



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

Volume 3 – B.6 (AS)

Great Britain

February 2022

Version History

When	What
November 2021	Initial DAR
February 2022	Updated post Expert Committee on Pesticides (ECP) Independent Scientific Advice (ISA) (November 2021 meeting)

Table of contents

B.6. TOXICOLOGY AND METABOLISM DATA.....	4
B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS	6
B.6.1.1. Absorption, distribution, metabolism and excretion by oral route	7
B.6.1.2. Absorption, distribution, metabolism and excretion by other routes	12
Summary of absorption, distribution, metabolism and excretion in mammals	12
B.6.2. ACUTE TOXICITY.....	13
B.6.2.1. Oral	14
B.6.2.2. Dermal.....	16
B.6.2.3. Inhalation	16
B.6.2.4. Skin irritation.....	21
B.6.2.5. Eye irritation	22
B.6.2.6. Skin sensitisation	22
B.6.2.7. Phototoxicity.....	24
B.6.3. SHORT-TERM TOXICITY	24
B.6.3.1. Oral 28-day study.....	26
B.6.3.2. Oral 90- day study.....	26
B.6.3.3. Other routes.....	33
B.6.4. GENOTOXICITY.....	36
B.6.4.1. <i>In vitro</i> studies.....	38
B.6.4.2. <i>In vivo</i> studies in somatic cells.....	41
B.6.4.3. <i>In vivo</i> studies in germ cells	41
B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS	41
B.6.6. REPRODUCTIVE TOXICITY	46
B.6.7. NEUROTOXICITY	46
B.6.7.1. Neurotoxicity studies in rodents	46
B.6.7.2. Delayed polyneuropathy studies	47
B.6.8. OTHER TOXICOLOGICAL STUDIES.....	47
B.6.8.1. Toxicity studies on metabolites and relevant impurities.....	47
B.6.8.2. Supplementary studies on the active substance - Immunotoxicity	47
B.6.8.3. Studies on endocrine disruption.....	47
B.6.9. MEDICAL DATA AND INFORMATION	47
B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies.....	47
B.6.9.2. Data collected on humans	48
B.6.9.3. Direct observation.....	48
B.6.9.4. Epidemiological studies	48
B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test.....	48
B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment	49
B.6.10. REFERENCES RELIED ON	49

B.6. TOXICOLOGY AND METABOLISM DATA

This assessment addresses the toxicology and metabolism of elemental iron, in the form [REDACTED] elemental iron. Elemental iron (Fe^0 , CAS No. 7439-89-6) is a new molluscicidal active substance developed by Adama Ltd for application on edible and non-edible crops grown in the field, greenhouses and indoors. The applicant's source of the active substance holds approval in the USA as a food-grade mineral supplement (US CFR (Code of Federal Regulation) 21 184.1375 for elemental iron). The applicant has provided information to support the compliance of their source with the specification for elemental iron [REDACTED] entry in the US Food Chemical Codex (Regulatory compliance statement July 2019). In the EU, elemental iron is one of a range of iron compounds authorised for use in food or food supplements (Regulations (EC) Nos. 1170/2009 and 53/2009, respectively). The representative product 'Final Bite' is a ready-to-use granular bait, containing 10 g/kg (1 % w/w) elemental iron in a nearly dust-free formulation. The product is used as a bait application during pre- and post-emergence of the crop.

The proposed mode of molluscicidal action of elemental iron in the representative product is as follows:

Following ingestion of the granular pellet, the acidic gastric environment in the target species causes liberation of solubilised, ionic iron, in the form of Fe^{2+} . In the representative product, the ionic form of iron is then available to form a soluble complex with a complexing agent that interferes with the oxygen transport capability of haemocyanin in molluscs - a mode of action irrelevant to humans since humans do not possess this target.

In considering the toxicological profile of elemental iron as a powdered active substance, it is acknowledged that exposure of humans to poorly soluble metal-containing particles may theoretically result in adverse effects due to the particle surface (particle size, form and surface reactivity), particle uptake or the release of metals from the particle. These potential hazards of elemental iron particles have been addressed by the submission of published literature on iron powders. As elemental iron is an existing food supplement substance with a significant amount of publicly available literature, the toxicological assessment of the substance is also extrapolated from these sources of information. In addition, the EU assessments of iron sulphate (FeSO_4 ; DAR UK 2008, EFSA conclusion 2012) and ferric phosphate (FePO_4 RAR, DE 2013, EFSA conclusion 2015) have been used to bridge to the toxicity potential of elemental iron. Iron sulphate, ferric phosphate and ferric pyrophosphate are approved in the EU for use as pesticidal active substances. Conclusions on the safety of elemental iron for the currently proposed molluscicidal use can be drawn from the literature on insoluble iron particulate matter and the scientific evaluations of these other – more soluble - forms.

The toxicological data on elemental iron (active substance) generated by the applicant is limited to an acute inhalation toxicity study, driven by concerns over the particle size of the active substance. Data from the literature on elemental iron are also available, but these, although evaluated by HSE where it might be considered relevant to address a data point, are of limited regulatory value. In view of the fact that, following external exposure to elemental iron, and regardless of the route of exposure, any subsequent systemic exposure of iron will be to its ionic forms (see below for justification), in the absence of either substance-specific literature or regulatory studies, the read-across from ferrous and ferric ionic forms has been performed where necessary for all routes of exposure:

- **Oral:** In humans, it is expected that solubilisation of elemental iron – presumed to be Fe^{3+} (ferric) - will occur in the acidic environment of the stomach and proximal duodenum, with subsequent uptake of inorganic, ferrous (Fe^{2+}) iron occurring via active, homeostatic processes, mainly in the duodenum and proximal jejunum. Further down the gastrointestinal tract, the alkaline environment of the jejunum reduces the solubility of iron by conversion to the ferric form, lowering the bioavailability of iron released from elemental iron (Hurrell *et al.*, 2002). There is no evidence that particulate or elemental iron will transfer into the blood.
- **Dermal:** Elemental iron (Fe^0) is poorly soluble in aqueous or organic solvents and hence negligible oxidation to either Fe^{2+} or Fe^{3+} is expected to occur following topical exposure at a skin sweat pH of approximately 4.2-6.5 (Hedberg *et al.*, 2010a; Stefaniak, A. B. *et al.*, 2014). Elemental iron is a micron-sized particulate substance, not a nanomaterial; therefore, it is unlikely that soluble or insoluble elemental iron will partition into the lipid matrix of dermal cellular membranes. A potential for

partitioning is even less likely to apply to elemental iron as a large granular PPP. Since it is well known that oral absorption of iron is actively physiologically regulated (Lynch *et al.*, 2018), and iron is ubiquitous in the human environment, the likelihood of passive migration through the stratum corneum to the dermis - and subsequent entry into the systemic circulation - is unlikely. Similarly, no local reactions from exposure to elemental iron in particulate form are predicted, as the surface of the material is not redox-active or corrosive (UK Iron DAR Vol 3 CA_B.2).

- **Inhalation:** Elemental iron (Fe^0) is incorporated into a nearly dust-free, solid plant protection product of non-respirable size ($> 50 \mu\text{m}$; see UK Iron DAR Vol 3 CP_B.2) and therefore any significant exposure via inhalation is considered unlikely. Any iron released from the solid matrix can be assumed to be either in an ionised form, or poorly soluble particles. If ionic iron penetrates the protective lung lining fluid – rich in antioxidants - systemic exposure to ionic iron is likely to be negligible due to the innate control of iron absorption. Furthermore, it is expected that insoluble deposited particulate matter will be captured by the mucociliary escalator for elimination via the GIT, with a subsequent solubilisation comparable to the oral route of exposure (described above).

No other data are supplied, and none are considered necessary, for the following reasons:

As iron is not easily metabolized by humans from food sources and since iron deficiency is a widespread condition, iron supplementation of food is a widely accepted practice. Those particularly vulnerable to iron deficiency are infants, toddlers, adolescents, menstruating and pregnant women, the elderly and those consuming foods high in iron absorption inhibitors. As a result, elemental iron (carbonyl, electrolytic and hydrogen-reduced) forms are currently approved for use in the EU in foods or as beneficial food supplements (Reg. (EC) No. 953/2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses). In accordance with this legislation, the single entry for elemental iron covers the three forms – reduced, carbonyl and electrolytic. HSE notes that the applicant's source of elemental iron is compliant with the US Food Chemical Codex specification, permitting its use as a food-additive in the USA, and is therefore suitable for human consumption. Similarly, ferric diphosphate (pyrophosphate), saccharate, ammonium citrate, sodium diphosphate; and ferrous ascorbate, bisglycinate, carbonate, citrate, fumarate, gluconate, lactate, L-pidolate and sulphate are already approved for use in foods (Reg. (EC) No. 953/2009).

Human exposure to iron and iron compounds is extensive. As highlighted during the EU reviews of iron sulphate and ferric phosphate, iron is ubiquitous in the environment and is essential for plant and animal function, including humans. Soil contains a range from 0.5 to 5 % of iron (Brady¹, 1974) and dietary sources such as liver, kidney, beef, ham, egg yolk and soybeans contain iron concentrations of the order of 30-150 mg Fe/kg fresh weight (Elinder², 1986). Iron is a natural constituent of the human body and is involved in oxygen transport, electron transfer, redox reactions, DNA synthesis and many other cellular functions. Iron deficiency leads to reduced levels of haemoglobin and myoglobin, with reduced cellular ATP. A lack of iron-dependent enzymes may impair RNA synthesis and neurotransmitter metabolism. Iron-deficiency anaemia increases the risk for low birth weight, while the cognitive deficiency symptoms observed with such anaemia include deficits in attention, perceptual motor speed, memory and verbal fluency. There is consensus on the importance of iron for cognitive function, with sufficient evidence of its role in cognitive development (EFSA, 2009).

Elemental iron is poorly soluble, of low bioavailability, non-volatile, and as the formulated product, elemental iron particles are incorporated in the product within a solid matrix that is of a non-respirable size. These physicochemical characteristics mean that systemic exposure via the oral, dermal and inhalation routes to the active substance from the product is low. Despite the low likelihood of exposure via the inhalation route from the representative product, due to the expected particle size of the active substance, the applicant has submitted a study on the acute inhalation toxicity of the active substance; no other data has been generated on the active substance. A data package on the representative product 'Final Bite' has been submitted, comprising of an *in vivo* dermal sensitisation (LLNA) study and *in vitro* dermal and ocular irritancy studies – these have been summarised in Volume 3CP_B6 document.

The information publicly available has been generated on [REDACTED] elemental iron, which differs from [REDACTED] iron in the manufacturing process, not in composition. Hurrell *et al.* (2002) refer to [REDACTED] elemental iron as

¹ Brady, NC, 1974, *The Nature and Properties of Soils*, 8th Ed., Macmillan Publishing, NY

² Elinder, CG, 1986, *Handbook on the toxicology of metals*, Friberg *et al.* (Eds), Elsevier

being sponge-like, irregular and porous, whereas [REDACTED] iron is smooth, dense and less liable to surface oxidation than [REDACTED] iron powder. Zhu *et al.* (2016) state that the [REDACTED] form of elemental iron is considerably larger than [REDACTED] elemental iron. Overall, this could imply that in a simple solution, [REDACTED] elemental iron is theoretically likely to be more bioavailable than carbonyl elemental iron. However, the applicant did not produce data supportive of this hypothesis and regardless of the method of manufacture, it is known that in complex biological media, elemental iron is of low bioavailability compared to ferrous sulphate. In considering the availability of inhalation toxicity data generated on particulate [REDACTED]-elemental iron, further information on the physicochemical properties of the particles would need to be known before [REDACTED] elemental iron is considered to be an acceptable surrogate for addressing particle-mediated toxicity of elemental iron.

The data from all scientific peer-reviewed sources, combined with the low toxicity of elemental iron and the proposed read-across of the systemic toxicity of elemental iron from data on ionic iron forms indicate that further testing is not necessary. For each endpoint, where reliable data on elemental iron are available, these will take priority in the evaluation. If these are not available, then data on the less soluble ferric (Fe^{3+}) ion form will be considered. If neither are available, then data on the more soluble and bioavailable ferrous (Fe^{2+}) ion form will be taken into account. This approach was supported by our advisory scientific committee, the ECP. Therefore, HSE concludes that an exemption from the requirements of the submission of further toxicological studies is justified.

B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS

Iron and iron salts are natural constituents of the human and animal diet, and the homeostatic processes involved in iron metabolism have been extensively published in peer-reviewed sources. Therefore, no new regulatory studies of the toxicokinetic characteristics of elemental iron in mammals have been included in the dossier supporting elemental iron. Instead, the applicant proposes a read-across from the more soluble ionic forms of iron for which agreed EU assessments are already available; this read-across has been supplemented by 5 peer-reviewed scientific publications, which are listed below.

Author	Title	Source	Supplemental to conclusions from EU reviews of FeSO_4 and FePO_4
Hurrell, R. F., 1999 (CA 5.1.1/01)	The mineral fortification of foods, Chapter 3: Iron	Review	N/A
Hurrell, R F., 2002 (CA 5.1.1/02)	Fortification: Overcoming technical and practical barriers	Review	N/A
Swain, J. H. <i>et al.</i> , 2003 (CA 5.1.1/03)	Bioavailability of elemental iron powders to rats is less than bakery grade ferrous sulfate and predicted by iron solubility and particle surface area	Original research article	Y
Hoppe, M. <i>et al.</i> , 2006 (CA 5.1.1/04)	The relative bioavailability in humans of elemental iron powders for use in food fortification	Original research article	Y
Swain, J. H. <i>et al.</i> , 2006 (CA 5.1.1/05)	An irradiated electrolytic iron fortificant is poorly absorbed by humans and is less responsive than FeSO_4 to the enhancing effect of ascorbic acid	Original research article	Y

N/A: not applicable owing to the conclusions of this article being reported under previous EU reviews of other iron-containing PPP active substances

B.6.1.1. Absorption, distribution, metabolism and excretion by oral route

The absorption of iron from the gastrointestinal tract is highly regulated in mammals and plays the leading role in the maintenance of iron homeostasis; necessarily so, since the capacity of the body to excrete iron is extremely limited. The absorption of iron is also dependent on the source of iron (heme or non-heme iron), the iron content of the diet and the presence of other dietary ingredients. Dietary iron is composed of 10 % heme iron and 90 % non-heme iron. The active substance - elemental iron – is a potential source of inorganic, non-heme iron. Non-heme iron is 2 to 3-fold less readily absorbed than heme iron and therefore it is less bioavailable than heme iron. In addition, unlike the heme-form of iron, the absorption of ionic non-heme iron is greatly affected by other dietary constituents. Since the absorption of heme-iron is irrelevant to the potential bioavailability of elemental iron, it is not considered further in this section.

The mechanism of the absorption of iron has been extensively studied - published in scientific peer-reviewed journals - over several decades. A summary of these processes is presented below:

Absorption of solubilized iron released from elemental iron is a two-step mechanism – initial transfer from the gut lumen, followed by subsequent transfer to the systemic circulation via the hepatic portal vein.

Iron crosses cell membranes exclusively in the ferrous (Fe^{2+}) state, through an active transport mechanism, not by passive diffusion. Ferric ions (Fe^{3+}) in food are liberated in the stomach by acid digestion, reduced to the ferrous state in the duodenum, and only thus made available for absorption. In routine absorption of dietary iron, the transport of ferrous compounds is by means of an ATP-dependent, saturable, carrier-mediated process that is reliant on divalent metal transporter protein (DMT1) binding of iron in the lumen of the gastro-intestinal tract. This process, which occurs mostly in the duodenum and upper jejunum enterocytes, is rate-limiting; however, it is noted that the entire intestinal tract can absorb some iron. Because free iron ions are toxic in cells - due to the creation of reactive oxygen species via the Fenton reaction – cellular iron is stored as bound ferritin. Once within the cell, ferrous iron is either converted to the trivalent (ferric) form and stored via attachment to ferritin protein, or it is transported out of the cell into the systemic circulation via ferroportin transporter in the intestinal mucosal cell basolateral wall, oxidised to the ferric Fe^{3+} form, which then binds to apotransferrin protein to form the plasma protein complex known as transferrin. Ferric iron in plasma transferrin is carried via the systemic circulation to the reticulo-endothelial cells of the bone marrow for use in haemoglobin synthesis, or to the liver or spleen for storage as intracellular ferritin or haemosiderin in the hepatocytes, Kupffer cells and splenic macrophages. Free iron does not cross the blood-brain barrier. There is also some storage in kidney, heart and skeletal muscle. Iron is incorporated into several different types of proteins for utilization in a variety of biological functions. Proteins that require iron for functionality, apart from haemoglobin and myoglobin for oxygen transfer, include cytochromes within the mitochondrial electron transport chain for cellular energy production and cytochrome P450 enzymes for the metabolism of endogenous and exogenous compounds.

The extent of absorption from the gut varies between individuals and is significantly influenced by several factors, both diet- and host-related. The various mechanisms whereby dietary substances affect iron absorption include chemical reactions such as chelation or changes in iron valency, effects on intestinal or mucosal function and competition with other minerals for transport protein. Ligands such as citric and ascorbic acid, fructose and amino acids, form soluble monomeric complexes with iron, preventing precipitation and polymerization, and thereby promoting absorption. Other chelating compounds, including polyphenols (containing alkyl groups), phosphates, carbonates and oxalates have an adverse effect on bioavailability, their inhibitory effect being usually due to the formation of large polymers. Meat, fish and poultry promote non-heme iron absorption; the mechanism for this is not yet certain but may plausibly involve the formation of iron complexes with amino acids such as cysteine or peptides, thereby counteracting luminal factors that inhibit absorption. Reducing agents such as ascorbic acid, change the valency of iron from Fe^{3+} to Fe^{2+} , which increases absorption, since Fe^{2+} is more soluble at $\text{pH} > 3$, as is found in the duodenum (Lynch, 2018). Dietary constituents that alter gut secretion and transit time affect the bioavailability of iron. For example, alcohol and meat promote gastric acid production, lowering the pH of the proximal small intestine and increasing the solubilization of iron, while calcium suppresses iron absorption.

The upper limit for absorption is determined by host factors such as iron status:

- Body iron stores (as measured by serum ferritin), which is the principal determinant

- Rate of erythropoiesis
- Level of iron to which the intestinal mucosal cells have been previously exposed
- Tissue hypoxia, if present

The absorption of non-heme iron requires prior solubilization of the iron salts in the upper gastrointestinal tract, ferrous salts being more readily absorbed than ferric salts, the latter being reduced - in the gastric lumen- to the ferrous Fe^{2+} form by the enzyme duodenal cytochrome B. HSE notes that the molluscicidal MOA of elemental iron is via postulated release of ferrous iron in the acidic gastric environment.

Typical daily intake of iron is approximately 6 to 14 mg, but only 10-18% of ingested iron is absorbed, from infant to adult and accounting for the extra demands of menstruation and pregnancy/lactation (EFSA, 2015^b). The excretion of iron is largely dependent on the production and degradation of erythrocytes, as most iron in the body is contained within erythrocytes. However, virtually all of the iron from erythrocytes is recycled for incorporation into haemoglobin and thus only a small amount of iron is excreted daily. The exception is where there is substantial blood loss. Daily basal iron losses are reported to be 0.2 mg in infants, 0.5 mg in children, 1.0 mg in men, 0.64 mg in non-menstruating women and 1.3 mg in menstruating women (EFSA 2015^b). Excretion of iron occurs predominantly via faeces, although trace amounts of iron are also excreted in the urine, desquamated gastrointestinal cells at the end of their lifespan, and bile.

Relevant peer reviewed scientific data related to elemental iron powder administered as an additive in human food are presented below. The studies are in general agreement with those referenced in the EU reviews of FeSO_4 and FePO_4 , which address comparative bioavailability of iron compounds, as used in fortification of foods. The relative bioavailability (RBV relative to the reference ferrous sulphate) are given for several compounds, including ferric orthophosphate and various other forms of elemental iron powder (Hurrell 1999, 2002 and Swain, 2003, Table 6.1-1). HSE notes that due to a lack of demonstrable effect upon iron status in humans i.e. minimal absorption, there is only weak support for the use of elemental iron as a food supplement (Hurrell, 2002).

Table 6.1-1: Bioavailabilities of various forms of iron in rats, relative to FeSO_4 , (CA 5.1.1/01, 02, 03)

Compound	Relative Bioavailability (%) compared to ferrous sulphate		
	Rats		Humans
	Hurrell 1999, 2002	Swain 2003	Hurrell 2002
Ferrous sulphate	100	100	100
Ferrous gluconate	97	ND	89
Ferrous lactate	ND	ND	106
Ferrous fumarate	95	ND	100
Ferrous succinate	119	ND	92
Ferrous citrate	76	ND	74
Ferric orthophosphate	6 – 46	ND	25 – 32
Ferric pyrophosphate	45 - 58	ND	21 - 74
Elemental iron powders:			
Electrolytic	16 – 70	44 – 60	75
H-reduced	13 – 54	39 – 49	13 – 148
CO-reduced	12 – 32	18 – 26	ND
Reduced	ND	21 – 29	ND
Carbonyl	35 – 66	62 – 71	5 – 20

ND = Not determined

The differences and variability depicted in Table 6.1-1 are influenced by factors including an individual's iron status and diet, and by particle surface area. Therefore, although the human data appear to suggest that the bioavailability of H-reduced iron can be up to 148 % that of ferrous sulphate, this is thought to be a consequence of the study design, including undefined dietary iron intakes.

The EFSA NDA³ panel in 2015 adopted values from 16-18 % for orally ingested iron that is absorbed, in the context of a serum ferritin value of 30 µg/L to represent iron status, and considering also the extra demands of menstruation, pregnancy and lactation. Average daily requirements and population reference intakes were determined to be 6 and 11 mg/day respectively for men, and 7 and 16 mg/kg for pre-menopausal women.

Under normal physiological circumstances – in the absence of anaemia or clinical iron overload disorders - approximately 10 % of ingested iron is absorbed (FeSO₄; DAR UK 2008, EFSA conclusion 2012; FePO₄; RAR DE 2013, EFSA conclusion 2015) and the risk of systemic iron overload from dietary sources is negligible with normal intestinal function (EFSA NDA, 2015).

