European Food Safety Authority

EFSA Journal 2010; 8(4):1585

SCIENTIFIC OPINION

Scientific Opinion on the safety of heme iron (blood peptonates) for the proposed uses as a source of iron added for nutritional purposes to foods for the general population, including food supplements[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion evaluating the safety of heme iron (blood peptonates) when added for nutritional purposes as a source of iron to food for the general population, including food supplements, and evaluating the bioavailability of iron from this source. The Panel concluded that iron from heme iron (blood peptonates) is bioavailable and absorbed to a significantly higher extent than iron from non-heme sources. No data on the toxicity of heme iron (blood peptonates) were provided by the petitioner except those from an acute toxicity study. The Panel is aware of the fact that heme iron is a constituent of the normal human diet and also an endogenous body constituent. However, given i) that the use levels of heme iron (blood peptonates) proposed by the petitioner result in exposure to iron at levels that are higher than the guidance value of 17 mg/day for supplemental intake of non-heme iron proposed by the EVM although they are in line with the Provisional Maximum Tolerable Daily Intake (PMTDI) value for iron of 0.8 mg/kg bw/day (50 mg/day for a 60 kg person) proposed by JECFA, ii) that the bioavailability of iron from heme iron as compared to iron from non-heme iron sources is significantly increased, iii) that epidemiological and animal model studies suggest that a high intake of heme iron may be associated with an increased risk of colon cancer, iv) that there are no genotoxicity data on heme iron (blood peptonates) but positive results reported for hemoglobin and hemin in a Comet assay in cells *in vitro*, and v) that there are no data from subchronic, reproductive, developmental, long-term toxicity and carcinogenicity studies on heme iron (blood peptonates), the Panel concludes that the available data are insufficient to demonstrate the safety of the proposed use and use levels of heme iron (blood peptonates) as a source of iron for nutritional purposes in foods intended for the general population, including food supplements.

KEY WORDS

Food supplements, iron, heme iron (blood peptonates).

1 On request from the European Commission, Question No EFSA-Q-2009-00375, adopted on 14 April 2010.

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Suggested citation: EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the safety of heme iron (blood peptonates) for the proposed uses as a source of iron added for nutritional purposes to foods for the general population, including food supplements. EFSA Journal 2010; 8(4):1585. [31 pp.]. doi:10.2903/j.efsa.2010.1585. Available online: www.efsa.europa.eu

SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to evaluate the safety of heme iron (blood peptonates) when added for nutritional purposes as a source of iron to food for the general population, including food supplements, and the bioavailability of iron from this source.

The present opinion deals only with the safety and bioavailability of a particular source of iron. The safety of iron, in terms of amounts that may be consumed, is outside the remit of this Panel.

The chemical form of iron is a main factor affecting its bioavailability. The Panel concluded that iron from heme iron (blood peptonates) is bioavailable and absorbed to a significantly higher extent than iron from non-heme sources. The bioavailability of iron from heme iron sources may be 2- to 7-fold higher than that of iron from non-heme sources.

The Panel is aware of the fact that heme iron is a constituent of the normal human diet and also an endogenous body constituent.

The petitioner indicates that hemoglobin is used worldwide as a food ingredient without any restrictions on its use or application and that the heme iron (blood peptonates) preparation of the present opinion has a substantial equivalence with porcine hemoglobin or with the heme iron present in red meat or liver.

The petitioner justifies the absence of toxicological data on the basis that the product is substantial equivalent to porcine haemoglobin, and/or the heme iron present in red meat or liver, which are part of the regular human diet.

Thus, no data on the toxicity of heme iron (blood peptonates) were provided by the petitioner except those from an acute toxicity study. This study revealed that the acute toxicity of heme iron (blood peptonates) in rats was low, with an LD_{50} greater than 2500 mg product/kg of bw, but the Panel notes that this study is not suitable to evaluate the safety in use of heme iron (blood peptonates) as a source of iron for the general population including food supplements.

The Panel noted that one study using the Comet assay reported that hemoglobin and hemin induced DNA damage in cells *in vitro*, and that epidemiological and animal model studies suggest that a high intake of heme iron, present in red meat, may be associated with an increased risk of colon cancer.

The recommended daily dose of heme iron (blood peptonates) proposed by the petitioner amounts to 2 g heme iron (blood peptonates) which is equivalent to 20 mg of elemental iron. The petitioner also indicated that they consider the maximum recommended daily intake to be 4.5-5 g heme iron (blood peptonates) which would be equivalent to 45-50 mg of elemental iron if it behaved like inorganic iron.

The Panel noted that these use levels of heme iron (blood peptonates) proposed by the petitioner result in exposure to elemental iron at levels that are higher than the guidance value of 17 mg/day for supplemental intake of non-heme iron proposed by the EVM, although they are in line with the Provisional Maximum Tolerable Daily Intake (PMTDI) value for iron of 0.8 mg/kg bw/day (50 mg/day for a 60 kg person) proposed by JECFA.

Overall, given:

i) that the use levels of heme iron (blood peptonates) proposed by the petitioner result in exposure to elemental iron at levels that are higher than the guidance value of 17 mg/day for supplemental intake of non-heme iron proposed by the EVM, although they are in line with the Provisional Maximum Tolerable Daily Intake (PMTDI) value for iron of 0.8 mg/kg bw/day (50 mg/day for a 60 kg person) proposed by JECFA,

ii) the significantly increased bioavailability of iron from heme iron as compared to iron from non-heme iron sources,

iii) that epidemiological and animal model studies suggest that a high intake of heme iron may be associated with an increased risk of colon cancer,

iv) the absence of genotoxicity data on heme iron (blood peptonates) but the positive results reported for hemoglobin and hemin in a Comet assay in cells *in vitro*,

v) the absence of subchronic, reproductive, developmental, long-term toxicity and carcinogenicity data on heme iron (blood peptonates),

the Panel concludes that the available data are insufficient to demonstrate the safety of the proposed use and use levels of heme iron (blood peptonates) as a source of iron for nutritional purposes in foods intended for the general population, including food supplements.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients.

The Commission has received a request for the evaluation of heme iron (blood peptonates) added for nutritional purposes to food for the general population, including food supplements. The relevant Community legislative measures are:

- Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements[4].
- Regulation (EC) 1925/2006 on the addition of vitamins and mineral and of certain other substances to foods[2].

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion, based on its consideration of the safety and bioavailability of heme iron (blood peptonates) as a source of iron added for nutritional purposes to food for the general population, including food supplements.

[4] OJ L 183, 12.7.2002, p.51
[2] OJ L 183, 30.12.2006, p.26

ASSESSMENT

1. Introduction

The present opinion deals only with the safety and bioavailability of a particular source of iron, intended to be used for nutritional purposes in foods intended for the general population, including food supplements. The safety of iron itself in terms of amounts that may be consumed is outside the remit of this Panel.

Heme iron (blood peptonates) is obtained by enzymatic hydrolysis of pig hemoglobin.

2. Technical data

2.1. Identity of the substance

The petitioner indicated that heme iron (blood peptonates) can be defined as a nutrient with a high iron content, which is reported to be 1%, while that of pig hemoglobin is reported to be 0.27-0.29%. The petitioner also indicated that the product is substantially equivalent to porcine hemoglobin or the heme iron present in red meat or liver, as in all cases the iron present in these products corresponds to heme iron with similar characteristics as the product. The petitioner indicates that the product can be defined as a ferrous peptonate.

A CAS Registry Number and a chemical name according to IUPAC (International Union of Pure and Applied Chemistry) nomenclature rules for heme iron (blood peptonates) are not available.

2.2. Specifications

The final product is described as a fine microgranulate dark-coloured/black powder with a characteristic odour and taste (meaty flavor).

