OPINION OF THE SCIENTIFIC PANEL ON DIETETIC PRODUCTS, NUTRITION AND ALLERGIES ON A REQUEST FROM THE COMMISSION RELATED TO THE TOLERABLE UPPER INTAKE LEVEL OF IRON

(REQUEST N° EFSA-Q-2003-018)
(ADOPTED ON 19 OCTOBER 2004)

SUMMARY

Iron is an essential trace element that has important metabolic functions, including oxygen transport and storage and many redox reactions. Insufficient intake results in the deficiency condition anaemia, adverse outcomes of pregnancy, impaired psychomotor development and cognitive performance and reduced immune function.

Case reports of accidental poisoning with medicinal iron, especially in young children, indicate acute damage of gastrointestinal, hepatic, pancreatic and cardiovascular structures after ingestion of very high doses. An acute oral dose of 60 mg iron/kg body weight can be lethal but oral doses below about 10-20 mg iron/kg body weight do not cause acute systemic toxicity.

Adverse gastrointestinal effects (e.g. nausea, epigastric discomfort, constipation) have been reported after short-term oral dosage at 50-60 mg daily of supplemental non-haem iron preparations, particularly if taken without food.

Iron overload with clinical symptoms, including liver cirrhosis, has been reported in individuals receiving long-term, high-dose medical treatment with iron (160-1200 mg iron/day). Iron overload with clinical symptoms has also been found in subjects homozygous for hereditary haemochromatosis (a genetic disorder of iron storage), even at normal dietary iron intakes. Bantu siderosis, with liver cirrhosis and diabetes, has been attributed to chronic excess intake of highly available iron (50-100 mg iron/day) in beer; however, these adverse effects may be confounded by chronic alcohol intake and possibly by a genetic disorder.

Although a proportion of the population has serum ferritin levels indicative of elevated iron stores (above 200 μg/L for women and 300 μg/L for men), the point at which an elevated serum ferritin level becomes associated with an increased risk of adverse effects (such as liver fibrosis) is not known. The risk of adverse effects from iron overload in the general population, including those heterozygous for hereditary haemochromatosis, is considered to be low.

Epidemiological studies have reported associations between high iron intake and/or stores with increased risk of chronic diseases such as cardiovascular disease, type II diabetes and cancer of the gastrointestinal tract. However, these data are conflicting and do not provide convincing evidence of a causal relationship between iron intake or stores and such chronic diseases.

The Panel considered that the available data are insufficient to establish a tolerable upper intake level for iron.

Based on estimates of current iron intakes in European countries, the risk of adverse effects from high iron intake from food sources, including fortified foods in some countries, but excluding supplements, is considered to be low for the population as a whole, except for those homozygous for hereditary haemochromatosis (up to 0.5% of the population). However, intake of iron from food supplements in men and postmenopausal women may increase the proportion of the population likely to develop biochemical indicators of high iron stores. Some groups at special risk for poor iron status, such as menstruating women or children, could benefit from additional iron intake and/or improved availability of dietary iron.

KEY WORDS

Iron, tolerable upper intake level, gastrointestinal effects.

BACKGROUND

In 2002, the European Parliament and the Council adopted Directive 2002/46/EC[1] related to food supplements containing vitamins and minerals.

In addition, and as announced in its White Paper on Food Safety, the Commission aims to put forward a proposal for harmonising legislation concerning the addition of vitamins and minerals to foods.

With a view to provide scientific support to the European Commission's legislative work in this field, the Scientific Committee on Food (SCF) issued, from October 2000 to April 2003, a series of opinions on tolerable upper intake levels of individual vitamins and minerals and safety factors in relation to their use in fortified foods and food supplements (available on the Internet at: http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html).

The SCF opinions covered 22 out of the 29 nutrients, which were considered to be within their mandate for this task. The SCF did not have sufficient time to adopt opinions for the following vitamins and minerals: vitamin C, chloride, fluoride, iron, phosphorus, potassium and sodium. In addition, during the decision making process for the adoption of Directive 2000/46/EC on food supplements the Parliament requested that boron, nickel, silicon, vanadium and tin should be allowed to be used in food supplements. Therefore, the European Food Safety Authority is asked to provide scientific opinions on the remaining 12 vitamins and minerals in accordance with the present terms of reference.

TERMS OF REFERENCE

With respect to the outstanding 12 vitamins and minerals, the European Food Safety Authority is asked 1) to review the upper levels of daily intakes that are unlikely to pose a risk of adverse health effects; 2) to provide the basis for the establishment of safety factors, where necessary, which would ensure the safety of fortified foods and food supplements containing the aforementioned nutrients.

ASSESSMENT

1. INTRODUCTION

Iron is a metal with an atomic mass of 55.8. It is present in biological systems in one of two oxidation states, and redox interconversions of the ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms are central to the biological properties of this mineral. Iron is an essential constituent of oxygen carriers, such as haemoglobin and myoglobin, and the iron contained within haem is essential for the redox reactions of numerous cytochromes.

2. NUTRITIONAL BACKGROUND, FUNCTION, METABOLISM AND INTAKE

2.1. Functions of iron, deficiency symptoms, and prevalence of iron-deficiency

Iron deficiency is associated with reduced function of an array of iron-dependent enzymes and proteins (Beard and Dawson, 1997). The most important effect of deficiency is impaired physical performance due to reduced levels of haemoglobin and myoglobin and lower activity of iron-dependent cytochromes, leading to reduced cellular concentrations of ATP. Lack of iron-dependent ribonucleotide-reductase and aminoacid-monoxygenases may impair RNA synthesis and neurotransmitter metabolism. Iron-deficiency anaemia increases the risk for low birth weight (Baynes and Bothwell, 1990), and appears to impair psychomotor development and cognitive performance (Grantham-McGregor and Ani, 2001). Cellular immune function and thermoregulation may also be impaired in severe iron-deficiency (Beard and Dawson, 1997).

1 - 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. OJ L 183. 12.7.2002, p. 51.

Iron deficiency anaemia is one of the most common nutritional disorders world-wide. It has the highest prevalence in women of childbearing age and in infants, affecting an estimated 25-46% of babies in the third world (DeMaeyer and Adiels-Tegman, 1985; Dewey *et al*, 1997). Anaemia is found in 2% of males and 5% of females in Germany (VERA-Schriftenreihe, 1995). In the USA the prevalence of iron-deficiency anaemia is 0.2%, 2.6% and 1.9% in men and in pre- and post-menopausal women, respectively (Cook *et al*, 1986); corresponding data from Northern Ireland are 0.5%, 6.6% and 4.6% respectively (Strain *et al*, 1990).

Groups vulnerable to iron deficiency are infants over 6 months, toddlers, adolescents and pregnant women (due to high requirements), older people and people consuming foods high in iron absorption inhibitors (see below) (due to poor absorption) and menstruating women or individuals with pathological blood loss (due to high blood losses) (EGVM, 2003).

2.2. Iron homeostasis

Ferrous iron in low molecular weight forms may increase oxidative stress, because reactive oxygen species (ROS) may convert superoxide anions into highly reactive hydroxyl radicals by an iron-catalysed Fenton reaction (McCord and Turrens, 1994). *In vitro* studies in cultured mammalian cells have demonstrated a direct interrelationship between the intracellular free, iron pool and oxidative stress. To minimise oxidative stress the body contains a number of high affinity iron-binding proteins so that there are very low levels of free non-bound iron in the circulation or within cells. The different binding proteins dominate the fate of iron in the body.

The adult human body contains 2.2-3.8 g iron under iron-adequate conditions (Lynch, 1984). Homeostatic mechanisms have evolved that can alter intestinal iron absorption and supply iron preferentially to functional compartments in response to deficiency or excess.

2.2.1. Iron absorption, distribution and regulation of tissue stores

Tissue concentrations and body stores of iron are controlled at three different levels:

i. Luminal iron: the extent of uptake of iron by the cells of the gastrointestinal tract affects how much remains unabsorbed and passes to the lower bowel, prior to elimination in faeces;

ii. Mucosal iron: the mucosa is the main site of regulation of iron uptake in relation to liver stores and ferritin levels;

iii. Post-mucosal iron: relates to the impact of iron intake on iron status and body stores.