Reference	CA 5.1.1/04: Hoppe, M, Hulthen, L and Hallberg, L (2006)
Article title	The relative bioavailability in humans of elemental iron powders for use in food fortification
Journal title	European Journal of Nutrition 45, 37-44
Test substance	Seven different elemental iron powders were evaluated, including H-reduced, carbonyl and electrolytic forms
Acceptability	Acceptable as a human volunteer study. <i>Note: Only the data relating to iron powder bioavailability have been summarized below. The publication also considered iron powder (electrolytic form) administered simultaneously with ascorbic acid (enhancing absorption), but this is not presented in the text below.</i>

Materials and Methods:

In a bioavailability study in humans, groups of 16 men were given (on separate occasions in a double-blind cross-over trial) a wheat roll fortified with iron sulphate (as a positive control) or with one of seven types of elemental iron powder which encompassed reduced, H-reduced, carbonyl and electrolytic iron powders. These powders were also the subject of a previous investigation in rats (see Swain, 2003; results summarised in Table 6.1-1). In the present study, the men were all regular blood donors, age range 35-61 years, mean age 50 years. Blood samples were collected every hour for 6 hours. Serum iron levels were measured using a validated method. The bioavailability of each iron powder relative to iron sulphate monohydrate was calculated from the area under the curve of the serum iron graph (AUC_{0-6h}), adjusted for personal diurnal variation.

Results:

Following consumption, serum iron concentrations peaked around 3-4 hours and were approximately 15-30 µM for the elemental powders (compared to 30-45 µM for iron sulphate). Baseline serum haemoglobin concentration and total iron binding capacity were not statistically significantly different between the iron sulphate and iron powder forms.

The relative bioavailability of the powders within 6 hours of administration (compared to iron sulphate; absolute bioavailability was not reported) ranged from 36-65% (Table 6.1-2).

³ Scientific Opinion on Dietary Reference Values for iron, EFSA Journal 2015;13(10):4254

Table 6.1-2: Bioavailabilities of various forms of iron in humans, relative to FeSO₄; (CA 5.1/04)

Elemental iron powders:	Relative bioavailability %
Electrolytic 'A-131'	65
Electrolytic	59
H-reduced 'AC-325'	56
H-reduced 'Hi-Sol'	50
Reduced 'Atomet 95SP'	36
Carbonyl 'CF'	37
Carbonyl 'Ferronyl'	58

Conclusion

In comparison to FeSO₄, a range of elemental iron powders currently available for commercial use are significantly less well absorbed in humans.

Reference	CA 5.1.1/05: Swain, J. H., Johnson, L. K. and Hunt, J. R. (2006)
Article title	An irradiated electrolytic iron fortificant is poorly absorbed by humans and is less responsive than FeSO ₄ to the enhancing effect of ascorbic acid.
Journal title	Journal of Nutrition 136, 2167 – 2174
Test substance	A-131 electrolytic iron powder (purity not reported), radiolabelled
Acceptability	Acceptable as a human volunteer study. However, the authors are unable to ascertain whether the prolonged storage of the test item led to radioactive decay and lower sensitivity of the study. <i>Note: Only the data relating to iron powder administered alone have been summarized below. The publication also considered iron powder administered simultaneously with ascorbic acid (enhancing absorption), with and without phytic acid (inhibits absorption).</i>

Materials and Methods:

Following fasting, 7 men and 13 women consumed a test meal containing 3 mg of radiolabelled electrolytic iron powder, within 24 hours of consuming a test meal containing 3 mg of iron as radiolabelled iron sulphate. The radiolabelled test substance had been stored under vacuum for 7-8 years; the study authors postulated that the irradiation procedure may have altered the physical form and diminished the bioavailability of the elemental iron powder. The radioactivity 2 weeks after dosing, compared to the radioactivity 1-3 hours after the meal, was used to estimate the absorption/retention of the iron from both sources (normalized to serum ferritin). The volunteers from this experiment and two further experiments involving additional administration of ascorbic acid (56 volunteers in total) were all healthy, age range 21-65 years (mean 37 y).

Results:

Two weeks after consumption, the absorption of iron from the administered iron powder was calculated to be 0.8%, as compared with a retention of 5.8% for iron sulphate. The absorption in humans of iron from irradiated electrolytic powder was only 5-15% of the absorption from FeSO₄.

Conclusion

Despite a much higher bioavailability of this source of electrolytic iron when tested previously by another research group (quoted within the current article as 50% relative to FeSO₄), its bioavailability was found to be relatively poor in humans by the present research group. It is possible that the method of radiolabelling the test article (irradiation) was a confounding factor in the current study; nonetheless, the evident low bioavailability of electrolytic elemental iron is consistent with other sources of information.

Reference	CA 5.9.2/01: Gordeuk, V.R., Brittenham, G. M., McLaren, C.E., Hughes, M. A. and Keating L. J. (1986)
Article title	Carbonyl iron therapy for iron deficiency anemia
Journal title	Blood, 67(3), 745-752
Test substance	Elemental iron (carbonyl form) in combination with ferrous sulfate
Acceptability	Considered acceptable, with restrictions, this is a human volunteer study. The test laboratory was identified as the Dept of Medicine, Cleveland Metropolitan General Hospital, USA. No technical specification details of the elemental iron are available. <i>Note: This reference also appears in the acute toxicity section 6.2 of this DAR chapter. Only the data relating to serum iron levels from single and repeat iron powder oral administration in non-anaemic or anaemic volunteers is summarised below.</i>

To determine the safety and efficacy of elemental carbonyl iron powder therapy for iron-deficiency anaemia, non-anaemic (healthy) and anaemic volunteers received 1000 to 10,000 mg of carbonyl iron (15 to 150 times the amount of iron in the usual dose of ferrous sulphate).

Materials and methods:

Nineteen healthy, non-anaemic adult volunteers received an oral dose of 100 mg iron sulphate + 100 mg ascorbic acid as a baseline reference solution before various doses of Fe⁰ were administered. Five subjects/group received carbonyl Fe⁰ iron at single doses of 100, 1000 and 5000 mg at approximately 1-week intervals; four subjects received a 10,000 mg dose. Blood sampling was taken at regular intervals for up to 6 h post-dose for serum iron content analysis. On a separate occasion, all volunteers received 3000 mg Fe⁰ and blood sampling was taken at regular intervals for up to 24 h post-dose for serum iron content analysis.

Thirty-two anaemic volunteers received short-term (7 – 28 d) oral courses of carbonyl iron powder at 500 or 1000 mg/d up to x3 daily, resulting in a cumulative dose of between 16 – 84 g Fe⁰ over the course of the study. Blood sampling was at 0, 1, 3, 6, 9 and 12 weeks for determination for serum iron content analysis and clinical chemistry measurements to identify haematological, hepatic or renal effects and are reported elsewhere in the current document.

Results:

Mean maximal increase in serum iron was at 3 h after the 100 mg reference dose of ferrous sulphate. With an equivalent dose of carbonyl iron, the rise was less abrupt, but more sustained, the peak increase being at 6 h and dose related in magnitude. The incremental doses of carbonyl iron led to a comparable increase in the serum iron content, with no saturation at the top dose of 10,000 mg Fe⁰, indicating that the serum iron-binding capacity was not exceeded.

In the healthy subjects dosed with a single dose at 3000 mg Fe⁰, serum iron change followed the same trend as the reference solution of 100 mg Fe²⁺, with the mean maximal change in serum iron content detected at 3 to 6 h, followed by a decline at 12 and 24 h for both the ionic and zero-valent doses.

In anaemic patients, there were indications that the absorption of Fe⁰ proceeds in a controlled physiological manner – there was a declining % absorption with increasing cumulative dose of Fe⁰ over an increased treatment time (see Table 6.1-3).

Table 6.1-3: Amount of iron absorbed and estimated percentage absorption in anaemic subjects, representing a human population prone to higher degrees of iron absorption.

Regimen* per d	Number of subjects	Cumulative dose (g)	Amount of Fe absorbed (mg/d)	Estimated absorption %
500 – 1000 mg, x2-x3, for 1 – 1.5 wks	3	16 – 21	47 ± 9	2.2 ± 0.2
500 mg x3 for 2 wks	6	21	27 ± 5	1.8 ± 0.3
1000 mg x2 for 2 wks	4	28	19 ± 5	0.9 ± 0.2
1000 mg x1 for 3 wks	5	21	12 ± 3	1.1 ± 0.3
500 mg x3 for 3 wks	8	31.5	14 ± 2	1.9 ± 0.1
1000 mg x3 for 4 wks	6	84	15 ± 2	0.5 ± 0.1

* administration frequency between meals

Conclusion

These human volunteer data indicate that the absorption of iron from carbonyl iron is under physiological control. Under single exposure conditions, a dose-dependent absorption was observed, however, a saturation of absorption was evident following repeated, relatively high cumulative oral exposure.

B.6.1.2. Absorption, distribution, metabolism and excretion by other routes

HSE considers that the likelihood of absorption and systemic exposure via the inhalation and dermal routes to elemental iron in the powder form (as supplied) is small, therefore, specific toxicokinetic studies by these routes are not required.

The dermal absorption of elemental iron from its representative product is addressed in CP_B6, where a dermal absorption value of 10% is proposed for solubilised, ionic iron following its dissolution into sweat.

Overall summary of ADME

The systemic toxicity of elemental iron has been extrapolated from the EU assessments of the existing approved substances, iron sulphate and ferric phosphate (EU approval of ferric pyrophosphate July 2020). Considering the poor solubility of elemental iron in both aqueous and organic solvent, the read-across is supported since both of these iron-based compounds are significantly more bioavailable substances in comparison to elemental iron and are also the most abundant forms in the systemic circulation following exposure to elemental iron.

Summary of absorption, distribution, metabolism and excretion in mammals

Oral exposure

Iron is an essential metal, and by virtue of its ability to undergo facile 1-electron loss or gain, it is involved in many critical cellular processes fundamental to life which require cytochromes, haemoglobin, myoglobin, metalloenzymes etc. These include oxygen transport, energy production, xenobiotic metabolism and DNA synthesis. Iron is toxic in its redox-active ‘free’ form and its availability is tightly regulated under normal homeostasis. There are no active routes of excretion and as there is no indication that iron accumulates in the body, it is evident that effective control at the point of absorption is vital. A fraction, estimated at 10 – 18 %, of dietary iron is absorbed, with several factors influencing absorption, mainly chemical form, solubility in the gastrointestinal tract (GIT), interaction with other dietary components and the systemic requirements of the body. In the EU assessments of the approved substances, iron sulphate and ferric phosphate (EU approval of ferric pyrophosphate July 2020), **an oral absorption value of 50% has been agreed**, based on human data, and is applicable to the risk assessment of elemental iron.

The mechanism of iron absorption is dependent on whether exposure is to heme- or non-heme sources, and in relation to elemental iron, the absorption of non-heme iron shall be the focus of this evaluation.

Inorganic, non-heme iron crosses cell membranes only in the ferrous (Fe^{2+}) state, through an active transport mechanism, not by passive diffusion; ferric ions (Fe^{3+}) in food have to be liberated in the stomach by gastric acid digestion, reduced to the Fe^{2+} state, and only thus made available for absorption. Alternatively, Fe^{3+} which arrives at the duodenum is reduced to the ferrous form by duodenal cytochrome B, a protein found on the surface of the enterocyte. The fraction of elemental iron solubilised in gastric acidic conditions is available for absorption into the intestinal mucosal cells by the divalent metal transporting protein (DMT1) which is expressed on the apical membrane of mature duodenal or proximal jejunum enterocytes.

Once inside the enterocyte, iron of either heme or non-heme form enters the same pool and is either stored intracellularly as ferritin (with up to 4500 ferrous ions per ferritin complex) or it is transported across the basement membrane via the ferroportin protein to the plasma carrier, apotransferrin, thereby forming transferrin. The main pools of ferritin are the liver and reticulo-endothelial cells. A small amount of absorbed iron may be retained within the enterocyte for processing into heme within mitochondria. The efflux of iron from enterocytes to apotransferrin is coupled to re-oxidation of Fe^{2+} to Fe^{3+} by the basement membrane-bound enzyme, hephaestin. Subsequently, transferrin transports ferric iron (2 ferric ions per transferrin molecule) to the liver

and reticulo-endothelial cells and releases its iron load into cells via transferrin receptors. Transferrin receptors are most prevalent in certain tissues – primarily the liver, erythroid cells, macrophages in the liver, spleen and bone marrow.

Regulation of systemic iron homeostasis is largely regulated by controlling the rate of iron delivery to circulating transferrin. This is achieved by adjustments to the expression of ferroportin on cell membranes through the action of circulating hepcidin. Hepcidin, a hormone produced in the liver, binds to ferroportin, causing the complex to be ubiquitinated, internalized and degraded in lysosomes. Hence, increased iron levels lead to a high concentration of circulating hepcidin, which will reduce the number of ferroportin molecules on enterocytes, eventually decreasing the amount of iron absorbed.

Homeostatic control of iron is largely dependent on the production and degradation of erythrocytes, as most iron in the body is contained within erythrocytes. Iron has no regulated excretion pathway, so absorbed iron is virtually completely utilized for functional or storage proteins. Virtually all of the iron from erythrocytes is recycled for incorporation into haemoglobin and thus only a small amount of iron is excreted daily, except where there is substantial blood loss. Daily basal iron losses are reported to be 0.2 mg in infants, 0.5 mg in children, 1.0 mg in men, 0.64 mg in non-menstruating women and 1.3 mg in menstruating women. Excretion of iron occurs predominantly via faeces, although trace amounts of iron are also excreted in the urine, desquamated gastrointestinal and urinary tract cells at the end of their lifespan, and bile.

Based on comparative absorption and bioavailability, elemental iron () is expected to be less hazardous than other forms of iron compounds, and thus data for any Fe^{2+} or Fe^{3+} compound is eligible for conservative extrapolation to the derivation of Fe^0 reference values for human risk assessment.

Dermal and Inhalation routes of exposure

The dermal absorption of elemental iron from its representative product is addressed in CP_B6, where a dermal absorption value of 10% is proposed for solubilised, ionic iron following its dissolution into sweat.

As one of the basic requirements for cellular life is to maintain osmotic homeostasis and compartmental integrity, the dermal ingress of relatively large, particulate elemental iron or ionised iron are not physiologically plausible. Noting that an extensive body of published literature on human physiology of systemic iron exposure is available from the applicant's review, the evidence indicate that the amount of iron passively shed by natural desquamation is in the order of magnitude of approximately 1 mg/day (Galaris and Pantopoulos, 2008).

The inhalation absorption of elemental iron is not an expected route of systemic exposure. Although a well-established and precautionary UK and EU approach taken with all plant protection product active substances is to assign a default inhalation absorption value of 100 %, this is not applicable to elemental iron in the form supplied. It is expected that insoluble deposited particulate matter will be captured by the mucociliary escalator for elimination via the GIT, with a subsequent dissolution and systemic exposure comparable to the oral route. Inhalation absorption is not expected to be greater than that of oral absorption (50%), therefore, if required, the value proposed for the oral absorption may be used for an inhalation exposure risk assessment.

B.6.2. ACUTE TOXICITY

No new regulatory studies generated with elemental iron have been performed to account for the acute irritation, sensitisation, oral or dermal toxicity of elemental iron. Instead, the applicant refers to the existing EU assessment of ferric phosphate (EFSA conclusion 2015). These studies consist of acute oral toxicity, primary eye irritation and primary skin irritation studies – a re-evaluation of those studies has not performed under the current assessment, but the results are reported below. The remaining endpoints of acute dermal toxicity and skin sensitization have been inferred from existing data and human experience summarised in the existing EU assessments of iron sulphate and ferric phosphate. Regarding the potential for acute inhalation toxicity, the active substance is not volatile, however a significant proportion of this commercial source of elemental iron powder is $< 22.4 \mu\text{m}$, therefore the applicant has submitted an acute inhalation toxicity study on the active substance in order to fulfil the data requirements under EU Reg 283/2013.

In addition to the extrapolation from the EU assessment of ferric phosphate, the applicant has provided additional peer-reviewed scientific literature on iron salts and elemental iron powder. Where the applicant has provided full papers, they have been summarised under each relevant section.

Regarding acute toxicity or local irritancy, HSE has also considered any potential for a read-across to FeSO₄ and this is discussed in greater detail under each data requirement point.

B.6.2.1. Oral

The applicant has submitted one original research article from the public domain and has referenced existing US EPA and EU reviews of ferric phosphate. Although not specifically addressed by the applicant, HSE has also reviewed the acute oral toxicity of iron sulphate (LD₅₀ 1185 – 1750 mg/kg bw, Acute Tox Cat 4; H302, EFSA final conclusion 2008) and considers that due to the significant differences in physico-chemistry between these two forms of iron, (i.e. poor solubility of elemental iron and resultant low bioavailability compared to iron sulphate), the data supporting ferric phosphate are more relevant to address this particular endpoint.

Based on several publications and the EU review of ferric phosphate, combined with the low oral absorption of elemental iron, the substance does not meet the criteria for classification for oral toxicity in accordance with Reg (EC) No. 1272/2008.

Table 6.2-1 Acute oral toxicity of elemental iron – summary of available data

Reference	Test item	Test species	Result	Previously referenced in
Whittaker P. <i>et al.</i> , 2002 (CA 5.2.1/01)	Fe ⁰ (carbonyl elemental iron)	Rat	LD ₅₀ > 50 g/kg bw, <i>i.e.</i> at least 45 times less toxic (in terms of iron) than that of iron sulphate (1100 mg/kg) Study summarized below, as it shows this direct comparison.	-
Gordeuk V. J. <i>et al.</i> (1986) (CA 5.9.2/01)	Fe ⁰ (carbonyl elemental iron)	Human	No evidence of toxicity at single doses of up to 10,000 mg Fe ⁰ (~ 160 mg/kg bw)	-
EPA Reregistration eligibility document (RED), Iron salts, 1993	Ferric sulphate Ferrous sulphate heptahydrate	Rat Rat, rabbit, mice	LD ₅₀ 1487 mg/kg bw (approx. 300 mg/kg of iron) LD ₅₀ rat 1389 mg/kg bw (approx. 280 mg/kg of iron); rabbit 2778 mg/kg (approx. 550 mg/kg of iron); mice 1520 mg/kg (approx. 300 mg/kg of iron)	-
FePO ₄ RAR 2013	FeIII (ferric phosphate)	Rat	LD ₅₀ > 5000 mg/kg bw (> 1850 mg/kg of iron)	EFSA Final Conclusion, 2015

Reference	CA 5.2.1/01: Whittaker, P., Ali, S. F., Imam, S. Z. and Dunkel, V.C. (2002)
Article title	Acute toxicity of carbonyl iron and sodium iron EDTA compared with ferrous sulfate in young rats
Journal title	Reg Toxicology and Pharmacology 36, 280- 286
Test substance	Elemental iron (carbonyl form) in comparison with ferrous sulfate
Acceptability	Considered acceptable, with restrictions. The test laboratory was identified as the 'Center for Food Safety and Applied Nutrition, FDA, USA'. No technical specification details of the elemental iron are available, but the study authors state that this form of elemental iron, produced by carbon monoxide reduction, yields a high-purity iron powder with very small particle size (average 5-6 µm). Note: Only the data relating to iron powder oral toxicity is summarised below. The authors also tested NaFeEDTA, but these data as less relevant to the current assessment.

Materials and methods: The oral toxicity of elemental (carbonyl) iron was investigated in a non-guideline study in rats; the main objective of the study authors was to compare the acute oral toxicity of elemental iron in comparison to ferrous sulfate (the latter being the most popular form used in dietary supplements). Groups of 8 ‘young’ male Sprague-Dawley rats (source: FDA NCTR colony) received single dosages of carbonyl iron at 40 or 50 g/kg body weight, or groups of 4 -8 rats received FeSO₄ at a range from 4.5-7.0 g/kg. The dosage ranges were selected on the basis of known LD₅₀ values from published literature.

Results: The pattern of mortality of the elemental iron and iron sulphate was as follows:

Table 6.2-2 Acute oral toxicity of elemental iron and iron sulphate (Whittaker *et al.*, 2002)

Fe compound	Number of animals	Compound dose (g/kg)	Iron dose (mg/kg)	Number of deaths
Control	8	0	0	0
FeSO ₄	8	4.5	900	1
	8	5.0	1000	2
	8	5.5	1100	4
	8	6.0	1200	7
	8	6.5	1300	8
	4	7.0	1400	4
Carbonyl Fe	8	40.0	40,000	0
	8	50.0	50,000	0

Conclusion

Under the conditions of this study, the LD₅₀ for ferrous sulphate was indicated to be in the region of 1100 mg Fe/kg, while for carbonyl iron, the LD₅₀ was in excess of 50 g/kg body weight. These data are in agreement with the EFSA final conclusions on iron sulphate and ferric phosphate. The acute oral toxicity of carbonyl (elemental) iron in rats was at least 45 times less than that of ferrous sulphate, indicating that elemental iron is of very low (negligible) toxicity via the oral route of administration.

Reference	CA 5.9.2/01: Gordeuk, V.R., Brittenham, G. M., McLaren, C.E., Hughes, M. A. and Keating L. J. (1986)
Article title	Carbonyl iron therapy for iron deficiency anaemia
Journal title	Blood, 67(3), 745-752
Test substance	Elemental iron (carbonyl form) in combination with ferrous sulfate
Acceptability	Considered acceptable, with restrictions, this is a human volunteer study. The test laboratory was identified as the Dept of Medicine, Cleveland Metropolitan General Hospital, USA. No technical specification details of the elemental iron are available. <i>Note: This paper also appears in various parts of this DAR chapter; only the data relating to iron powder acute oral administration are summarised below.</i>

To determine the safety and efficacy of elemental carbonyl iron powder therapy for iron-deficiency anaemia, non-anaemic (healthy) and anaemic volunteers received 1000 to 10,000 mg of carbonyl iron (the authors state this is 15 to 150 times the amount of iron in the usual dose of ferrous sulphate).

Materials and methods:

Nineteen healthy, non-anaemic adult volunteers subjects received a 3000 mg dose of carbonyl iron approximately 1 week after a reference dose of 100 mg ferrous sulphate co-administered with ascorbic acid, with blood sampling at regular intervals for up to 24 h post-dose for serum iron content analysis (summarised in the ADME section B.6.1 of this document) and clinical chemistry parameters were measured prior to dosing and at weeks 1 and 3 post-dosing. Clinical symptoms were monitored.

Results:

In the 19 subjects receiving 3000 mg carbonyl iron after the FeSO₄ reference dose, mean maximal increases in serum iron were comparable for both types of iron, occurring at 3 h for ferrous sulphate and 6 h for carbonyl iron. Side-effects were limited to gastrointestinal symptoms and unpleasant taste, all described as ‘mild’ by all subjects. A significantly greater incidence of diarrhoea and abdominal cramping were noted with carbonyl iron in comparison with ferrous iron. There was no evidence of hepatic or renal toxicity as judged by lack of effect upon WBC, platelets, bilirubin, AST, ALT, ALP and creatinine measurements.