The protein content is $\geq$ 70%, the total iron content is approximately 1%, the heme iron content is 96% of the total iron haematin quantification. The water content is $\leq$ 8%, ash $\leq$ 10%, solubility < 50%, pH (10%) 7-9, density 0.33-0.38 g/cc, crude fat < 0.3% and crude fibre < 1%.

The concentrate retains the porphyrin ring bound to iron in its reduced form.

Microbiological characteristics and an amino acid profile were also provided by the petitioner.

2.3. Manufacturing process

The petitioner indicated that the product is obtained by enzymatic hydrolysis of pig hemoglobin. After enzymatic hydrolysis of hemoglobin, the hydrolysed globin is removed, leaving a concentrate of the hemoglobin's porphyrin complex, which contains the heme group. This concentrate retains globin residues and the porphyrin ring bound to iron in reduced form with an approximate iron content of 1%.

The petitioner indicated that the blood is obtained solely from pigs slaughtered at authorized Spanish abattoirs and that consequently, the product is a 100% porcine product.

The petitioner also provided data on the average, standard deviation and standard error for different parameters that determine the end-product's quality: these data revealed that there is little variation between production batches.

2.4. Methods of analysis in food

The petitioner indicated that total iron is analysed by atomic absorption (AOAC 995.13/968.08 method), and that the heme iron content can be analysed by an adaption of the Haematin quantification method (Hornsey, 1956).

2.5. Stability, reaction and fate in food

The petitioner indicated that when stored in its original pack (heat-sealed aluminium bag containing 1 kg of product) in a cool, dry place, the product remains stable for at least two years after its production date. Being a powdered product, with very low water content, it is not affected by bacterial or fungal growth during storage.

The heme iron is stable at high processing temperatures, without a reduction in the amount of hematin detected when treated at 240 ºC for 10 minutes.

The petitioner stated that due to its iron content (approximately 1%), care should be taken when adding the product to food with a high fat content, to prevent fat oxidation and rancidity. When the product was added to chocolate filling with a total fat content of 10.2 and 6.3%, respectively, the products were not seen to become rancid when stored for 1 year in their original packs under correct temperature (i.e. room temperature, 20-22 ºC) and humidity conditions (dry).

The petitioner indicated that there are numerous articles in the literature that describe the administration of hemoglobin or heme iron concentrates both alone (in capsules or tablets) or combined with various types of food (baby food in jars, biscuits, mixed with cereals, pâtés or meat products), without detecting any incompatibility of the product with the various foods it was used to fortify (Eskeland *et al.*, 1997; Fernández *et al.*, 2000; Hertrampf *et al.*, 1990; Martínez *et al.*, 2000; Pallarés *et al.*, 1996; Salinas *et al.*, 1998).

The petitioner also stated that there is positive experience with the addition of the product both to biscuit filling (Quintero, 2003; Quintero *et al.*, 2008) and to sausages (internal tests).

Due to the product's deep black colour, one expected effect of its use in food supplements or enriched food will be a darkening of the end food product's colour, depending on the type of food it is added to and the concentration used.

2.6. Case of need and proposed uses

The product is intended to be marketed as a heme iron concentrate in microgranulate powder to be used directly in the form of capsule or pills with or without adequate excipients.

The product can be also included directly in the formulation of different food products to be enriched with heme iron, such as chocolate fillings for pastry products, jams, baby food in jars, meat products such as pâtés, sweets, dairy desserts, bread-making products, drinks, etc.

The petitioner indicated that the recommended daily dose amounts to 2 g heme iron (blood peptonates), which is equivalent to 20 mg of elemental iron if it behaved like inorganic iron.

The petitioner also indicated that they consider the maximum recommended daily intake to be 4.5-5 g heme iron (blood peptonates), which would be equivalent to 45-50 mg of elemental iron if it behaved like inorganic iron.

Proposed use levels in food were not provided by the petitioner.

The petitioner argued that the inclusion of heme iron (blood peptonates) is particularly indicated for those population groups vulnerable to iron deficiency or which have higher iron requirements. It can also be given to population groups with a low meat protein intake or in whom the presence of iron absorption inhibitors in the diet may increase the risk of iron deficiencies (fibre- or phytate-rich diets).

2.7. Information on existing authorisations and evaluations

Iron

For iron, the Scientific Committee on Food (SCF) recommended daily intakes of 6 mg and 4 mg for infants aged 0.5-1 year and 1-3 years, respectively, assuming 15% absorption of the daily intake. For adults, assuming 10% absorption, the recommended dietary iron intake has been estimated to be between 8 and 10 mg iron/day in adult males, and 15 to 20 mg iron/day in adult females of reproductive age (SCF, 1993; IOM, 2001; D-A-CH, 2000).

Regarding upper limits, EFSA's Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA) concluded in 2004 that the available data were insufficient to establish a Tolerable Upper Intake Level (UL) for iron. The opinion states that adverse gastrointestinal effects (e.g. nausea, epigastric discomfort, constipation) have been reported after short-term oral dosage of 50-60 mg daily of supplemental non-heme iron preparations, particularly if taken without food. It was also concluded that an acute oral dose of 60 mg/kg bw can be lethal, but that lower oral doses of about 10-20 mg iron/kg bw do not cause acute systemic toxicity (EFSA, 2004). The US Food and Nutrition Board (FNB) established a UL of 45 mg iron/day for individuals aged 14 years and older, and 40 mg iron/day for younger age groups (IOM, 2001).

The Expert Group on Vitamins and Minerals (EVM) concluded that there were insufficient appropriate data to establish a Safe Upper Level for iron (EVM, 2003). It was also stated that for guidance purposes only, a supplemental intake of approximately 17 mg/day (equivalent to 0.28 mg/kg bw/day for a 60 kg adult) would not be expected to produce adverse effects in the majority of people.

JECFA (1983) established a Provisional Maximum Tolerable Daily Intake (PMTDI) for iron of 0.8 mg/kg bw/day based on the safe long-term intake of ferrous supplements of 50 mg/day (for a 60 kg individual). The following iron compounds were evaluated by JECFA as food additives and/or nutrient sources: iron oxides, ferrous gluconate, ferrous sulphate, ferrous glycinate, ferric ammonium citrate, sodium iron (III) ethylenediaminetetraacetate and FAP (JECFA, 1983).

Heme iron (blood peptonates)

In EFSA's opinion on the maximum tolerable levels of iron intake published in 2004, it was stated that heme iron absorption was not regulated by other substances in the diet and absorption was higher than that for inorganic iron, being regulated only by the levels of iron already present in the body.

The petitioner indicated that hemoglobin is used worldwide as a food ingredient without any restrictions on its use or application and that the petitioning company and other European and international companies have been marketing hemoglobin-derived products for different uses in

nutrition (mainly for meat products, etc.) for more than 30 years. The European Directive 77/99/EEC[5], which established the sanitary requirements for meat products, already included in January 1977 dried or salted animal blood and animal plasma within the definition of other animal origin products. The European Directive 92/5/EEC[6], published in 1992, also acknowledged animal blood as being included in other animal origin products.

Subsequently, Regulation (EC) 853/2004[7] which establishes specific hygiene requirements for animal origin food, includes blood in the definition of meat (definition 1.1 in Appendix I of this regulation). Furthermore, the definition of offal (definition 1.11 in the same Appendix) also includes blood. The use of hemoglobin derivates in the European market, including in blood sausage or black puddings, is traditional across Europe.

The use of a heme iron concentrate has been customary since the publication in 1981 (Vilhelm, 1981) describing the enzymatic hydrolysis of pig hemoglobin by means of proteases (subtilisins) obtained from *Bacillus subtilis*.