2.2.1.1. Luminal iron

Non-haem iron is present in foods largely as salts, which are made soluble in the stomach, and absorption from foods depends on its dissolution as ferric salts and subsequent reduction to the ferrous form. Any elemental iron in the diet is probably absorbed as non-haem iron following its dissolution in the acid stomach contents. The absorption of non-haem iron can be increased substantially by the presence of ligands, such as ascorbate, citrate and fumarate, as well as the presence of amino acids (e.g. cysteine) and oligopeptides resulting from meat digestion (Mulvihill *et al*, 1998). In contrast, very stable complexes, for example with phytates, phosphates and oxalates, impair non-haem iron absorption. Depending on the concentration of supportive or inhibitory ligands in the intestinal lumen the absorption of non-haem iron can vary by a factor of 10 in single-meal studies, but the effects are less pronounced in more long-term studies (Hallberg and Rossander, 1984; Rossander, 1987; Hunt and Roughead 2000).

2.2.1.2. Mucosal iron

In addition, homeostatic regulation will influence the extent of non-haem iron absorption. Body iron content is linked to demand by regulated intestinal non-haem iron absorption which, in turn, is regulated to a major extent by the uptake of iron into the cells of the intestinal mucosa (Schümann *et al*, 1999a and b). This step is mediated by the divalent metal transporter (DMT-1) (Gunshin *et al*, 1997), the expression of which is higher in iron deficient animals (Fleming *et al*, 1999) and in hereditary haemochromatosis (Byrnes *et al*, 2002). The activity of DMT-1 decreases after a period of high oral iron intake (Oates *et al*, 2000), and such down-regulation of iron uptake may be regarded as protection against iron overload. There is evidence of iron-zinc interaction at the mucosal level. Large doses of supplemental non-haem iron inhibit zinc absorption if they are both taken without food, but do not inhibit zinc absorption if they are consumed with food (FNB, 2001).

Transfer of information on hepatic iron stores to the gut may be mediated via an acute phase hepatic protein, hepcidin (Nicolas *et al*, 2001), or possibly the pro-hormone form pro-hepcidin (Kulaksiz *et al,* 2004), which influences the extent of iron absorption (Leong and Lonnerdal, 2004) and can result in a mucosal block. Hepcidin levels are inappropriately low in patients with hereditary haemochromatosis in relation to their iron stores and ferritin levels (Robson, 2004), and genetic variants of which may contribute to iron accumulation in some types of haemochromatosis (Bridle *et al*, 2003; Ganz, 2003).

Haem iron enters the mucosa via a pathway that is not regulated by DMT-1, but involves interaction of iron in a porphyrin complex with a haem-receptor (Tenhunen *et al*, 1980; Roberts *et al*, 1993). Haem iron absorption is less regulated (Finch, 1994), and varies between 15% in iron-replete individuals and 35% in iron-deficient subjects. The increased haem-iron absorption in iron-deficiency is partly due to higher haem-binding to the brush-border (Roberts *et al*, 1993) and partly to induction of mucosal haem-oxygenase, an enzyme that cleaves the porphyrin ring and liberates iron for transfer into the body. Haem-iron absorption is largely independent of dietary ligands, although a high luminal calcium concentration reduces absorption (Hallberg *et al*, 1992).

A recent study showed that iron absorption does not appear to be altered in heterozygotes for hereditary haemochromatosis; HFE C282Y-heterozygous subjects did not absorb dietary iron more efficiently, even when foods were highly fortified with iron from ferrous sulfate and ascorbic acid, than did control subjects (Hunt and Zeng, 2004).

Due to the various exogenous and endogenous factors affecting iron absorption, a clear relationship is generally not found between total iron intake and iron status. Total daily iron absorption is about 0.9 mg in males and 0.5 and 0.6 mg higher in menstruating women and blood donors. The mucosal barrier can be overwhelmed by high iron doses, such as occurs in acute iron intoxication (Ellenham and Barceloux, 1988).

2.2.1.3. Post-mucosal iron

The concentration of free iron in blood is extremely low due to the high affinity binding of iron to transferrin. Transferrin is an 80 kDa plasma protein that binds two Fe3+ ions per molecule with high affinity (binding constant: 10^{-30}). The total iron binding capacity (TIBC) of transferrin in the plasma of healthy adults is approximately 56µmol/L. About 30% and 10% of TIBC is occupied in normal iron status and in iron deficiency respectively. The plasma iron pool is approximately 3 mg, but its daily turnover is more than 30 mg/day (Bothwell *et al*, 1979). Free iron, or non-transferrin-bound iron (NTBI), can occur in the presence of normal transferrin saturation values, and this has been demonstrated using the method of Hider (2002) in both homozygotes (Loreal *et al*, 2000; Gosriwanata *et al*, 1999) and in heterozygotes for hereditary haemochromatosis (de Valk *et al*, 2000).

A low iron concentration in plasma is essential for the bacteriostatic and bactericidal systems in blood and lymph (Ward *et al*, 1996). Iron overload, with its associated increased plasma iron levels, suppresses immune cell function, allows increased growth rates of infectious organisms and increases the risks of morbidity and mortality due to infectious disease (Collins, 2003; Failla, 2003; Ward *et al*, 1996).

Iron is transferred from the plasma to the tissues via binding of iron-loaded transferrin to transferrin receptors (TfR) at the cell surface, which are subsequently internalised within endocytotic vesicles. High serum TfR values indicate high activity of cellular iron uptake, which in most cases is due to high erythropoietic iron demand. Transferrin releases its iron under acidic conditions in the endocytotic vesicles, and the transferrin and TfR return subsequently to the cell surface.

Within cells, iron is preferentially distributed to iron-dependent enzymes and iron-binding proteins. Excessive intracellular iron is sequestered in the storage protein ferritin (500 kDa) or in its degradation product called haemosiderin. One ferritin molecule can store up to 4500 iron atoms as ferric hydroxyphosphate micelles. Such iron can be remobilised and utilised on demand. The serum ferritin concentration is an indication of body iron stores, except when high levels arise due to inflammation (Ponka *et al*, 1998).

Homeostatic mechanisms involving cytosolic iron-regulatory proteins (IRPs) have developed to maintain low concentrations of free iron, in order to provide iron for essential functions and to protect the cells from oxidative damage. IRP-1 is activated by cellular iron-deficiency, but also by reactive oxygen species (ROS). At normal cellular levels of free iron, IRP-1 contains a 4Fe-4S cluster but in iron-deficient cells the 4Fe-4S cluster is lost and the molecule binds to specific base loops of mRNAs for DMT-1,

TfR and ferritin, called “iron-response elements”. In deficiency IRP binding to TfR-mRNA increases TfR expression, while the ferritin mRNA is broken down more rapidly, which restores the intracellular free iron concentration by a combination of increased uptake and decreased sequestration. IRP activity is also induced by ROS and during hypoxia-reoxygenation stress (Rouault and Klausner 1997; Hanson and Leibold, 1999) when any increase in free iron could lead to increased oxidative damage (Martins *et al*, 1995; Hentze and Kühn, 1996; Pantopoulos and Hentze, 1995; Mueller *et al*, 2001; Rouault and Klausner, 1997, Hanson and Leibold, 1998; 1999). This is prevented, because the tendency towards an increased free intracellular pool causes an increase in the expression of ferritin and a decrease in TfR expression (Kakhlon *et al*, 2001), thereby reversing the effects of IRP activation and decreasing both the free intracellular pool and oxidative stress (Picard *et al*, 1998).

The low pH values in inflamed tissues may mobilise free iron from ferritin and from its haem-bound form (Britton *et al*, 1994; Biemond *et al*, 1984; Koishinako *et al*, 1996). Iron-related increases in ROS production may contribute to pathogenic processes, such as myocardial infarction and intermittent claudication, which can follow hypoxia and re-oxygenation, (see Schümann, 2001), and also in the induction of cirrhosis, as exemplified in iron-overloaded rat livers (Bacon *et al*, 1983). Hepcidin (see above) is an acute phase protein and its increased release from the liver may result in a mucosal block, thereby contributing to the effects of inflammation on iron homeostasis.