Conclusion:

A single dose of 3000 mg (approx. 50 mg/kg bw) carbonyl elemental iron was well tolerated in a group of healthy human volunteers, with only mild gastrointestinal side effects.

General conclusion on acute oral toxicity:

In a published research article, the administration of 50 g/kg Fe⁰ caused no mortality in rats (Whittaker *et al.*, 2002). In a human volunteer study, a single dose of 3000 mg carbonyl Fe⁰ was tolerated by non-anaemic human volunteers with no evidence of toxicity other than mild gastrointestinal side effects.

Although the applicant also referenced 3 other peer-reviewed publications, these were not submitted to HSE; therefore, they are not tabulated below. HSE notes that those publications date from 1950 – 1986 and provide LD₅₀ values within range of the magnitude reported by Whittaker *et al.*, 2002.

It can be reasonably concluded that elemental iron is not acutely toxic by the oral route, and in accordance with Regulation (EC) 1272/2008, does not meet the criteria for classification for acute oral toxicity or STOT-SE.

B.6.2.2. Dermal

Due to the predicted low oral toxicity of elemental iron summarised above – in excess of 2000 mg/kg bw/day – and in accordance with Reg No. 283/2013, a study on the acute dermal toxicity is not required. In addition, Fe⁰ is not expected to penetrate the skin to any biologically relevant extent (a significantly low dermal absorption value of 10 % is proposed, see CP_B6 for further details), it is very poorly soluble (technically impossible to measure using standard physicochemical analytical techniques), and vertebrate data (generated on Fe³⁺ and Fe²⁺, more bioavailable forms of iron) contained in US EPA and EU reviews, add further justification to the waiver for this data point.

Table 6.2-3 Acute dermal toxicity of elemental iron – summary of available data

Reference	Test item	Test species	Result	Previously referenced in
EPA Reregistration Eligibility Document (RED), Iron salts, 1993	Ferric sulphate Fe ₂ (SO ₄) ₃	Rabbit	LD ₅₀ >2000 mg/kg bw	FePO ₄ RAR 2013

General conclusion on acute dermal toxicity:

It can be reasonably concluded that elemental iron is not acutely toxic by the dermal route, and in accordance with Regulation (EC) No. 1272/2008, does not meet the criteria for classification for acute dermal toxicity or STOT-SE.

B.6.2.3. Inhalation

Exposure to iron in the representative product by the inhalation route for the proposed use is considered highly unlikely in practice, as the elemental iron is a non-volatile powder incorporated in the product within a solid matrix that is nearly dust-free, non-respirable, with no particles <50 µm (Vol 3 CP B.2 Physical and chemical properties of the PPP), and the solid product is not applied by spraying.

However, as this assessment should address the toxicological properties of the active substance elemental iron *per se* and due to uncertainties in the expected particle size distribution of the active substance as supplied to the applicant, in which 50 % of the particulate matter are stated to present a median diameter < 22.4 µm (D50, 22.4 µm; D90, 40.8 µm Applicant Doc M), the applicant has submitted an acute inhalation toxicity study (OECD 403). The study was performed on the commercial source of elemental iron powder, which the applicant states to be representative of the specification for which approval is being sought. In addition to this study, the applicant has referenced existing EU and US EPA reviews of ionic iron salts. In addition, HSE considers that 4 out of 5 research articles identified by the applicant from the published literature on elemental iron are informative on this endpoint. The publications are summarised below; however, as reliable data on elemental iron itself are available, the references to the ionic forms have not been considered further.

Reference	CA 5.2.3/01: [REDACTED] (2018)
Title	Elemental iron powder: Acute inhalation toxicity in rats
Date performed	3 -17 January 2018
Test facility	Product Safety Labs, USA
Report reference	Report no. 47151
Guideline(s)	OECD 403 (2009), OPPTS 870.1300 (1998), EC Method B.2 (2014)
GLP	Yes
Published	No
Test substance	Elemental iron powder, batch 2290598, purity 98%
Acceptability	Yes

An acute inhalation toxicity study (GLP-compliant; OECD 403) was conducted in rats, to determine the potential for elemental iron to produce toxicity from a single concentration of inhaled test article.

Materials and Method:

Male and female SD rats (5/sex/group) were exposed, nose-only, to the maximum practicable concentration of the test item. Elemental iron was administered as an aerosol, at a single concentration of 5.15 mg/L, measured gravimetrically, for a 4 h continuous exposure period. The average mass median aerodynamic diameter was 2.86 µm ± 2.70. A pre-test trial had been conducted to establish the aerosol generation procedures to achieve the desired chamber concentration (5 mg/L of air) and particle size distribution (1-4 µm diameter). The test atmospheres were sampled - for gravimetric analysis - from the breathing zone of the animals at 6 intervals during the 4 h exposure period. This method of analysis (weight of the material on the filter) although not considered fully validated, is generally accepted as fit for purpose. An additional 2 samples were withdrawn from the breathing zone of the animals at two intervals to measure particle size distribution.

At the end of the exposure period, residual test material was wiped from the fur and the animals were returned to their home cages. Clinical observations for all animals were made upon removal from the exposure tube, at 1 h post-removal and at least once daily for 14 days following exposure. Body weights were recorded prior to exposure and again on Days 1, 3, 7 and 14 (terminally). The animals were sacrificed on Day 15 and subjected to detailed macroscopic necropsy.

Results:

The particle size distribution indicates an adequately inhalable aerosol was produced (Table 6.2-5).

Table 6.2-5 Acute inhalation toxicity study - exposure conditions of elemental iron powder

Parameter	Value (±SD)
Target concentration	5 mg/l
Mean achieved concentration	5.15 ± 0.28 µm
Mass Median Aerodynamic Diameter	2.86 µm
Geometric Standard Deviation	2.70 µm
Inhalable fraction (< 9 µm)	89 %

With the exception of irregular respiration - which was only noted in the first hour of removal from the test chambers, there were no adverse clinical signs in any animal. Due to the transient nature of these clinical signs,

these were not considered to be a substance-specific adverse effect of the elemental iron exposure. Bodyweight was unaffected and there no gross abnormalities noted for any of the animals at necropsy.

Conclusion

Under the conditions of this acute inhalation toxicity study, the single-exposure (4 h) acute inhalation LC₅₀ of elemental iron powder in rats was in excess of 5.15 mg/L of air. Therefore, there is no requirement for its classification for acute inhalation toxicity or STOT-SE in accordance with Regulation (EC) No. 1272/2008.

Reference	CA 5.2.3/02: Sayes, C. M., Reed, K. and Warheit, D. B. (2007)	
Article title	Assessing toxicity of fine and nanoparticles: Comparing <i>in vitro</i> measurements to <i>in vivo</i> pulmonary toxicity profiles	
Journal title	Toxicological Sciences 97(1), 163-180	
Test substance	Elemental iron (carbonyl) as a negative comparative control, in comparison with PBS.	
Guideline	None	GLP: No
Acceptability	<p>Considered acceptable, with restrictions. The test laboratory was identified as DuPont Haskell Laboratory for Health and Environmental Sciences, USA. The data are supplemental to the key study summarised at 5.2.3/01.</p> <p><i>Note: Carbonyl elemental iron powder was used as a negative comparative control. Only the data relating to iron powder toxicity is summarised below and is viewed as being complimentary to the results of Warheit 2007a and b (5.2.3/04 and /05, respectively).</i></p>	

Materials and methods:

In the *in vivo* component of this study, 5 male CrI:CD (SD) IGS BR rats/group were exposed by intratracheal instillation to 1 or 5 mg/kg of carbonyl iron (0.8 – 3 µm; supplied by BASF, > 99 % purity) or phosphate-buffered saline (PBS vehicle). Animals were approximately 8 weeks old. Bronchio-alveolar lavage (BALF) fluid parameters were analysed at 24 h, 1 week, 1 and 3 months, for inflammation (neutrophil recruitment) and cytotoxicity (lactate dehydrogenase, LDH release as a general marker of cellular injury).

For the *in vitro* component, cultures of rat L2 lung epithelial cells, or rat primary alveolar macrophages (AMs), or co-cultures of both, were incubated with a range of concentrations of carbonyl iron from 0.001 µg/mL to 30 mg/mL (0.052 to 520 µg/cm², where 52 and 520 µg/cm² were considered overload doses of low-toxicity dusts). Culture fluids were evaluated for inflammatory cytokines (MIP-2, TNF-α and IL-6) and cytotoxicity markers (LDH) and the MTT tetrazolium reduction assay (mitochondrial activity), and for at periods up to 48 h. The potential haemolytic activity of the particles was examined in human erythrocytes at up to 15 mg/mL.

Results:

In the *in vivo* exposure component of this publication, elemental iron produced no significant or notable differences from PBS vehicle control in BALF LDH activity, or cell numbers, while only at 24 h was there a rise in % neutrophils, which was clearly transient - most likely due to the instillation procedure – and fully reversible by the 1-week time point.

In the *in vitro* components of this study, there was no inflammatory cytokine induction nor was there any evidence of a haemolytic potential of the carbonyl elemental particles. Noting that the mechanism by which erythrocytes are lysed is thought to include either direct surface reactivity (e.g. jagged edges), or by production of reactive oxygen species, the absence of haemolytic activity indicates a low surface reactivity of respirable carbonyl elemental iron. There were no meaningful effects on mitochondrial (MTT) activity in any cell line. At overload concentrations (at 52 µg/cm² and above), elemental iron produced slightly elevated LDH levels at up to 4 h in the L2 cell line, and at 24 h in the co-culture and AM cell line treatments. Due to these transient observations, and being evident only at overload concentrations, the effect in LDH is not considered to be of toxicological significance.

Conclusion

In a non-guideline yet acceptable study, there was no biologically significant effect on rat lungs of intra-tracheally instilled carbonyl iron at up to 5 mg/kg body weight. Both *in vivo* and *in vitro*, there were no indications of a specific oxidative or inflammatory effect of carbonyl elemental iron.

Reference	CA 5.2.3/04: Warheit, D. B., Webb, T.R. and Reed, K. L. (2007a)	
Article title	Pulmonary toxicity screening studies in male rats with M5 respirable fibers and particulates	
Journal title	Inhalation Toxicology 19, 951-963	
Test substance	Elemental iron (carbonyl) as a negative comparative control, in comparison with PBS	
Guideline	None	GLP : No
Acceptability	<p>Considered acceptable, with restrictions. The data are supplemental to the key regulatory acute toxicity study summarised at 5.2.3/01. Unknown group size or test substance purity. The test laboratory was identified as DuPont Haskell Laboratory for Health and Environmental Sciences, USA and is likely to follow the same group sizes as per Sayes <i>et al.</i>, (2007) at 5.2.3/02.</p> <p><i>Note: Carbonyl elemental iron powder was used as a negative comparative control and used to bridge across separate studies from the same laboratory. Only the data relating to iron powder toxicity is summarised below and is viewed as being complimentary to the results of Sayes et al., 2007 and Warheit 2007b (5.2.3/02 and /05, respectively). Positive responses were elicited by silica quartz in the study, confirming the sensitivity of the model.</i></p>	

Materials and methods:

Groups of - an unknown number of - male Crl:CD (SD) IGS BR rats were exposed by intratracheal instillation at 5 mg/kg of carbonyl iron (0.8 – 3 µm; supplied by GAF Corp.) or phosphate-buffered saline (PBS vehicle). Animals were approximately 8 weeks old. Bronchio-alveolar lavage (BALF) fluid parameters were analysed at 24 h, 1 week, 1 and 3 months, for inflammation (neutrophil recruitment) and cytotoxicity (LDH, ALP – measure of epithelial alveolar (Type II) cell injury and vascular protein release – measure of lung permeability in the form of damage to alveolar and capillary membranes). Lung weights and morphology (pulmonary BrdU cell proliferation and histopathology) were also assessed.

Results:

In the three months following a single exposure of elemental iron, there were no notable differences - compared to PBS vehicle control - in any of the endpoints assessed: BALF inflammatory cell numbers, LDH and ALP levels. Regarding the lung tissue analyses, rats exposed to elemental carbonyl iron presented with lung weights, lung parenchymal cell proliferation rates and pulmonary histopathology which were comparable to PBS-exposure groups.

Conclusion

In a non-guideline yet acceptable study in male rats, in which carbonyl iron was deployed as a negative control, there were no indications of either an acute or delayed adverse pulmonary inflammatory, pathological or cytotoxic response following a single intratracheal administration of elemental carbonyl iron at 5 mg/kg bw.

Reference	CA 5.2.3/05: Warheit, D. B., Webb, T.R., Colvin, V. L., Reed, K. L. and Sayes, C. M. (2007b)	
Article title	Pulmonary bioassay studies with nanoscale and fine-quartz particles in rats: toxicity is not dependent upon particle size but on surface characteristics	
Journal title	Toxicological Sciences 95(1), 270-280	
Test substance	Elemental iron (carbonyl) as a negative comparative control, in comparison with PBS	
Guideline	None	GLP : No
Acceptability	<p>Considered acceptable, with restrictions. The data are supplemental to the key regulatory study summarised at 5.2.3/01. The test laboratory was stated as DuPont Haskell Laboratory for Health and Environmental Sciences, USA.</p> <p><i>Note: Carbonyl elemental iron powder was used as a negative comparative control and used to bridge across separate studies. Only the data relating to iron powder toxicity is summarised below and is viewed as being complimentary to the results of Sayes et al., 2007 and Warheit 2007a (5.2.3/02 and /04, respectively). Positive responses across multiple endpoints were induced by 5 mg/kg bw silica-quartz, confirming the sensitivity of the model.</i></p>	

Materials and methods:

Groups of 4 or 5 male Crl:CD (SD) IGS BR rats/group were exposed by intratracheal instillation at 5 mg/kg of carbonyl iron (0.8 – 3 µm; unknown purity, supplied by GAF Corp.) or phosphate-buffered saline (PBS vehicle). Animals were approximately 8 weeks old. In 5 rats per time point, bronchio-alveolar lavage (BALF) fluid parameters were analysed at 24 h, 1 week, 1 and 3 months, for inflammation (neutrophil recruitment) and cytotoxicity (LDH, ALP – measure of epithelial alveolar (Type II) cell injury and vascular protein release – measure of lung permeability in the form of damage to alveolar and capillary membranes). In an additional 4 rats per timepoint, lung weights and morphology (pulmonary BrdU cell proliferation and histopathology) were also assessed. Statistical analyses per time point were performed (ANOVA and Bartlett's test).

Results:

In the three months following a single exposure of elemental iron, there were no notable differences - compared to PBS vehicle control - in any of the endpoints assessed: BALF inflammatory cell numbers, protein content, LDH and ALP levels. There were no indications at any time point assessed, of increased cell turnover in the tracheobronchial or parenchymal regions, which indicates an absence of inflammatory, fibrotic and potential tumourigenic processes. In addition, normal lung architecture was evident following exposure to elemental iron – there was no increase in alveolar macrophage number which may have otherwise indicated an increase in lung clearance, nor was there any thickening of pulmonary tissue. The absence of alterations in these endpoints substantiates the low potential of carbonyl elemental iron to elicit inflammatory or fibrotic responses in the lung.

Conclusion

In a non-guideline yet acceptable study in male rats, in which carbonyl iron was deployed as a negative control, there were no indications of either an acute or delayed adverse pulmonary inflammatory, pathological or cytotoxic response following a single intratracheal administration of elemental carbonyl iron at 5 mg/kg bw.

Reference	CA 5.2.3/06: Kiranmai, G. and Reddy A. R. N. (2012)	
Article title	Antioxidant status in MgO nanoparticle-exposed rats	
Journal title	Toxicology and Industrial Health 29(10), 897-903	
Test substance	Elemental iron (carbonyl) as a negative comparative control, in comparison with PBS	
Guideline	None	GLP : No
Acceptability	Considered acceptable, with restrictions. <i>Note: Carbonyl elemental iron powder was used as a negative comparative particle control. Only the data relating to iron powder toxicity is summarised below and is viewed as supplemental to the acute inhalation regulatory toxicity study. Positive findings were elicited by exposure to quartz particles, confirming the sensitivity of the methodology.</i>	

The aim of this study was to assess the antioxidant status of rats following inhalation exposure to magnesium oxide nanoparticles. Elemental carbonyl iron was included in the study as a particulate negative control. This study is informative in addressing the potential for Fe to induce toxicity by the formation of reactive oxygen species.

Materials and methods:

Groups of 6 male Wistar rats were exposed by intratracheal instillation of 1 or 5 mg/kg of carbonyl iron (4.5 – 5.2 µm, purity > 98 % Sigma-Aldrich) or phosphate-buffered saline + 1 % Tween (PBS vehicle). Animals were approximately 8 weeks old, comparable to other publications included in this dossier. Blood was collected from each animal at intervals of 24 h, 1 week and 1 month after dosing. Serum catalase, erythrocyte superoxide dismutase (SOD) and serum total antioxidant status were assessed. Statistical analyses were performed (ANOVA and Dunnett's test).

Results:

Following a single exposure of carbonyl elemental iron at either 1 or 5 mg/kg bw there were no discernible decreases in catalase activity, SOD levels or total antioxidant status over the course of a month post-instillation. All responses were sufficiently comparable to that of the PBS control group.

Conclusion

The results indicate that there was no reduction in antioxidant defence mechanisms in the rat, following exposure to either 1 or 5 mg carbonyl Fe/kg bw.

General conclusion on acute inhalation toxicity:

In an OECD 403 test guideline-compliant acute inhalation toxicity study, elemental iron powder did not induce any mortality or morbidity; the single-exposure (4 h) acute inhalation LC₅₀ of elemental iron powder in rats was in excess of 5.15 mg/L of air. Therefore, there is no requirement for its classification for acute inhalation toxicity or STOT-SE in accordance with Regulation (EC) No. 1272/2008. In addition to these data, the applicant has identified several papers from the public domain, mainly on carbonyl elemental iron following intratracheal administration, which investigated several endpoints, including the potential of elemental iron to cause oxidative stress, inflammation and fibrosis. The absence of any notable findings following exposures of up to 5.15 mg Fe⁰/kg bw in a guideline-compliant regulatory study adds support to the conclusion that elemental iron is of low toxicity via the inhalation route.

Table 6.2-4 Acute inhalation toxicity of elemental iron – summary of available data

Reference	Test item	Test species	Result	Previously assessment (source)
██████ 2018 (CA 5.2.3/01)	Elemental iron powder (NutraFine RS®)	Rat	LC ₅₀ 4h > 5.15 mg/L nose-only	None
Sayes <i>et al.</i> , 2007 (CA 5.2.3/02)	Fe ⁰ (carbonyl; 0.8-3 µm)	<i>in vivo</i> : Rat <i>in vitro</i> : rat primary and immortalised cell cultures, human erythrocytes.	No adverse effects	None (published literature)
Warheit <i>et al.</i> , 2007a (CA 5.2.3/04)	Fe ⁰ (carbonyl; 0.8-3 µm)	Rat	No adverse effects	None (published literature)
Warheit <i>et al.</i> , 2007b (CA 5.2.3/05)	Fe ⁰ (carbonyl; 0.8-3 µm)	Rat	No adverse effects	None (published literature)
Kiranmai and Reddy (2012) (CA 5.2.3/06)	Fe ⁰ (carbonyl; 4.5-5.2 µm)	Rat	No adverse effects	None (published literature)

B.6.2.4. Skin irritation

Elemental iron is widely used (e.g. in jewellery) and does not have any known direct skin effects. There were no positive reports of dermal irritation identified by the applicant's literature review. The EU review of ferric phosphate concluded that FePO₄ is not irritating to the skin in an acute irritation study in rabbits, and therefore there was no requirement for classification or labelling under EU criteria (RAR, 2013).

The applicant proposes that that dermal exposure of elemental iron will result in oxidation to Fe₂O₃ 'rust' due to the reaction of elemental iron with ambient oxygen and that although the oxide layer will not prevent further oxidation, it will limit direct interaction of elemental iron with biological membranes. Since skin has a pH which is only slightly acidic (~ pH 4.5 to 7), the oxide is expected to be relatively stable and will be of limited solubility (Hedberg *et al.*, 2010a, b). In addition, HSE cannot rule out the possibility of other ionised iron species being formed in the presence of sweat on the apical surface of the skin; these are likely to be ferric in form. Therefore, in addition to an absence of dermal-effects findings in the literature, a read-across to the data generated on ferric phosphate is appropriate for this endpoint.

With regards to a read-across to iron sulphate - considered to be significantly more soluble than elemental iron - the EU review (FeSO₄ DAR 2008) concluded there were no indications of skin irritancy in the published literature. The EU review did however conclude that due to its highly water-soluble nature, FeSO₄ may have the potential to be irritating within a localised dermal microenvironment and hence the EFSA final conclusion proposed a classification as a Cat 2 skin irritant. Since the postulated mechanism of action is by direct

physiological damage to cell membranes from a low pH microenvironment caused by dissociation of the iron sulphate salt, this conclusion is not extrapolated to elemental iron, as elemental iron is significantly less soluble than iron sulphate.

General conclusion on acute dermal irritation:

The physico-chemical and surface characteristics of elemental iron determine the metal bioavailability and potential toxicity of its particles. Elemental iron is poorly soluble, and it can be reasonably concluded that there is no need for a specific study with elemental iron and read-across from data generated on FePO_4 is appropriate. Overall, elemental iron is not a skin irritant and there is no requirement for its classification for skin corrosion/irritation in accordance with Regulation (EC) No. 1272/2008.

B.6.2.5. Eye irritation

Elemental iron does not have any known direct eye effects, other than the possibility of transient mechanical irritation effects that would result from any fine particulate material getting under the eyelids. There were no positive reports of ocular irritation identified by the applicant's literature review. The EU review of ferric phosphate concluded that FePO_4 is not irritating to the eye in an acute irritation study in rabbits, and therefore there was no requirement for classification or labelling under EU criteria (RAR, 2013).

Considering the read-across to the EU review of iron sulphate, in which limited human data indicate some ocular irritation potential of that ferrous salt, this conclusion is less relevant to Fe^0 since it is expected that topical exposure of elemental iron will result in oxidation to Fe_2O_3 and therefore the read-across to the data generated on ferric phosphate is more appropriate for this endpoint. HSE also notes that iron sulphate is significantly more soluble than elemental iron and the UK DAR indicates the formation of a local acidic environment is critical to the irritant potential of the sulphate salt; these conditions are highly unlikely to result from ocular exposure to elemental iron, since Fe^0 is not a mineral salt, and thus will not dissolve into ionic form.

