200/300, EFV 250, EFV 250/400, EO 5A, Ferronyl, Ferrous Iron, Ferrovac E, Ferrum, GS 6, [REDACTED] EH. NC 100, PZh-1M3, PZh-2, PZh1M1, PZh2M, PZh2M1, PZh2M2, PZh3, PZh3M, PZh4M, PZhO, Remko, SUY-B 2 or E1UOL152H7 and powder.

Search result:

Rapid result: Based on the abstracts, the following topics were used to classify publications as being obviously irrelevant: efficacy, analytical method development, new ways of synthesis, studies on a molecular level which cannot be related to environmental risk assessment, non-EU monitoring studies, abstracts which refer to a conference contribution not containing data and full text not available, and studies with target organisms with

missing information.

Detailed assessment: Publications which passed the rapid assessment were then evaluated based on their full text versions using the following assessment criteria:

Not relevant because:

- Target substance not a test item
- Conversion into units useful for RA not possible
- Study design / test system not sufficiently described
- Study design / test system not adequate
- Study design / test system not relevant to EU data requirements
- Test system not relevant to representative uses/GAPs
- Test method does not cover the right targets
- Test material deviates from composition of active ingredient / product
- Findings not related to a certain test system
- No endpoint can be derived
- Observations are not attributable (i.e. ecotox) to a specific substance
- Effects are caused by a non-relevant route of exposure
- Observations cannot be transferred into an endpoint

There were 176 publications related to residues which were considered not relevant to the risk assessment after detailed assessment of the full text documents. No publications were considered relevant to the area of metabolism and residues in plants and animals. The methodology used in the search, and determination of publications as not relevant is considered acceptable.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CA 7.2.1/01	Rout G. R., Sahoo S.	2015	Role of iron in plant growth and metabolism N/A Reviews in Agricultural Science, 3:1-24, 2015. GLP: No Published: Yes	N	N	-	Published literature.	n/a
CA 7.2.1/02	Saenchai C., Prom-u-thai C., Lordkaew S., Rouached H., Rerkasem B.	2016	Distribution of iron and zinc in plant and grain of different rice genotypes grown under aerobic and wetland conditions N/A Journal of Cereal Science, Volume 71, September 2016, pages 108-115 GLP: No Published: Yes	N	N	-	Published literature.	n/a
CA 7.2.1/03	Graham R., Stangoulis C. R.	2003	Trace Element Uptake and Distribution in Plants N/A The journal of nutrition GLP: No Published: Yes	N	N	-	Published literature.	n/a
CA 7.2.1/04	Garnett T. P.	2005	Distribution and Remobilization of Iron and Copper in Wheat N/A Annals of Botany, volume 95, Issue 5, April 2005 GLP: No Published: Yes	N	N	-	Published literature.	n/a
CA 7.2.2/01	Ohira Y., Hegenauer J.,	1981	Distribution and Metabolism of Iron in Muscles of Iron-Deficient Rats	N	N	-	Published literature.	n/a

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
	Saltman P., Edgerton V. R		N/A Biological trace element research, Vol. 4, Issue 1, 45-56 (1982) GLP: No Published: Yes					
CA 7.2.2/02	Rouault T. A.	2003	How Mammals Acquire and Distribute Iron Needed for Oxygen-Based Metabolism N/A PLoS Biol 1(3): e79; https://doi.org/10.1371/journal.pbio.0000079 GLP: No Published: Yes	N	N	-	Published literature.	n/a
CA 7.2.2/03	Papanastasiou DA, Vayenas DV, Vassilopoulos A., Repanti M.	2000	Concentration of iron and distribution of iron and transferrin after experimental iron overload in rat tissues in vivo: study of the liver, the spleen, the central nervous system and other organs. N/A Pathol Res Pract. 2000, 196(1): 47-54 GLP: No Published: Yes	N	N	-	Published literature	n/a
CA 7.2.5/01	Zhao L., Xia Z., Wang F	2014	Zebrafish in the sea of mineral (iron, zinc, and copper) metabolism N/A Front Pharmacol. 2014; 5: 33. GLP: No Published: Yes	N	N	-	Published literature	n/a
CA 7.2.5/02	Carriquiriborde P., Handy R. D., Davies S. J.,	2003	Physiological modulation of iron metabolism in rainbow trout (Oncorhynchus mykiss) fed low and high iron diets	N	N	-	Published literature	n/a

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			N/A The Journal of Experimental Biology 207, 75-86 GLP: No Published: Yes					
CA 7.3/01	Vitosh M.L., Wamcke D.D., Lucas R.E.	1994	Secondary and Micronutrients for Vegetables and Field Crops N/A Michigan State University Extension, Departement of Crop and Soil Sciences, E- 486, Revised August 1994 GLP: No Published: Yes	N	N	-	Published literature	n/a
CA 7.3/02	Chen Y., Barak P.	1982	Iron nutrition of plants in calcareous soils N/A Advances in Agronomy, 35, 217 – 240 GLP: No Published: Yes	N	N	-	Published literature	n/a
CA 7.3/03	Vose P. B.	1982	Iron nutrition in plants: a world overview Iron nutrition of plants in calcareous soils N/A Journal of Plant Nutrition, 5, 233 GLP: No Published: Yes	N	N	-	Published literature	n/a
CA 7.3/04	Pasian C. C.	2001	Micronutrient disorders N/A Ohio State University Fact Sheet HYG- 1252-98 GLP: No Published: Yes	N	N	-	Published literature	n/a
CA 7.3/05	Nand Kumar Fageria, Baligar V.C., Wright R.J.,	1990	Iron nutrition of plants: an overview on the chemistry and physiology of its deficiency and toxicity N/A	N	N	-	Published literature	n/a

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			Pesq. agopec. bras., Brasília, 25(4):553-570, abr. 1990 GLP: No Published: Yes					
CA 7.6/01	Kobayashi T., Nishizawa N. K.	2003	Iron Uptake, Translation, and Regulation in higher plants N/A Annu. Rev. Plant Biol. 2012. 63:131–52 GLP: No Published: Yes	N	N	-	Published literature	n/a
CA 7.6/02	Banin A., Navrot J.	2008	Pattern of iron distribution in the soil-plant system and its possible relation to iron-chlorosis N/A Journal, Communications in Soil Science and Plant Analysis, volume 3, 1972 – Issue 3 GLP: No Published: Yes	N	N	-	Published literature	n/a
CA 7.7/01	Anon.	2008	Iron in Drinking-water N/A WHO/SDE/WSH/03.04/08 GLP: No Published: Yes	N	N	-	Published literature	n/a