1 **DRAFT SCIENTIFIC OPINION**

2 Scientific Opinion on Dietary Reference Values for copper[1]

3 **EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2,3]**

4 European Food Safety Authority (EFSA), Parma, Italy

5 **ABSTRACT**

6 Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies
7 (NDA) derived Dietary Reference Values (DRVs) for copper. Due to the absence of appropriate biomarkers of
8 copper status and the limitations of available balance studies, the Panel was unable to derive Average
9 Requirements (ARs) and Population Reference Intakes (PRIs). Hence, Adequate Intakes (AIs) were derived
10 based on mean observed intakes in several European Union (EU) countries, given that there is no evidence of
11 overt copper deficiency in the European population. Data from balance studies were used as supportive evidence.
12 For adults, AIs of 1.6 mg/day for men and 1.3 mg/day for women are proposed. For children, AIs are 0.7 mg/day
13 for children aged 1 to < 3 years, 1 mg/day for children aged 3 to < 10 years, and 1.3 and 1.1 mg/day for boys and
14 girls aged 10 to < 18 years, respectively. For infants aged 7–11 months, based on mean observed intakes in four
15 EU countries, an AI of 0.4 mg/day is proposed, which is supported by upward extrapolation of estimated copper
16 intake in exclusively breast-fed infants. For pregnant and lactating women, an increment of 0.2 mg/day is
17 estimated to cover the amount of copper deposited in the fetus and the placenta over the course of pregnancy and
18 in anticipation of the needs for lactation, and the amount of copper secreted with breast milk, respectively. Thus,
19 for pregnant and lactating women, the Panel derived an AI of 1.5 mg/day.

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22 **KEY WORDS**

23 copper, balance, observed intake, Adequate Intake, Dietary Reference Value

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[1] On request from the European Commission, Question No EFSA-Q-2011-01210, endorsed for public consultation on 12 June 2015.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Dietary Reference Values for Minerals: Peter Aggett, Carlo Agostoni, Susan Fairweather-Tait, Marianne Geleijnse, Ambroise Martin, Harry McArdle, Androniki Naska, Hildegard Przyrembel and Alfonso Siani for the preparatory work on this scientific opinion.

Suggested citation: EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 20XX. Draft Scientific opinion on Dietary Reference Values for copper. EFSA Journal 20YY;volume(issue):NNNN, 52 pp. doi:10.2903/j.efsa.20YY.NNNN
Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on Dietary Reference Values (DRVs) for the European population, including copper.

Copper is an essential micronutrient required for electron transfer processes. It is a central component of many enzymes, including those involved in neurotransmitter synthesis, in energy metabolism and in collagen and elastin cross-linking.

The main food group contributing to the copper intake of all population groups except infants was grains and grain-based products. Another important contributor to copper intake was the food group meat and meat products. The food groups starchy roots or tubers and products thereof and sugar plants; coffee, tea and infusions; and fish, seafood, amphibians, reptiles and invertebrates were also important contributors, the latter only in certain European populations.

Based on balance studies and other studies, the Panel considered that copper absorption from the diet is around 50 % for all age and life-stage groups.

The primary site of copper absorption is the upper small intestine. Uptake is through a carrier protein, Ctr1, and once in the cell, the copper is directed towards its target via one of a series of chaperone proteins that ensure the metal is present in a non-toxic form. In the gut, the major pathway of secretion is via a Cu-ATPase, ATP7A. In the portal circulation, copper is bound either to histidine, albumin or possibly transcuprein, and transported to the liver, where it is incorporated into ceruloplasmin, which is then secreted into the systemic circulation. It is taken up into the liver through Ctr1, and if it is not incorporated into ceruloplasmin, it is stored as metallothionein. Excess copper is excreted in bile after transport across the apical membrane of the hepatocytes via another ATPase, ATP7B. This copper is not reabsorbed. In humans, between 80–95 % of the copper in plasma is ceruloplasmin, with the remainder being a low molecular weight form. It is not certain which of these two pools, ceruloplasmin or low-molecular weight copper complexes, makes the major contribution to uptake by organs other than the liver, though it is more likely to be low molecular weight copper than ceruloplasmin, which plays a major role in release of iron from the liver.

If the dietary supply of copper is less than adequate, the body upregulates transfer systems to make more copper available. If these are not able to rectify the problem, then copper deficiency results. Clinical symptoms are not common in humans, and generally are seen as a consequence of mutations in the genes involved in copper metabolism. Symptoms of copper deficiency include anaemia that is refractory to iron supplementation, neurological defects and cutis laxa (“floppy” skin). There are also changes in hair colour and texture, and an increased risk of aneurysm as a consequence of impaired collagen and elastin synthesis.

The Panel noted that there are no biomarkers of copper status that are sufficiently robust, sensitive and specific to be used for deriving requirements for copper. The Panel also considered whether health outcomes can be used to derive DRVs for copper. However, it was concluded that the limited evidence available on copper intake and cardiovascular disease-related outcomes and cancer cannot be used for setting DRVs for copper.