General conclusion on acute eye irritation:

It can be reasonably concluded that there is no need for a specific study with elemental iron and that read-across from data on FePO_4 is appropriate. Overall, elemental iron is not an eye irritant and there is no requirement for its classification for eye irritation in accordance with Regulation (EC) No. 1272/2008.

B.6.2.6. Skin sensitisation

Elemental iron is widely used (e.g. in jewellery) and no direct skin effects have been reported in the applicant's literature review of elemental iron. The EU review of iron sulphate concluded that FeSO_4 was not a skin sensitizer based on chemical structure, the absence of human or animal adverse effects in published literature. , There was no requirement for classification or labelling under EU criteria (FeSO_4 DAR, 2008). HSE also notes that the EFSA final conclusion on ferric phosphate concludes that there are no indications of sensitising potential for ferric phosphate based on a Guinea pig study on ferric sulphate (US EPA RED, 1993).

General conclusion on acute dermal sensitisation:

It can be reasonably concluded that there is no need for a specific study with elemental iron and that in the absence of other relevant information, read-across from data on FeSO_4 is appropriate. Overall, elemental iron is not a skin sensitizer and there is no requirement for its classification for skin sensitisation in accordance with Regulation (EC) No. 1272/2008.

Overall summary of acute toxicity:

The acute toxicity of elemental iron powder has been assessed on the basis of information in the public domain, extrapolation from specific studies on ferric phosphate and/or iron sulphate and an acute inhalation toxicity study on a representative source of the active substance. These data confirm that elemental iron is of extremely low oral, dermal and inhalation toxicity and is not a dermal or eye irritant or skin sensitizer. The table below provides an overview of the acute toxicity, irritation and skin sensitisation potential of elemental iron:

Table 6.2-5 Acute toxicity of elemental iron – overall summary

Guideline, reference	Test item	Species	Result	Classification
Acute Oral toxicity				
Whittaker P. <i>et al.</i> , 2002 5.2.1/01 Publication	Fe ⁰ (carbonyl elemental iron)	Rat	LD ₅₀ >50 g/kg bw, <i>i.e.</i> at least 45 times less (in terms of iron) than that of iron sulphate (1100 mg/kg)	None
EPA Reregistration eligibility document (RED), Iron salts, 1993 Publication	Ferric sulphate Ferrous sulphate heptahydrate	Rat Rat, rabbit, mice	LD ₅₀ 1487 mg/kg bw (approx. 300 mg/kg of iron) LD ₅₀ rat 1389 mg/kg bw (approx. 280 mg/kg of iron); rabbit 2778 mg/kg (approx. 550 mg/kg of iron); mice 1520 mg/kg (approx. 300 mg/kg of iron)	None
FePO ₄ RAR 2013 OECD 423	FeIII (ferric phosphate)	Rat	LD ₅₀ >5000 mg/kg bw (>1850 mg/kg of iron)	None
Acute Dermal toxicity				
EPA Reregistration Eligibility Document (RED), Iron salts, 1993	Ferric sulphate	Rabbit	LD ₅₀ >2000 mg/kg bw	None
Acute Inhalation toxicity				
■ 2018 OECD 403	Elemental iron powder (NutraFine RS®)	Rat,	LC ₅₀ 4h > 5.15 mg/L nose-only	None
EPA Reregistration Eligibility Document (RED), Iron salts, 1993 Publication	Ferric sulphate	Rat	LC ₅₀ >1.1 mg/L	None
Sayes <i>et al.</i> , 2007 Publication	Fe ⁰ (carbonyl; 0.8-3 µm)	<i>in vivo</i> : Rat <i>in vitro</i> : Rat primary and immortalised cell cultures, Human erythrocytes.	No adverse effects	None
Warheit <i>et al.</i> , (2007a) Publication	Fe ⁰ (carbonyl; 0.8-3 µm)	Rat	No adverse effects	None
Warheit <i>et al.</i> , (2007b) Publication	Fe ⁰ (carbonyl; 0.8-3 µm)	Rat	No adverse effects	None
Kiranmai and Reddy (2012) Publication	Fe ⁰ (carbonyl; 4.5-5.2 µm)	Rat	No adverse effects	None

Guideline, reference	Test item	Species	Result	Classification
Skin Irritation				
FePO ₄ RAR 2013 OECD 404	Ferric phosphate	Rabbit	No observable irritancy	None
Eye Irritation				
FePO ₄ RAR 2013 OECD 405	Ferric phosphate	Rabbit	No observable irritancy	None
Skin Sensitisation				
FePO ₄ RAR 2013 and FeSO ₄ DAR 2008.	none	none	Weight of evidence, absence of positive reports from the published literature for these more soluble forms of iron.	None
EPA Reregistration Eligibility Document (RED), Iron salts, 1993 Publication	Ferric sulphate	Guinea Pig	Not sensitising	None

B.6.2.7. Phototoxicity

Based on the known physical and chemical properties of elemental iron, no data on phototoxicity are required. The substance does not meet the criteria which would otherwise have triggered the need for information on this data point. Elemental iron is not soluble in either aqueous or organic solvent, rendering it unfeasible to acquire the UV or molar extinction coefficient.

B.6.3. SHORT-TERM TOXICITY

No standard short-term regulatory studies on elemental iron are available. Instead, this data requirement has been fulfilled by a read-across case to the existing EU assessments of the more bioavailable forms of iron - iron sulphate and ferric phosphate. HSE notes that the repeat dose toxicity profiling of both iron sulphate and ferric phosphate are based on published literature, with the ADI and AOEL for both substances being extrapolated from the starting point of 50 mg/day, based on food supplement programmes in pregnant and non-pregnant populations (Evaluation of certain food additives and contaminants (27th report of the Joint FAO/WHO Expert Committee on Food Additive. WHO Technical Report Series, No. 696, 1983).

This read-across has been supplemented by 6 peer-reviewed scientific publications addressing the sub-chronic toxicity of elemental iron in rats and mice by the oral and inhalation routes – these are listed below. These studies are limited in their design and reporting. However, considering that elemental iron is poorly soluble in either aqueous or organic solvent, and that systemic exposure following external exposure to elemental iron is to ionised iron, the read-across from these more soluble (and bioaccessible) ionic forms of iron has been performed where necessary. Therefore, no regulatory vertebrate short-term studies with elemental iron are required.

Table 6.3-1: Short-term toxicity of elemental iron – summary of available animal data

Reference	Route of exposure and duration	Test item, doses	LOAEL mg/kg bw/d	NOAEL mg/kg bw/d	Species	Main adverse effects
Whittaker P. <i>et al.</i> , (1996) Publication	Oral 84 d	Fe ⁰ (carbonyl) 35 (control), 350, 3500, 20,000 mg iron/kg diet Approximately equivalent to 3.2, 35, 350, 1850 mg/kg bw/d	35	3.2	Rat	<u>3.2 mg/kg bw/d</u> No observed effects <u>35 mg/kg bw/d</u> Liver iron deposition (haemosiderosis) and lipid peroxidation <u>350 mg/kg bw/d</u> Mortality 2/10, liver iron deposition (haemosiderosis) and lipid peroxidation, cardiomyopathy, splenic and pancreatic atrophy <u>1850 mg/kg bw/d</u> Mortality 5/18, iron deposition (haemosiderosis), cardiomyopathy , splenic and pancreatic atrophy
Zhu Q. <i>et al.</i> , (2016) Publication	Oral 90 d	Fe ⁰ (carbonyl) 0, 100 or 200 mg/kg bw/d	> 200	200	Rat	No adverse effects observed on a variety of parameters
Akhtar S. <i>et al.</i> , 2011a Publication	Oral 56 d	Fe ⁰ (carbonyl) 0 or 1.5 mg/kg bw/d	> 1.5	1.5	Rat	No observed adverse effects on clinical chemistry
Akhtar S. <i>et al.</i> , 2011b Publication	Oral 28 & 56 d	Fe ⁰ (carbonyl) 0 or 1.5 mg/kg bw/d	> 1.5	1.5	Rat	No observed adverse effects on hepatic enzymes and thyroid hormones
Domitrović R., <i>et al.</i> , 2008 Publication	Oral 21 d	Fe ⁰ (carbonyl) 600 mg/kg bw/d	n/a	n/a	Mice	Mechanistic study, not clearly investigating markers of toxicity. Accumulation of iron in the liver, induction of lipid peroxidation, some evidence of redistribution of antioxidants to the liver.

Warheit <i>et al.</i> , 1997	Inhalation	Fe ⁰ (carbonyl) 0, 5, 50 or 250 mg/m ³	5 mg/m ³	50 mg/m ³	Rat	<p><u>5 mg/m³</u> No observed adverse effects</p> <p><u>50 mg/m³</u> Mild and transient pulmonary inflammation (neutrophilic infiltration), pulmonary cell proliferation, deficits in alveolar macrophage function, and particle-laden macrophage aggregates at alveolar/alveoli bifurcations associated with adverse histopathology (mild)</p> <p><u>250 mg/m³</u> Lung overload – sustained pulmonary inflammation (neutrophilic infiltration), pulmonary cell proliferation (terminal bronchioles and pulmonary parenchyma), impaired particle clearance, deficits in macrophage function and large numbers of particle-laden macrophage aggregates at alveolar/alveoli bifurcations associated with adverse histopathology (widespread)</p>
Publication	5 d/wk, 4 weeks					
Considered not relevant to elemental iron						

B.6.3.1. Oral 28-day study

No data available.

B.6.3.2. Oral 90- day study

Reference	CA 5.3.2/01: Whittaker, P., Hines, F. A., Robl, M. G. and Dunkel V. C. (1996)	
Article title	Histopathological evaluation of liver, pancreas, spleen, and heart from iron-overloaded Sprague-Dawley rats	
Journal title	Toxicologic Pathology 24, 558-563	
Test substance	Elemental iron (carbonyl form)	
Guideline	None	GLP : No
Acceptability	Supplementary information, due to the shortcomings in reporting (e.g. no data on bodyweight or food consumption), it is not wholly reliable for risk assessment. The test laboratory was identified as the Center for Food Safety and Applied Nutrition, FDA, USA. No technical specification details of the elemental iron are available.	

The aim of this study was to induce an iron-overloaded rat model via oral exposure – as a comparison to the human condition, ‘genetic haemochromatosis’.

Materials and Methods:

Groups of ‘weanling’ male SD rats (bodyweight unpublished, applicant conversion submitted September 2019) were fed diets supplemented with carbonyl iron (purity unknown, ISP Technologies, USA) at 35, 350, 3500 or 20,000 mg iron/kg of diet (11, 10, 10 and 18 rats per group, respectively), for 12 weeks. The applicant has calculated (based on default values for SD rat bodyweights and food intakes) that these dietary levels convert to an estimated intake range of 3.2 to 1850 mg/kg bw/day. HSE accepts the applicant’s range, as these dose levels are broadly in line with standard conversion ratio in use by the OECD, JMPR and previous UK assessments (IPCS Env. Health Crit. Monograph 70, WHO (1987)). In addition, HSE has calculated the intermediate dose

levels to be 35 and 350 mg/kg bw/d, using the OECD conversion factors. The concentration level of 35 mg/kg diet (~3.2 mg Fe/kg bw/day) represented a 'control' exposure and the data from the three 'treatment' groups (approximately 35, 350 and 1850 mg/kg bw/d) were compared to the control group. At termination, all animals were subjected to the following investigations: necropsy with a focus on the liver, heart, pancreas and spleen; non-heme iron content and lipid peroxidation in the liver, apoptosis in the pancreas and histopathology (Prussian blue staining for Fe stored as haemosiderin, and general hematoxylin & eosin staining).

Results (Table 6.3-2):

Test-substance related mortality was observed at 350 and 1850 mg/kg bw/d, 2/10 and 5/18, respectively. Five of the seven early decedents presented with heart damage which was related to iron overload. There was a statistically significant and concentration-related increase in non-heme Fe detected in the liver and heart at 35 mg/kg bw/d and above. Histopathology of the liver revealed a small amount of Fe-positive material in sinusoidal macrophages in controls, while intensely stained Fe/haemosiderin occurred in the periportal hepatocytes of all rats in a dose related manner, accompanied by a portal-to-central gradient of Fe. Haemosiderosis was also observed in the heart and spleen but to a minimal amount in the pancreas. Lipid peroxidation in the liver demonstrated a concentration-related statistically significant increase of conjugated dienes in all treatment groups. Atrophy was evident in the spleen and pancreas at 350 and 1850 mg Fe⁰/kg bw/d. The incidence and severity of cardiomyopathy increased at 350 and 1850 mg/kg bw/d and were characterised by myofiber degeneration/necrosis, fibrosis and inflammation.

Table 6.3-2: Effect of elemental carbonyl iron on the liver, pancreas, spleen and heart (12 week dietary study; Whittaker *et al.*, 1996)

Parameter	3.2 mg/kg bw/d	35 mg/kg bw/d	350 mg/kg bw/d	1850 mg/kg bw/d	<i>p</i> -value
Survival	11/11 (100%)	10/10 (100%)	8/10 (80%)	13/18 (72%)	
Liver					
Nonheme Fe ($\mu\text{g/g}$) ^a	112 \pm 5*	234 \pm 21*	911 \pm 45 [†]	3,501 \pm 163 [‡]	<0.001
Prussian blue reaction	4/11 (36%)	9/10 (90%)	10/10 (100%)	18/18 (100%)	
Hepatocytes, grade ^b	0	1.0	2.0	3.8	
Kupffer cells, grade	1.0	1.0	1.5	1.5	
Conjugated diene, $\mu\text{mol/mg}$ ^a	0.019 \pm 0.001*	0.023 \pm 0.001*	0.032 \pm 0.002 [†]	0.039 \pm 0.002 [‡]	<0.001
Heart					
Nonheme Fe ($\mu\text{g/g}$) ^a	34.4 \pm 0.7*	39.2 \pm 2*, [†]	40.5 \pm 2.49*, [†]	44.4 \pm 1.1 [†]	0.001
Prussian blue reaction	0/11 (0%)	0/10 (0%)	2/10 (20%)	15/18 (83%)	
Grade	0	0	1.0	1.0	
Cardiomyopathy	0/11 (0%)	1/10 (10%)	7/10 (70%)	12/17 (71%)	
Grade	0	1.0	1.6	2.3	
Spleen					
Prussian blue reaction	10/11 (100%)	10/10 (100%)	10/10 (100%)	18/18 (100%)	
Lymph follicle, grade	0	1.0	1.0	1.0	
Interstitial macrophages, grade	1.5	2.0	2.4	4.0	
Atrophy, white pulp	0/10 (0%)	0/10 (0%)	8/9 (89%)	15/15 (100%)	
Grade	0	0	1.9	2.1	
Pancreas					
Prussian blue reaction					
Acinar cells	0/11 (0%)	0/10 (0%)	3/10 (30%)	2/18 (11%)	
Grade	0	0	1.9	2.0	
Connective tissue	0/11 (0%)	0/10 (0%)	5/10 (50%)	15/18 (83%)	
Grade	0	0	1.0	1.2	
Atrophy	0/10 (0%)	0/10 (0%)	8/9 (89%)	15/15 (100%)	
Grade	0	0	3.6	3.8	

a Values are the mean \pm SEM; n = 10 rats/treatment group except for the group receiving 3,500 $\mu\text{g Fe/g}$ diet (n = 8). Means for a variable not sharing a common symbol (*, †, ‡) are significantly different ($p < 0.05$), as determined by the Scheffé multiple-comparison method, which was applied only if significant differences were determined to exist by ANOVA. Data from Whittaker *et al.* (27).

b Average histological grade among animals with positive Prussian blue reaction or lesion

Conclusion:

This published study confirms that large overdoses of carbonyl elemental iron in rats exposed for 90 d may lead to an exceedance of the iron-binding capacity of transferrin, leaving free iron circulating in the plasma and being deposited in target organs (liver, spleen, pancreas and heart). Iron deposition in the liver was seen from a dose of ~ 35 mg Fe /kg bw/day, the lowest dose tested in comparison to control animals exposed to ~ 3.2 mg Fe /kg bw/day. Adverse histopathology, including atrophy, of the spleen, pancreas and heart was noted from ~ 350 mg Fe/kg bw/d. These data also demonstrate the ability of iron overload to initiate hepatic lipid peroxidation in the form of conjugated dienes.

Reference	CA 5.3.2/02: Zhu, Q., Qian, Y., Yang, Y., Wu, W., Xie, J. and Wei, D. (2016)	
Article title	Effects of carbonyl iron powder on iron deficiency anemia and its subchronic toxicity	
Journal title	Journal of Food and Drug Analysis 24, 746-753	
Test substance	Elemental iron (carbonyl form)	
Guideline	None	GLP: No
Acceptability	Considered acceptable, with limitations. The laboratory did not deploy specific histopathological staining against iron deposits (Prussian blue for haemosiderin). The standard hematoxylin and eosin staining and light microscopy used is able to detect tissue damage. Dept of Food Science and Technology, East China University of Science and Technology, Shanghai. No technical specification details of the elemental iron are available.	

The study authors were investigating the applicability of Fe⁰ as a dietary supplement and this publication also reports the restorative effect of carbonyl Fe⁰ on iron deficiency anaemia. However, for the purposes of this UK assessment, the summary below addresses only the 90-day subchronic toxicity component of the study.

Materials and Methods:

Groups of 10 male Wistar rats received daily oral gavage doses of 0 (control diet), 100 or 200 mg/kg bw carbonyl Fe⁰, for 13 weeks. The study identifies these as groups 7, 8 and 9, respectively. Bodyweight and food consumption were monitored twice weekly. Blood samples were taken at termination for haematology and clinical chemistry investigations. Following complete necropsy, the weights of liver, kidneys, spleen and testes were recorded and subsequent histopathology of these organs, stomach and ileum was performed.

Results:

There were no mortalities and no abnormal signs were observed in terms of behaviour, breathing, sensory responses or gastrointestinal effects. Food consumption was slightly reduced for both dosages of carbonyl elemental Fe⁰ to a similar degree, which was reflected in body weight gain, without a dose-response relationship. There were no significant changes to organ weights (absolute or relative to bw), nor any treatment-related findings in the gross or histopathological investigations. There were no adverse effects on haematological or clinical chemistry parameters.

Conclusion

Based on an absence of adverse effects at up to the maximum dose administered, the NOAEL for carbonyl iron in rats gavage-dosed for 13 weeks was > 200 mg/kg body weight.

Reference	CA 5.3.2/03: Akhtar, S., Anjum, F. M., Rehman, Z. U., Sultan, M. T., Riaz, M. and Ahmed, A. (2011a)	
Article title	Effect of mineral fortification on plasma biochemical profile in rats	
Journal title	Biological Trace Element Research 143, 1594-1606	
Test substance	Elemental iron (unspecified form)	
Guideline	None	GLP: No
Acceptability	Considered acceptable with restrictions. Investigations were limited to clinical chemistry and bodyweights at the start and at study termination. No dietary intake data are available; however, the single dietary dose level of 30 mg Fe ⁰ /kg is comparable to the 'control' dietary elemental iron concentration of Whittaker <i>et al.</i> , 1996	

	<p>(5.3.2/01).</p> <p>This publication is linked to the next study from the same authors, summarised at 5.3.2/04. The test substance ‘elemental iron’ can be presumed to the [REDACTED] form, akin to the test article identified in the linked study (Akhtar <i>et al.</i> 2011b; 5.3.2/04).</p> <p>Study performed at Dept of Food Science and Technology, Bahauddin Zakariya University, Pakistan.</p>
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The main objective of the study authors was to investigate the changes in clinical chemistry in rats exposed to Zn or Fe dietary supplements. Only the results pertinent to elemental iron Fe⁰ are discussed below.

Materials and Methods:

Groups of 5 male SD rats (10 weeks old, approx. 216 g) were given diets containing unfortified wheat flour (group code T0), or wheat flour fortified with elemental Fe⁰ powder (96 % purity, Micronutrient Initiative), providing approximately 30 mg/kg diet ~ 1.5 mg iron/kg bw/day* (group code T2), for 56 days. The animals were terminated on Day 57, terminal bodyweights were measured, and blood was sampled for clinical chemistry analyses (total protein, albumin, glucose, triglycerides, cholesterol, HDL and LDL).

* The applicant has calculated the dietary intake level of elemental iron (September 2019) as follows:

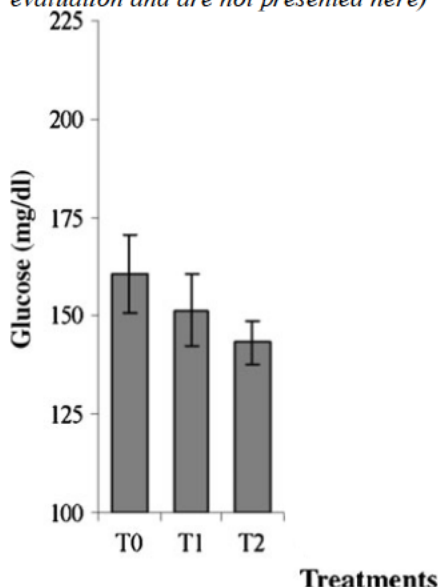
“Iron level in the fortified wheat flour = 40 mg/kg, and the wheat flour subsequently constituted 750 g/kg diet, therefore iron in the diet = 30 mg/kg. A rat estimated to weigh 350 g and to eat 18 g of food/day would consume a daily amount of 0.54 mg of iron from the fortified wheat flour, equivalent to 1.54 mg/kg bw/day”

HSE notes that the applicant’s reference bodyweight is over 50% higher than that of the experimental animals, leading to uncertainty in these calculated dietary concentrations. Nevertheless, the fortified diet concentration of 30 mg Fe⁰/kg can be compared as being similar to the ‘control’ 35 mg Fe⁰/kg dietary concentration of Whittaker *et al.*, 1996 (5.3.2/01).

Results:

There were no alterations in any clinical chemistry parameter, with the exception of a small reduction in mean plasma glucose as compared to the control group (see Figure 6.3.1, group T2; approximately -11% *cf* control, not statistically significant). In isolation, this was not considered to be of toxicological significance.