Under physiological conditions iron status is almost exclusively regulated by adaptation of intestinal iron absorption according to the demand (see 2.2.1.2). In healthy iron-replete humans with iron stores of 800-1200 mg, non-haem iron absorption increases when iron stores are depleted and vice versa (Finch, 1994). This mode of regulation is highly predictable in normal subjects, and iron stores as determined by plasma ferritin values have been used to predict iron absorption (Magnusson *et al*, 1981). Duodenal non-haem iron absorption is linked to body iron status via the supply of serum iron to intestinal crypt cells. At adequate supply levels, the activity of IRPs in these cells is low, whereas in iron-deficiency IRP activity in the enterocytes is increased and intestinal iron absorption is high. Iron absorption adapts to changes in plasma iron concentration with a lag time of 48h, which corresponds to the time required for young enterocytes to express enzymes and transport proteins, such as DMT-1 and iron regulated gene (IREG), and to migrate up the villi to the site of absorption (Schümann *et al*, 1999b).

In anaemia, an undefined and unidentified “erythrocyte regulator” can increase iron absorption to 20-40 mg Fe/day from oral iron preparations (Finch, 1994). In addition iron absorption is increased by an unknown mechanism to up to 66% in pregnant women and returns to normal at 16-24 weeks after delivery (Barrett *et al*, 1994). Iron absorption is also increased during lactation and growth.

Regulation of non-haem iron absorption protects from iron overload at normal and moderately increased iron-intake levels. A group of 12 Swedish male blood donors and 19 non-donors received standard meals with radioactively labelled non-haem iron (12 mg Fe/day) and haem-iron (2 mg/day). Total iron absorption increased when serum ferritin concentrations were less than 60 µg/L, but decreased when serum ferritin exceeded this level, and the homeostatic equilibrium point of the system was estimated to be 60 µg ferritin/L (Hallberg *et al*, 1997). This study did not investigate the maximum regulatory capacity by the administration of high levels of additional iron, but such data are available from fortification studies. Fortification of curry powder with sodium-iron-EDTA giving additional intakes of 7.5 mg Fe/day for 2 years in iron-replete male subjects (Ballot *et al*, 1989) did not increase serum ferritin levels. There were no changes in iron stores as estimated by serum ferritin following the addition of 10 mg Fe/day as ferrous sulphate to the food of a healthy male subject for 500 days, or as determined via serial phlebotomies after the end of the iron substitution period (Sayers *et al*, 1994). In these two studies serum ferritin levels did not change despite long-term challenge with 7.5-10 mg Fe/day in addition to normal dietary iron intake of approx. 10 mg Fe/day. Iron status in the elderly, as indicated by elevated serum ferritin levels, was increased after intake of an additional 30 mg Fe/day as supplements (Fleming *et al*, 2002), in a study in which subjects with abnormal results for blood leukocytes, C-reactive protein (CRP), and 3 liver enzymes, as indicators of inflammation and liver diseases, were excluded. Theoretical calculations of the accumulation of iron in a fertile woman given different daily intakes (Borch-Iohnsen and Petersson Grawe, 1995) indicated that a daily intake of 60 mg for 5 years would lead to a serum ferritin value close to that seen in iron overload.

2.2.2. Iron excretion

Iron excretion via the kidneys is very low, and body iron is highly conserved. Renal elimination is not controlled as part of iron homeostasis or the control of excess body stores. Normally, only about 0.1 mg is lost daily in urine. The sloughing of mucosal enterocytes results in elimination of absorbed iron before it reaches the systemic circulation and accounts for the loss of 0.6mg per day into the intestinal

lumen. About 0.2-0.3 mg is lost daily from the skin. The total daily loss is equivalent to about 0.05 % of body iron content (Green *et al*, 1968). Menstrual losses are variable and may be almost as high as the total loss in non-menstruating women (FNB, 2001).

2.2.3. Biomarkers for the characterisation of iron status

Approximately 70% of body iron content is present in haemoglobin (Hb) and the Hb concentration closely reflects the amount of iron utilised in the organism. Serum iron concentration, total iron binding capacity (TIBC), serum transferrin and transferrin saturation may be used to indicate the level of iron supply to the tissues. Because serum transferrin binds iron very tightly, TIBC is essentially a measure of the amount of serum transferrin. Transferrin saturation gives the percentage of TIBC that is occupied by serum iron. Serum transferrin and TIBC are increased in iron-deficiency, during infancy and in pregnancy.

Ferritin binds and sequesters intracellular iron which is not utilised in the functional iron pool. Small amounts of ferritin are also found in the serum and can be used as an indicator of body iron stores (Halliday *et al*, 1994). Changes in serum ferritin correlate with changes in iron-stores (Skikne *et al*, 1990). Because of this reproducible relationship, ferritin is regarded as the most reliable indirect measure of iron stores over a wide range (Finch, 1994), and 1 µg ferritin/L in the serum represents 8-10 mg of storage iron (Walters *et al*, 1973; Finch *et al*, 1986).

Although it is an indicator for body iron stores serum ferritin concentration is influenced by other factors, such as the presence of inflammation or cancer (Fodinger and Sunder-Plassmann, 1999). Therefore, epidemiological studies on the relationship of serum ferritin and chronic diseases (see later) need to determine parameters for inflammation along with the ferritin values in order to exclude inflammation as a cause of elevated serum ferritin levels. Moreover, serum ferritin showed associations with a variety of parameters (Milman and Kirchhoff, 1999), such as serum fibrinogen and albumin (Danesh *et al*, 1998). The correlation between serum ferritin and alcohol consumption may partly be due to higher iron absorption, liver damage (Milman and Kirchhoff, 1996) or to an independent effect of alcohol (Meyer *et al*, 1984). Significant positive correlations have been reported between serum ferritin and a number of cardiovascular risk factors including body mass index, serum triglycerides and systolic and diastolic blood pressures; there was no association between serum ferritin and physical activity, serum total cholesterol or serum HDL cholesterol, but a negative association with cigarette smoking (Halle *et al*, 1997; Milman and Kirchhof, 1999). A negative association reported between serum ferritin and aspirin consumption may have arisen from increased occult blood losses and/or suppression of cytokine-mediated inflammation (Fleming *et al*, 2001a). There are also correlations between serum ferritin and high income and nature of employment, but not with social class (Milman and Kirchhof, 1996 and 1999). In light of the above there is a need for caution in the interpretation of associations of ferritin with chronic disease.

The serum concentration of the transferrin receptor (TfR) represents the extent to which this receptor is expressed in different parts of the body; approximately 80% of serum TfR derives from the erythroid marrow (Cook, 1999). Elevated serum TfR is directly proportional to the severity of iron storage depletion, as determined by serial phlebotomies (Skikne *et al*, 1990).

2.3. Requirements and recommended intakes

The recommended daily intakes for different groups of the population are based on the amount of ingested iron necessary for absorption of the estimated average amounts of iron lost each day.

During the first year of life the body requires approximately 260 mg of iron for metabolism and growth, i.e. 0.6-0.8 mg Fe/day, which corresponds to a dietary intake of 6-8 mg Fe/day, assuming 10% absorption. These data are the rationale for the recommendation of 1 mg Fe/kg body weight per day for children between the 4th month and the 3rd year of life (Oski, 1993). The Scientific Committee on Food 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 (SCF, 1993).

An adult male loses approx. 1 mg Fe/day, mostly from the intestine (Green *et al*, 1968), and a daily intake of approx. 10 mg Fe is needed to replace these basal losses, assuming 10% absorption, and the recommended dietary iron intake has been estimated as between 8 and 10 mg Fe/day (SCF, 1993; FNB, 2001; Arbeitsgruppe Referenzwerte für Nährstoffzufuhr, 2000).

Menstrual iron losses are below 1.6 mg Fe/day in 95% of women, which leads to an average total loss of approx. 2.5 mg Fe/day (Baynes and Bothwell, 1990). Assuming 10-20% absorption in iron

deficiency, an intake of 15-20 mg Fe/day is recommended for women of reproductive age (SCF, 1993; FNB, 2001; Arbeitsgruppe Referenzwerte für Nährstoffzufuhr, 2000).

During pregnancy, 450 mg Fe is needed to allow increased erythropoiesis, while 270-300 mg and 50-90 mg are transferred to the foetus and placenta, which gives a total extra demand of 770-840 mg. This demand corresponds to approx. 3 mg Fe/day and will be provided by an intake of 30 mg Fe/day and is the rationale for the recommended higher iron intake in pregnancy (FNB, 2001; Arbeitsgruppe Referenzwerte für Nährstoffzufuhr, 2000).