There have been several balance studies examining the relationship between copper intake and losses in men, but few in women and children. Studies differed with regard to experimental conditions, and many studies had limitations and their results varied. Nevertheless, the Panel considered that they may be used, in conjunction with data on observed intakes in the European Union, to inform the setting of DRVs for copper.

The Panel decided to derive Adequate Intakes (AIs) based on observed intakes in several EU countries. Mean copper intakes in eight EU countries range from 1.47 to 1.67 mg/day in men and from

1.20 to 1.44 mg/day in non-pregnant women. The Panel noted that midpoints of ranges for intake estimates in three age groups of adults and in both sexes are in good agreement with medians, for the respective sex and age groups, of the average intakes estimated per survey. The Panel noted that there is, at present, insufficient evidence to set different DRVs according to age in adults, but decided to set different AI values for women and men since intakes are lower for women. For men, based on observed intakes and taking into account that zero copper balance was reported at a copper intake of approximately 1.6 mg/day in men, the Panel proposed an AI of 1.6 mg/day. For women, based on observed intakes, the Panel proposed an AI of 1.3 mg/day.

For infants aged 7–11 months, based on results from four surveys in infants, the Panel proposed an AI of 0.4 mg/day. The Panel noted that upward extrapolation by allometric scaling of estimated copper intake in exclusively breast-fed infants aged 0–6 months results in an estimated intake at 7–11 months of 0.36 mg/day, which supports the AI of 0.4 mg/day.

For boys and girls aged 1 to < 3 years, considering the absence of a strong basis for a distinct value according to sex and the distribution of observed mean intakes of 0.60–0.86 mg/day in boys and 0.57–0.94 mg/day in girls, the Panel selected the midpoint of average intakes and set an AI of 0.7 mg/day. In children aged 3 to < 10 years, mean observed intakes range from 0.92 to 1.44 mg/day in boys and from 0.82 to 1.30 mg/day in girls. The Panel selected the midpoint of average intakes and set an AI of 1.0 mg/day for boys and girls aged 3 to < 10 years. In children aged 10 to < 18 years, mean observed intakes range from 1.16 to 1.59 mg/day in boys and from 0.98 to 1.41 mg/day in girls. Considering the rather large differences in intakes of boys and girls, the Panel decided to set separate AI values. Taking into account the distribution of observed average intakes, the Panel proposed an AI of 1.3 mg/day for boys and of 1.1 mg/day for girls aged 10 to < 18 years.

In pregnancy, taking into account the requirement for the developing fetus and its placenta, the additional requirement for copper was calculated to be 0.06 mg/day. Considering a fractional copper absorption of 50 %, and in anticipation of copper requirements for lactation, the Panel proposed that the AI of non-pregnant women be increased by 0.2 mg/day during pregnancy.

For lactation, taking into account a fractional absorption of copper of about 50 %, an increment of 0.56 mg/day would be required to compensate for copper losses in breast milk. The Panel assumed that this can be mitigated in part by the increased AI in pregnancy. Thus, the Panel proposed that the AI of non-pregnant women be increased by 0.2 mg/day during lactation.

101 **TABLE OF CONTENTS**

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and, if necessary, to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on the nutrient and energy intakes for the European Community.[4] The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context the EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1)(a) and Article 31 of Regulation No. 178/2002,[5] the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on population reference intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, *trans* fatty acids;

[4] Scientific Committee for Food, Nutrient and energy intakes for the European Community, Reports of the Scientific Committee for Food 31st series, Office for Official Publication of the European Communities, Luxembourg, 1993.

[5] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1-24.

- Protein;
- Dietary fibre.

Following on from the first part of the task, EFSA is asked to advise on population reference intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

ASSESSMENT

1. Introduction

In 1993, the Scientific Committee for Food adopted an opinion on the nutrient and energy intakes for the European Community (SCF, 1993). For copper, the SCF (1993) set an Average Requirement (AR) of 0.8 mg/day and a Population Reference Intake (PRI) of 1.1 mg/day for adults. An additional intake of 0.3 mg/day was advised for lactating women, but no extra intake was set for pregnant women. A PRI of 0.3 mg/day for infants aged 6–11 months was set and for children PRIs were interpolated between the PRIs for infants and adults. A Lowest Threshold Intake was also set at 0.6 mg/day for adults.

2. Definition/category

2.1. Chemistry

Copper is a transition metal and has the atomic number 29 and a standard atomic weight of 63.5 Da. There are two stable copper isotopes, ^{63}Cu and ^{65}Cu (abundance ratio 70 and 30 %, respectively (Rosman and Taylor, 1998)). Copper plays a significant role in biology through its capacity to have two oxidation states; it mainly exists either as Cu(I) or Cu(II) and this ability to gain or lose an electron underpins its role in energy transfer processes in biological systems. It also has 27 radioactive isotopes; two of which, ^{64}Cu and ^{67}Cu, with half-lives of 12.7 hours and 61.8 hours, respectively, have been used in biological studies.