Figure 6.3.1: Effect on plasma glucose following 56 days of dietary administration of elemental Fe⁰ at 30 mg/kg diet ~ 1.5 mg/kg bw/d. T0 = Control; T2 = Elemental iron (T3 to T8 are irrelevant to current evaluation and are not presented here)



Conclusion

Following the sub-chronic dietary administration of elemental iron at 30 mg/kg diet (approximately 1.5 mg/kg bw/d) to rats for 56 days, there were no adverse effects upon clinical chemistry. This dose level appears to have been too low to induce general toxicity.

Reference	CA 5.3.2/04: Akhtar, S., Anjum, F. M., Rehman, Z. U., Riaz, M., Arshad, M., Basit, A. and Ismail, T. (2011b)	
Article title	Effect of Zinc and Iron fortification of the feed on liver and thyroid function.	
Journal title	Biological Trace Element Research 144 , 894 - 903	
Test substance	Elemental iron (reduced form)	
Guideline	None	GLP: No
Acceptability	<p>Considered acceptable with restrictions. Linked to the previous study from the same research group summarised at 5.3.2/03.</p> <p>Test item identified as elemental iron produced by either a H-reduction or electrolytic process.</p> <p>No dietary intake data are available; however, the single dietary concentration of 30 mg Fe⁰/kg is comparable to the 'control' dietary concentration of Whittaker <i>et al.</i>, 1996 (5.3.2/01). Investigations were limited to specific liver and thyroid clinical chemistry parameters and start and termination bodyweights.</p> <p>Study performed at Dept of Food Science and Technology, Bahauaddin Zakariya University, Pakistan.</p> <p><i>Note: Study authors terminology</i> glutamate oxaloacetate transaminase (GOT) = AST; glutamate pyruvate transaminase (GPT) = ALT</p>	

The main objective of the study authors was to perform targeted investigations into the liver and thyroid clinical chemistry (hepatic enzymes and thyroid hormones) of rats exposed to Zn or Fe dietary supplements. Only the results pertinent to elemental iron Fe⁰ are presented below.

Materials and Methods:

Groups of 10 male SD rats (10 weeks old, approx. 216 g) were given diets containing unfortified wheat flour, or wheat flour fortified with elemental Fe⁰ powder (96 % purity, Micronutrient Initiative), at approximately 30 mg/kg diet ~ 1.5 mg iron/kg bw/day*) for either 4 or 8 weeks. Five animals from each group were terminated after 4 weeks. The remaining 5 animals/group were sacrificed after 8 weeks. Terminal bodyweights were measured and blood (plasma) was sampled for clinical chemistry analyses - AST, ALT, T3 and T4.

* See preceding study summary 5.3.2/03 for rationale supporting applicant's dietary intake rates.

Results:

There were no statistically significant or biologically relevant differences between the treated group and control, for any of the liver or thyroid parameters measured.

Table 6.3-3: Effect of dietary administration of Fe⁰ to male rats (4 and 8-week dietary study; mean ± SD)

Parameter	Interval (n = 5)	Control No fortification	Elemental iron 30 mg Fe⁰/kg diet
AST (IU/L)	Week 4	102.17 ± 1.60	112.44 ± 8.09
	Week 8	188.00 ± 3.92	180.60 ± 8.62
ALT (IU/L)	Week 4	48.69 ± 2.63	53.55 ± 3.83
	Week 8	133.41 ± 4.57	124.55 ± 5.75
Plasma T4 (µg/dL)	Week 4	7.92 ± 0.55	7.92 ± 0.66
	Week 8	6.54 ± 0.35	5.98 ± 0.38
Plasma T3 (ng/mL)	Week 4	1.04 ± 0.25	1.21 ± 0.26
	Week 8	1.28 ± 0.15	1.16 ± 0.11

Conclusion

Following the sub-chronic dietary administration of elemental iron at 30 mg/kg diet (approx. 1.5 mg/kg bw/d) to rats for either 4 or 8 weeks, there were no adverse effects upon AST, ALT, T3 or T4. Although this study is considered to be a very specific (limited) investigation, the data presented indicates no adversity to the liver or thyroid at a relatively low dose level of approximately 1.5 mg/kg bw/d. This dose level appears to have been too low to induce toxicity.

Reference	CA 5.3.2/05: Domitrović R., Tota, M. and Milin, C. (2008)	
Article title	Differential effect of high dietary iron on α -tocopherol and retinol levels in the liver and serum of mice fed olive oil- and corn-enriched diets	
Journal title	Nutrition Research 28, 263-269	
Test substance	Elemental iron (carbonyl form)	
Guideline	None	GLP: No
Acceptability	Considered to be supplemental information. Only a small number of parameters were measured, limiting the utility of the study. No relevant tabulated results are available, in general, data are reported in graphical format in the original publication, therefore specific quantitative differences are difficult to ascertain. Statistical differences are reported, however, there was no evidence of adjustment of the statistical tests for the numerous multiple comparisons made, limiting containment of possible false positive results.	

The main objective of the authors was to investigate the effects of a high- Fe^0 , lipid-rich diet on antioxidant status – specifically α -tocopherol and retinol - in mice, and whether a lipid-enriched diet would affect liver iron levels. The results of the Fe^0 supplemented lipid-enriched groups, compared to lipid-enriched groups, or basal control diet are discussed below. The study did not include a basal control diet enriched with Fe^0 iron.

Materials and Methods:

Groups of 6 male Balb/c mice were fed diets with or without enrichment by corn oil or olive oil at 5% by weight. All diets had a basal iron concentration of 96 mg/kg diet (providing approximately 12 mg/kg bw/day) and oil-enriched diets contained either no additional iron or additional carbonyl iron at 1% by weight, such that the total iron intake in the supplemented groups was approximately 600 mg Fe^0 /kg bw/day. A control group received a basal diet with neither lipid or iron supplementation. After 3 weeks of feeding, the animals were sacrificed, their livers excised, and blood was taken for analysis. The following parameters were measured in the liver: iron content, α -tocopherol, retinol and thiobarbituric acid-reactive substances (TBARS – as a measure of lipid peroxidation); in the serum, analyses were limited to α -tocopherol and retinol levels.

Results:

In comparison to either the control or oil-enriched diets, the iron content in livers of animals fed a Fe^0 -supplemented lipid-enriched diet was approximately twice the content of either basal or lipid-enriched diets (statistically significant). There was no difference in iron uptake between the corn oil or olive oil-enriched diets.

The supplementation of corn oil and olive oil-rich diets with Fe^0 resulted in statistically significant decreases in serum retinol, and a similar effect was observed on serum α -tocopherol in mice fed an olive-oil rich Fe^0 -diet. However, no effect on serum α -tocopherol was observed in the mice fed a corn-oil enriched Fe^0 -diet. In the liver, increases in retinol and α -tocopherol were observed in mice fed either an olive (statistically significant) or corn-oil enriched Fe^0 -diet. Liver TBARS levels were statistically significantly increased with both the olive or corn-oil enriched Fe^0 -diets, confirming a promotion of lipid peroxidation in the presence of iron; the effect was more severe with a corn-oil enriched diet, which the authors propose as being due to the different lipid profile between the two types of oil. No effect on liver TBARS was observed in the absence of elemental iron, in the control or 'oil-only' enriched diets. There was no correlation between liver TBARS and either of the hepatic antioxidant levels.

Conclusion

The number of parameters investigated was very limited, meaning that only inferred conclusions can be made. Under the conditions of this study in mice – 3 week exposure to 600 mg Fe^0 /kg bw/d – the lipid content of the diet did not appear to impact dietary iron uptake. The supplementation of iron to a lipid-enriched diet resulted in an influx of both of α -tocopherol and retinol antioxidant from the serum to the liver, more evident in mice fed an

olive oil-enriched diet compared to those fed a corn oil-enriched diet. Hepatic lipid peroxidation was statistically significantly increased by iron supplementation, less so with a corn-oil enriched diet, indicating the capacity of Fe⁰ to facilitate a state of oxidative stress.

B.6.3.3. Other routes

The applicant has identified a single peer-reviewed original research article on the short-term (sub-acute) inhalation toxicity of the carbonyl form of elemental iron, and this is summarised on the basis of information available in the EU REACH registration dossier.

Reference	CA 5.3.3/01: Warheit, D. B., Hansen, J. F., Yuen, I.S., Kelly, D. P., Snajdr, S. I and Hartsky, M. A. (1997)	
Article title	Inhalation of high concentrations of low toxicity dusts in rats results in impaired pulmonary clearance mechanisms and persistent inflammation	
Journal title	Toxicology and Applied Pharmacology 145, 10-22	
Test substance	Elemental iron (carbonyl form)	
Guideline	None	GLP: No
Acceptability	<i>Considered acceptable with restrictions. The aim of this study was to investigate the pulmonary effects of exposure to high concentrations of poorly-soluble, low-toxicity particulate matter. The focus of the publication is on TiO₂, hence the level of reporting is lacking/absent for some parameters on elemental (carbonyl) iron. Investigations were localised to respiratory system; no systemic toxicity was assessed. No exposure group sizes were provided. A parallel test group was exposed to titanium dioxide and a sham control group was included. Data relating to carbonyl iron powder toxicity is summarised below. The article is useful in illustrating the potential effects of particle overload.</i>	

Materials and Methods:

Groups of – an unknown number of – male Crl:CD BR rats were exposed (nose-only) for 6 h/day, 5 d/week, for 4 weeks at 5, 50 or 250 mg Fe⁰/m³ air (carbonyl elemental iron, 0.2-2.0 µm, MMAD 2.9-3.4 µm, purity unknown; supplied by GAF Corp.). This was followed by a 6-month recovery period. Animals were approximately 8 weeks old. Bronchio-alveolar lavage (BALF) fluid was evaluated for markers of inflammation (granulocytes), LDH and protein (*data not presented in the publication*), cell proliferation (BrdU-labelling), alveolar macrophage (AM) chemotactic and phagocytic function *in vitro*, and histopathology (presence and translocation of iron in alveolar macrophages and lymph nodes) at 0 hr, 1 week, and 1, 3- and 6-months post-exposure. Statistical analyses were performed (Students t-test).

Results:

Exposure was adequately demonstrated by atmospheric chamber analyses (Table 6.3-4) and no mortalities were reported. The authors report that at 250 mg/m³, a total lung burden of 17 mg/lung was achieved, and this is considered to be a high-load dust burden in the lung, by which the authors intended to reproduce a generic response, unspecific to the chemical.

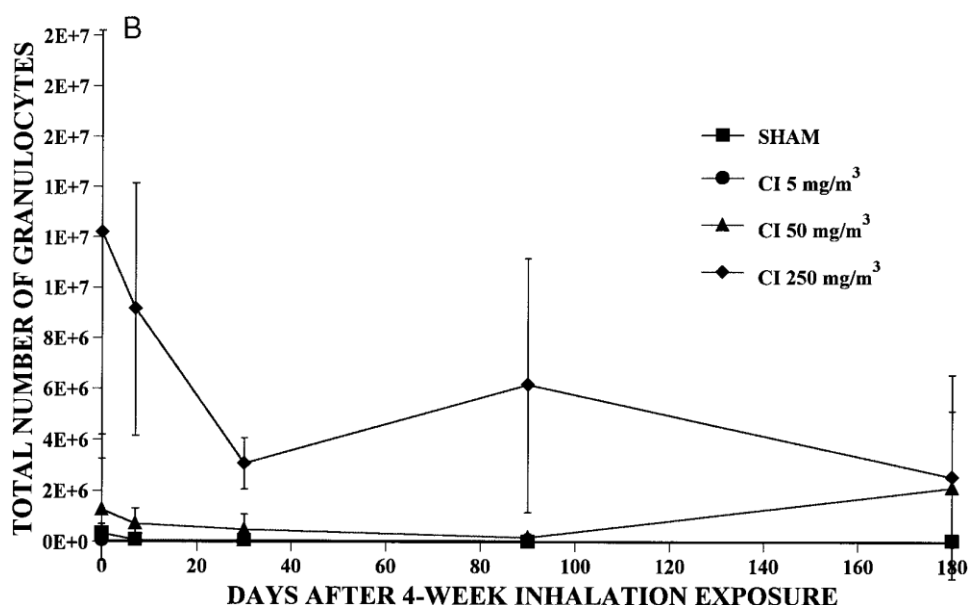
Table 6.3-4: Achieved atmospheric concentrations and MMADs (4-week inhalation study; rat)

Target concentration (mg/m ³)	Mean achieved for 20 exposures (mg/m ³)	MMAD (µm)
5	4.8 ± 2	3.4
50	51.8 ± 15	3.2
250	243.6 ± 90	2.9

There was significant exposure-related accumulation of particles in the lungs, with nearly 100 % of lavage fluid AM at 250 mg/m³ presenting with particulate matter at 6 months post-exposure. At this concentration level, AM chemotactic and phagocytic function was adversely affected and accompanied by pulmonary granulocytic

(neutrophilic) inflammation at 3 months post-exposure; at this point, inflammation was negligible at 50 and 5 mg/m³. Similarly, LDH and protein values in BALF were only elevated at 250 mg/m³ (*data not presented in the publication*). There were no significant increases in BALF alkaline phosphatase or N-acetylglucosaminidase levels at any of the exposure levels (*data not presented in the publication*). These BALF clinical chemistry results indicate that there was no detectable damage to alveolar cells or pulmonary capillary membranes.

Figure 6.3-2: Pulmonary inflammatory response in BALF of rats exposed to elemental iron (4-week inhalation rat study)



Note: Increase at 50 mg/m³ after 6 months reportedly only occurred in only one animal

At 50 mg/m³ and above, functionally deficient AM were still detected at study termination, albeit to a minimal degree (*data not presented in the publication*). The deficit in AM motility may have been due at least in part to particle overloading and/or the inflammation present.

At 50 and 250 mg/m³, free granular pigment of Fe⁰ was present on the mucosal surfaces of bronchioles and bronchi immediately after exposure, with particle-laden AM in the air spaces and dense aggregates of such macrophages in the alveoli and alveolar ducts. Cellular hypertrophy and hyperplasia were evident at alveolar wall and duct bifurcations, adjacent to macrophage aggregates, but not in the bronchial region. There was no indication of pre-fibrotic lesions at any exposure level.

Rats exposed to 250 mg Fe⁰/m³ showed substantial increases in pulmonary cell BrdU proliferation indices, measured on lung parenchymal and terminal bronchiolar surfaces, which were sustained for 3-6 months post-exposure (Table 6.3-5). Cell proliferation could be an indication of pre-fibrotic lesions, noting that there was no detectable fibrosis of pulmonary structures. The investigators did, however, detect an occasional alveolar duct bifurcation with some indications of immature collagen which was associated with free dust particles, suggesting a causal relationship. Translocation of the inhaled CI particles to the tracheobronchial lymph nodes was also noted at the highest dose level, which may be expected as part of the intrinsic particle clearance mechanisms in place.

There was evidence of iron particle overloading of alveolar macrophages, indicating a potential for solubilisation to ionic forms by lysosomal action under conditions of high levels of particle exposure. However, the intracellular excesses of iron are sequestered as an unreactive form in lysosomes, thereby minimising the risk of ROS-induced iron toxicity.

Table 6.3-5: BrdU labelling index for lung cells exposed to elemental iron (% labelled cells; 4-week inhalation rat study)

Exposure level (mg/m ³)	Days after 4-week exposure to CI				
	0	7	30	90	180
Proximal parenchymal cells					
Sham	0.50 ± 0.10	0.48 ± 0.09	0.61 ± 0.03	0.58 ± 0.10	0.59 ± 0.09
5	0.39 ± 0.03	0.53 ± 0.28	0.58 ± 0.05	0.50 ± 0.02	0.50 ± 0.27
50	0.91 ± 0.38	1.02 ± 0.09*	0.63 ± 0.31	1.09 ± 0.47	0.61 ± 0.21
250	1.62 ± 0.08*	1.67 ± 0.62	1.17 ± 0.50	2.09 ± 0.28*	1.11 ± 0.17*
Terminal bronchiolar cells					
Sham	0.59 ± 0.09	0.53 ± 0.06	0.46 ± 0.05	0.50 ± 0.08	0.60 ± 0.10
5	0.43 ± 0.23	0.77 ± 0.59	0.50 ± 0.15	0.87 ± 0.07	0.46 ± 0.10
50	0.93 ± 0.33	1.08 ± 0.57	0.78 ± 0.14	0.89 ± 0.05*	0.59 ± 0.09
250	1.14 ± 0.14*	1.08 ± 0.51	1.05 ± 0.25	1.08 ± 0.15*	0.75 ± 0.12

* p<0.05 (Student's *t*-test)**Conclusion**

The results of this study clearly demonstrate that exposure of rats to high dust concentrations of carbonyl Fe⁰ for 4 weeks produced sustained pulmonary inflammation (an influx of granulocytic neutrophils), enhanced proliferation of pulmonary cells, impairment of particle clearance, deficits in macrophage function, and the appearance of macrophage aggregates at sites of particle deposition from a concentration of 50 mg/m³, becoming more pronounced at the top concentration of 250 mg/m³. The evident impairment of clearance and recruitment of inflammatory cells into the lung are characteristic of “lung overload”. A NOAEC was identified at 5 mg/m³, with a LOAEC of 50 mg/m³. Following consultation with the ECP, due to the potential for differences in physico-chemistry (including particle size, porosity and other particle characteristics) between [REDACTED] forms of elemental iron particles in the respiratory environment, the reliability of the read-across from [REDACTED] iron for the inhalation route of exposure was deemed too uncertain. Overall, when combined with the shortcomings in the reporting of the data (including information on immunological parameters in the lungs) from a single publication, derivation of a local effects AOEC for [REDACTED] elemental iron was not supported.

Overall summary for short-term toxicity

The assessment of the short-term toxicity of elemental iron relies primarily on the EU assessments of the more bioavailable forms of iron, FeSO₄ and FePO₄, summarised below.

Short-term toxicity summary of iron sulphate (excerpt taken from Vol.1 FeSO₄ DAR 2008):

In a published 49-day study in mice at doses of 120, 5000 and 8000 ppm corresponding to 20, 833 or 1333 mg Fe/kg body weight or 100, 4165 or 6665 mg FeSO₄·7H₂O/kg body weight there was evidence of liver toxicity at dose levels of ≥ 5000 ppm whilst the significance of any findings at 120 ppm were not clearly reported.

Corrosive effects can be expected following large doses related to poisoning and changes in gut flora has been observed with large iron intakes. The effects of iron on the intestinal flora have been noted to be in line with a reported human cohort study in Chile [Yeary et al. (1966)]. In this study, adverse effects on the gut flora, including more frequent diarrhoea, have been observed when children consumed iron-fortified milk (12 mg Fe/l) while the control group drank normal cow milk with a content of 1 mg Fe/l.

Short-term toxicity summary of ferric phosphate (excerpt taken from Vol. 1 FePO₄ RAR 2013):

In a study with schoolchildren approximately 52.5 mg ferric phosphate per capita were consumed for a period of up to 18 months without adverse effects. Most information on ferric phosphate and other iron salts are based on acute and chronic observations. A special subchronic risk of ferric phosphate has not been identified.

The applicant has supplemented the read-across to the existing approved iron-based substances by the provision of peer-reviewed scientific literature on elemental iron, which confirm that data on the more soluble, ionic forms represents a worse-case scenario. The published literature generally focussed on iron-overload conditions in rats or mice via the oral or inhalation routes of exposure.

The main toxicity of iron is secondary to free radical formation, and the generation of ROS; comparable iron toxicity has not been reported under conditions of normal intestinal function. Iron toxicity has been reported under conditions of hereditary genetic disorders and some of the key initiating pathology can be replicated in short-term vertebrate studies.

Under exaggerated oral exposure of elemental iron, there is evidence of accumulation of iron in the liver and an exceedance of the iron-binding capacity of transferrin, leaving free iron circulating in the plasma. These may then reach target organs, resulting in wider tissue damage from increased levels of reactive oxygen species, e.g. hepatic lipid peroxidation. In one publication, rats dosed for 90 days demonstrated no adverse effects up to a dose of 200 mg/kg bw/d. In contrast, data from another research group indicate increased unbound iron and insoluble deposition (haemosiderosis) in the liver from approximately 35 Fe/kg bw/d in rats, with more widespread organ damage including atrophy at higher dose levels (heart, pancreas and spleen); the NOAEL from this study was 3.2 mg/kg bw/d.

Under excessive lung overload conditions induced at high concentration levels in a published sub-acute inhalation study (on the carbonyl form of elemental iron) in rats, effects on sensitive biomarkers of pulmonary toxicity were seen, including pulmonary inflammation, impaired particle clearance, deficits in macrophage function, particle-loaded macrophage aggregation at bifurcations of the lower respiratory tract, and adverse histopathology (hypertrophy and cellular proliferation). These findings were noted from 50 mg/m³. At 250 mg/m³, there was an increase in severity of several of the findings, (notably grade and persistence of adverse histopathology and inflammation), accompanied by translocation of particulate matter to tracheobronchial lymph nodes. Hence in addition to overwhelming the normal clearance mechanisms (mucociliary escalator towards oral elimination via the GIT), it appears there is some potential for particle retention within the respiratory system following exposure to high load dust burdens of insoluble elemental iron. The LOAEC was established at 50 mg/m³, leading to a NOAEC at 5 mg/m³. Following consultation with the ECP, due to the potential for differences in physico-chemistry (including particle size, porosity and other particle characteristics) between [REDACTED] forms of elemental iron particles in the respiratory environment, the reliability of the read-across from [REDACTED] iron for the inhalation route of exposure was deemed too uncertain. Overall, when combined with the shortcomings in the reporting of the data (including information on immunological parameters in the lungs) from a single publication, derivation of a local effects AOE for [REDACTED] elemental iron was not supported.

There are no reports of adverse pulmonary effects from the medical surveillance at the manufacturing site from which the 'free' (technical material) active substance is sourced (see Section B.6.9), nor any evidence from the applicant's literature search.

In the absence of any evidence of significantly altered pathology or functional deficiency, the HSE does not consider these effects to be sufficiently severe to support classification for specific target organ toxicity in accordance with Regulation (EC) No. 1272/2008.

B.6.4. GENOTOXICITY

It is widely accepted that following ionisation, transition metals in particulate form, are capable of generating reactive oxygen species (ROS) and inducing oxidative stress, a well-known mechanism of genotoxicity. Fundamental to the potential for ROS generation from elemental iron, is the Haber-Weiss and Fenton reactions, which require oxidation from the zerovalent state. However, there is no evidence from the applicant's literature search including an *in vitro* Comet assay (see below), that production of ROS occurs from elemental iron. The applicant's literature search yielded a single, relevant publication on genotoxicity, and HSE are satisfied that the applicant's literature search is of an acceptable standard. The applicant identified an *in vitro* comet assay with an inhalation perspective, on elemental iron particles in the published literature and this study is summarised below.