2.4. Food sources and intake

The iron content of food varies greatly, and factors such as the soil, climate conditions and processing can influence the iron content of similar foods. The results can also be affected by differences in analytical methods (Becker 1996). Foods rich in total iron include liver and offal, game and beef; cereals, cereal products and pulses also contain moderate to high levels. Poor sources of iron include milk and dairy products, whereas pork, poultry, and green vegetables contain intermediate concentrations.

Average iron intakes for populations in 8 European countries are given in Table 1. The recommended intakes for males are met by the mean values and exceeded more than two-fold at the 97.5th percentile.

3. HAZARD IDENTIFICATION

3.1. In vitro and animal toxicity data

3.1.1. In vitro data

Ferrous lactate, ferric pyrophosphate, ferric orthophosphate and sodium ferric pyrophosphate were negative in *Saccharomyces cerevisiae* strain D4 and *Salmonella typhimurium* strains TA1535, TA1537 and TA1538 without metabolic activation in plate- and suspension-tests. Ferrous sulphate was active in suspension tests with metabolic activation and ferrous gluconate was mutagenic for the indicator strain TA1538 in activation tests with primate liver preparations, but was inactive in other tests (Litton Bionetics, 1975, 1976 a and b). Iron in the presence of haemoglobin induces DNA strand breaks and oxidised bases in the human colon tumor cell line HT 29, clone 19A, as determined by single cell microelectrophoresis (comet assay) (Glei *et al*, 2002). The concentrations used in this study were similar to those found in human faeces after oral supplementation of 19 mg Fe/day (Lund *et al*, 1999).

Table 1. The daily intakes of iron in EU countries (mg/day)

	Population	N	Method	Supplements	Mean	97.5%
Austria[a]	Individual (M,F)	2488	24 h recall	Not defined	13	25
Germany[b]	Individual (M) Individual (F)	1763 2267	Computer-assisted dietary interview	Not defined	14-19 12-14	20-27 16-20
Ireland[c]	Individual (M) Individual (F) Individual (M) Individual (F)	662 717 662 717	7-day record	- - + +	14 10 14 14	26 19 29 72
Italy[d]	Household	2734	7-day record	+	13	22
Netherlands[e]	Individual (M,F)	5958	2-day record	-	11	19
Norway[f]	Individual (M) Individual (F) Individual (M) Individual (F)	1298 1374 1298 1374	Quantitative food frequency questionnaire	- - + +	12 10 13 12	23 17 27 27
Sweden[g]	Individual (M) Individual (F) Individual (M) Individual (F)	1897 2223 770 1655	8-day record	- - + +	17 13 22 18	29 21 41 37
UK[h]	Individual (M) Individual (F) Individual (M) Individual (F)	833 891 833 891	7-day record	- - + +	13 10 14 12	23 18 28 27

[a] Elmadfa *et al* (1998)
[b] Mensink *et al* (2002) - values are 90th percentile.
[c] IUNA (2001)
[d] Turrini (INRAN)
[e] Hulshof and Kruizinga (1999)
[f] Johansson and Solvoll (1999)
[g] Elmstahl *et al* (1994) - values are the median and 95th percentile. These data relate to a period when sifted flour was fortified with iron; this ceased in 1995, since when intakes from food have been on average 2-3mg/day lower.
[h] Henderson *et al* (2003) - values are the mean for total iron

3.1.2. Animal toxicity data

A problem with the use of animal data for the hazard characterisation of iron is that there are large species and strain differences in response to dietary iron overload, e.g. between rats and mice and between different mouse strains (Whittaker *et al*, 1997; Lebeau *et al*, 2002). Animal models can be useful in some circumstances, for example hepatic iron accumulation is significantly increased in HFE knockout mice (a model for hereditary haemochromatosis) (Fleming *et al*, 2001b).

Acute toxicity occurred in mice after oral doses of ferrous compounds in the range 200-650 mg Fe/kg body weight, with ferrous sulphate being the most toxic and ferrous fumarate the least toxic. Administration of 50 and 100 mg Fe/kg body weight/day for 12 weeks decreased growth rates in male rats with potency in the order ferrous sulphate > succinate > fumarate > gluconate. An emetic effect was found in cats and an irritant effect on the gastric mucosa in rabbits (Berenbaum *et al*, 1960).

The presence of iron in the lumen of the colon may act as a catalyst for the production of free radicals by bacteria (Babbs, 1990). Feeding diets containing 29 mg or 102 mg Fe/kg dry weight of the diet increased free radical generation and lipid peroxidation in rat colon and caecum, and administration of 100 mg Fe/kg diet caused a small but significant increase in cell proliferation in the rat colon (Lund *et al*, 1998). Iron acts as a tumour promotor in the mouse colon after exposure to the genotoxic carcinogens dimethylhydralazine or azoxymethane (Siegers *et al*, 1988). Dietary phytate, which forms a stable ligand complex with iron, prevented the promotion of colon carcinogenesis in experimental animals (Nelson *et al*, 1989), and protects the pig colon from lipid peroxidation caused by high iron intakes (Porres *et al*, 1999).

Oxidative damage of DNA is believed to be the cause of renal adenocarcinoma in rats treated with an intraperitoneal dose of the iron chelate of nitrilotriacetic acid (Ebina *et al*, 1986; Hamazaki *et al*, 1989a). The induction of renal cancer was associated with lipid peroxidation (Hamazaki *et al*, 1989b) and this could be reduced significantly by vitamin E administration (Hamazaki *et al*, 1988). The relevance of these observations to normal dietary forms of iron is unclear, but the observations indicate that administration of synthetic chemical forms of iron may lead to redox-related damage.

3.2. Human toxicity

3.2.1. Acute effects of iron

Acute poisoning with oral pharmaceutical-like iron preparations causes mucosal erosion in the stomach and intestine, with young children particularly at risk (Anderson, 1994). Side effects of oral iron preparations and acute iron toxicity may be mediated directly by the presence of high concentrations of 'free iron' in the gastrointestinal mucosa (Engle *et al*, 1987). Absorption of high quantities of iron causes shock symptoms due to vasodilatation, capillary leakage and heart failure, and can result in CNS and kidney damage (Anderson, 1994), and hepatocellular necrosis with bleeding disorders and hepatic failure (Engle *et al*, 1987).

Side effects of oral iron preparations at therapeutic dose levels of 50-220 mg Fe/day include nausea, vomiting, heartburn, epigastric discomfort, diarrhoea and intractable constipation (Blot *et al*, 1981; Brock *et al*, 1985; Coplin *et al*, 1991; Frykman *et al*, 1994; Ganzoni *et al*, 1974; Hallberg *et al*, 1966; Liguori, 1993; Reddaiah *et al*, 1989). The side effects in the upper gastrointestinal tract depend on the local iron concentrations and are due to irritation of the mucosa, alteration of gastrointestinal motility and/or rapid transfer of iron into the circulation (Cook *et al*, 1990).

3.2.2. Chronic effects of iron

Information about the chronic effects of iron predominantly found in adults can be divided into:

i. adverse effects reported in subjects with iron overload, and

ii. associations reported between increased iron intakes or elevated markers of body stores and specific diseases.

3.2.2.1. Adverse effects reported in subjects with iron overload

The main causes of chronic iron overload are:

i. genetic defects in the mechanisms involved in iron homeostasis, of which the most important is hereditary haemochromatosis;

ii. excessive dietary intake associated with ethanol ingestion; and

iii. iatrogenic excessive intake, for example for the treatment of haemoglobinopathies.