Copper in biological systems is rarely found as a free ion, but is normally chelated to amino acids, primarily histidine or, in mammals, to proteins with imidazole residues, such as albumin. The interaction with amino acids is significant in terms of the biology of copper, since it is probably one of the major factors in determining uptake and processing of the metal. In most mammalian plasma, including humans, copper binds to the N-terminal three amino acids of albumin. This binding is critically dependent on the ante-penultimate amino acid being histidine.

2.2. Function of copper

2.2.1. Biochemical functions

Copper serves as an electron donor and acceptor, in a similar chemical reaction to that for iron. It is part of the catalytic centre in many enzymes, especially those involved in neurotransmitter synthesis. There are about twelve cupro-enzymes in humans. Table 1 gives a partial list of the enzymes and their role in metabolism, giving an idea of the spectrum of functions served by cupro-enzymes.

Table 1: Examples of copper-dependent enzymes

Enzyme	Role in metabolism
Amine oxidase	Deamination of primary amines
Ceruloplasmin, GPI-ceruloplasmin, hephaestin, zyklopen (multi-copper ferroxidases)	Iron metabolism ferroxidases
Cu/Zn superoxide dismutase (SOD)	Superoxide dismutation
Cytochrome c oxidase	Electron transport, energy metabolism
Dopamine B-monooxygenase	Dopamine to norepinephrine conversion
Lysyl oxidase	Collagen and elastin cross linking
Peptidylglycine α-amidating monooxygenase	α-amidation of neuropeptides
Tyrosinase	Melanin synthesis

GPI, glycosylphosphatidylinositol

2.2.2. Health consequences of deficiency and excess

2.2.2.1. Deficiency

The effect of copper deficiency on haematological function was first recognised in the early 20th century, when anaemia that was refractive to iron supplementation was shown to be cured by giving ashed food containing copper (see Fox (2003) for a comprehensive review of this early literature). Sheep in Western Australia were also shown to develop a disorder called swayback as a consequence of copper deficiency. The symptoms included neurological deficit, "floppy" (lax) skin, and muscular weakness. In addition, the wool was changed in a very characteristic fashion. The similarity to the hair changes in babies born with an X-linked disorder called Menkes disease was noted by Danks and colleagues in Melbourne, who showed that Menkes was indeed induced by functional copper deficiency (Danks et al., 1972).

Menkes disease is an X-linked recessive disorder of ATP7A, one of two copper pumps involved in transferring copper across cell membranes (Mercer et al., 1993; Vulpe et al., 1993). In the case of Menkes disease, the copper is not taken across the gut membrane, so the deficiency is throughout the body. Boys born with Menkes have neurological deficits, very lax skin (cutis laxa) and "kinky" hair – hence the name kinky hair syndrome (Danks et al., 1972). Importantly, the lamina propria of the large vessels is disrupted, and the boys will often die early from aortic aneurysms. Attempts to rectify the disorder by injecting copper, either in babies at term, induced early or directly into the cerebrospinal fluid, have had limited success (Kaler et al., 1996; Kaler, 1998; Kaler, 2014). There are many different mutations recorded in ATP7A, some of which do not have lethal consequences, such as those that cause occipital horn syndrome, and the functions of the different parts of the enzyme have been deduced from the phenotype demonstrated from these mutations (Proud et al., 1996).

Given the wide range of enzymes that use copper as a co-factor (Section 2.2.1), the symptoms of copper deficiency are diverse. They include normocytic and hypochromic anaemia, hypercholesterolaemia, skin and hair hypopigmentation, leukopenia, neutropenia, myelodysplasia and, in the majority of patients, neurological findings, most commonly due to neuromyelopathy (human swayback). Osteoporosis, scoliosis and scorbutic-like changes have also been reported in copper-deficient infants and children (Williams, 1983; Danks, 1988; Lukasewycz and Prohaska, 1990; Prohaska, 1990; Klevay, 2000).

Further, there is evidence that copper deficiency is associated with alterations in immune function (Kelley et al., 1995; Turnlund et al., 2004) and possibly bone function (Baker et al., 1999a), although in a further study Baker et al. (1999b) failed to confirm their earlier results. However, all these symptoms can occur in other diseases, making it very difficult to identify copper deficiency from the phenotype.

2.2.2.2. Excess

Under normal circumstances, copper homeostasis ensures that copper overload does not occur. The SCF (2003) set a No Observed Adverse Effect Level (NOAEL) of 10 mg/day on the basis of a supplementation study lasting 12 weeks, which showed the absence of adverse effects on liver function at this dose (Pratt et al., 1985). Using an uncertainty factor of two, a Tolerable Upper Intake Level (UL) of 5 mg/day was established for adults, but not for pregnant and lactating women due to the absence of adequate data. For children, the UL of adults was extrapolated based on body weight.