According to the information available, the Japanese government has approved the use of a heme iron concentrate, known as Heme Iron Polypeptide or HIP, which is obtained by enzymatic hydrolysis and subsequent concentration from pig hemoglobin (a similar process to that described in this document) and marketed as a FOSHU food. FOSHU is the Japanese term for a functional food or a food with a specific health application. It is recognised in this FOSHU designation that heme iron is a highly bioavailable source of iron in human nutrition. The HIP product is currently being marketed in the United States.

2.8. Exposure

Exposure estimates were not provided by the petitioner.

The petitioner proposes a recommended daily dose amounting to 2 g heme iron (blood peptonates), equivalent to 20 mg of elemental iron if it behaved like inorganic iron. This would amount to 0.33 mg/kg bw/day for a 60 kg person. The petitioner also indicated that they consider the maximum recommended daily intake to be 4.5-5 g heme iron (blood peptonates), which would be equivalent to 45-50 mg of elemental iron if it behaved like inorganic iron, amounting to 0.75 to 0.83 mg/kg bw/day for a 60 kg person.

Given an iron content of 1% in the heme iron (blood peptonates), the guidance value set by the EVM (2003) for supplemental intake of approximately 17 mg iron/day (equivalent to 0.28 mg/kg bw/day for a 60 kg adult) would necessitate an intake of 1.7 gram/day of heme iron (blood peptonates) equal to 28 mg heme iron (blood peptonates)/kg bw/day for a 60 kg person.

According to the SCF the average and 97.5th percentile iron intakes from food in European countries vary from 10 to 17 mg/day and 17 to 29 mg/day, respectively. Including the intake from food supplements high percentile values are in the range from 27 to 72 mg/day (SCF, 2003).

Since use levels for uses in food were not provided by the petitioner an exposure estimate for intake resulting from proposed uses and use levels in food cannot be made.

[5] Council Directive 77/99/EEC of 21 December 1976 on health problems affecting intra-Community trade in meat products. OJ L 26 31.1.1977, p. 85–100.
[66] Council Directive 92/5/EEC of 10 February 1992 amending and updating Directive 77/99/EEC on health problems affecting intra-Community trade in meat products and amending Directive 64/433/EEC. OJ L 57, 2.3.1992, p. 1–26.
[7] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p. 55–205.

3. Biological and toxicological data

3.1. Bioavailability

Absorption of non-heme iron (present mainly in plant-based foods and food supplements) is determined mainly by its luminal solubility, which diminishes as the pH of the gastric content approaches neutral. During digestion, the ferric complexes are reduced to the ferrous form, which binds to soluble low molecular weight complexes. Hydrochloric acid and organic acids contained in foods, such as lactic, ascorbic and citric acid, some sugars such as fructose and sorbitol, amino acids such as cysteine, lysine and histidine, help to stabilise iron in its soluble and more absorbable ferrous form. However, other compounds contained in the diet, such as carbonates, oxalates, phytates, phosphates, tannins, polyphenols, some proteins such as albumin and proteases, egg yolk, some inorganic nutrients (calcium, manganese, copper, cadmium and cobalt) and fibre (although it has modest inhibitory capacity) make the absorption of iron more difficult (Brune, 1992; Craig, 1994; Claydesdale, 1983; Layrisse, 2000).

In a review of the uses of hemoglobin in nutrition (Wismer-Pedersen, 1983) the author highlighted the advantages of using the heme group in meat products as a way of supplementing these foods with a highly bioavailable source of iron.

Heme iron (derived mainly from hemoglobin and myoglobin in animal tissues and also present at high concentration in the liver) is an important dietary source of iron because it is absorbed more efficiently than non-heme iron and also because it enhances the absorption of the latter.

Absorption of heme iron takes place by a different process from non-heme iron; however, regulation of the quantity absorbed does not differ between the two forms of iron and, apparently, depends on the body's iron requirements (Hallberg, 1997).

Heme iron enters the mucosa through a different route from the absorption of non-heme iron and, therefore, it is not regulated by the Divalent Metal Transporter (DMT-1); instead, regulation involves interaction of the iron in the porphyrin complex with an iron receptor (Tenhunen *et al.*, 1980; Roberts *et al.*, 1993). Heme iron absorption is less regulated than non-heme iron absorption (Finch, 1994) and ranges between 15% in individuals with full iron reserves and 35% in individuals with iron depletion. The increased heme iron absorption in iron deficiency conditions is due in part to iron's greater affinity for membrane microvilli (Roberts *et al.*, 1993) and in part to the induction of the enzyme hemooxygenase in the membrane, which breaks the porphyrin ring and releases the iron for transport into the cells. Iron absorption is independent of other compounds in the diet, although high concentrations of luminal calcium reduce absorption (Hallberg *et al.*, 1992; EFSA, 2004).

Hallberg *et al.* (1991, 1992) studied the effect of calcium on heme iron absorption and observed a significant reduction in absorption when high calcium doses were used (300-600 mg calcium), suggesting that calcium interferes with the iron carrier in the cell membrane which, as is known, is common to both heme iron and non-heme iron absorption.

Heme iron is more easily absorbed than inorganic iron from the diet and absorption takes place by different routes: proteolytic digestion of hemoglobin and myoglobin causes release of the heme group, which is kept in soluble form by globin degradation products, which makes it available for absorption. The iron enters the enterocyte as an intact metalloporphyrin, probably by means of an endocytic vesicular system or through the action of an integrin-like binding protein; the iron is then released within the cell as inorganic iron (Fe^{2+}) through the action of the enzyme hemooxygenase. It appears that from that point onwards, the path followed by heme iron is similar to that for inorganic iron (Conrad, 1967; Wheby, 1970; Uzel, 1998).

In 1966, Conrad *et al.* indicated that the degradation products of hydrolysed hemoglobin improved iron absorption. However, solutions containing bicarbonate reduced heme iron absorption.

Conrad *et al.* (1967) indicated that the iron contained in hemoglobin was selectively absorbed in greater quantities than non-heme iron and stated that the porphyrin ring contained in the heme group was absorbed intact into the cells of the duodenal mucosa and that it was inside the cells where the iron transported to the plasma was subsequently released. They also indicated that the greatest degree of iron absorption took place when the porphyrin ring was accompanied by peptone residues from globin degradation and the iron was not in a purified or chemically prepared form. This means that the globin peptone residues bound to the porphyrin ring played an important role in iron absorption by the mucosal cells.

Recently, Latunde-Dada *et al.* (2006) indicated in their review of the absorption of heme iron that the Heme Carrier Protein 1 (HCP-1) has been recently cloned and characterised as the carrier in the apical region of the duodenum responsible for the absorption of heme iron into intestinal cells. Its expression seems to be regulated pre- and post-transcriptionally in hypoxic and iron-deficient rats, respectively. Its identification has revealed the extensively studied mechanism by which heme iron is absorbed from the diet into the intestine.

3.1.1. Animal data

Wheby *et al.* (1970) showed that absorption of the heme iron contained in hemoglobin was increased in iron-deficient rats compared with rats with high iron levels, thereby showing that, as happens with the absorption of inorganic iron, the absorption of heme iron is regulated by the individual iron reserves and that intercellular iron transport is probably similar for heme iron and inorganic iron and, therefore, it is the regulation point of iron absorption for both sources.

Pallarés *et al.* (1996) and Lisbona *et al.* (1999) administered respectively a cereal diet supplemented with elemental iron (ferric citrate) for 7 days or a mixture containing elemental iron and heme iron (as hemoglobin) in a 80/20 proportion providing a total iron content of 35 mg/kg of diet for 10 days to normal or anemic rats. They observed that in the rats receiving the diet supplemented with heme iron, supplementation did not interfere with the absorption of other minerals such as calcium, phosphorus, and magnesium, which did happen when only non-heme iron (i.e. ferric citrate) was used for supplementation.