3.2.2.1.1. Hereditary haemochromatosis

a. Homozygotes for hereditary haemochromatosis

Homozygotes for hereditary haemochromatosis are a sub-population that is highly sensitive to iron overload. This disease is due to a defect in the HFE (formerly HLA) gene that results in increased iron absorption, and has been reported to occur in 0.3-0.5% of the Caucasian population (Powell *et al*, 1994). Recent studies on the genetic basis of haemochromatosis have identified five iron-overload conditions that are described as haemochromatosis (OMIM, 2004). There are three important HFE mutations, C282Y, H63D and S65C, of which the H63D variant is more common than the C282Y mutation in New Zealand and the USA (Burt *et al*, 1998; Steinberg *et al*, 2001). The distributions of these two mutations differ within Europe, C282Y being limited to those of north-western European ancestry, and H63D being found at allele frequencies of more than 5% in countries bordering the Mediterranean, in the Middle East, and in the Indian subcontinent (Rochette *et al*, 1999). Milman and Pedersen (2003) linked the distribution of the C282Y mutation to the spread of the Vikings, since the highest frequencies (5.1 to 9.7%) are in populations in the northern part of Europe, such as Denmark, Norway, Sweden, Faroe Islands and Iceland, intermediate allele frequencies (3.1 to 4.8%) are found in central Europe, and low allele frequencies (0 to 3.1%) occur in populations in southern Europe and the Mediterranean.

The male/female ratio among homozygotes is 1:1, but males are at greater risk of serious manifestations (Olynyk and Bacon, 1994), because women have higher iron losses via menstruation. Disorders associated with chronic iron overload include hepatomegaly, joint inflammation, diabetes mellitus, cardiomyopathy and hepatoma. Although there have been conflicting reports, recent data show that subjects homozygous for C282Y show increased incidences of arthritis and liver fibrosis (Bulaj *et al*, 2000; Ajioka and Kushner, 2003), although many homozygous subjects appear asymptomatic (Asberg *et al*, 2001; Beutler, 2003). Mutation S65C appears to result in a mild to moderate influence on iron uptake and accumulation (Holmström *et al*, 2002). The penetrance of the C282Y mutation, which is the most frequent mutation causing hereditary haemochromatosis, has recently been estimated to be in the region of 1% (Beutler *et al*, 2002) to 25% (European Haemochromatosis Consortium, 2002). A recent paper indicates that the apparent low penetrance may be due to the non-specific nature of early symptoms because about 50% of subjects homozygous for C282Y had biochemical indicators of iron overload (as defined by a transferrin saturation >52% combined with a serum ferritin >300 µg/L for men and >200 µg/L for women (Ryan *et al*, 2002).

b. Heterozygotes for hereditary haemochromatosis

The frequency of heterozygotes in Caucasians is estimated to be 13% (9.5-18%) (Nelson *et al*, 2001). In recent screening studies 5-15% were heterozygous for the HFE gene mutation C282Y (Burt *et al*, 1998; Distante *et al*, 1999; Rochette *et al*, 1999; Steinberg *et al*, 2001; Moirand *et al*, 2002; Milman and Pedersen, 2003) and 5% of patients with suspected iron overload had the S65C mutation (Holmström *et al*, 2002). Heterozygous subjects tend to show a greater incidence of biochemical indicators of high body loads of iron, such as serum ferritin, transferrin and transferrin saturation. However, iron absorption does not appear to be altered in heterozygotes (Hunt and Zeng, 2004). Overall there is inconsistent evidence of an increased risk of adverse effects associated with iron overload in heterozygous subjects (see below).

3.2.2.1.2. Secondary haemochromatosis

There have been rare anecdotal cases of patients who developed secondary haemochromatosis and died of cirrhosis, diabetes or cardiac failure after ingesting 160-1200 mg/day of therapeutic doses of iron over more than a decade (see Turnberg, 1965; Johnson, 1968; Green *et al*, 1989). Subjects with hereditary haemochromatosis may have contributed to these early case reports.

3.2.2.1.3. Bantu siderosis

Bantu siderosis is caused by excess oral iron intake with home-brewed beer fermented in iron drums in sub-Saharan Africa. Regular consumption of such beer leads to hepatic cirrhosis (Isaacson *et al*, 1961) and diabetes (Seftel *et al*, 1961). The bioavailability of such iron is high, but the alcohol intake with the beer may have contributed to the development of cirrhosis (Tsukamoto *et al*, 1995). A genetic difference, distinct from HFE-linked haemochromatosis, has been hypothesised to increase intestinal iron absorption in affected Bantu subjects (Gordeuk *et al* 1992; Mc Namara *et al*, 1998). This possibility was supported by analyses using transferrin saturation (Moyo *et al*, 1998), although the blood samples were taken from non-fasting individuals at different times of the day which makes transferrin saturation values unreliable. Iron absorption in affected Bantu subjects, who had high iron stores, was lower than that in healthy white volunteers, which argues against a genetic alteration of iron absorption and its regulation (Bothwell *et al*, 1964). Despite the presence of possible genetic differences and alcohol intake as confounders, iron is clearly the underlying causative agent of liver cirrhosis in Bantu siderosis.

3.2.2.1.4. Serum ferritin, liver iron and risk of liver fibrosis

There is a significant positive correlation between serum ferritin and liver iron concentrations (Asberg *et al*, 2001) and biochemical indicators of iron overload (a serum ferritin >300 µg/L for men and >200 µg/L for women) are equivalent to liver iron concentrations of about 158 µmol/g protein, although there was very wide variability in the relationship. Severe hepatic fibrosis (cirrhosis) is associated with hepatic iron concentrations above 400 µmol Fe/g (22.3 mg per g) dry weight (Bassett *et al*, 1986). It is difficult to relate serum ferritin to the risk of liver disease. Different degrees of fibrosis were found in 32 patients in a survey of 120 patients with haemochromatosis (Bell *et al*, 2000); the median serum ferritin levels in patients with grade 1 (n=8), grade 2 (n=12), grade 3 (n=8) and grade 4 (cirrhosis; n=5) liver disease were 858, 1441, 2764 and 4094 µg/L respectively, although there was considerable overlap between the groups, so that a clear cut-off cannot be defined.

3.2.2.2. Associations reported between increased iron intakes or elevated markers of body stores and specific diseases

3.2.2.2.1. Iron and cancer risk

Because of its potential pro-oxidant effects, there has been extensive research into possible links between iron and cancer development. Homozygotes for hereditary haemochromatosis with hepatic cirrhosis have an increased risk for hepatocellular carcinoma (Powell, 1970), and this association is supported by an increased risk in Black Africans with dietary iron-overload, after controlling for alcohol, hepatitis B and C and exposure to aflatoxin B1 (Mandishona *et al*, 1998). Evidence for a possibile increased incidence of extrahepatic malignancies in hereditary haemochromatosis is inconsistent (see Niederau *et al*, 1985; Hsing *et al*, 1995; Fracanzani *et al*, 2001).

The incidences of tumours in the upper digestive tract (Freng *et al*, 1998; Zhang *et al*, 1997) have been reported to be negatively associated with dietary iron intake.

Evidence supporting a link between high iron stores/intakes and cancer of the lower bowel includes a significant association between the recurrence of colorectal adenoma after their removal and serum ferritin concentrations, dietary iron intake and meat intake (Tseng *et al*, 2000). A case-control study from Switzerland showed that the risk of colorectal cancer was positively associated with iron intake (Levi *et al*, 2000). In agreement with these studies a nested case-control study on 105 cases with colorectal cancer and 523 controls with a follow up of 4.7 years showed a significantly increased risk for colorectal cancer associated with higher total iron intake (Kato *et al*, 1999). In another case-control study with 433 cases of rectal adenocarcinoma, iron intake normalised for meat consumption was associated with an added risk ratio of 3.2 (Deneo-Pellegrini *et al*, 1999).

A number of studies have indicated an association between markers of body iron stores and cancer of the lower bowel. In the general population Stevens *et al* (1988 and 1994) found an increased frequency of cancer in the oesophagus, the bladder, and in the colorectal area at transferrin saturation levels above 40%. In one study, the risk of colorectal cancer correlated with increasing iron stores (Knekt *et*

al, 1994), while in a different study serum iron concentration was significantly increased in individuals with colorectal cancer (Wurzelmann *et al*, 1996), although the differences were small. A case-control study of 264 men and 98 women, which was controlled for smoking, gender and alcohol consumption, showed an association between serum ferritin concentration and adenoma of the colon (Nelson *et al*, 1994). The relative risk for colorectal cancer, colonic adenoma and haematological malignancies was increased in heterozygotes for hereditary haemochromatosis; the risk for stomach cancer was also increased in this study (Nelson *et al*, 1995), which is inconsistent with an association with low iron stores in other studies (Knekt *et al*, 1994).