An excess of copper has been recorded and shown to cause problems only under certain specific conditions, notably genetic disorders such as Wilson disease (Tanzi et al., 1993; Tao et al., 2003). Wilson disease is caused by a mutation in ATP7B, a transport protein similar to ATP7A, but expressed in different tissues (Tanzi et al., 1993). In Wilson disease, the protein is defective in the liver, and excretion is inhibited so that copper accumulates initially in the liver, followed by the brain, heart, kidney and eyes. Over time, hepatic damage results in cirrhosis, and can also develop into fulminant liver disease. The latter occurs following the breakdown of the copper storage systems, with

subsequent release of copper into the blood, and hence a positive feedback of cell damage and copper release. Copper toxicosis has been recorded in dogs, but not in humans, and is caused by mutations in a protein termed MURR1, which interacts directly with ATP7B (Tao et al., 2003). To date, no equivalent mutations have been detected in humans.

Excessive copper accumulation has also been recorded in Indian Childhood cirrhosis (Tanner, 1998). Milk heated in copper vessels was found to contain very high amounts of copper. Some children accumulated large amounts of copper in their liver and suffered from symptoms of copper overload. A similar disorder was recorded in families from the Austrian Tyrol (Muller et al., 1998). The Tyrolean cirrhosis was mapped to one particular village and to one progenitor in that village. More recently, however, a comprehensive study of Indian Childhood cirrhosis has concluded that, while copper may have some role to play in the development of the disorder, it may also have a genetic predisposition (Nayak and Chitale, 2013).

2.3. Physiology and metabolism

2.3.1. Intestinal absorption

Copper in the lumen of the gut is largely bound to amino acids. It is taken up across the apical membrane of the enterocyte, probably through a protein called Ctr1 (see Figure 1 in Section 2.3.3). Whether it is absorbed as Cu(II) or Cu(I) is not clear. Once in the cell, the copper is directed, through unknown mechanisms, to one of a family of copper chaperones. Atox1 carries the copper to either ATP7A or ATP7B, depending on the tissue. In the gut, it is ATP7A and, in patients with Menkes disease, where ATP7A is defective, the copper will accumulate within the gut cell (Schaefer and Gitlin, 1999).

There is a limited number of studies on copper absorption in humans. Most of them measured apparent absorption only, which may differ from true absorption because endogenous losses are not considered.

In two studies in adults, faecal excretion of absorbed copper was taken into account to estimate true copper absorption from dietary copper intakes ranging from 0.7 to 6 mg/day, and true copper absorption ranged from 45 to 49 % (Harvey et al., 2003; Harvey et al., 2005).

The effect of diet composition on copper absorption has been examined, though care must be exercised in interpretation of results since apparent rather than true absorption was generally measured. In adults, apparent copper absorption (in %) tended to be higher with omnivorous diets than with lacto-ovo-vegetarian diets (Hunt et al., 1998; Hunt and Vanderpool, 2001). However, this difference was compensated for by the higher copper content of the lacto-ovo-vegetarian diets compared to the omnivorous diets, resulting in similar amounts of copper absorbed daily.

The Panel considers that absorption of copper from a mixed diet is around 50 %.

2.3.2. Transport in blood

Once released from the gut, the copper binds to either albumin, or possibly a protein termed transcuprein. This copper is accumulated by the liver. Around 40 % of copper is taken up into the liver in the first pass. Once taken up, the copper is stored in metallothionein, incorporated into ceruloplasmin or excreted in the bile.

Most of the copper in blood is bound to ceruloplasmin, with values varying from 80 to 95 % (Wirth and Linder, 1985; Hellman and Gitlin, 2002). Ceruloplasmin is a ferroxidase, one of a family of multi-copper ferroxidases. Each molecule has six atoms of copper which are not exchangeable, with a seventh that may be loosely bound. It plays a critical role in iron release from the liver (see Section 2.3.3) but has also been proposed as a copper delivery mechanism. Putative ceruloplasmin receptors have been identified in various tissues (Hilton et al., 1995; Sasina et al., 2000), but the protein has never been isolated and therefore its mechanism has not yet been elucidated.

The remainder of the copper in plasma is present predominantly as histidine-bound or albumin-bound copper. This relatively small fraction is the one most likely to form the pool for transfer to tissues. However, it is very difficult to measure, though various approaches are being developed (Beattie et al., 2001; Venelinov et al., 2004; Zhang et al., 2014).

2.3.3. Distribution to tissues

Copper uptake into cells is a passive process, probably through carrier-mediated diffusion (McArdle, 1995; Gambling et al., 2008). The Cu(I) is taken into the cell through Ctr1, much in the same way as described for absorption in the gut (see Figure 1). Following uptake, copper binds to one of a series of chaperones (CCS, Atox1, Cox17). From CCS, copper is incorporated into SOD1 (Cu/Zn SOD). From Cox17, copper goes to the mitochondria where it is incorporated into the electron transport chain enzymes. Atox1, the first chaperone identified, traffics copper to either ATP7A or ATP7B, depending on the tissue.