Vaghefi *et al.* (2000, 2002) showed that when pig hemoglobin was hydrolysed using a procedure very similar to that used to prepare the heme iron (blood peptonates) preparation of the present opinion, the increase in iron absorption (analysed using an *in vitro* system (chamber model) in rats), was significantly higher than that from whole hemoglobin. Iron absorption depended on the degree of hydrolysis and the enzyme used and was higher when the enzymatic hydrolysis was performed with subtilisin (alcalase) than when it was performed with pepsin. They observed a higher iron absorption when the degree of hydrolysis attained values greater than 10% (as is the case with heme iron (blood peptonates)) compared with the absorption of intact, unhydrolysed hemoglobin. Their results highlighted the importance of globin peptide residues around the heme group for increasing the absorption of this form of iron.

Vaghefi *et al.* (2005) confirmed higher absorption of heme iron in rats compared with a hydrolysate obtained by pepsin and also showed that the iron concentration had no effect on its absorption.

Heme iron extracted from pig hemoglobin, with an iron content of 1%, was included in the filling (chocolate flavour) of biscuits without observing any palatability problems. These heme iron-enriched biscuits were used in a study with piglets (as animal model) in which a mild anaemia had been induced. The piglets were divided into three groups which were given ferrous sulphate, ferrous lactate and heme iron (biscuits with the heme iron-supplemented filling), respectively. The quantity of iron supplemented was similar in all three groups and the supplements were given for 12 weeks; blood samples were obtained every two weeks throughout the 12 week period. At the end of the iron supplementation period, it was observed that the animals that had consumed heme iron weighed more

and had grown more than the animals belonging to the other two study groups (50 kg increase for the animals that consumed heme iron compared with 43 and 37 kg for the animals that consumed ferrous lactate and ferrous sulphate, respectively). Although at the end of the iron supplementation period, all of the animals, irrespective of the type of supplement, recovered normal hemoglobin values, the animals that had consumed heme iron had the highest levels of body iron (hemoglobin, liver reserves and circulating iron). The bioavailability of the heme iron in the biscuit filling was 23% greater than that observed for ferrous sulphate and 13% greater than that recorded for ferrous lactate (Quintero *et al.*, 2008).

In a subsequent study performed in Mexico with growing sows as animal model for iron absorption, the diets of 10-week-old sows with a starting weight of 14 kg were supplemented with a chocolate-flavoured biscuit filling containing 16% heme iron (blood peptonates) (equivalent to 1176 mg iron /kg of filling) compared with another group supplemented with a similar biscuit filling containing 20% ferrous sulphate (equivalent to 1640 mg iron/kg of filling). Iron supplementation was performed for 16 weeks, administering 70 g of both fillings until the sow weighed 50 kg, and then 210 g and 151 g of filling containing heme iron and ferrous sulphate, respectively, to provide 247 mg iron/day until the end of supplementation (100 kg live weight). The blood parameters were monitored at the start and end of supplementation and it was observed that the increases in hemoglobin, haematocrit, red blood cells and serum iron were statistically greater in the group supplemented with heme iron than in the group that received ferrous sulphate. The bioavailability of the heme iron included in the biscuit filling as heme iron (blood peptonates) was 32% greater than that observed for ferrous sulphate, again confirming the results obtained in the first study described above.

3.1.2. Human data

In a study performed with healthy volunteers, Björn-Rasmussen *et al.* (1974) showed that absorption of the heme iron present in a complete diet was significantly (5- to 7-fold) higher than that of non-heme iron, with average absorption of non-heme iron being 5.3% of total intake compared with 37.3% for heme iron.

Reizenstein (1980) also stated that heme iron absorption was 2-3.6 times greater for all types of meals and food combinations compared with the absorption of the ferrous (non-heme) ion.

Martínez-Torres *et al.* (1981) observed that cysteine improved absorption of non-heme iron in humans when it was given during meals. However, although it increased the absorption of heme iron, this increase was much less than the increase observed for non-heme iron, indicating that the absorption of heme iron is less affected by other substances present in the diet.

In a study performed in humans, Bezwoda *et al.* (1983) observed that the absorption of non-heme iron was enhanced by the presence of heme iron in the diet, while the absorption of heme iron remained stable for all meals, irrespective of the food eaten and the concentration of heme iron.

Monsen (1988) stated that the absorption of heme iron present in the diet ranged between 15 and 35%, depending on individual iron reserves, while the absorption of non-heme iron was less, between 2 and 20% and depended not only on individual iron reserves but also on the presence or absence of promoters or inhibitors in the diet. Likewise, Bothwell *et al.* (1989) stated that the absorption of non-heme iron is highly influenced by the presence of promoter or inhibitor substances in the diet.

Studies performed in Cuba to assess heme iron-enriched food in human nutrition showed that in the group that consumed the iron-enriched food (8 mg/day, of which 75% heme iron), the percentage of individuals with heme deficiency (hemoglobin < 12 g/dl) at the start and at the end of the study (6 months) showed a very significant reduction in all the risk groups studied, with a 2.5 to 3-times lower incidence of anaemia in children aged 1-4 years and women aged 15-59 years, respectively (Fernández, 1991).

In another study performed in Cuba, on pregnant women aged 20-30 years, Fernández (1991) identified pregnant participants with anaemia (hemoglobin < 11 g/dl) at the start of heme iron supplementation. Two study groups were formed, composed of pregnant women with and without anaemia at baseline. One of the groups was given food enriched with ferrous fumarate from the time of recruitment (before the 18th week of pregnancy) to birth. The other group received food enriched with heme iron. In the pregnant women with anaemia at baseline, supplementation with heme iron progressively diminished the anaemia until the time of delivery, which did not happen in the pregnant women with anaemia who ate food supplemented with ferrous fumarate (Fernández, 1991).

Olivares *et al.* (1990) observed that biscuits enriched with 6% bovine hemoglobin were associated with a good iron absorption (19.7%). Also, in a study with 215 schoolchildren, it was observed that the hemoglobin-enriched biscuit was readily accepted. Although no differences were observed in hemoglobin content between the control (non-enriched) and enriched groups, as in both cases the children were in a good state of health, the enriched group had higher iron reserves, as measured by the serum ferritin level. This same research group (Hertrampf *et al.*, 1990) also observed that when 4-month old babies were given a cereal formula enriched with bovine hemoglobin, the number of babies with iron-deficiency anaemia at 12 months of age was significantly reduced.

However, Martínez *et al.* (1998) did not observe any differences in iron absorption from baby food contained in jars and enriched with heme iron in the form of hemoglobin, compared with the same product enriched with ferrous sulphate.

Walter *et al.* reported in 1993 on the results of a study performed for three years on approximately 1000 Chilean schoolchildren who were given biscuits enriched with bovine hemoglobin, compared with a non-supplemented control group. The results clearly showed significant differences in hemoglobin and serum ferritin levels between the two groups, in favour of the hemoglobin supplemented group, even though the study subjects were not anaemic at the starting time of supplementation.

Ekman and Reizenstein (1993) compared absorption of heme iron in the form of hemoglobin and of inorganic iron in the form of ferrous sulfate in healthy and anaemic pregnant women, and observed that heme iron absorption was significantly higher for both groups (16.1 and 22.0%, respectively) than absorption of inorganic iron (4.6 and 9.4%, respectively).

In a double-blind study, Frykman *et al.* (1994) gave 49 healthy volunteers a supplement containing a mixture of heme iron obtained from pig hemoglobin containing 1.2 mg of iron and 8 mg ferrous fumarate, and compared results with a control group (45 healthy subjects) who were given a supplement containing 60 mg of iron as ferrous fumarate only. After two months of supplementation, the serum ferritin and hemoglobin levels were similar in both supplemented groups, showing that less iron as heme iron was required to maintain blood iron levels.