Hepatocellular carcinoma is a recognised risk for subjects homozygous for hereditary haemochromatosis, and is responsible for about one-third of deaths in affected homozygotes (OMIM, 2004). Hepatocellular carcinoma occurs in other situations associated with hepatic degenerative and fibrotic changes, such as alcohol-induced hepatic cirrhosis.

Overall, these results indicate the possibility of a role of luminal exposure to excessive iron in the development of colon carcinoma, but the evidence is limited and not convincing. There are few data available for other cancers and the evidence is not convincing.

3.2.2.2.2. Iron and cardiovascular risk

a. Iron stores and cardiovascular risk

In a study on risk factors for ischemic heart disease, in which 51 out of 1931 men experienced myocardial infarction (AMI), a serum ferritin concentration >200 µg/L was associated with a 2.2 fold higher risk for AMI, after adjusting for cigarette smoking, systolic blood pressure, blood glucose, HDL-cholesterol, apolipoprotein, triglycerides and leukocyte (Salonen *et al*, 1992 and 1994), with the strongest association in those with high LDL cholesterol. The study was criticised because there was not adequate control of effects of inflammation on serum ferritin and because the relationship should have been analysed in tertiles and not with a cut-off value of 200 µg/L ferritin which is in the high range of normal values (FNB, 2001; Vera-Schriftenreihe, 1995; Claeys *et al*, 2002).

Following this study, a considerable number of epidemiological investigations were performed between 1994 and 1997, involving a total of 7800 cases of coronary heart disease (CHD). A meta-analysis undertaken by Danesh and Appleby (1999) reported a combined risk ratio of 1.0 for the five studies that compared ferritin values below and above 200 µg/L in 570 CHD cases. A combined relative risk ratio of 0.9 was reported for the comparison between top and bottom tertiles for transferrin saturation, which included 6194 cases of CHD in 5 studies. The combined risk ratios were 1.0, 0.8, and 0.8 for total iron-binding capacity, serum iron and total dietary iron intake. All of these findings show the absence of a relationship between iron stores and cardiovascular risk, although the control for confounders, such as liver disease, inflammation and cancer, was insufficient in some of the studies (Sullivan, 1999; Kiechel *et al*, 1997).

The data from the NHANES II study (1976-1980) have been analysed for the risk for AMI, with an average follow-up of about 14 years, in relation to iron status as determined by serum-ferritin concentrations (Sempos *et al*, 2000). Cholesterol, blood pressure, BMI, diabetes and smoking were included as confounders, but the analysis did not control for inflammation. There was a U-shaped distribution of risk increment, with increased risk for white women and black males with the lowest and highest iron status, but the numbers in these groups were too low and statistical significance was not reached.

A recent prospective study with 177 AMI cases and 89 controls from Switzerland (Claeys *et al*, 2002) showed an increased risk for AMI in subjects with serum ferritin concentrations over 300 µg/L, but statistical significance was lost when the data were adjusted for smoking, hypertension, diabetes, BMI and total cholesterol by multiple regression analysis. The authors concluded that high serum ferritin concentrations appear to be a weak risk factor, but that the major risk factors may correlate with high iron stores thereby complicating the analysis of dependent co-variables.

A nested case-control study with 60 AMI cases and 112 controls (Klipstein-Grobusch *et al*, 1999) analysed subjects more than 55 years of age, with a mean follow up of 4 years. The cohort was chosen to assess risk factors for disabling cardiovascular, neurodegenerative and locomotor diseases. In this study the possible effects of inflammation were controlled using blood sedimentation rate and serum C-reactive protein, while iron status was assessed by serum ferritin, serum iron, serum transferrin concentration and total iron intake. Serum ferritin was not associated with a statistically significant increased risk of AMI, or with the other measures of iron status. Smoking, hypercholesterolaemia and

diabetes were confounders that increased AMI risk, while no association was found for sex, age, BMI and systolic blood pressure.

A prospective, nested, case-control study analysed the risk for heart attacks during 1982-1992 (Toumainen *et al*, 1998). The average follow-up period on 99 male cases of AMI and 98 age-matched controls was 6.4 years, and the study controlled for inflammation and liver diseases by determination of serum C-reactive protein and γ-glutamyl-transferase activity. The relative risks for AMI in the highest and middle tertiles were significantly increased compared to the lowest tertile of iron status, as determined by the TfR/ferritin ratio.

Cardiovascular disease is not part of the recognised syndrome of hereditary haemochromatosis and epidemiological studies investigating the possibility of an increased risk of cardiovascular disease in heterozygous subjects have yielded inconsistent results. Nassar *et al* (1998) reported that higher concentrations of plasma ferritin were associated with a higher risk for early-onset acute myocardial infarction (AMI), but the increased risk for heterozygous subjects was not statistically significant. A prospective cohort study from Finland, which controlled for 13 confounders (Tuomainen *et al*, 1999), reported that the risk of AMI in male heterozygotes for haemochromatosis was nearly twice as high as in controls, and that smoking increased the risk significantly. The findings of a retrospective study (Franco *et al*, 1998) did not support the hypothesis of increased cardiovascular risk because the fraction of heterozygotes with late-onset atherosclerosis was lower than in corresponding controls; however the cases had been selected on the basis of pre-existing atherosclerosis, and the results may have been influenced by a survival bias. Roest *et al* (1999) found nearly twice as many heterozygous women among cardiovascular cases than among controls (7.2% vs. 4.1%), but the difference was most apparent for cerebrovascular death rather than for AMI. A more recent case-control study in middle aged men and women involving 243 cases and 535 controls found a slightly increased risk of coronary heart disease in heterozygotes for C282Y mutation (RR: 1.6; 95% CI, 0.9-2.9). The association was statistically significant (RR 2.70, 95% CI: 1.2-6.1) after adjustment for age, gender, smoking, diabetes, hypertension, LDL and HDL cholesterol and triglycerides although the estimates were based on small numbers subjects in some comparisons (Rasmussen *et al*, 2001). In contrast no association was found between the severity of coronary atherosclerosis and the presence of C282Y and H63D mutations in heterozygotes in a prospective case-control study on 174 patients with angiographically documented stenosis or history of AMI, 187 controls and 142 blood donors (Battiloro *et al*, 2000). Although H63D carriers, but not C282Y carriers, were more numerous among subjects with atherosclerotic plaques in the AXA study (Hetet *et al*, 2001), analysis of the ECTIM and GENIC studies found no significant impact of H63D or C282Y mutations on the risk of myocardial and brain infarction. A prospective case control study from Switzerland with 177 patients surviving AMI found no direct association between haemochromatosis mutations and AMI events (Claeys *et al*, 2002). A recent study investigated ferritin levels and C282Y mutations in 546 patients with angiographically confirmed severe coronary atherosclerotic disease and 303 individuals mostly with valvular disease, who were shown angiographically to be free of coronary atherosclerosis (Bozzini *et al*, 2002). There was no difference in serum ferritin levels after adjustment for C-reactive protein and sex, and the incidences of heterozygotes for C282Y were 4.8% in those with severe coronary atherosclerotic disease and 6.6% in those without coronary atherosclerosis. The authors concluded that their data do not support a role for iron stores as predictors of coronary atherosclerotic disease.

Most epidemiological studies have correlated the iron-related health risk directly to the cumulative iron stores, the determination of which relies on proxy parameters. Some of these parameters, such as transferrin saturation and serum iron, represent the turnover of the haemoglobin iron pool rather than the size of iron stores (Bothwell *et al*, 1979). These parameters together with the TIBC showed a low correlation to cardiovascular risk (Klipstein-Grobusch *et al*, 1999). Serum ferritin levels represent iron stores more directly, but this parameter may be confounded by inflammatory processes and other influences. Therefore, the better controlled epidemiological studies used parameters, such as serum C-reactive protein, blood sedimentation rate or liver enzymes, to control for inflammation (Klipstein-Grobusch *et al*, 1999). Serum TfR is less confounded by inflammation, and although this parameter responds better to deficiency states than to overload, it can be used to control increased ferritin values for iron deficiency (see Tuomainen *et al*, 1998). The studies of Tuomainen *et al* (1998) and Klipstein-Grobusch *et al* (1999) appear to be the best controlled, and these do not show a clear or consistent relationship between iron status and cardiovascular risk.