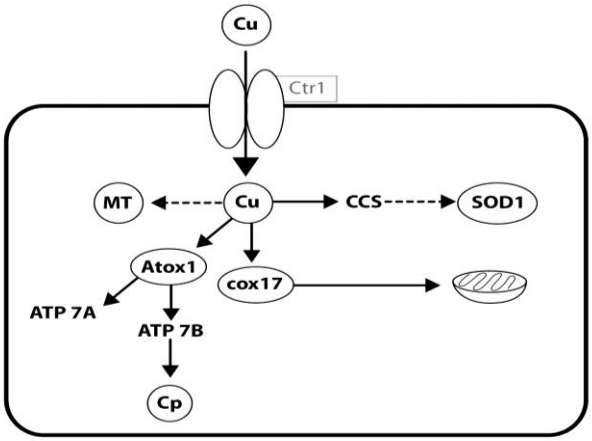

Figure 1: Copper metabolism in the liver. Cu(I) is transferred into the cell through Ctr1. It binds to chaperones (CCS, Atox1, Cox17), is stored in metallothionein (MT), or released as ceruloplasmin (Cp). The copper is then transferred to the final target protein, depending on the chaperone. In the case of ATP7A and B, the copper can be transferred out of the cell, into the bile or fetal circulation, depending on the tissue, or can be incorporated into ceruloplasmin (in the liver with ATP7B).

Once copper is taken up into the liver, it is either stored, incorporated into ceruloplasmin or excreted into the bile. In order to synthesise ceruloplasmin, copper is transported across the endoplasmic reticulum by ATP7B and is added to the apo-protein during synthesis. When copper is present in excess, the ATP7B relocates to the bile canaliculus membrane, and the copper is transported into the bile. It is thought that this copper is not re-absorbed, presumably because it is complexed to bile salts or other moieties and is not available (see review by Wang et al. (2011)).

Ceruloplasmin synthesis is regulated by many factors. Copper deficiency or excess itself does not change mRNA levels, but the apo-protein is unstable and is catabolised. However, levels are increased by steroids, including oral contraceptives, and in the inflammatory response. Its main function is to act as a ferroxidase, oxidising iron released from the liver from Fe^{2+} to Fe^{3+} prior to incorporation into transferrin (Prohaska, 2011). However, as mentioned in Section 2.3.2, there are also some data suggesting that ceruloplasmin may act as a copper transport protein (Hilton et al., 1995).

Copper is essential for normal fetal development and deficiency during pregnancy results in a wide spectrum of problems. Transfer from mother to fetus occurs across the placenta. The mechanism of transport is largely similar to that described in liver and other cells, except that both ATP7A and B seem to be involved. At present, the results suggest that ATP7A transports copper out of the placenta to the fetal side, while ATP7B may have a role in returning excess copper back to the maternal blood

(Hardman et al., 2004; Hardman et al., 2006). How these two are regulated in tandem is not known. The amount of copper transported across the placenta increases as gestation proceeds. The expression of the copper genes outlined in Figure 1 has been measured in a rat model (Lenartowicz et al., 2014). The pattern is different for the different genes, but tends to drop from about day 17 of gestation to term (21.5 days), thereafter increasing in the postnatal period (Lenartowicz et al., 2014). The expression in humans has not been determined, but given that copper metabolism is similar in both species, it is not likely to be very different.

2.3.4. Storage

Copper is largely stored in the liver. In one study, it is suggested that there is no increase in copper concentration in the fetal liver as gestation proceeds (Donangelo et al., 1993), but that copper content rises as the liver grows. Estimates of copper concentration in infant liver are quite variable, and some references state that it is similar to adult levels at about 40 µg/g dry weight (Dorea et al., 1987), while others suggest the neonatal to adult ratio is as high as 16 to 1 (Meinel et al., 1979). It is also proposed that the concentration varies within the liver, which may be related to differential functions in different parts of the liver (Meinel et al., 1979). In the fetus, copper accumulates mostly during the latter third of gestation. This may be needed to provide stores during the perinatal period, when copper supplies from maternal milk are quite low (see Section 2.3.6.3), but it may also reflect the fact that the bile ducts are not patent prenatally, and the major excretion pathway is blocked. Copper concentration in the liver reaches about 40 µg/g dry weight in adults (Gurusamy and Davidson, 2007). The concentration of free copper in a cell is very low, probably no more than a few atoms per cell, but total levels can be quite varied.

2.3.5. Metabolism

Copper uptake is largely regulated on a cellular level, operating through passive diffusion pathways. Most of the copper in blood is bound to ceruloplasmin (Section 2.3.2). Ceruloplasmin is an acute phase protein, and is also increased in plasma by steroid hormones and by oral contraception. In a cell model of placental function, it was shown that insulin and oestrogen upregulate expression of ATP7A, the Menkes protein, and decrease levels of ATP7B, the protein associated with Wilson disease (Hardman et al., 2007). Whether this occurs in other cells is not known. There are few data on possible endocrine effects of membrane copper transporters, although one group has reported differential localisation of Ctr1 in mammary cells depending on the degree of differentiation (Freestone et al., 2014), possibly through the action of prolactin (Kelleher and Lönnerdal, 2006).