Hurrell (1997) reviewed the prevention of iron deficiency through food fortification and, in his table of the relative bioavailability of different organic and inorganic iron compounds for humans, he highlighted that hemoglobin-derived iron can be up to 7-fold more bio-available than ferrous sulphate. Hurrell (1997) also stated that the absorption of heme iron was not affected by inhibitor substances in the diet but it was affected by the individual's iron reserves.

Eskeland *et al.* (1997) performed a double-blind study on pregnant women who were supplemented during the second half of pregnancy with a mixture containing heme and non-heme iron (27 mg iron in total), compared with a product containing an equal quantity of iron in purely non-heme form and vitamin C, and a non-supplemented placebo group. The results indicated that the percentage of women who were anaemic after giving birth was 25% for both supplemented groups, while it was 52% for the placebo group. Better values in the iron parameters were observed in the group supplemented with the heme iron mixture, where the percentage of women with low post-partum iron reserves (8%), was less than in the other group supplemented with non-heme iron (27%) or the placebo group (52%).

Salinas-Piélago *et al.* (1998) reported on the study performed on 53 children who received heme iron-supplemented biscuits for 6-8 weeks, determining the children's intellectual status by means of various intelligence and concentration tests and compared the results with 55 children in the non-enriched control group. Compared to the non-supplemented group, the results indicated a significant improvement in the children in the group supplemented with heme iron as regards the intelligence and concentration tests performed before and after supplementation. Salinas-Piélago *et al.* (1998) concluded that fortification with heme iron could improve school performance in children from families with limited financial resources.

Martínez *et al.* (1999) also stated that heme iron absorption is less affected by substances in the diet than non-heme iron, indicating that heme iron absorption is determined mainly by the body iron levels and, to a very minor extent, by dietary factors, with two exceptions: meat increases heme iron absorption and calcium inhibits it, although this influence is several orders of magnitude higher for non-heme iron.

In a study in healthy volunteers (n=57), Roughead and Hunt (2000) observed that the absorption of non-heme iron changed in response to supplementation with ferrous sulphate (50 mg iron/day) for 12 weeks. Thus, absorption of this non-heme iron diminished during the supplementation period, while high body iron reserves (serum ferritin) were maintained. However, the absorption of heme iron remained stable during the entire supplementation period.

In the course of treating Cuban children aged 6-36 months with iron-deficiency anaemia and intolerance for iron salt supplements, Fernández *et al.* (2000) observed a recovery of hemoglobin values in 86% of the cases when 14 children were treated with a product based on heme iron and small quantities of inorganic iron (8 mg total iron/kg/day).

Seligman *et al.* (2000) reported the results of a clinical study performed using a product obtained from the enzymatic hydrolysis of hemoglobin, and a product with similar properties to those indicated for heme iron (blood peptonates) in this dossier, called HIP (Heme Iron Polypetide). HIP was obtained from pig hemoglobin digested with proteolytic enzymes, which produced a highly soluble heme iron concentrate with small globin polypeptide chains and an iron content in excess of 1%. In their study on 14 healthy subjects, they determined iron absorption during a meal and three and six hours after taking a supplement containing 20 mg of HIP, and iron absorption after taking a supplement containing the same quantity of ferrous fumarate. Results were compared with a placebo group that had received 20 mg of glucose. It was observed that iron absorption in the HIP-supplemented group was at least 2 mg, while iron absorption in the ferrous fumarate group was less than 1 mg.

In the review of iron absorption in humans by Conrad and Umbreit in 2002, they stated that in countries with high meat consumption, heme iron made up one third of the iron in the diet, but accounted for two thirds of the iron absorbed by the body. This was stated to be due to the higher iron absorption from heme iron, since heme iron is soluble at the pH in the small intestine and absorption by the enterocytes is not affected adversely by components in the diet, as occurs with inorganic iron. In this review, Conrad and Umbreit also stated that absorption of heme iron by the enterocytes was not adversely affected by dietary components, as was the case for inorganic iron. Likewise, they confirmed that the body's iron reserves were balanced via transfer receptors in the absorptive cells' basolateral membrane.

Pizarro *et al.* (2003) showed in 27 healthy women of childbearing age (aged 28-50 years) that heme iron absorption from hemoglobin was saturable, with a daily maximum amount absorbed of 2 mg of iron. They suggested that the saturability of heme iron absorption may be a protective factor to avoid iron overload when iron intake is provided primarily by consumption of meats or blood.

Swain *et al.* (2004) reported data on the bioavailability of an iron concentrate similar to the heme iron (blood peptonates) of the present opinion, which was obtained from bovine blood. In their study, 52 premenopausal women with moderate body iron levels received either 5 mg of iron as heme iron, taking two capsules per day, or 50 mg of electrolytic iron, reduced iron or iron in the form of ferrous

sulphate in pastry products. The supplements were administered for 12 weeks. They observed changes in body iron reserves by analysing the serum transferrin/serum ferritin receptor ratio, which is the most sensitive detection method of iron reserves. Body iron (mg/kg bw) increased with all four sources of iron analysed when compared with a placebo group that received no iron supplements. The results indicated that the electrolytic and reduced iron were 50 and 85% as effective as iron sulphate and that just 5 mg of iron as heme iron was half as effective as 50 mg of iron in the form of iron sulphate.

The petitioner also provided results of a study performed last year in the Mexican State of Morelos. The study included female teenagers (n=112) aged 12-15 years that were enrolled in telesecondary education. After obtaining the parents' or guardians' informed approval, the girls were distributed equally into three groups. The first group (n=35), called Placebo, received no iron supplements; the girls with the highest hemoglobin values at the start of the study were included in this group. These girls received non-iron supplemented biscuits or Suavicremas (Gaufrette style cookies). The second group (n=40) received biscuits or Suavicremas supplemented with heme iron, and the third group (n=37) received biscuits or Suavicremas supplemented with iron sulphate.

It was only possible to give iron supplements during 7 weeks, instead of the 16 weeks initially planned. The study had a double-blind design; neither the person distributing the biscuit nor the teachers knew to which treatment was each girl allocated. The quantity of iron provided with the cookies was intended to provide 50% of the Recommended Daily Allowance (RDA) for women aged 14-18 years, which is 15 mg iron/day, according to the National Academy of Sciences (NAS, 2001).

The interim results of the study, recently completed, show that the girls included in the study, with an average age of 14.5 years and an average weight of 52.1 kg, had an average hemoglobin level of 14.1 g/dl and an average haematocrit of 36.6%, which indicated that the girls were within suitable reference ranges for their age and therefore were non-anaemic. Of the teenagers 65% had a suitable nutritional status; the other teenagers were distributed in equal proportions between overweight (19%) and obese (16%).

The girls included in the two iron-supplemented groups received a total of 380 mg of iron supplements during the period. This is equivalent to approximately 7.7 mg of iron/day, which is 50% of the recommended daily intake of iron requirements for this population group.

The results of the baseline and final hemoglobin concentration, depending on the type of biscuit eaten, indicated a decrease in the teenagers in the control group, although, even so, this group still had the highest average. Hemoglobin levels increased in the other two groups and the differences were statistically significant.

The study interim results indicated that supplementation with heme iron-enriched biscuits was readily accepted by the girls, who could not distinguish by taste between the iron-supplemented biscuits and the unsupplemented biscuits. The supplemented biscuits were consumed for only seven weeks, which should be taken into account when assessing the results. In any case, in spite of this short supplementing period, the iron levels improved significantly in both supplemented groups, providing evidence of the heme iron bioavailability. The petitioner stated that this fact is very interesting as it must be remembered that the teenagers included in the study had iron levels within the accepted normal range for this age group. Since iron absorption, as already reported, depends on the body's iron requirements, a significant increase in iron absorption is not to be expected when there are normal iron levels in the body.