Overall, epidemiological data on associations between iron status and increased risks of cardiovascular disease are contradictory and unconvincing at the present time.

b. Iron intake and cardiovascular risk

Data on correlations between dietary iron intake and the risk of AMI are difficult to interpret. In dietary studies it is difficult to define the contribution of iron intake *per se* to any reported associations, because iron intake, especially haem iron, usually correlates with the increased intakes of other important dietary risk factors.

Dietary iron intake, estimated by a 4-day food recording, was associated with a 5-8% increase in the risk of AMI for each milligram of daily iron intake (Salonen *et al*, 1992; Tuomainen *et al*, 1998), but there must be doubts about the validity of a short-term diary to represent long-term intake and the contributions of confounding dietary factors. Assessment of total habitual dietary iron intake during the previous year showed no significant variations in coronary mortality in quintiles of dietary iron intake (Reunanen *et al*, 1995). There was no association between total iron and the risk for AMI in a study that used a semiquantitative food questionnaire to assess habitual food intake over the past year, although haem intake, estimated as 40% of total iron intake, was significantly associated with myocardial risk (Klipstein-Grobusch *et al*, 1999). Results from a food frequency questionnaire in a US study showed a decreased risk for AMI at high total iron intake, but a significantly increased the risk of AMI at high haem iron intakes (Ascherio *et al*, 1994). Such studies do not provide convincing evidence of a causal relationship between iron intake and risk of AMI.

If iron-related oxidative stress is a contributory factor for increased cardiovascular risk, the overall risk will depend also on other pro-oxidative factors and the strength of antioxidative defence mechanisms. Variations in such interfering factors may have contributed to the inconsistent outcomes of the different epidemiological trials on iron and cardiovascular risk, and could either have masked a real effect or generated a spurious apparent effect. Overall the epidemiological data on iron and cardiovascular risk remain controversial (Schümann *et al*, 2002) and there is no consistent evidence on this topic.

3.2.2.2.3. Iron and risk of type II diabetes mellitus

Recent studies have reported a positive association between serum ferritin concentration, haem iron intake from red meat sources and the risk of developing type II diabetes mellitus (Jiang *et al*, 2004a and 2004b). One study was a prospective nested case-control study within the Nurses' Health Study. From the cohort of 32826 women, 698 developed diabetes mellitus during 10 years of follow-up (Jiang *et al*, 2004a). A logistic regression analysis of the data corrected for potential confounding factors showed that there was an increasing risk across the quintiles of ferritin concentration, which was not altered by correction for markers of inflammation. The second study was a prospective cohort study among American health professionals (40-75 years old males; n=38.394) (Jiang *et al*, 2004b). After adjustment for age, BMI and other diabetes risk factors, intake of haem iron from red meat sources, but not total iron intake, was found to be associated with increased diabetes risk across the quintiles of haem iron intake (RR=1.63; P for trend <0.001). These studies do not provide convincing evidence of a causal relationship between iron intake or stores and type II diabetes.

4. DOSE-RESPONSE ASSESSMENT

4.1. Acute oral iron intoxication

There have been numerous reports of accidental poisoning with medicinal iron, especially in young children (FNB, 2001). An acute oral dose of 60 mg Fe/kg body weight can be lethal; characteristically poisoned subjects initially show nausea, vomiting and lethargy or coma, then an asymptomatic period for up to 24 hours, which is followed by gastrointestinal perforation, coma, convulsions, cardiovascular collapse and hepatic and renal failure (Merrill *et al*, 2001). Oral doses below about 10-20 mg Fe/kg body weight do not cause acute systemic toxicity (FNB, 2001).

4.2. Gastrointestinal effects of oral iron preparations

The side effects of oral iron preparations increase with increase in dosage, but there are fewer side effects with slow delivery systems or if the iron is taken with food (Brock *et al*, 1985; Reddaiah *et al*, 1989). The adverse gastrointestinal effects are related to the concentration of iron in the intestinal lumen (Cook *et al*, 1990).

A daily dose of 50 mg of iron produced a higher incidence of gastrointestinal effects in subjects given conventional ferrous sulphate compared with subjects given the same amount in a wax-matrix (Brock *et al*, 1985), and also in subjects given ferrous sulphate compared with subjects given the same amount

of iron as bis-glycino iron (Coplin *et al*, 1991). Neither of these studies included a placebo group, and therefore the association with iron is based on different responses to different preparations. A higher incidence of side effects was reported in subjects given 60 mg of iron as iron fumarate daily compared with placebo; daily doses of 120 mg of iron as fumarate for 8 weeks given to 19 young women in a double blind cross-over study resulted in gastrointestinal effects in 5 subjects while receiving iron, compared with 2 while taking placebo (Frykman *et al*, 1994).

Overall a dose level of 50 mg Fe/day (Brock *et al*, 1985) or 60 mg Fe/day (Frykman *et al*, 1994) appears to be the LOAEL for transient gastrointestinal side effects in humans. This hazard applies to supplemental forms of non-haem iron.

4.3. Iron overload

Iron overload with clinical symptoms has been found in adult subjects homozygous for hereditary haemochromatosis, those under long-term, high-dose medical treatment with iron, and those given repeated blood transfusions.

A prolonged intake of 160-1200 mg Fe/day in the form of a highly absorbable therapeutic iron preparation (Green *et al*, 1989) has been shown to cause secondary haemochromatosis and may result in death. The reports are scarce and anecdotal, and the possible contribution of hereditary haemochromatosis was not excluded in the older reports.

The point at which an elevated serum ferritin level becomes associated with an increased risk of adverse effects is not known, although serum ferritin values above 200 µg/L for women and 300 µg/L for men have been considered as indicative of biochemical iron overload (Holmström *et al*, 2002). Data for a small number of patients with haemochromatosis suggest that grade 1 hepatic fibrosis may develop at ferritin levels of above approximately 500 µg/L (Bell *et al*, 2000). Although a proportion of the population have serum ferritin levels indicative of biochemical iron overload, the risk of symptomatic clinical iron overload in the general population, including those heterozygous for hereditary haemochromatosis, is considered to be low.

Serum ferritin levels were not increased by fortification giving an additional intake of 7.5 mg Fe/day for 2 years in iron-replete male subjects (Ballot *et al*, 1989) or following the addition of 10 mg Fe/day as ferrous sulphate to the food of a healthy male subject for 500 days (Sayers *et al*, 1994). These studies indicate no effects of 7.5-10 mg Fe/day in addition to normal dietary iron intake of approximately 10 mg Fe/day. Serum ferritin was increased in the elderly after intake of an additional 30 mg Fe/day or more as supplements (Fleming *et al*, 2002). Theoretical calculations of the accumulation of iron in a fertile woman given different daily intakes (Borch-Iohnsen and Petersson Grawe, 1995) indicated that a daily intake of 60 mg for 5 years could lead to a serum ferritin value close to that seen in iron overload. Similar calculations were not made for iron replete males.

Chronic excess intake of highly available iron with beer was observed in Bantu siderosis, with intake levels between 50-100 mg Fe/day associated with liver cirrhosis and diabetes (Bothwell *et al*, 1964). There is little doubt that iron intake was an essential causative factor for this disease. A threshold of 5 mg Fe/g wet weight of liver was established for the development of cirrhosis (Bothwell and Bradlow, 1960), which is almost identical to the threshold for iron-induced cirrhosis in hereditary haemochromatosis (Basset *et al*, 1986). The hepatic and pancreatic damage in Bantu siderosis may be confounded by chronic alcohol intake and possibly by a genetic disorder (see above), and these observations cannot be used as a basis for the derivation of an UL.

Overall there is a poor correlation between iron intake and biochemical indicators of iron status, between biochemical indicators and actual body stores, or between body stores and adverse clinical effects. In consequence, the dose-response relationship between iron intake and the adverse effects of iron accumulation has not been defined adequately.

4.4. Cancer

Studies have reported associations between iron intake, or iron stores, and cancer of the gastrointestinal tract, especially the lower bowel. However, the reported associations do not provide a consistent body of evidence, and do not demonstrate causality between high iron intakes and cancer development.

4.5. Cardiovascular disease and diabetes mellitus

Epidemiological data on associations between iron status and increased risks of cardiovascular disease are contradictory and unconvincing at the present time. Two epidemiology studies have reported an association between indicators of body iron intake or stores and the incidence of diabetes mellitus. These studies do not provide convincing evidence of a causal relationship between iron intake or stores and type II diabetes.