No new regulatory studies have been submitted, since the potential for genotoxicity following systemic exposure to elemental iron has been extrapolated from the EU assessments of FeSO_4 (EFSA conclusion, 2012) and FePO_4 (EFSA conclusion, 2015). Both of these iron-based compounds are significantly more bioavailable substances in comparison to elemental iron and are also the most abundant forms in the systemic circulation following exposure to elemental iron.

B.6.4.1. *In vitro* studies

Reference	CA 5.4.1/01: Hedberg, Y., Gustafson, J., Karlsson, H. L., Moller, L. and Wallinder, I.O. (2010b)	
Article title	Bioaccessibility, bioavailability and toxicity of commercially relevant iron- and chromium-based particles: <i>in vitro</i> studies with an inhalation perspective	
Journal title	Particle and Fibre Technology 7, 23	
Test substance	Elemental iron (carbonyl form)	
Guideline	None	GLP: No
Acceptability	<p><i>Considered acceptable, considering that the publication is not intended to fulfil the requirements of TG489. Shortcomings include: lack of metabolic activation, however, HSE can find no evidence of the inclusion of S9 fraction in the regular use of the comet assay in particle toxicology; data only available as graphs not tabulated.</i></p> <p><i>In addition to the Comet assay, the paper informs on aspects of particle surface reactivity of iron powder. No tabulated data are available in the publication. Elemental iron was supplied by the manufacturer of the active substance as proposed by the applicant.</i></p>	

The main objective of the study was an assessment of the potential health hazards resulting from the inhalation of chromium- and iron-based occupational exposures. This included the assessment of iron release in various synthetic media, *in vitro* investigations of particle surface reactivity in a haemolysis assay and genotoxicity in a cultured human lung cell line comet assay.

Materials and Methods:

Comet assay: In an alkaline version of the Comet assay, a human alveolar type II epithelial cell line (A549 sourced from ATCC) were exposed to elemental iron Fe⁰ in DMEM cell culture medium supplemented with 10% inactivated FBS. Negative control cell cultures were exposed to the supplemented medium only. The pure iron source was stated to be 99.96 % pure (). Elemental iron was received from the manufacturer and milled to less than 20 µm in order to create a “fine” sample which could be compared to a raw “coarse” sample. HSE notes that only the quantitative data for total metal release (measured by flame atomic absorption spectroscopy) in ALF are presented in the publication.

For the Comet assay, 0.16×10^6 A549 cells were seeded and allowed to grow to 90 % confluence over 24 h. The test item was added at 80 µg particulate Fe⁰/mL (40 µg/cm² in the plate well) and incubated for 4 hours. HSE notes that this is in line with the exposure time recommended by Tice *et al.* (2000)⁴. For genotoxicity assessment, cells were subjected to alkaline single cell gel electrophoresis for the detection of DNA damage. The essential steps of the comet assay were successively layering of cells mixed with low melting point agarose (over coated glass microscope slides), lysis (to lyse the cell and nuclear membranes and other proteins), unwinding of DNA, electrophoresis, neutralization, staining and scoring, mainly for single-strand breaks, alkali-labile sites and double-strand breaks. The % DNA in the tail of the comets of 70 cells per concentration (35 cells were evaluated in duplicate) and each experiment was conducted in triplicate. HSE notes that this exceeds the minimum recommended by Tice *et al.*, (2000) of 25 cells/slide prepared from a minimum of 50 cells/culture). NiO nanoparticles were included as a positive control.

For the cytotoxicity assay, 0.08×10^6 A549 cells were seeded and allowed to grow to 50 % confluence over 24 h, prior to incubation with the particulate matter for a further 24 h. Cytotoxicity was measured by the Trypan blue dye exclusion technique after 24 h of exposure and counting of stained cells (100 cells per sample in triplicate).

Cells were maintained at 37 °C, 5 % CO₂ for all incubations.

Bioaccessibility: As an indicator of bioavailability in mammalian systems, the release of iron (no information on ionic speciation) from Fe⁰ was investigated by incubating -with shaking - particulate matter for a week in either

⁴ Tice R. R *et al.*, Single cell gel/Comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environmental and molecular mutagenesis 35: 206 -221 (2000)

artificial lysosomal fluid (ALF pH 4.5; simulates intra-cellular inflammatory conditions in the lung cells following phagocytosis), artificial sweat (ASW; pH 6.5); Gamble's solution to mimic the pulmonary interstitial fluid (GMB ; pH 7.4) and artificial tear fluid (ATF; pH 8.0).

Haemolysis assay: To investigate the potential for Fe⁰ particles to present ROS-mediated surface reactivity, either fine (achieved diameter less than 5 µm) or coarse forms – sourced from [REDACTED] were incubated (with shaking) with fresh human erythrocytes at 0.67, 1.33 and 2.67 mg/ml for 30 min before centrifugation to isolate the cell-free supernatant. Saline and 0.1 % Triton detergent were used as negative and positive control substances, respectively. Released haemoglobin in the isolated supernatant was measured in duplicate, via OD₅₄₀ nm. HSE notes that only the quantitative data for the highest concentration of 2.67 mg fine Fe⁰/ml are presented in the publication.

Results:

Genotoxicity, cytotoxicity and particle bioreactivity: There were no meaningful biological or statistical increases in DNA damage (% DNA tail migration) from exposure to either fine or coarse Fe⁰ particulate matter (Figure 6.4.1-1). There was a clear increase in DNA damage from exposure to the nanoparticulate NiO positive control and a very low background level of DNA damage from exposure to the vehicle-only negative control. There was no evidence of cytotoxicity, nor of biologically relevant haemolysis (Figure 6.4.1-2).

Figure 6.4.1-1: DNA damage (% DNA in tail) and cytotoxicity (non-viable cells %) following exposure of fine and coarse elemental iron to alveolar cell line Comet assay *in vitro*

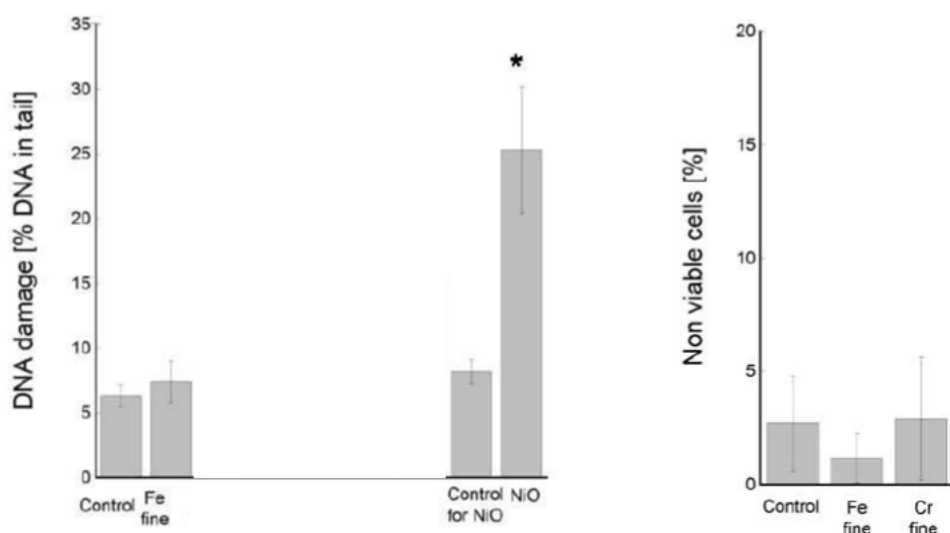
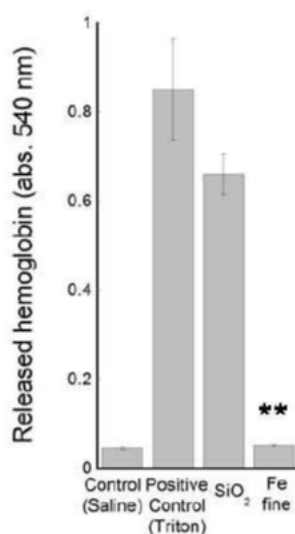


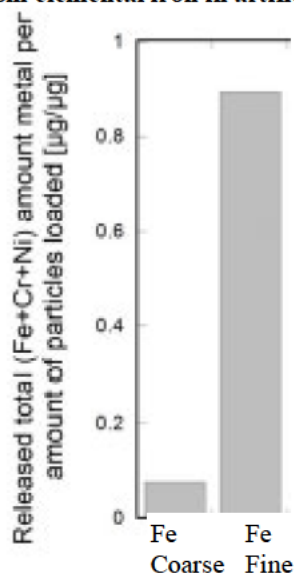
Figure 6.4.1-2: Released haemoglobin as a marker of particle surface reactivity following exposure of fine elemental iron to human erythrocytes (*p*-value 0.01 vs. controls)**



Although the extent of haemolysis induced by fine elemental iron was statistically significant in comparison to the saline control (Figure 6.4.1-2), the authors conclude this is a biologically irrelevant response.

Bioavailability: The release of iron from particulate elemental iron Fe⁰ after 1 week in ALF (~ pH 4.5) was essentially complete at $\approx 1 \mu\text{g Fe}/1 \mu\text{g Fe}$ from fine particulate matter, with notably less being released from the coarse particles (7.6 %; Figure 6.4.1-3). The authors imply that there was negligible release in the pH neutral and weakly alkali media investigated, however, no further details are provided in the current publication.

Figure 6.4.1-3: Bioaccessibility of iron from elemental iron in artificial lysosomal fluid (ALF)



Conclusion

Under the conditions of this *in vitro* alkaline Comet assay, ‘fine’ particulate Fe⁰ (described as < 20 μm , no further details provided) was neither genotoxic or cytotoxic.

It is well-known within particle inhalation toxicology, that low-solubility particles can elicit toxicity via their surface reactivity. In this study, fine particulate Fe⁰ iron did not cause any biologically relevant damage to erythrocyte cell membranes, indicating neither direct particle-cell interaction, or generation of ROS. In addition, considering that the haemolysis assay has been shown to be a good predictor for the *in vivo* inflammation

potential of inhaled particulate matter, it seems unlikely that Fe⁰ poses a similar hazard. The relatively low toxicity of the Fe⁰ is in agreement with other findings from this study.

The immersion of elemental iron in ALF is intended to simulate – in simple terms -a state of intracellular inflammation in lung cells, following phagocytosis. This is relevant to a scenario of lung particulate overload, where particles may be engulfed by alveolar macrophages, (see Section B.6.3.3 Warheit *et al.*, 1997), and indicates that exposure to lysosomal fluid will increase the amount of iron potentially bioavailable *in vivo*. Any iron released will be subject to normal homeostatic physiological controls to prevent free metal ions in solution which could otherwise promote toxicity. HSE notes that a particle overload by any route of exposure of elemental iron from the representative product is highly unlikely. It is also indicated that there is likely to be negligible release of iron into lung lining fluid, and very low release into sweat (Hedberg *et al.*, 2010a).

B.6.4.2. In vivo studies in somatic cells

No *in vivo* genotoxicity studies in somatic cells are submitted, and none are required.

B.6.4.3. In vivo studies in germ cells

No *in vivo* genotoxicity studies in germ cells are submitted, and none are required.

Summary of genotoxicity:

Elemental iron is approved for use as a human food supplement in the EU and in comparison with ionic forms, it is relatively insoluble and hence of low potential bioavailability. Following exposure to elemental iron, systemic exposure is expected to be to the ionic forms of iron and read-across from the more soluble forms - to address the potential for *in vivo* systemic genotoxicity – has been performed. The EU reviews for both iron sulphate and ferric phosphate conclude that on a range of *in vitro* and *in vivo* data, neither substance is considered mutagenic. Furthermore, the applicant has identified an *in vitro* Comet assay generated on elemental iron in an alveolar cell line, which yielded negative results.

Particle genotoxicity can arise as a function of the particle surface reactivity and is relevant to assessing the potential for local toxicity of elemental iron; however, no such concerns have been identified for elemental iron in the published literature. In a haemolysis assay in human erythrocytes (Hedberg *et al.*, 2010b), there were no biologically relevant increase in haemoglobin release, indicating low surface activity- either by direct interaction, or by generation of ROS. In the same study, no genotoxicity was observed in an alveolar cell line using the Comet assay.

It is widely known that iron salts can produce strand breaks in DNA when incubated with purified DNA or isolated mitochondria, and iron overloaded rats show an increased number of strand breaks in hepatic DNA. The mechanism of strand breakage is likely via iron-catalysed production of hydroxyl radicals that can abstract hydrogen atoms from both the ribose moiety and purine/pyrimidine bases (Winterbourn, 1995). The oxidative/catalytic mechanisms responsible for these events require the presence of iron atoms with free coordination linkages and cannot be mediated by transferrin-bound iron, or by iron resident within the core of ferritin. Consequently, these potentially genotoxic/carcinogenic reactions are only likely to occur when iron levels exceed the binding capacity of ferritin to transferrin. Saturation of transferrin occurs only in severe iron overload from high-levels of oral exposure.

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

No new regulatory studies have been submitted. The applicant has identified a limited number of articles – largely reviews - from the public literature which inform on these toxicological endpoints, noting that no new significant epidemiological peer-reviewed research articles or animal studies have been published since the EU PPP assessments of FeSO₄ and FePO₄. The information on the relationship between iron and chronic disease is largely based on epidemiological studies; the methodology for the literature review is presented elsewhere in this document (Section B.6.9). Epidemiological studies themselves have limitations, with the most critical one being the lack of reliable quantification of iron intake/exposure with food, dietary supplements, lifestyle of the participants and small population sizes. The latter results in low statistical power of the data obtained.

In addition to the published information submitted by the applicant, HSE are aware of a comprehensive review of the role of iron in human nutrition “Iron and Health”, published by the UK Scientific Advisory Committee on Nutrition (SACN, 2010)⁵. The following text is taken directly from the “Iron and Health” report, which focussed predominantly, on dietary heme-sources of iron. Nevertheless, certain conclusions on the health consequences of high iron intakes are relevant to the current evaluation of (non-heme) elemental iron, following oral exposure:

“Iron and cancer

7.30 Although it is uncertain whether, or how, iron might be carcinogenic, it has been suggested that oxygen free radical formation catalysed by iron might play a role in this process (Toyokuni, 1996; Okada, 1996). Two main pathways have been suggested: increased DNA damage induced either directly or indirectly by impeding DNA repair, and modulation of nuclear redox sensitive transcriptional regulators through signal transduction mechanisms (Galaris and Evangelou, 2002). Haem iron, but not inorganic iron, also increases production of N-nitroso compounds in the lumen of the gastrointestinal tract (Cross et al, 2003); many N-nitroso compounds have been shown to be human and animal carcinogens (IARC, 1978). In addition, iron is a limiting nutrient for the growth and replication of cancer cells in the human body (Weinberg, 1984)”

The SACN report focused on the relationship of iron intakes and systemic iron, with cancer and cardiovascular disease (CVD) risk since these were considered to be the two main issues of public health concern in the UK. Since most dietary iron is not absorbed, it was postulated that luminal exposure to excessive dietary iron would overwhelm the mechanisms of absorption and may result in direct oxidative damage to the colorectal lumen (Huang, 2003). However, the SACN concluded that overall, there are insufficient data on the association between intakes of total dietary iron, heme iron, ferritin concentration and colorectal cancer or CVD risk, to reach clear conclusions.

The EFSA Panel on Dietetic Products, Nutrition and Allergies recently published a Scientific Opinion on dietary reference values for iron (2015^b), which concluded that the risk of systemic iron overload from dietary sources is negligible. Specific clinical conditions and genetic disorders (e.g. haemochromatosis or repeated blood transfusions) may lead to iron overload but as yet, there has been no evidence of iron storage being overwhelmed, nor of oxidative architectural and functional tissue damage in either heterozygous individuals or within a standard EU population with normal intestinal function.

The role of iron in a wide range of cancers has been the subject of much interest over the past few decades, however in most instances – with the exception of pre-existing clinical conditions such as haemochromatosis or thalassaemia - causality remains unproven. There has been extensive research on the association of liver iron overload and hepatocellular carcinoma. The liver is the main site of iron storage and metabolism and it has been hypothesised that iron overload is an important factor in the development of hepatic carcinomas. As a human model, the hereditary condition of haemochromatosis may provide indication of a mode of action. Hereditary haemochromatosis is an inherited iron overload disease, characterised by excessive levels of ferritin (soluble iron) and haemosiderin (insoluble iron). Haemochromatosis secondary to haemosiderosis is also possible, from a non-genetic aetiology. A typical case of haemochromatosis is characterised by inappropriately high levels of absorption of dietary iron, and efflux of iron from reticuloendothelial macrophages; the result is elevated plasma iron and serum ferritin. Excessive accumulation in parenchymal hepatocytes (and other tissues) then overwhelms the liver storage capacity. Clinically relevant iron overload has been described as total body iron of more than 5 g, noting that the normal range is 35 – 40 mg and 50 – 60 mg/kg in females and males, respectively (Huang, 2003). This leads to a disruption of the redox balance, resulting in chronic oxidative stress which may then cause mutational changes in genomic DNA, or modulate signalling networks, including those related to inflammation, fibrosis and malignant transformation (Benhar *et al.*, 2002; Toyokuni, 2002; Galaris and Pantopoulos, 2008). In clinical terms, potentially fatal hepatic iron toxicity in haemochromatosis manifests as fibrosis, progressing to cirrhosis and an increased relative risk of developing hepatocellular carcinoma. Unlike other metalloid/metals such as arsenic, chromium and nickel, there is no evidence that iron itself is directly carcinogenic. Patients who suffer from haemochromatosis also sustain injury to the heart, endocrine pancreas, hypogonadism (delayed onset puberty) and arthritis. However, the SACN concluded that under normal intestinal function, there was no evidence that dietary iron is associated with cardiovascular disease, arthritis, diabetes mellitus or neurodegenerative disease.

⁵ ‘Iron and Health’ Scientific Advisory Committee on Nutrition, pub. TSO, 2010

An imbalance in ROS leading to a state of oxidative stress can play multiple roles in tumour initiation, progression and maintenance, either by direct disruption of biomolecules, or by modulating transcription factors and signalling pathways e.g. stress activated protein kinases. The following text is taken directly from the “Iron and Health” SACN (2010) report, which describes the potential generation of ROS from free iron:

“The role of iron as a pro-oxidant

7.16 The ability of iron to gain or lose single electrons makes it an efficient catalyst for free-radical reactions. Fe^{2+} (ferrous form) and Fe^{3+} (ferric form) which have four and five unpaired electrons in each configuration respectively, and Fe^{4+} (ferryl species), can exist in biological systems. Ferryl species are generated when certain haem moieties react with hydrogen peroxide (H_2O_2).

7.17 Haemoglobin and myoglobin contain haem iron and undergo oxidation to form superoxide and Fe^{3+} protein; both can initiate damage when they react with peroxide. Haemoglobin and myoglobin will degrade to haem and iron ions, which can in turn stimulate lipid peroxidation, protein oxidation and DNA oxidation. Haem iron can also catalyse the decomposition of pre-existing lipid peroxides to alkoxy and/or peroxy radicals, causing cellular damage and leading to cell death (McCord et al, 1998).

7.18 Cells have evolved protective mechanisms to remove free radicals. These include superoxide dismutase which converts superoxide to H_2O_2 , and catalase, peroxidase and glutathione peroxidase that reduce peroxides and H_2O_2 . Under normal conditions these defence mechanisms are effective at scavenging free radicals generated by iron dependent and iron-independent mechanisms.”

The applicant’s literature search revealed two publications which address specific cancer endpoints and a single comprehensive systematic review and meta-analysis of the epidemiological evidence surrounding iron and cancer risk. The main findings from all three articles are summarised in the section below.

Reference	CA 5.9.4/01: Choi, J-Y <i>et al.</i> , (2008)	
Article title	Iron intake, oxidative stress-related genes (MnSOD and MPO) and prostate cancer risk in CARET cohort	
Journal title	Carcinogenesis 29(5) 964-970	
Guideline	None	GLP: No
Acceptability	<p>Considered acceptable as scientifically-peer reviewed, however, unclear relevance to the current assessment of elemental iron.</p> <p>Limitations:</p> <ul style="list-style-type: none"> Participants were all heavy smokers/occupationally exposed to asbestos, putting them under high baseline levels of oxidative stress and minimising the relevance of extrapolation of the conclusion to the general population. Iron intake was assessed using a questionnaire but did not include measurement of blood or serum biomarkers of iron stores, nor was there any assessment of the use of dietary iron supplements in the study’s participants. The data revealed a potential confounding effect of the MnSOD genotype status and iron intake. 	

Summary:

In a nested case-control prospective epidemiological investigation, exploring the relationship between iron intake and prostate cancer in approximately 2000 subjects, a borderline association between iron intake and an aggressive form of prostate cancer was detected. Data was collected from heavy smokers between 1985 – 2005, including former heavy smokers, and asbestos-exposed workers in the USA. Controls were men who were free of cancer. Blood was sampled for genotyping of manganese superoxide dismutase (MnSOD) and myeloperoxidase (MPO) and iron intake was calculated from a dietary questionnaire. The dietary questionnaire was also used to obtain intake levels of antioxidant-rich foods such as fruit and vegetables. Those in the highest dietary iron intake tertile (≥ 15.8 mg/day) were more likely to develop the disease during the 20-year follow-up than those in the lowest tertile (< 10.7 mg/day). The association was statistically significant for participants with low (below-median) intakes of fruit, vegetables and vitamin C. Genotyping analysis indicated that genetic variation can modify the association between iron intake and risk of developing an aggressive form of prostate cancer, but there was no association with the non-aggressive form of prostate cancer, nor with all-prostate cancers. The TT homozygosity in the MnSOD (manganese superoxide dismutase; reduced activity) gene and GG homozygosity in the MPO (myeloperoxidase; higher activity) gene, were risk factors for the high-iron intake

group. Among aggressive cancer cases with the TT genotype, higher iron intake level was associated with an approximate 2-fold increase in risk (OR = 2.3, 95% CI = 1.0-4.9), whereas there was no association among men with CC genotypes (OR = 0.9, 95% CI = 0.4-2.3). Although interactions were not significant, there were similar patterns for MPO genotype, iron intake and risk. The authors proposed that a reduction in MnSOD activity (TT genotype), and an increase of MPO activity (GG genotype) may lead to elevated ROS via the Fenton and Haber-Weiss reactions, thereby promoting oxidative damage. In conclusion, HSE considers the generation of oxidative stress to be a plausible mode of action, noting no supporting mechanistic data are presented in the publication. Overall, these epidemiological data suggest that in subjects consuming lower-than-average amounts of fruit and vegetables, high-iron intake may be associated (borderline) with clinically aggressive prostate cancer; however, this was observed in the presence of confounding factors of diet, genetic polymorphism and basal iron exposure, calling the robustness of the association into question.