2.3.6. Elimination

2.3.6.1. Faeces

Copper is primarily excreted through the bile. Excess copper is transported across the canalicular membrane by ATP7B (Prohaska and Gybina, 2004). In the bile, the copper forms complexes that prevent re-absorption and is presumably linked to bile salts, because current evidence suggests it is not re-absorbed in the gut and passes through to the faeces (Prohaska and Gybina, 2004). Faecal copper increases almost linearly with dietary intake and ranges from 0.33 mg/day (for a dietary copper intake of 0.38 mg/day) to 2.17 mg/day (for a dietary copper intake of 2.49 mg/day) (Turnlund et al., 1998).

2.3.6.2. Urine

Urinary copper shows little or no variation with dietary copper intake and ranges between 11 µg/day and 60 µg/day (Turnlund et al., 1990; Turnlund et al., 1998; Milne et al., 2001; Turnlund et al., 2005). This is very small (between 1 and 2 %) (Turnlund et al., 2005) in relation to total turnover and is ignored in most balance studies.

2.3.6.3. Skin and sweat

Sweat and dermal losses in adults have been reported to vary between 120–150 µg/day (two daily collections in three men) (Milne et al., 1990) and 340 ± 240 µg/day (88 daily collections in 13 men)

(Jacob et al., 1981). Several different factors may alter sweat losses for copper. For example, Chinevere et al. (2008) showed, in a study in eight healthy young men, that heat acclimation resulted in a lower sweat rate and a lower loss of copper as a consequence of exercise, from 0.41 mg × h^{-1} to 0.22 mg × h^{-1}. However, later studies (Ely et al., 2013) suggested that the results may at least in part be explained as an artefact of the collection procedure. In studies of athletes on bicycle ergometers, as much as 0.83 mg copper could be lost in sweat, measured from whole body washdown (Baker et al., 2011). However, results are very variable from exercise period to period, and between individuals (Aruoma et al., 1988).

The Panel considers that copper losses in sweat and skin may be significant, but the results are variable and subject to many confounding factors. Dermal losses are not usually measured in balance studies, and this may limit the value of balance studies in terms of deriving requirements for copper.

2.3.6.4. Breast milk

In a comprehensive review of breast milk copper concentrations in women around the world at stages of lactation between one day and nine months, Dorea (2000) reported concentrations ranging from about 200 to 1 000 µg/L over the course of lactation, with most values in the order of 300–400 µg/L. Breast milk copper concentration did not correlate with dietary copper intake or with serum copper concentration, but decreased with time of lactation. Ceruloplasmin was identified in human milk and may carry up to 25 % of total milk copper. In general, copper concentrations in breast milk were quite variable in different studies, but did not correlate with social status, nutrient intake or copper levels in the diet.

Comprehensive searches of the literature published from January 1990 to February 2012 (Bost et al., 2012) and from January 2011 to January 2014 (LASER Analytica, 2014) were performed as preparatory work to this assessment in order to identify data on breast milk copper concentration. Appendix A reports data from 12 studies on the mean copper concentration of human milk from healthy lactating mothers of term infants, published after the review of Dorea (2000).

Milk copper concentrations significantly decrease with advancing lactation (Dörner et al., 1989; Lönnerdal, 1998; Dorea, 2000; Wünschmann et al., 2003). Copper concentrations in breast milk were reported to decrease from 420 ± 58 µg/L at day 1 to 344 ± 93 µg/L at day 36 post partum in British women (Hibberd et al., 1982), from 450 ± 110 µg/L at day 0–4 to 270 ± 90 µg/L at days 10–30 in Polish women (Wasowicz et al., 2001), from 1 084 (80 % range 752–1 488) µg/L fore-milk at 2 weeks to 680 (80 % range 395–1 156) µg/L fore-milk at 16 weeks (Dörner et al., 1989), and from 1 040 ± 54 µg/L at days 4–7 to 847 ± 38 µg/L at days 30–45 in women in the USA (Feeley et al., 1983). In a sample of 23 women-infant pairs from Germany, Poland and the Czech Republic studied over 2–8 weeks, Wünschmann et al. (2003) observed that the copper intake of breast-fed infants < 4 months was 250 µg/day (range 150–320 µg/day), while it decreased to 105 µg/day (range 66–210 µg/day) in infants aged > 4 months (highest age was 16.4 months at the start of the study).

Maternal diet does not seem to influence milk copper concentration (Wünschmann et al., 2003). The mechanisms governing the transfer of copper from blood to breast milk are not fully understood, but they do not seem to depend on maternal intake or maternal copper reserves. Serum copper concentration does not seem to influence the uptake of copper by the mammary gland and specific maternal conditions that markedly alter copper metabolism, such as Wilson disease, do not affect milk copper concentrations (Dorea, 2000).