CONCLUSIONS AND RECOMMENDATIONS

1. DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

The Panel considered that the adverse GI effects which have been reported after short-term oral dosage at 50-60 mg daily of supplemental non-haem iron preparations are not a suitable basis to establish an UL for iron from all sources.
An UL cannot be established for iron based on iron overload due to a poor correlation between iron intake and biochemical indicators of iron status, between biochemical indicators and actual body stores, or between body stores and adverse effects.

An UL cannot be established for iron (including haem iron) based on increased risk of chronic diseases such as cardiovascular disease, diabetes and cancer, due to the lack of convincing evidence of a causal relationship between iron intake or stores and chronic diseases.

2. RISK CHARACTERISATION

Adverse gastrointestinal effects (i.e. nausea, epigastric discomfort, constipation) have been reported after short-term oral dosage at 50-60 mg daily of supplemental non-haem iron preparations, particularly if taken without food.

The limited data indicate that supplemental intakes of non-haem iron at levels of 30 mg/day or more (in addition to iron intake from food) can be associated with indicators of high iron stores (e.g elevated serum ferritin) in older adults. However, the point at which an elevated serum ferritin level becomes associated with an increased risk of adverse effects (such as liver fibrosis) is not known. Furthermore, epidemiological associations between high iron intake and/or stores and increased risk of chronic diseases such as cardiovascular disease, type II diabetes and cancer of the gastrointestinal tract are conflicting and do not provide convincing evidence of a causal relationship between iron intake or stores and such chronic diseases.

Based on estimates of current iron intakes in European countries, the risk of adverse effects from high iron intake from food sources, including fortified foods in some countries, but excluding supplements, is considered to be low for the population as a whole. However, intake from food supplements in men and postmenopausal women may increase the proportion of the population likely to develop biochemical indicators of high iron stores. Some groups at special risk for poor iron status, such as menstruating women or children, could benefit from additional iron intake and/or improved availability of dietary iron.

A particularly sensitive subpopulation (up to 0.5% of the population) are homozygotes for hereditary haemochromatosis, who are susceptible to iron overload even at normal dietary iron intakes. Such individuals should avoid iron-supplements and highly iron-fortified foods. The majority of homozygotes are not diagnosed or identified, and they are not aware of their greater susceptibility until sufficient iron has accumulated to produce adverse effects.

3. RECOMMENDATIONS FOR FURTHER WORK

1. Despite the numerous studies that have used biochemical indicators of iron status, there remains a need for data on the relationship between dietary intake of iron, as haem iron, non-haem iron and supplements, and iron status and also between iron status and iron stores in different age groups.

2. The available data on associations between iron status and disease are inconsistent and complex. There is a need for studies defining the relationships between dietary iron intake, as haem iron, non-haem iron and supplements, and diseases such as cancer and cardiovascular disease.

4. REFERENCES

Ajioka RS and Kushner JP (2003). Rebuttal to Beutler. Blood 101: 3358.

Anderson AC (1994). Iron poisoning in children. Curr Opin Pediatr 6: 289-294.

Arbeitsgruppe: Referenzwerte für die Nährstoffzufuhr (2000). Umschau Braus Verlag, Frankfurt, pp174-179.

Asberg A, Hveem K, Thorstensen K, Ellekjter E, Kannelonning K, Fjosne U, Halvorsen TB, Smethurst HB, Sagen E, Bjerve KS (2001). Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,238 persons. Scand J Gastroenterol 36: 1108-1115.

Ascherio A, Willett WC, Rimm EB, Giovannucci EL, Stampfer MJ (1994). Dietary iron intake and risk of coronary disease among men. Circulation 89: 969-974.

Babbs CF (1990). Free radicals and the etiology of colon cancer. Free Radic Biol Med 8: 191-200.

Bacon BR, Tavill AS, Brittenham GM, Park CH, Recknagel RO (1983). Hepatic lipid peroxidation in vivo in rats with chronic iron overload. J Clin Invest 71: 429-239.

Ballot DE, MacPhail AP, Bothwell TH, Gillooly M, Mayet FG (1989). Fortification of curry powder with NaFe(III) EDTA in an iron-deficient population: Report of a controlled iron-fortification trial. Am J Clin Nutr 49: 156-161.

Barrett JFR, Whittaker PG, Eilliams JG, Lind T (1994). Absorption of non-haem iron from food during normal pregnancy. Br Med J 309: 79-82.

Bassett ML, Halliday JW, Powell LW (1986). Value of hepatic iron measurements in early haemochromatosis and determination of the critical iron level associated with fibrosis. Hepatology 6: 24-29.

Battiloro E, Ombres D, Pascale E, D´Ambrosio E, Verna R, Arca M (2000). Haemochromatosis gene mutations and risk of coronary artery disease. Eur J Hum Gen 8: 389-392.

Baynes RD and Bothwell TH (1990). Iron deficiency. Ann Rev Nutr 10: 133-148.

Beard JL and Dawson HD (1997). Iron. In: Handbook of nutritionally essential elements, O'Dell BL, Sunde RA, eds., Marcel Dekker Inc, New York, pp. 275-334.

Bell H, Berg JP, Undlien DE, Distante S, Raknerud N, Heier HE, Try K, Thomassen Y, Haug E, Raha-Chowdhury R, Thorsby E (2000). The clinical expression of hemochromatosis in Olso, Norway. Excessive iron intake may lead to secondary hemochromatosis even in HFE C282Y mutation negative subjects. Scan J Gastroenterol 35: 1301-1307.

Berenbaum MC, Child KJ, Davis B, Sharpe HM, Tomich EG (1960). Animal and human studies on ferrous fumarate, an oral hematinic. Blood 15: 540-550.

Beutler E (2003). Penetrance in hereditary hemochromatosis. The *HFE* Cys282Tyr mutation as a necessary but not sufficient cause of clinical hereditary hemochromatosis. Blood 101: 3347-3350.

Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T (2002). Penetrance of 845G – A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 359: 211-218.

Biemond P, Eijk HGN, Swaak AJG, Koster JF (1984). Iron mobilization from ferritin by superoxide derived from stimulated PMN leukocytes. J Clin Invest 73: 1576-1579.

Blot I, Papiernik E, Kaltwasser JP, Werner E, Tchernia G (1981). Influence of routine administration of folic acid and iron during pregnancy. Gynecol Obstet Invest 12: 294-304.

Borch-Iohnsen B and Ptersson Grawe K (1995). In Risk evaluation of essential trace elements. Ed. Oskarsson A. Nordic Council of Ministers, Copenhagen, 1995, pp 67-117.

Bothwell TH and Bradlow BA (1960). Siderosis in the Bantu. A combined histopathological and chemical study. Arch Path 70: 279-292.

Bothwell TH, Seftel H, Jacobs P, Torrance JD, Baumslag N (1964). Iron overload in Bantu subjects; studies on the availability of iron in Bantu beer. Am J Clin Nutr 14: 47-51.

Bothwell TH, Charlton RW, Cook JD, Finch CA (1979). Internal iron kinetics In: Iron metabolism in man. Blackwell Scientific Publishers, Oxford, London, pp 327-353.

Bozzini C, Girelli D, Tinazzi E, Olivieri O, Stranieri C, Bassi A, Trabetti E, Faccini G, Pignatti PF, Corrocher R (2002). Biochemical and genetic markers of iron status and the risk of coronary artery disease: an angiographically-based study. Clin Chem 48: 622-628.

Bridle KR, Frazer DM, Wilkins SJ, Dixon JL, Purdie DM, Crawford DH, Subramaniam VN, Powell LW, Anderson GJ, Ramm GA (2003). Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. Lancet 361: 669-673.

Britton RS, Tavill AS, Bacon BR (1994). Mechanisms of iron toxicity. In: Brock JH, Halliday JW, Pippard MJ, Powel LW, eds, Iron metabolism in health and disease. Philadelphia, WB Saunders, pp 211-351.

Brock C, Curry H, Hama C, Knipfer M, Taylor L (1985). Adverse effects of iron supplementation: A comparative trial of wax-matrix iron preparation and conventional ferrous sulfate tablets. Clin Ther 7: 568-573.