Reference	CA 5.9.4/03 Polesel, J., Talamini, R., Maurizio, M., dal Maso, L., Crovatto, M., Parpinel, M., Izzo, F., Tommasi, L., Serraino, D., La Vecchia, C. and Fransechi, S. (2007)	
Article title	Nutrients intake and the risk of hepatocellular carcinoma in Italy	
Journal title	European Journal of Cancer 43 , 2381-2387	
Guideline	None	GLP: No
Acceptability	<i>Acceptable as supplemental information.</i> <ul style="list-style-type: none"> <i>Limitations: Relatively small number of cases and controls (185 cases of hepatocellular carcinoma and 412 cancer-free controls),</i> <i>Iron intake was assessed using a questionnaire, and although blood was sampled for antibody screening, there was no measure of blood or serum biomarkers of iron stores, nor was there any assessment of the use of dietary iron supplements in the study's participants.</i> <i>Potential selection bias of hospital controls (they may not be representative of the general population in relation to dietary habits, although care was taken to minimize this potential).</i> 	

Summary:

In order to explore the relationship between hepatocellular carcinoma risk and iron (amongst several other macro- and micronutrients), a case-control study (hence subject to recall biases) was conducted, in the context of the Mediterranean diet. The subjects were patients at an Italian hospital, admitted between 1999-2002. Cancer-free controls were selected from patients admitted to the hospital, excluding those admitted for alcohol- or smoking-related conditions. Iron intakes were assessed by a dietary questionnaire. The study authors reported that an estimated iron intake of 13.9 mg/day was associated with increased hepatocellular carcinoma risk (OR=3.00; 95% CI: 1.25-7.23), but the association was considerably reduced and no longer statistically significant, when iron from wine was excluded, indicating a dominant role of wine intake in the perceived association between dietary iron and HCC (OR=1.61; 95% CI: 0.78-3.30). HSE notes that an intake of 50 mg/day is recommended for pregnant women.

Reference	CA 5.9.4/04: Fonseca-Nunes, A., Jakszyn, P. and Agudo, A. (2014)	
Article title	Iron and cancer risk - a systematic review and meta-analysis of the epidemiological evidence.	
Journal title	Cancer Epidemiol. Biomarkers Prev. 23(1) 12-31	
Guideline	None	GLP: No
Acceptability	<i>Considered acceptable and the meta-analysis was adequately defined.</i> <i>Limitations: Based on a Pubmed search, data from a mixture of prospective and case-control studies, the latter being subject to recall bias. Differing methodologies for determining heme iron intake. No accounting for use of iron supplementation in most studies. Early studies did not consider serum ferritin as a marker of iron status. Generally variable quality and completeness of data. Neither elemental iron or non-heme iron were included as search terms in the Pubmed search.</i>	

Summary:

A review was conducted of 59 epidemiologic studies, published between 1995 and 2012, reporting information on total iron, dietary iron, heme iron, and biomarkers of iron status, with various different cancer types e.g. colorectal, gastric, oesophageal, breast and lung cancers. A meta-analysis was performed for colorectal [relative risk (RR) 1.08; 95% confidence interval (CI) 1.00–1.17], colon (RR 1.12; 95% CI 1.03–1.22), breast (RR 1.03; 95% CI 0.97–1.09), and lung cancer (RR 1.12; 95% CI 0.98–1.29), for an increase of 1 mg/day of heme iron intake. Overall, on the basis of the systematic review and meta-analysis, a higher intake of heme iron showed a tendency for positive association with cancer risk. Meanwhile, evidence regarding high levels of biomarkers of iron stores, such as serum ferritin, serum iron, transferrin saturation and total iron binding capacity, suggested no effect on cancer risk.

Other routes - literature on long-term toxicity and carcinogenesis:

The applicant has also identified three peer-reviewed scientific publications (surplus to their literature search) on the potential for airborne iron to influence chronic disease and mortality. Two of these papers - from a single research group (Ostro *et al.*, 2007 and 2008) - related to ambient airborne PM_{2.5} and a third publication related to the estimated exposure to iron and welding fumes in the occurrence of lung cancer (Siew *et al.*, 2008). Following close examination of all three articles by HSE, none have been deemed relevant for detailed summary in the dossier for several reasons:

- Relatively limited (in number) samples of PM_{2.5} or population numbers all three publications
- no analytical or speciation analysis of the iron in the estimated exposures
- co-exposure was a confounding issue and it is possible that associations with mortality and lung cancer may be a result of exposures to other substances with which the iron component is highly correlated.

As the inhalation route is not relevant to the exposure of elemental iron as a molluscicide, no further data are required to address this specific route of exposure.

Summary of long-term toxicity and carcinogenesis

Despite the poor bioavailability of elemental iron as a nutritional food supplement, it has been widely used in the EU and on a global scale for several decades, primarily due to its chemical stability in food items, combined with its relatively low cost of bulk production. The applicant's source of elemental iron is currently approved for use as a food-grade supplement in the USA (GRAS).

In the absence of substance-specific data on elemental Fe⁰ iron in the public domain, the potential for long term toxicity and carcinogenesis - following exposure to elemental iron - has been extrapolated from the approved EU PPP active substance assessments of FeSO₄ and FePO₄ (EFSA conclusions 2012 and 2015, respectively); both of which - in comparison to elemental iron - are more bioavailable forms of iron and are the forms systemically available following exposure to elemental iron. The WHO (WHO/JECFA, 1983⁶) proposed a provisional maximum tolerable daily intake for a 60 kg human of **0.8 mg/kg bw/day**, based on observations that healthy individuals have taken dietary supplements of **50 mg Fe/day (ferrous iron)** for long periods and that females can - during pregnancy and lactation - meet requirements for iron supplementation with dosages of 30 – 60 mg/day. This therapeutic dose has formed the basis for the EU long-term dietary risk assessments of FeSO₄ and FePO₄, as approved pesticidal active substances, however details of the WHO database are unavailable to HSE. In relation to the potential local effects of iron particles in the lungs following inhalation exposure, a NOAEC of 5 mg/m³ was identified in rats exposed to elemental (carbonyl) iron dust for 4 weeks (see short-term toxicity section).

Disorders of iron metabolism – as is seen in hereditary haemochromatosis whereby patients have an excessive gastrointestinal absorption of dietary iron – is directed towards the liver, therefore hepatotoxicity is the most common finding in those affected. Several mechanisms plausibly link iron overload to hepatic toxicity and hepatocellular carcinoma – an excessive accumulation of iron in the liver leads to the generation of ROS from 'free' iron, resulting in tissue damage and modification of proteins and DNA; the generation of ROS depletes the antioxidant status and suppresses host immune defences, thus inducing chronic diseases such as fibrogenesis

⁶ Evaluation of certain food additives and contaminants (27th report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.

and cirrhosis; iron may also promote the proliferation of tumour cells by increasing the activity of iron-containing enzymes and proteins, which catalyse cellular metabolism thereby accelerating growth. There has been no evidence to suggest that these are likely adverse health outcomes following exposure to elemental iron under normal intestinal function, nor even in subjects heterozygous for the genetic condition. Considering the extensive human database available on iron intake, and the recent work by EFSA on establishing dietary reference values for iron (2015), it is notable that no reference values for iron have been derived from identifiable adverse health outcomes. Overall, this is due to a variety of uncertainties - inaccurate estimates of iron intake, iron status, impact of iron deficiency, confounding by dietary and lifestyle factors, concurrent infection and inflammation – all of which prevent the determination of dose–response relationships and the assessment of risks associated with iron excess.

There is no convincing evidence of either chronic toxicity or carcinogenicity from the long-term use of elemental iron in the epidemiological data provided by the applicant. Elemental iron is very poorly absorbed, therefore with limited systemic exposure from either dietary or non-dietary routes, chronic iron overload is an unlikely outcome of the approval of elemental iron as a molluscicide plant protection product.

B.6.6. REPRODUCTIVE TOXICITY

No regulatory reproductive or developmental toxicity studies performed with elemental iron have been submitted by the applicant, nor have any relevant publications been identified by the applicant's literature search. In the absence of substance-specific data on ferric phosphate, the conclusions on this endpoint have been extrapolated from the EU assessment of the more soluble FeSO₄.

Due to the inefficient and variable absorption of iron from GIT, rather than a risk of excess iron intake, pregnant women are more susceptible to iron deficiency and related anaemia. Iron plays a fundamental role in all cellular functions critical to the reproduction and development of healthy offspring. As well as increased iron requirements before and during pregnancy –vital for oxygen transport, electron transport chain, DNA synthesis, xenobiotic metabolism – adaptive physiological processes take place, leading to an increased efficiency of iron absorption. However, for some women, dietary iron intake is insufficient to meet the exponentially increased demand of the developing foetus. To meet a total iron demand of approximately 1000 mg during pregnancy (the vast majority of which is destined for the foetus), iron supplementation of 50 mg ferrous iron/day is recommended for all women as standard practice (EFSA 2015^b). The WHO report (WHO/JECFA, 1983) cites the results of studies on the influence of iron and its compounds on reproduction, which show that no maternal toxicity or developmental effects were observed for doses up to 60 mg/d in pregnant women.

Based on the information presented and noting that elemental iron (along with the read-across compound FeSO₄) is one of the substances approved for use as a dietary supplement, it can be concluded that further vertebrate testing for this endpoint is not warranted.

B.6.7. NEUROTOXICITY

Elemental iron has been used as a food additive for several decades in the EU. No regulatory neurotoxicity studies performed with elemental iron have been submitted in support of its use as a plant protection product. The applicant has identified a single epidemiological research article from their literature search, which concluded that total iron intake (dietary and supplements) was not associated with increased risk of Parkinson's disease (Logroschino *et al.*, 2008; CA 5.9.4/02). The SACN 2010 report on "Iron and health" concludes that there is no convincing evidence that dietary iron is associated with any neurodegenerative disease.

Elemental iron has been approved for use as a food additive for several years in the EU, with no known neurotoxic effects, and the US FDA have placed elemental iron on the list of substances "generally known as safe". Elemental iron is insoluble in aqueous and organic solvents and the EU assessments of the FeSO₄ and FePO₄ - both of which are significantly more bioavailable than elemental iron - did not require neurotoxicity studies. In the presence of sufficient information in the public domain which indicates no known potential for neurotoxicity resulting from exposure to elemental iron, no specific neurotoxicity regulatory studies are required to support the approval of elemental iron as a pesticide active substance.

B.6.7.1. Neurotoxicity studies in rodents

No studies are required.

B.6.7.2. Delayed polyneuropathy studies

No studies are required.

B.6.8. OTHER TOXICOLOGICAL STUDIES**B.6.8.1. Toxicity studies on metabolites and relevant impurities**

No residual metabolites resulting from the use of elemental iron as a molluscicide, are expected from either dietary or non-dietary routes. The expected oxidation from the zerovalent state to the ferrous and ferric states is addressed by read-across to the existing EU assessments of FeSO₄ (EFSA conclusion 2012) and FePO₄, respectively (EFSA conclusion 2015).

For the assessment of relevant impurities, please refer to Volume 4 (confidential information).

B.6.8.2. Supplementary studies on the active substance - Immunotoxicity

No supplementary studies on the active substance have been submitted. The available evidence in the public domain points to the fundamental role of iron for normal development of the immune system, and no indication of iron overload in a normal human population, leading to immunotoxic effects, has been found in the public domain. No specific immunotoxic events have been identified in patients suffering from clinical conditions of iron overload, nor in individuals taking dietary iron supplements (SACN, 2010). Elemental iron is very poorly absorbed, therefore with limited systemic exposure from either dietary or non-dietary routes, chronic iron overload is a negligible outcome of the authorisation of elemental iron as a molluscicidal plant protection product. In conclusion, no specific vertebrate testing for immunotoxicity is required to support the current evaluation.

B.6.8.3. Studies on endocrine disruption

No specific studies investigating endocrine disruption have been submitted. A large database of human data is available on iron, including its use as a dietary supplement, and no relevant papers presenting positive indications of endocrine disruption were identified from the applicant's literature review. The lack of endocrine disrupting properties of elemental iron in mammals is supported by human data. In accordance with Reg. (EU) No 609/2013, elemental iron is approved for use in baby food for infants and young children, processed cereal-based foods and food for children, food for special medical purposes, and total diet replacement. In addition, the US FDA positively assessed elemental iron placing it on the list of substances generally recognised as safe (GRAS).

Although substantial and prolonged iron overload, as seen for example in patients suffering haemochromatosis or thalassaemia, can lead to damage of the pancreas resulting in Type II diabetes, this is a non-specific, secondary consequence of the general toxicity of iron overload mediated by oxidative damage to cells. The damage leads to architectural and hence functional damage to pancreatic endocrine cells. Therefore, organ damage following iron overload is not viewed as specific endocrine-mediated adverse event.

Considering the low toxicity in the available database, its poor solubility and its use as a human food supplement, it is reasonable to conclude that elemental iron does not pose a hazard to the endocrine system. An assessment in line with the ECHA/EFSA (2018) guidance is not considered necessary and no further data are required.

B.6.9. MEDICAL DATA AND INFORMATION**B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies**

According to the data requirements (EU) 283/2013, reports of occupational health surveillance programs and monitoring studies shall be submitted. Such reports should include number of persons, nature of exposure, including data relevant to the known mode of action.

The elemental iron source for which approval is being sought, is currently manufactured as [REDACTED] iron powder, from [REDACTED]. HSE UK notes that no medical reports supporting this statement have been submitted by the applicant. Instead, an overarching statement from the production site in [REDACTED], states that elemental iron has been produced there since 2005, with an annual tonnage of approximately 1633 metric tonnes over the last 3 years. On average, 25 workers per year have been involved with synthesis of this source of elemental iron. Exposure of workers in its production involves incidental skin contact during milling, packaging and transport. The workers do not undergo regular general medical examination by medical personnel, other than annual pulmonary function testing, respirator fit testing and hearing testing.

The medical monitoring programme appears to be a general health check-up, and this is considered acceptable, since there is no consistent disease pattern - known or suspected - with exposure to elemental iron powder.

B.6.9.2. Data collected on humans

According to the data requirements Reg. (EU) 283/2013, where available, reports of studies conducted in humans shall be submitted. No new human data, specifically generated with elemental iron, has been submitted by the applicant. Any epidemiological or observational information on humans has been presented elsewhere in this document. The applicant's literature search did not identify any major adverse effects.

B.6.9.3. Direct observation

According to the data requirements Reg. (EU) 283/2013, available reports from the open literature relating to clinical cases and poisoning incidents shall be submitted. A single case study from the published literature has been submitted by the applicant to address this data point.

Reference	CA 5.9.7/01: Tam, Y., Chan, Y.C. and Lau, F. L. (2008)	
Article title	A case series of accidental ingestion of hand warmer	
Journal title	Clinical Toxicology 46, 900-904	
Test substance	Elemental iron (unknown form)	
Guideline	None	GLP: No
Acceptability	<i>Considered acceptable</i>	

Four cases were reported in which elderly subjects ingested the contents of hand-warmers containing iron powder (respective doses were in general unascertained, though may be up to 60g) resulted in either in no effects or mild and transient effects (for example, temporary elevated serum iron levels). The lack of significant toxicity is consistent with the current knowledge on elemental iron.

B.6.9.4. Epidemiological studies

According to the data requirements Reg. (EU) 283/2013, any relevant epidemiological studies shall be submitted. The information submitted by the applicant has been presented in several places elsewhere in this document, to inform on the relevant endpoint. In addition to the published information submitted by the applicant, HSE is aware of a comprehensive review of the role of iron in human nutrition "Iron and Health", published by the UK Scientific Advisory Committee on Nutrition (SACN, 2010). See Section B.6.10 for a description of the methods employed in the literature review.

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

No specific human symptoms diagnostic of iron toxicity are known for adults, but iron poisoning is a serious life-threatening condition and a significant cause of morbidity and mortality in young children, associated with accidental excessive consumption of iron supplements containing soluble iron salts such as ferrous sulphate. Fatalities in children have occurred following ingestions of the equivalent of 1200 to 4500 mg of elemental iron from soluble iron salts. The applicant has identified no published reports of serious or fatal poisoning from the ingestion of carbonyl elemental iron products. The toxicity (limited to iron salts) is due to free radical formation, when the normal protective transport and storage mechanisms, binding to transferrin and ferritin, are

overwhelmed by high doses which cause localised damage to the gastrointestinal mucosa, leading to unregulated absorption. The clinical manifestations and time course of iron poisoning are shown in the table below.

Clinical Stages of iron poisoning:

Stage	Symptoms	Time from ingestion
1	Vomiting, diarrhoea, gastrointestinal blood loss due to ROS formation and damage to GIT mucosa	0-6 hours
2	Transient resolution of gastrointestinal symptoms in some cases. Progressive organ cellular damage continues,	12-24 hours
3	Recurrence of gastrointestinal symptoms as iron suppresses the coagulation cascade, metabolic acidosis due to excessive systemic ferric iron, shock, acute respiratory syndrome	24-48 hours
4	Hepatotoxicity due to the hepatocytes high metabolic activity predisposing the liver to free radical and subsequent ROS damage	48+ hours
5	Vomiting, gastric outlet obstruction due to significant intestinal mucosal scar tissue formation.	2-4 weeks

From Madiwale and Liebelt (2006)

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

According to the data requirements Reg. (EU) 283/2013, first aid measures to be used in the event of poisoning shall be provided. The applicant has submitted a SDS for elemental iron, in which a symptomatic treatment is recommended. Supplementary to the SDS, the following first aid measures and medical treatment for more serious is stated:

In more serious cases of acute (oral route) intoxication, the following is recommended:

- Emesis induction and/or lavage with isotonic NaHCO₃ solution.
- Insert in stomach (via lavage tube), 5-10 g of desferroxamine in 50 to 100 mL water.
- In case of an intake larger than 60 mg Fe/kg body weight, or when symptoms of iron poisoning are present, desferroxamine (1-2 g in 10 to 20 ml of sterile water) should be given intramuscularly. Desferroxamine given perorally complexes with iron ions and prevents absorption from the gut. One gram of desferroxamine binds 85 mg of iron. Parenterally administered desferroxamine markedly increases urinary excretion of iron

B.6.10. REFERENCES RELIED ON

LITERATURE SEARCH

A literature review on elemental iron was performed by the applicant in accordance with the EFSA guidance (EFSA Journal 2011;9(2):2092). The applicant conducted a single literature review encompassing all areas of the assessment: Toxicology; Ecotoxicology; Metabolism; Residues and Environmental Fate and Behaviour (dated . The search was designed to capture any data published between January 2006 and November 2016. A top-up search was conducted to cover the period from November 2016 to December 2017 (dated February 2018). In response to a request from HSE to include the search term “elemental iron”, the applicant conducted a further literature search (dated November 2018) and this resulted in 2 literature reviews reports being generated in support of the main dossier. Appropriate search terms were used and appropriate databases were searched. All the hits identified by the search were assessed manually for relevance. Relevant publications and publications of unclear relevance were fully considered, evaluated for reliability and submitted to the HSE. In relation to toxicology, 15 relevant publications from the literature search were included in the doc CA B6

The table below shows the databases used by the applicant to perform the search.