The Panel notes that the available data indicate that during the first six months of lactation mean/median copper concentrations in mature breast milk of populations from Western countries are variable and are reported to range between 100 and 1 000 µg/L. The Panel selected a value of 350 µg/L as breast milk copper concentration of mature milk (Dorea, 2000; EFSA NDA Panel, 2013) and, based on a mean milk transfer of 0.8 L/day (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA

NDA Panel, 2009) during the first six months of lactation in exclusively breastfeeding women, the Panel estimates a loss of 280 µg/day of copper in breast milk during the first six months of lactation.

2.3.7. Interaction with other nutrients

2.3.7.1. Copper and iron

Copper is required for normal functioning of the multi-copper ferroxidases, hephaestin, ceruloplasmin, GPI-anchored ceruloplasmin and zyklopen (Gambling et al., 2008). These are all critical for iron release on the basolateral side of epithelia. Hephaestin knockout mice die from iron deficiency, while patients with aceruloplasminaemia have iron overload in liver and brain, and will suffer from cirrhosis and psychiatric problems (Harris et al., 1998). To date, there are no recorded mutants of the GPI-anchored ceruloplasmin, and zyklopen knockout mice are not available.

Although copper is largely taken up by Ctr1, it is possible that some copper enters through DMT1, the iron transport channel. As such, high levels of iron in the diet can reduce copper absorption (Sharp, 2004).

2.3.7.2. Copper and zinc

It is well established that high levels of dietary zinc can affect copper absorption and that chronic high zinc intake can result in severe neurological diseases attributable to copper deficiency (Hedera et al., 2009). A No Observed Adverse Effect Level (NOAEL) for zinc of 50 mg/day was based on the absence of any adverse effect on a wide range of relevant indicators of copper status in controlled metabolic studies (SCF, 2002). High levels of zinc, in rats at least, induce metallothionein. This sequesters both copper and zinc, but has a higher affinity for copper. Thus, when copper is absorbed, it displaces the zinc, which in turn induces more metallothionein. This results in blocking of copper absorption.

Two studies examined the interactions between zinc and copper directly. They found that high intake of zinc (53 mg/day) can reduce copper absorption and change copper balance (Taylor et al., 1991; Milne et al., 2001). High levels of zinc (i.e. above the UL for zinc (SCF, 2002)) can be used to treat Wilson disease. This treatment acts to prevent absorption of copper, rather than removing it from intracellular stores as for other treatments (Brewer et al., 1998).

2.3.7.3. Copper and molybdenum

In ruminants, copper molybdenosis has been recognised for many years but this has not been observed in humans (Nederbragt et al., 1984; Ladefoged and Sturup, 1995).

In four adult males on two sorghum diets providing daily 2.4 mg of copper and 166 µg or 540 µg of molybdenum, respectively, faecal copper excretion was comparable and apparent copper absorption unaffected by molybdenum intake (Deosthale and Gopalan, 1974).

2.3.7.4. Conclusions on interactions with other nutrients

The Panel considers that zinc, iron and molybdenum at levels occurring in the normal diet do not interfere with copper metabolism and hence interactions do not need to be taken into consideration when setting DRVs for copper.

2.4. Biomarkers

2.4.1. Serum/plasma copper concentration

Traditionally, serum/plasma copper concentration is taken as the best indicator of status, but it reflects ceruloplasmin concentration rather than copper stores, and this can vary during the acute phase reaction, in infection, and is also increased by steroid hormones and use of oral contraceptives (Harvey and McArdle, 2008). The Panel considers that serum and plasma copper concentrations are equivalent.

Based on a systematic review to evaluate the usefulness of biomarkers of copper status, Harvey et al. (2009) concluded that serum copper concentration responds to copper supplementation depending on copper status, with a greater response to supplementation in copper-deficient subjects (two studies) compared to copper-replete subjects (five studies). Less information was available regarding the response of plasma copper concentration to supplementation.

In one trial, a small but significant decrease in plasma copper concentration was observed in young healthy men at the end of a copper depletion period compared to the preceding equilibration period or the subsequent repletion period (Turnlund et al., 1997; Werman et al., 1997). In contrast, other studies showed no difference in plasma copper concentration following a period of depletion (Milne et al., 1990; Turnlund et al., 1990; Milne and Nielsen, 1996; Baker et al., 1999b; Milne et al., 2001; Araya et al., 2003; Davis, 2003; Harvey et al., 2003).

The Panel considers that plasma and serum copper concentrations are of limited value as a biomarker of copper status in individuals, especially in relation to copper overload, but notes that low concentrations may indicate copper depletion.

2.4.2. Ceruloplasmin concentration and ceruloplasmin activity

Ceruloplasmin is the major copper carrier in the plasma. Plasma ceruloplasmin concentration and ceruloplasmin activity significantly declined when dietary copper intake was reduced from 0.66 mg/day for 24 days to 0.38 mg/day for 42 days in one controlled trial involving male subjects (Turnlund et al., 1997; Werman et al., 1997). However, plasma ceruloplasmin and ceruloplasmin activity did not increase after a repletion period providing 2.49 mg copper/day for 24 days.