Both the control group and the group that ate biscuits enriched with ferrous sulphate showed a decrease in ferritin concentration, with the difference being statistically significant in the treated group, that is, the decrease in ferritin could be real in the teenagers that ate biscuits enriched with ferrous sulfate. The group that ate biscuits enriched with heme iron showed a steady value for this parameter. This could be interpreted as the existence of higher iron reserves in the group supplemented with heme iron.

It was also observed that some parameters were increased in the heme and ferrous sulphate groups, such as hematocrit or serum iron, however, these differences were not statistically significant. Statistically significant differences were found for MCH (Mean Corpuscular Hemoglobin) and MCHC (Mean Corpuscular Hemoglobin Concentration) between the two groups. The results of these experiments are presently being adapted for its publication in a peer review scientific journal

The petitioner also indicated that at present, a joint study with the Food Hygiene Department at the Autonomous University of Barcelona and the Paediatrics Department at the Hospital General de Catalunya is being performed. In this study, children aged 3-6 years with recurrent upper airways infections, recurrent otitis media or even frequent diarrhea, not associable with any specific disease and with little appetite, are undergoing a nutritional study in which a placebo group given the usual treatment by the hospital Paediatrics Department is compared with another two study groups that receive bakery products (biscuits, Suavicremas or chocolate bars) enriched with heme iron (blood peptonates) or ferrous sulphate.

As inclusion criterion, the study accepted children with hemoglobin levels below 12 mg/dl. The supplements are given for 12 weeks and it is intended to supplement the diet daily with at least 60% of the RDA. In addition to obtaining general data concerning age, weight, medical history, etc, blood samples are obtained from the children at the start and end of the supplementation period; these are analysed to determine their iron profile and immune status from the white blood cell index. A nutritional study of the child's weekly diet is also performed using a diary and approximate nutritional measures. So far, data on the first 15 children, seven from the group supplemented with ferrous sulphate, three from the group supplemented with heme iron and five from the unsupplemented placebo group are available. In both supplemented groups, hemoglobin levels have increased to above 12 g/dl. This did not happen for the placebo group, in which the value fell during the study period. It is interesting to note that not only the blood hemoglobin levels improved in the children receiving iron supplements but also their physical condition changed, with significant weight gains during the supplementation period. The group with the greatest increase in body weight was the heme iron-supplemented group, with an average gain of 2.3 kg; average weight gains were 1.93 kg in the iron sulphate-supplemented group and only 0.48 kg in the placebo group.

Table 1 presents an overview of the main characteristics of both forms of iron in the diet.

Table 1: Overview of the main characteristics of both forms of iron in the diet

Non –heme iron	Heme iron
Absorption 1-15%	Absorption 20-35%
Absorption affected by other components in the diet	Absorption not affected by other components in the diet
Source: vegetables and ferrous salts	Source: Myoglobin and hemoglobin
Percentage consumed very high (> 85%)	Percentage consumed very low (< 15%)

The Panel concluded that iron from heme iron (blood peptonates) is bioavailable and absorbed to a significantly greater extent than iron from non-heme sources. The bioavailability of iron from heme iron sources may be 2- to 7-fold higher than that of iron from non-heme sources (Quintero *et al*. 2008; Björn-Rasmussen *et al*., 1974; Reizenstein 1980; Monsen 1988; Ekman and Reizenstein 1993; Hurrell 1997; Seligman *et al*., 2000; Swain *et al.,* 2004).

3.2. Toxicological data

The petitioner justifies the absence of toxicological data on the basis that the product is substantial equivalent to porcine haemoglobin, and/or the heme iron present in red meat or liver, which are part of the regular human diet. The petitioner indicates that the substantial equivalence of the heme iron (blood peptonates) is porcine hemoglobin; the heme iron present in red meat or liver can also be

considered, as in both cases the iron present in these products corresponds to heme iron with similar characteristics as the product evaluated in the present opinion.

The petitioner also argues that hemoglobin is used worldwide as a food ingredient without any restrictions on its use or application. The company and other European and international companies have been selling hemoglobin-derived products for different uses in nutrition (mainly for meat products, etc.) for more than 30 years.

3.2.1. Acute oral toxicity

Within the dossier the petitioner provided data from a study that revealed that the acute toxicity of iron-heme (blood peptonates) in rats was low, with an LD_{50} being greater than 2500 mg product/kg bw.

3.2.2. Short-term and subchronic toxicity

No data on heme iron (blood peptonates) were provided by the petitioner.

3.2.3. Genotoxicity

No data on heme iron (blood peptonates) were provided by the petitioner.

Glei *et al.* (2006) reported that hemoglobin and hemin induced DNA damage in human colon tumor cells HT29 clone 19A and in primary human colonocytes. DNA damage was investigated using the Comet assay.

3.2.4. Chronic toxicity and carcinogenicity

No data on heme iron (blood peptonates) were provided by the petitioner.

3.2.5. Reproductive and developmental toxicity

No data on heme iron (blood peptonates) were provided by the petitioner.

3.2.6. Human studies

Ekman and Reizenstein (1993) compared the absorption of heme iron in the form of hemoglobin and inorganic iron in the form of ferrous sulphate in healthy and anaemic pregnant women. The authors indicated that as the heme iron group required less iron for supplementation due to the improved absorption, there were less side effects of iron administration due to the smaller quantities of free ferric ions in the intestinal lumen.

In a double-blind study, Frykman *et al.* (1994) gave to 49 healthy volunteers a supplement containing a mixture of heme iron obtained from pig hemoglobin containing 1.2 mg of iron and 8 mg of ferrous fumarate, compared with a control group (45 healthy subjects) who were given a supplement containing 60 mg of iron as ferrous fumarate only. After two months of supplementation, less iron was required in the heme iron-supplemented group to maintain blood iron levels and the number of

patients reporting side effects such as stomach ache, constipation, nausea or diarrhea during supplementation was significantly reduced.

Based on these studies, the petitioner concluded that heme iron has no side effects associated with its consumption and that it does not cause nausea, gastric irritation, vomiting or diarrhea. The petitioner also indicated that administration of heme iron (blood peptonates) either alone in capsules, pills, or combined with food, does not cause the characteristic side effects of non-heme iron intake (i.e. nausea, stomach irritation, vomiting or diarrhoea).

3.2.7. Other studies

Epidemiological and animal model studies suggest that a high intake of heme-iron, present in red meat, may be associated with an increased risk of colon cancer (Balder *et al.*, 2006; Oates and West, 2006; de Vogel *et al*., 2008; Sawa *et al*., 1998; Lee *et al*., 2005; Larsson *et al.*, 2005a, 2005b; Santarelli *et al*., 2008), and prostate cancer (Sinha *et al*., 2009) and lung cancer (Tasevska *et al*., 2009).

3.2.7.1. Animal studies

Sawa *et al.* (1998) examined the possible implication of lipid peroxyl radicals generated from fatty acids and heme-iron in DNA damage, and hence in the possibility of colon cancer development. F344 female rats were given *N*-nitroso-*N*-methylurea six times during a 2-week period and then fed diets containing different amounts of safflower oil and hemoglobin (rich in iron) for 36 weeks; the occurrence of colon cancer was determined by hematoxylin and eosin stain (H&E) staining. In this animal model, simultaneous feeding of a fat diet and heme-iron produced a significant increase ($P < 0.05$) in the incidence of carcinomas in the colon, compared with a diet without hemoglobin. The authors concluded that lipid peroxides and heme components generate peroxyl radical species that exert DNA-cleaving activity, and that lipid peroxyl radicals thus generated, may contribute, at least in part, to the high incidence of colon cancer.

Oates and West concluded that previous studies have shown that heme irritates the epithelium of the colon as evidenced by mild diarhhoea (Sesink *et al*., 1999, 2000). Sesink *et al.* (1999) reported that feeding heme but not non-heme iron to rats results in significant increased proliferation of colonic mucosa.