Table B.6.10-1: Bibliographic databases included in the applicant's literature searches

Database	Applicant's Justification	Subject coverage
AGRICOLA	Consists of worldwide literature citations for journal articles, monographs, proceedings, theses, patents, translations, audio-visual materials, computer software, and technical reports pertaining to all aspects of agriculture and related fields.	<ul style="list-style-type: none"> -Agriculture (general) - Agriculture (products, engineering, information systems) - Animal sciences -Biotechnology -Botany - Chemical conservation - Cytology - Agricultural economics, energy, entomology, and history - Farm management - Feed science -Fertilisers - Fibre and textiles - Food and nutrition - Forestry - Horticulture - Human ecology - Human nutrition - Hydrology - Microbiology - Natural History - Natural resources - Pesticides - Physiology - Plant sciences - Pollution -Public health - Rural sociology - Soil sciences - Veterinary medicine - Water quality - Weather and climate - Wildlife - Zoology
Analytical Abstracts	Covers all aspects of analytical chemistry in a wide variety of areas including general applications, biochemistry and clinical chemistry, industrial and applied science, environmental science, agriculture and food, pharmaceuticals and instrumentation.	<ul style="list-style-type: none"> - General - Inorganic - Organic - Industrial - Biochemical - Pharmaceutical - Food - Agricultural and environmental - Computer handling of analytical data - Instrumentation
BIOSIS ® Toxicology	Subset of BIOSIS ® Previews with a focus on toxicology and related topics. Records are drawn from journal articles, conference papers, monographs and book chapters, notes letters, and reports, as well as original research. U.S. patent records are also included.	<ul style="list-style-type: none"> -Agriculture - Bacteriology -Biochemistry - Biophysics - Biotechnology -Botany - Cell biology - Clinical medicine - Drugs - Environmental biology - Environmental science

Database	Applicant's Justification	Subject coverage
		<ul style="list-style-type: none"> - Experimental medicine - Genetics - Immunology - Microbiology - Nutrition - Occupation health - Parasitology - Pathology - Pharmacology - Physiology -Public health - Radiation Biology - Systematic biology - Veterinary science - Virology
CAB Abstracts	Coverage of worldwide literature on agriculture and allied fields, including veterinary medicine, human nutrition, horticulture, forestry, leisure, recreation, recreation and tourism, crop science, crop protection, breeding and genetics, animal production, animal nutrition, parasitology, soils, land use, agricultural engineering, agricultural economics, and biotechnology. Publication types are journals, monographic series, theses, technical reports, conferences, selected patents, books and annual reports.	<ul style="list-style-type: none"> -Agricultural biotechnology - Agricultural economics and rural sociology - Agricultural engineering - Animal health and veterinary medicine - Animal production and genetics -Biodeterioration and biodegradation - Crop production - Crop protection - Dairy science - Environmental degradation, conservative and amelioration - Forestry - Genetic resources - Horticulture - Human nutrition and diet-related disorders - Human parasitic diseases - Leisure, recreation and tourism - Plant breeding and genetics - Postharvest science - Rural development - Soil science - Sugar industry
Embase ®	Bibliographic coverage of literature on drugs and pharmacology and all other aspects of human medicine and related discipline. Embase is a key resource for biomedical evidence, from published, peer-reviewed literature, in-press publications and conference abstracts.	<ul style="list-style-type: none"> - Drug research - Pharmacology - Pharmacoeconomics - Pharmaceuticals - Toxicology - Human medicine - Basic biological research - Health policy and management - Public, occupational and environmental health - Substance dependence and abuse - Psychiatry - Forensic science - Biomedical engineering and instrumentation - Medical devices
Environment Abstracts	Encompasses all aspects of the impact of people and technology on the environment and the effectiveness of remedial policies	<ul style="list-style-type: none"> -Agriculture - Air pollution -Control technologies

Database	Applicant's Justification	Subject coverage
	and technologies. The database covers journals, conference papers and proceedings, special reports from international agencies, non-governmental organisations, universities, associations and private corporations. Other materials selectively indexed include significant monographs, government studies and newsletters.	<ul style="list-style-type: none"> -Endangered species - Energy - Environmental design - Environmental education - Environmental law and policy - Environmental safety - Geophysical and climate science - Global warming - International environmental policy - Land use and pollution - Marine pollution - Noise pollution - Population - Population studies - Radiological contamination - Resource management - Solid and toxic waste - Sustainable development - Toxicological effects - Transportation - waste management - Water pollution - Wildlife/ biodiversity
Medline ®	US National Library of Medicine premier bibliographic database. It contains references to journal articles in life sciences with a concentration on biomedicine and health. This is broadly defined to encompass those areas of the life sciences, behavioural sciences, chemical sciences, and bioengineering need by health professionals and other engaged in basic research and clinical are, public health, health policy development, or related educational activities. Medline also covers life science vital to biomedical practitioners, researchers, and educators, including aspects of biology, environmental science, marine biology, plant and animal science as well as biophysics and chemistry.	<ul style="list-style-type: none"> - Clinic and preclinical medicine - Dentistry - Nursing - Population and reproductive biology - Pharmacology and pharmaceutics - Psychiatry and psychology - Environmental, public and occupational health - Veterinary medicine - Nutrition - Pathology - Anatomy and physiology - Toxicology - Genetics - Microbiology - Pathology - Biomedical technology - Health planning and administration - Space life science
Toxfile ®	Covers the toxicological, pharmacological, biochemical and physiological effects of drugs, pesticides and other chemicals. Typical areas of coverage include drug reactions, chemically induced diseases, carcinogenesis, mutagenesis, teratogenesis, environmental pollution, waste disposal, radiation, and food contamination.	<ul style="list-style-type: none"> - Adverse drug reaction - Air pollution - Animal venom - Antidotes - Carcinogenesis via chemicals - Chemically induced diseases - Drug evaluation - Environmental pollution - Food contamination - Metagenesis - Occupation - Pesticides - Radiation - Teratogenesis - Toxicology

Database	Applicant's Justification	Subject coverage
		- Waste disposal
Toxicology Abstracts	Covers issues from social poisons and substance abuse to natural toxins, from legislation and recommended standards to environmental issues.	<ul style="list-style-type: none"> - Pharmaceuticals - Food, additives and contaminants - Agrochemicals - Cosmetics, toiletries and household products - Industrial chemicals - Metals - Toxins and other natural substances - Social poisons and drug abuse - Polycyclic hydrocarbons - Nitrosamines and related compounds - Radiation and radioactive materials - Methodology - Legislation and recommended standards
TOXLINE ®	Toxicology reference database that provides bibliographic information for journal articles on the effects of drugs and other chemicals.	<ul style="list-style-type: none"> - Biochemistry - Pharmacology - Physiology - Toxicology
TRACE	Includes information from peer-reviewed toxicology and nutrition journals as well as secondary sources and websites.	- Chemical toxicology

Search strategy and terms

The applicant implemented a single concept search strategy, using the CAS number (7439-89-6) for iron, its synonyms and name fragments, along with the form-specific term “powder”. This approach captured all data for all subject areas in a single search. This is acceptable according to the EFSA 2011 guidance for submission of scientific peer-reviewed open literature.

Search terms used for the single concept search strategy for elemental iron

Subject areas	Search terms
Toxicology, metabolism, residues, environmental fate and behaviour, ecotoxicology.	(“7439-89-6” or “iron” or “Ancor B” or “Ancor en 80/150” or “Armco iron” or “Atomel 28” or “Atomel 300M200” or “Atomel 500M” or “Atomel 95” or “Atomiron 44MR” or “Atomiron 5M” or “Atomiron AFP 25” or “Atomiron AFP 5” or “ATW 230” or “ATW 432” or “carbonyl iron” or “DSP 1000” or “DSP 1288” or “DSP 135” or “DSP 135C” or “DSP 138” or “EF 1000” or “EF 250” or “EFV 200/300” or “EFV 250” or “EFV 250/400” or “EO 5A” or “Ferronyl” or “Ferrous iron” or “Ferrovac E” or “Ferrum” or “GS 6” or “ XXXXXXXXXX EH” or “NC 100” or “PZh-1M3” or “PZh-2” or “PZh1M1” or “PZh2M” or “PZh2M1” or “PZh2M2” or “PZh3” or “PZh3M” or “PZh4M” or “PZhO” or “Remko” or “SUY-B 2” or “E1UOL152H7”) AND (powder)

During the course of the evaluation, HSE considered that the specific term for the active substance i.e. “elemental iron” should be included in the search strategy for completeness. Furthermore, HSE considered that as pure iron (Fe) is highly likely to form ionic compounds in the presence of water and oxygen under environmental conditions, this should be taken into consideration in the search terms selected. The following question was asked:

Please include the search terms “ferric” and “elemental iron” in the search strategy.

Specific points to address in reply to HSE CRD request: It is not considered appropriate to exclude all forms of iron other than pure iron powder from the search. As you have noted in the dossier, elemental iron (Fe) is highly likely to oxidise to ferric iron (Fe 3+) in the natural environment and oxides or hydroxides under mammalian physiological conditions. Therefore, the HSE evaluator considers the term “ferric” should also be included in the search strategy. Please also include the specific name of the active substance for which approval is being sought (elemental iron).

Applicant’s response:

“Preliminary searches on the term “elemental iron” for the date range specified in the existing Literature Review Report (LRR) (excluding references already identified by the searches described in the LRR) indicate that slightly more than 500 references would require a rapid filter for potential relevance.

However, similar searches using the term “ferric” returns nearly 80,000 references requiring rapid filter. This would be a substantial undertaking requiring several months to consider the titles only and produce a “short-list” of potentially relevant references. (Please note that this is a preliminary stage, and further work would subsequently be required to evaluate the “short-list”, on the basis of abstract and/or full text, for relevance and reliability)

It may be worth considering at this stage that “ferric” is not a particularly useful search term, in that it will not necessarily identify references relevant to iron in its 3+ oxidation state. For example, ferric oxide would (arguably) be more commonly referred to as “iron oxide”; ferric hydroxide as “iron hydroxide”; therefore, searches for “ferric” would not necessarily identify all the publications that use those terms to identify the test substance. To avoid this pitfall, we would recommend a different approach – this is, the identification of specific compounds and/or physical forms of interest, for which the searches to be undertaken would include CAS Registry Numbers.

We would be pleased to discuss this with you and hear your opinion about the above.”

HSE accepts that including the references for ‘ferric’ would be cumbersome and not materially add to the assessment. HSE has requested that the additional 500 references identified in a preliminary search for the specific term ‘elemental iron’ are considered in the literature review for completeness. The results of the search process for the specific term ‘elemental iron’ are included in Table B.6.10-2.

Initial rapid assessment

The applicant carried out an initial rapid assessment of relevance, using the titles of publications alone. Anything considered to have potential relevance (including tenuous, fleeting or even undecipherable relevance) to the fields of environmental fate, toxicology, ecotoxicology, metabolism or residues, were not rejected at this stage. Abstracts were not used during the rapid assessment.

“Toxicity” (in the context of the rapid assessment) was interpreted to encompass ALL biological effects of relevance to human health risk assessment. This, by default, includes all *in vivo* mammalian toxicology; it also includes *in vitro* studies conducted using mammalian cells, *in vitro* genotoxicity/mutagenicity studies conducted in any cells (including bacteria/yeast) and *in vitro* and *in vivo* studies that could, in any way, be construed as relevant to mammalian endocrine disruption. [There is currently no expert group consensus for defining endocrine disruption, and so potentially-relevant studies could include, for example, oestrogen/androgen receptor-binding/competitive receptor-binding/agonist/antagonist studies, endocrine-relevant gene expression studies, relevant receptor transactivation assays and cell proliferation assays in any potentially-relevant cell type (including, but not limited to, mammalian cells); potentially-relevant studies would also include certain assays in, for example, zebrafish/goldfish/amphibians/etc.]

Criteria for relevance in the selection of toxicology and metabolism literature:

Of those publications deemed to be of potential relevance, the following criteria were applied:

- Appropriate test substance (excluding salts and other iron compounds⁵); in contrast to the parent LRR on iron powder, in this instance the specific physical form of powder for the test substance was not a primary criterion for evaluation of potential relevance, and
- Appropriate test species for mammalian toxicological risk assessment (i.e. mammalian species for all endpoints except genotoxicity and endocrine disruption), and
- Relevant route of administration (i.e. oral, dermal or inhalation for all endpoints except genotoxicity and endocrine disruption), and
- Information relating to human or animal health, toxicology or metabolism (excluding nutrient efficacy/benefit and some metabolic studies⁶, unless the title and/or available preview suggested that adverse effects may also be under assessment; also excluding studies where deficiency was induced in order to evaluate effects of then administering sufficient dietary iron)
- OR epidemiological studies associating health effects with iron exposure were considered potentially relevant due to their possible implications if assessed as part of a weight-of-evidence evaluation, in that the information presented may be directly applicable to elemental iron⁷. Estimates of dietary intakes, occupational exposures or exposures via the iron content of atmospheric pollutants – although inherently imprecise – would all be considered to be sufficient surrogate exposure indicators in these cases.

Criteria for reliability

The applicant has stated that the reliability assessment for all relevant publications was done according to the criteria outlined by Klimisch *et al.* (Klimisch HJ, Andreae E and Tillmann U (1997). A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory Toxicology and Pharmacology 25) and HSE considers this to be a widely accepted scoring system. A Klimisch score of at least 2 (reliable with restriction) was required for inclusion in the applicant's dossier.

The number of records retrieved for each stage of the process are shown below for the main review (2006 to 2016), the top-up review (2016 to 2017) and the supplemental search term “elemental iron” (Nov 2019; the search method used was identical to that described above for the main literature review). Following a full text relevance assessment, HSE has considered 18 of the publications in the toxicology and metabolism assessment. The results of all three literature review searches are tabulated below.

Stage of study selection process	Number of summary records retrieved and date performed		
	Nov 2016	Dec 2017	Nov 2019
	1/1/2006 – 11/2016	28/11/2016 – 18/12/2017	‘elemental iron’
Total number of summary records retrieved:	11,002	2145	710
Number of summary records excluded from the search results after rapid assessment for relevance:	10,609	2061	431
Total number of summary records remaining after filtering out those of clear irrelevance and manual de-duplication:	393	84	279

Total number of full-text documents assessed in detail:	38	6	37
Number of studies excluded from further consideration after detailed assessment for relevance and/or reliability:	24	6	31
Number of studies not excluded for relevance or reliability after detailed assessment:	14	0	5*
Number of studies not excluded for relevance or reliability after detailed assessment, that were relevant to the Toxicology assessment:	10	0	5
<i>Number of relevant and reliable publications identified by the applicant's literature review which are included in the HSE CRD evaluation of elemental iron</i>	<i>18**</i>	<i>0</i>	<i>0</i>

* Applicant's text: "This figure is not "6", as may be expected from taking into account [of] the two rows directly above. This is because a single study not "excluded" after detailed assessment (Yameen *et al.*, 2013), was nevertheless eventually awarded an evaluation indicating that it did not achieve a suitable standard of reliability ("Klimisch score 4 - not assignable")."

** Discrepancy from the applicant's Doc MCA literature review and the original literature search performed by the bibliographic consultancy.

During the evaluation, HSE has excluded a small number of publications from the DAR on the basis that they:

- are secondary, supportive literature linking to primary literature which was already discussed under the pertinent data point
- do not describe toxicity or physiological parameters which can be considered toxicologically relevant
- focus on the therapeutic use of iron as a food supplement.

Reference	Author	Year	Title Test facility Report No. GLP [Y/N] Published [Y/N]	Vertebrate Study [Y/N]	Data Protection claimed [Y/N]	Justification if data protection is claimed	Owner	Previous evaluation
General Intro.	Brady N.C.	1974	The Nature and Properties of Soils, 8th Ed., Macmillan Publishing, NY GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
General Intro.	Elinder C.G.	1986	Handbook on the toxicology of metals, Friberg et al. (Eds), Elsevier GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.

Reference	Author	Year	Title Test facility Report No. GLP [Y/N] Published [Y/N]	Vertebrate Study [Y/N]	Data Protection claimed [Y/N]	Justification if data protection is claimed	Owner	Previous evaluation
General Intro.	Hedberg R. <i>et al.</i>	2010a	Particles, Sweat, and Tears: A comparative Study on bioaccessibility of ferrochromium alloy and stainless steel particles, the Pure metals and their metal oxides, in simulated skin and eye contact GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
General Intro.	Stefaniak, A.B. <i>et al.</i>	2014	Dissolution of the metal sensitizers Ni, Be, Cr in artificial sweat to improve estimates of dermal bioaccessibility. GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
CA 5.1.1/01	Hurrell, R F	1999	The mineral fortification of foods, Chapter 3: Iron GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
CA 5.1.1/02	Hurrell, R F	2002	Fortification: Overcoming technical and practical barriers GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
CA 5.1.1/03	Swain, J H <i>et al.</i>	2003	Bioavailability of elemental iron powders to rats is less than bakery grade ferrous sulfate and predicted by iron solubility and particle surface area GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.
CA 5.1.1/04	Hoppe, M <i>et al.</i>	2006	The relative bioavailability in humans of elemental iron powders for use in food fortification Dept. of Clinical Nutrition, Institute of Internal Medicine, Göteborg University, Sweden n/a GLP: No Published: Yes	Y (human)	N	-	Pub. Lit.	N.A.
CA 5.1.1/05	Swain, J H <i>et al.</i>	2006	An irradiated electrolytic iron fortificant is poorly absorbed by humans and is less responsive than FeSO ₄ to the enhancing effect of ascorbic acid US Dept of Agriculture, Grand Forks, ND, USA n/a GLP: No Published: Yes	Y (human)	N	-	Pub. Lit.	N.A.

Reference	Author	Year	Title Test facility Report No. GLP [Y/N] Published [Y/N]	Vertebrate Study [Y/N]	Data Protection claimed [Y/N]	Justification if data protection is claimed	Owner	Previous evaluation
CA 5.2.1/01	Whittaker, P <i>et al.</i>	2002	Acute toxicity of carbonyl iron and sodium iron EDTA compared with ferrous sulfate in young rats Center for Food Safety and Applied Nutrition, FDA, USA n/a GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.
CA 5.2.3/01	██████	2018	Elemental iron powder: Acute inhalation toxicity in rats Product Safety Labs, USA 47151 GLP: Yes Published: No	Y	Y	Article 59(1) & (2) of Regulation (EC) 1107/2009 applies	Adama	N.A.
CA 5.2.3/02	Sayes, C M <i>et al.</i>	2007	Assessing toxicity of fine and nanoparticles: Comparing <i>in vitro</i> measurements to <i>in vivo</i> pulmonary toxicity profiles DuPont Haskell Laboratory for Health and Environmental Sciences, USA n/a GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.
CA 5.2.3/04	Warheit, D B <i>et al.</i>	2007a	Pulmonary toxicity screening studies in male rats with M5 respirable fibers and particulates DuPont Haskell Laboratory for Health and Environmental Sciences, USA n/a GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.
CA 5.2.3/05	Warheit, D B <i>et al.</i>	2007b	Pulmonary bioassay studies with nanoscale and fine-quartz particles in rats: toxicity is not dependent upon particle size but on surface characteristics DuPont Haskell Laboratory for Health and Environmental Sciences, USA n/a GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.
CA 5.2.3/06	Kiranmai, G and Reddy, A R N	2012	Antioxidant status in MgO nanoparticle-exposed rats Dept of Pharmacology, Vaageswari College of Pharmacy, Karimnagar, India n/a GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.

Reference	Author	Year	Title Test facility Report No. GLP [Y/N] Published [Y/N]	Vertebrate Study [Y/N]	Data Protection claimed [Y/N]	Justification if data protection is claimed	Owner	Previous evaluation
CA 5.3.1/01	Akhtar, S, <i>et al.</i>	2010	Bioavailability of Iron and Zinc Fortified Whole Wheat Flour in Rats. Department of Food and Horticultural Sciences, University College of Agriculture, Bahauddin Zakariya University, Multan, Pakistan (SA) n/a GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.
CA 5.3.1/03	Warheit, D B, <i>et al.</i>	1997	Inhalation of high concentrations of low toxicity dusts in rats results in impaired pulmonary clearance mechanisms and persistent inflammation Central Research and Development, DuPont Haskell Laboratory, USA n/a GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.
CA 5.3.2/01	Whittaker, P <i>et al.</i>	1996	Histopathological evaluation of liver, pancreas, spleen, and heart from iron-overloaded Sprague- Dawley rats Center for Food Safety and Applied Nutrition, FDA, Washington, USA n/a GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.
CA 5.3.2/02	Zhu, Q <i>et al.</i>	2016	Effects of carbonyl iron powder on iron deficiency anemia and its subchronic toxicity Dept of Food Science and Technology, East China University of Science and Technology, Shanghai n/a GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.
CA 5.3.2/03	Akhtar, S <i>et al.</i>	2011a	Effect of mineral fortification on plasma biochemical profile in rats Dept of Food Science and Technology, Bahauddin Zakariya University, Pakistan n/a GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.

Reference	Author	Year	Title Test facility Report No. GLP [Y/N] Published [Y/N]	Vertebrate Study [Y/N]	Data Protection claimed [Y/N]	Justification if data protection is claimed	Owner	Previous evaluation
CA 5.3.2/04	Akhtar, S <i>et al.</i>	2011 b	Effect of zinc and iron fortification of the feed on liver and thyroid function in rats Dept of Food Science and Technology, Bahauaddin Zakariya University, Pakistan n/a GLP: No Published: Yes	Y	N	-	Pub. Lit.	N.A.
CA 5.3.2/05	Domitrovic R <i>et al</i>	2008	Differential effect of high dietary iron on α -tocopherol and retinol levels in the liver and serum of mice fed olive oil- and corn oil – enriched diets. University of Rijeka, Croatia n/a GLP: No Published: Yes	Y	N		Pub. Lit.	N.A.
CA 5.4.1/01	Hedberg, Y <i>et al.</i>	2010 b	Bioaccessibility, bioavailability and toxicity of commercially relevant iron- and chromium-based particles: <i>in vitro</i> studies with an inhalation perspective Division of Surface and Corrosion Science, Royal Institute of Technology, Sweden n/a GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
CA 5.4	Winterbourn	1995	Toxicity of iron and hydrogen peroxide: the Fenton reaction	N	N	-	Pub. Lit.	N.A.
CA 5.5/01	Benhar, M <i>et al.</i>	2002	ROS, stress-activated kinases and stress signaling in cancer Dept of Biological Chemistry, The Hebrew University of Jerusalem, Israel EMBO Reports 3(5) GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
CA 5.5/02	Toyokuni, S	2002	Iron and carcinogenesis: from Fenton reaction to target genes Dept of Pathology and Biology of Diseases, Kyoto University, Japan n/a GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.

Reference	Author	Year	Title Test facility Report No. GLP [Y/N] Published [Y/N]	Vertebrate Study [Y/N]	Data Protection claimed [Y/N]	Justification if data protection is claimed	Owner	Previous evaluation
CA 5.5/03	Huang, X	2003	Iron overload and its association with cancer risk in humans: evidence for iron as a carcinogenic metal Dept of Environmental Medicine, NYU School of Medicine, NY, USA n/a GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
CA 5.5/04	Galaris, D & Pantopoulos, K	2008	Oxidative stress and iron homeostasis: Mechanistic and health aspects Laboratory of Biological Chemistry, University of Ioannina, Greece n/a GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
CA 5.9.1/01	[REDACTED]	2017	Statement on medical supervision of [REDACTED] [REDACTED] [REDACTED] [REDACTED] n/a GLP: No Published: No	N	N	-	Adama	N.A.
CA 5.9.2/01	Gordeuk, V R <i>et al.</i>	1986	Carbonyl iron therapy for iron deficient anemia Dept of Medicine, Cleveland Metropolitan General Hospital, Cleveland, USA n/a GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
CA 5.9.4/01	Choi, J-Y <i>et al.</i>	2008	Iron intake, oxidative stress-related genes (MnSOD and MPO) and prostate cancer risk in CARET cohort Dept of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, USA n/a GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
CA 5.9.4/02	Logroscino G <i>et al.</i>	2008	Dietary iron intake and risk of Parkinson's disease Dept of Neurology and Psychiatry, University of Bari, Italy n/a GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.

Reference	Author	Year	Title Test facility Report No. GLP [Y/N] Published [Y/N]	Vertebrate Study [Y/N]	Data Protection claimed [Y/N]	Justification if data protection is claimed	Owner	Previous evaluation
CA 5.9.4/03	Polesel, J <i>et al.</i>	2007	Nutrients intake and the risk of hepatocellular carcinoma in Italy Unit of Epidemiology and Biostatistics, Istituto Nazionale Tumori, Aviano, Italy	N	N	-	Pub. Lit.	N.A.
CA 5.9.4/04	Fonseca-Nunes, A <i>et al.</i>	2014	Iron and cancer risk – A systematic review and meta-analysis of the epidemiological evidence Unit of Nutrition, Environment and Cancer, L'Hospitalet de Llobregat, Barcelona, Spain n/a GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.
CA 5.9.5	Madiwale, T and Liebelt, E	2006	Iron: not a benign therapeutic drug. Current opinions in paediatrics 18:174-179	N	N	-	Pub. Lit.	N.A.
CA 5.9.7/01	Tam, A Y B <i>et al.</i>	2008	A case series of accidental ingestion of hand warmer Accident and Emergency Dept, United Christian Hospital, Hong Kong, China n/a GLP: No Published: Yes	N	N	-	Pub. Lit.	N.A.