In addition, Pierre *et al*. (2003, 2004) reported that in rats, the incidence of Aberrant Crypt Foci (ACF) and Mucin-Depleted Foci (MDF) increased as the heme content of the diet increased. A heme breakdown product rather than heme or iron *per se* might be responsible for the inflammation and ACF formation (Sesink *et al*., 1999, 2000).

De Vogel *et al.* (2008) reported a study in rats fed a purified, humanized, control diet or a similar diet supplemented with 0.5 mmol heme/kg for 14 days. Dietary heme induced a more than ten-fold increased cytolytic activity of the fecal water and a hundred-fold lower excretion of host DNA. Colons of heme fed rats showed injured surface epithelium and an approximately 25% increase in crypt depth. Futhermore, dietary heme doubled colonocyte proliferation, shown by all three markers, but inhibited colonic mucosal apoptosis. The authors concluded that the results demonstrate that dietary heme injures colonic surface epithelium, which is overcompensated by inhibition of apoptosis and hyperproliferation of cells in the crypts, which might explain why intake of dietary heme is associated with an increased risk of colon cancer.

3.2.7.2. Human studies

Balder *et al*. (2006) reported a cohort study on heme and chlorophyll intake and risk of colorectal cancer in the Netherlands. Multivariate rate ratios for quintiles of heme iron intake and colon cancer were 1.00, 0.98, 1.04, 1.13 and 1.29 (*P*trend = 0.10) among men and 1.00, 1.31, 1.44, 1.18 and 1.20 (*P*trend = 0.56) among women, respectively. No consistent associations were observed for rectal cancer. Rate ratios for colon cancer increased across successive quintiles of the ratio of heme/chlorophyll among men only (1.00, 1.08, 1.01, 1.32 and 1.43; *P*trend = 0.01). No consistent associations were observed between fresh meat and colorectal cancer. The authors concluded that their data suggest an elevated risk of colon cancer in men with increasing intake of heme iron and decreasing intake of chlorophyll.

Previous studies have established that red meat, but not white meat, stimulates endogenous N-nitrosation in humans, and heme iron, specifically, can produce the same effect (Hugues *et al.*, 2001; Bingham *et al.*, 2002; Cross *et al.*, 2003).

Lee *et al.* (2004), reported a positive association of heme iron intake and an inverse association of zinc intake with the risk of colon cancer among women who consumed alcohol. During 15 years of follow-up, 34,708 postmenopausal women, aged 55–69 years at baseline who completed a food-frequency questionnaire for the Iowa Women's Health Study, were followed for incidence of colon cancer. After adjusting for each micronutrient, the relative risks for proximal colon cancer increased more than 2-fold across categories of heme iron intake (*P*trend = 0.01). The positive association with heme iron was stronger among women who consumed alcohol than among those who did not.

In the same cohort, Lee *et al.* (2005) also examined associations among dietary heme iron as a possible pro-oxidant and the incidence of upper digestive tract cancer; 34,708 postmenopausal women, aged 55–69 years at baseline who completed a food frequency questionnaire, were followed 16 years. There were 75 upper digestive tract cancer cases (52 gastric cancer and 23 oesophageal cancer). Heme iron intake was positively associated with the risk of upper digestive tract cancer. After adjusting for age, total energy intake, cigarette smoking and alcohol consumption, relative risks for quintiles of heme iron intake were 1.0, 1.53, 2.15, 3.05 and 2.83 (*P*trend = 0.06). It was concluded that higher intake of heme iron is associated with higher risk of upper digestive tract cancer.

Larsson *et al.* (2005a, 2005b) analysed data from the population-based Swedish Mammography Cohort of 61,433 women aged 40 – 75 years and without cancer at baseline in 1987-1990 (Larsson *et al.*, 2005a). In this large population-based cohort study, both heme iron and red meat intake, when mutually adjusted, showed a positive association with the risk of colon cancer among women who consumed alcohol. The authors concluded that these findings suggest that heme iron may only partly explain the apparent increased risk of colon cancer associated with a high red meat consumption and that other factors in red meat may be implicated in colon carcinogenesis.

In contrast, a cohort study of dietary iron and heme iron intake (excluding iron supplements) and risk of colorectal cancer in 49,654 Canadian women concluded that overall there was no association of intake of iron, heme iron, or iron from meat with risk of colorectal cancer or of any of the subsites (Kabat *et al*. 2007).

Santarelli *et al.* (2008) published a review of epidemiologic and experimental evidence of the relationship between processed meat and colorectal cancer. The authors stated that epidemiologic studies published to date conclude that, compared with non-eaters of processed meat, the excess risk in the highest category of processed meat-eaters is between 20 and 50%. In addition, the excess risk per gram of intake is clearly higher than that of fresh red meat. The authors also summarise several hypotheses, mainly based on studies carried out on red meat that may explain why processed meat intake is linked to cancer risk. The hypotheses that have been tested experimentally are (i) that high-fat diets could promote carcinogenesis via insulin resistance or fecal bile acids; (ii) that cooking meat at a high temperature forms carcinogenic heterocyclic amines and polycyclic aromatic hydrocarbons; (iii) that carcinogenic *N*-nitroso compounds are formed in meat and endogenously; and (iv) that heme iron

in red meat can promote carcinogenesis because it increases cell proliferation in the mucosa, through lipidperoxidation and/or cytotoxicity of fecal water. Nitrosation might increase the toxicity of heme in cured products.

Sinha *et al*. (2009) reported a study in meat and meat-related compounds and risk of prostate cancer in a large prospective cohort study in the United States. The authors examined associations between meat consumption (type, cooking method and related mutagens), heme iron, nitrite/nitrate, and prostate cancer in a cohort of 175,343 US men aged 50-71 years. During 9 years of follow-up (1995-2003), they ascertained 10,313 prostate cancer cases (1,102 advanced) and 419 fatal cases. Hazard ratios comparing the fifth intake quintile with the first revealed elevated risks associated with red and processed meat for total (red meat: hazard ratio (HR) = 1.12, 95% confidence interval (CI): 1.04, 1.21; processed meat: HR = 1.07, 95% CI: 1.00, 1.14) and advanced (red meat: HR = 1.31, 95% CI: 1.05, 1.65; processed meat: HR = 1.32, 95% CI: 1.08, 1.61) prostate cancer. Heme iron, was positively associated with total (HR = 1.09 (95% CI: 1.02, 1.17), and advanced (HR = 1.28 (95% CI: 1.03, 1.58), disease. There were no clear associations for fatal prostate cancer. The authors concluded that red and processed meat may be positively associated with prostate cancer via mechanisms involving heme iron, nitrite/nitrate, grilling/barbecuing, and benzo[a]pyrene.

Tasevska *et al*. (2009) reported a prospective study of meat, cooking methods, meat mutagens, heme iron, and lung cancer risks. It was prospectively investigated whether meat type, cooking method, doneness level, and intake of specific meat mutagens and heme iron are associated with lung carcinoma. Men (n = 278,380) and women (n = 189,596) from the National Institutes of Health-AARP Diet and Health Study with no history of cancer at baseline were monitored for 8 y. Diet was assessed with a 124-item food-frequency questionnaire. A meat-cooking module was used to estimate the intake of individual heterocyclic amines, benzo(a)pyrene, and heme iron. Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% CIs. In a comparison of quintiles 5 with 1 (Q5vsQ1), a high intake of red meat was associated with an increased risk of lung carcinoma in both men (HR(Q5vsQ1): 1.22; 95% CI: 1.09, 1.38; P for trend = 0.005) and women (HR(Q5vsQ1): 1.13; 95% CI: 0.97, 1.32; P for trend = 0.05). In an analysis stratified by smoking status, the authors observed a tendency for an increased risk with red meat intake in never smoking men and women; however, the risks were not statistically significant. Heme iron intake increased the risk of lung carcinoma in both men (HR(Q5vsQ1): 1.25; 95% CI: 1.07, 1.45; P for trend = 0.02) and women (HR(Q5vsQ1): 1.18; 95% CI: 0.99, 1.42; P for trend = 0.002). The authors concluded that they observed a moderate association between meat consumption and lung carcinoma, which might be explained by heme iron intake, high-temperature cooking, and associated mutagens.

3.2.7.3. Allergenicity

The petitioner provided results from a study investigating the ability of heme iron (blood peptonates) to cause skin or eye irritation. It was concluded that the product does not cause skin inflammation or any significant eye injury.

4. Discussion

The present opinion deals only with the evaluation of the safety of heme iron (blood peptonates) as a source of iron and the safety of that source when added for nutritional purposes in foods intended for the general population, including food supplements. The safety of iron, in terms of amounts that may be consumed, is outside the remit of this Panel.

The chemical form of the iron is the main factor affecting its bioavailability. Iron is naturally present in two forms: non-heme iron and heme iron, with the latter having the greater bioavailability.

The EFSA opinion on the Tolerable Upper Intake Level for iron published in 2004, stated that heme iron absorption was not regulated by other substances in the diet and absorption was higher than that for inorganic iron, being regulated only by the levels of iron already present in the body.

The Panel concluded that iron from heme iron (blood peptonates) is bioavailable and absorbed to a significantly greater extent than iron from non-heme sources. The bioavailability of iron from heme iron sources may be 2- to 7-fold higher than that of iron from non-heme sources (Quintero *et al*. 2008; Björn-Rasmussen *et al*., 1974; Reizenstein 1980; Monsen 1988; Ekman and Reizenstein 1993; Hurrell 1997; Seligman *et al*., 2000; Swain *et al.*, 2004).

The petitioner indicated that hemoglobin is used worldwide as a food ingredient without any restrictions on its use or application and that the product has a substantial equivalence with porcine hemoglobin or with the heme iron present in red meat or liver.

The Panel is aware of the fact that heme iron is a constituent of the normal human diet and also an endogenous body constituent.

The petitioner justifies the absence of toxicological data on the basis that the product is substantial equivalent to porcine haemoglobin, and/or the heme iron present in red meat or liver, which are part of the regular human diet.

Thus, no data on the toxicity of heme-iron (blood peptonates) were provided by the petitioner except for an acute toxicity study. This study revealed that the acute toxicity of heme iron (blood peptonates) in rats was low, with an LD_{50} greater than 2500 mg product/kg bw. However, the Panel notes that this study is not suitable to evaluate the safety in use of heme iron (blood peptonates) as a source of iron in foods for the general population, including food supplements.

Glei *et al.* (2006) reported that hemoglobin and hemin induced DNA damage in human colon tumor cells HT29 clone 19A and in primary human colonocytes. DNA damage was investigated using the Comet assay.

The Panel noted the fact that epidemiological and animal model studies suggest that a high intake of heme iron, may be associated with an increased risk of colon cancer (Balder *et al.*, 2006; Oates and West, 2006; de Vogel *et al*., 2008; Sawa *et al*., 1998; Lee *et al*., 2005; Larsson *et al*., 2005a, 2005b; Santarelli *et al*., 2008) and prostate cancer (Sinha *et al*., 2009) and lung cancer (Tasevska *et al*., 2009). The Panel noted that other risk factors may be involved.

The recommended daily dose of heme iron (blood peptonates) proposed by the petitioner amounts to 2 g heme iron (blood peptonates) equivalent to 20 mg of elemental iron.

The petitioner also indicated that they consider the maximum recommended daily intake to be 4.5-5 g heme iron (blood peptonates), which would be equivalent to 45-50 mg of elemental iron if it behaved like inorganic iron.

The Panel noted that in terms of bioavailability, heme-iron does not behave like inorganic iron and is bioavailable to a higher extent, and that the use levels of heme iron (blood peptonates) proposed by the petitioner result in levels of exposure to elemental iron that are higher than the guidance value of 17 mg/day for supplemental intake of non-heme iron proposed by the EVM, although they are in line with the Provisional Maximum Tolerable Daily Intake (PMTDI) value for iron of 0.8 mg/kg bw/day (50 mg/day for a 60 kg person) proposed by JECFA.

Altogether, given:

i) that the use levels of heme iron (blood peptonates) proposed by the petitioner result in exposure to elemental iron at levels that are higher than the guidance value of 17 mg/day proposed by the EVM for supplemental intake of non-heme iron, although they are in line with the Provisional Maximum Tolerable Daily Intake (PMTDI) value for iron of 0.8 mg/kg bw/day (50 mg/day for a 60 kg person) proposed by JECFA

ii) the significantly increased bioavailability of iron from heme iron as compared to iron from non-heme iron sources,

iii) that epidemiological and animal model studies suggest that a high intake of heme iron may be associated with an increased risk of colon cancer,

iv) the absence of genotoxicity data on heme iron (blood peptonates) but the positive results reported for hemoglobin and hemin in a Comet assay in cells *in vitro*,

v) the absence of subchronic, reproductive, developmental, long-term toxicity and carcinogenicity data on heme iron (blood peptonates),

the Panel considered that the submitted data are insufficient to demonstrate the safety of the proposed use and use levels of heme iron (blood peptonates) as a source of iron for nutritional purposes in foods intended for the general population, including food supplements.

CONCLUSIONS

The Panel concluded that iron from heme iron (blood peptonates) is bioavailable and absorbed to a significantly higher extent than iron from non-heme sources. The bioavailability of iron from heme iron sources may be 2- to 7-fold higher than that of iron from non-heme sources.

The Panel concluded that the available data are insufficient to demonstrate the safety of the proposed use and use levels of heme iron (blood peptonates) as a source of iron for nutritional purposes in foods intended for the general population, including food supplements.

DOCUMENTATION PROVIDED TO EFSA

1. Document for notifying the product APROFER-1000 as a food supplement according to the Directive 2002/46/EC of the European Parliament and of the Council. Submitted by APC EUROPE July 2009.
2. Document for notifying the product APROFER-1000 as a food substance that can be added to foods according to the Regulation 1925/2006 of the European Parliament and of the Council. Submitted by APC EUROPE May 2009.

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GLOSSARY / ABBREVIATIONS

8-iso-PGF2A	8-iso-prostaglandin-F2A
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Foods
AOAC	Association of Analytical Communities
ATF	Aberrant Atypical Foci
bw	body weight
CAS	Chemical Abstracts Service
DHN-MA	1,4-dihydroxynonane mercapturic acid
DMT-1	Divalent Metal Transporter
DNA	Deoxyribonucleic Acid
EC	European Commission
EFSA	European Food Safety Authority
EVM	Expert group on Vitamins and Minerals
FDA	U.S. Food and Drug Administration
FNB	Food and Nutrition Board
FOSHU	Foods for Specific Health Use (Japan)
GLP	Good Laboratory Practice
Hb	Hemoglobin
HCP-1	Heme Carrier Protein 1
HDL	High-Density Lipoprotein
HIP	Heme Iron Polypeptide
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50% i.e. dose that causes death among 50% of treated animals
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MDF	Mucin-Depleted Foci
MPCEs	Micronucleated polychromatic erythrocytes
NDA	Scientific Panel on Dietetic Products, Nutrition and Allergies
NOAEL	No Observable Adverse Effect Level
PRI	Population Reference Intake
RDA	Recommended Daily Amount
SCF	Scientific Committee on Food
TDS	Total Diet Study
UL	Tolerable Upper Intake Level
WHO	World Health Organization