No effect of dietary copper (either by depletion to 0.7 mg/day or supplementation to levels as high as 7 mg/day) on plasma ceruloplasmin concentration was reported in other trials (Milne et al., 1990; Milne and Nielsen, 1996; Baker et al., 1999b; Davis et al., 2000; Kehoe et al., 2000; Turley et al., 2000; Milne et al., 2001; Araya et al., 2003; Harvey et al., 2003; Turnlund et al., 2004). Further, ceruloplasmin is an acute phase protein, and is elevated with oral contraceptive use, so that its value as a biomarker is somewhat restricted.

The Panel considers that ceruloplasmin is of limited value as a biomarker of copper status in individuals.

2.4.3. Erythrocyte superoxide dismutase (SOD)

In animal studies, SOD levels are decreased in copper deficiency (West and Prohaska, 2004). The effect of dietary copper on the activity of the erythrocyte Cu/Zn SOD has been assessed in five controlled trials and one balance study (Turnlund et al., 1997; Davis et al., 2000; Kehoe et al., 2000; Milne et al., 2001; Harvey et al., 2003). Four out of these six trials reported no change in erythrocyte SOD in response to dietary copper. In a randomised controlled trial (RCT) involving 17 males, Davis (2003) did not observe any difference in erythrocyte SOD activity when changing from a diet containing 0.59 mg/day for six weeks to one containing 2.59 mg/day for six weeks. In another trial in 12 young males, no difference in SOD was observed at the end of the three feeding periods with "low" (0.66 mg/day for 24 days), "very low" (0.38 mg/day for 42 days) and "high" (2.49 mg/day for 24 days) copper intakes, respectively (Turnlund et al., 1997). No change in SOD activity was observed by Kehoe et al. (2000) or Harvey et al. (2003) with copper intakes varying between 1.59 and 6 mg/day.

In one study in postmenopausal women the authors reported an increase in erythrocyte SOD when copper intake increased from 1 to 3 mg/day (Milne et al., 2001). However, in this study dietary zinc was either very low (3 mg/day) or very high (53 mg/day), which may have affected the response of erythrocyte SOD to dietary copper. Moreover, a systematic review on biomarkers of copper status concluded that erythrocyte SOD is not a suitable biomarker of copper status (Harvey et al., 2009).

The Panel considers that erythrocyte SOD cannot be used as a biomarker of copper status.

2.4.4. Diamine oxidase (DAO)

Serum DAO was increased after copper supplementation with 3 mg/day for six weeks in two RCTs including 24 participants each (Kehoe et al., 2000; O'Connor et al., 2003). However, the Panel considers that the studies were at high risk of bias due to incomplete reporting of outcomes. One systematic review included three other supplementation studies (RCTs) assessing serum DAO activity (Harvey et al., 2009). According to the authors, no conclusions can be drawn regarding the usefulness of DAO activity as a biomarker of copper status.

The Panel considers that DAO cannot be used as a biomarker of copper status.

2.4.5. Skin lysyl oxidase

One controlled trial carried out in 12 young men (Werman et al., 1997) observed a decrease in skin lysyl oxidase activity when changing from a diet providing 0.66 mg copper/day to a diet providing 0.38 mg copper/day and an increase in its activity after a repletion period (2.48 mg copper/day), which suggests that lysyl oxidase activity declines when dietary copper intake is inadequate. However, the Panel considers that this study is at high risk of bias (not randomised, blinding not reported) and does not enable to conclude that skin lysyl oxidase can serve as a useful indicator of copper status. In their systematic review, Harvey et al. (2009) were unable to identify suitable data to evaluate the usefulness of skin lysyl oxidase as a biomarker of copper status.

The Panel considers that skin lysyl oxidase cannot be used as a marker of copper status.

2.4.6. Other biomarkers

In a systematic review on possible biomarkers of copper status, Harvey et al. (2009) stated that no conclusions can be drawn on the usefulness of erythrocyte and platelet copper, leukocyte SOD, erythrocyte, platelet, and plasma glutathione peroxidase, platelet and leukocyte cytochrome-c oxidase, total glutathione, and urinary pyridinoline. There was a lack of data for novel biomarkers such as CCS (copper chaperone for SOD) (Harvey et al., 2009; de Romana et al., 2011), and peptidyl glycine α-amidating monooxygenase (PAM) activity (Harvey et al., 2009; Bousquet-Moore et al., 2010) as biomarkers of copper status. More recently, it has been suggested that CCS mRNA may be related to copper status (Araya et al., 2014), but more work is needed to test if it can be a biomarker in the general population.

The Panel considers that other biomarkers of copper status are either not sensitive or specific enough to be used for setting DRVs for copper.

2.5. Effects of genotype

ATP7A and ATP7B have many recorded polymorphisms (Thomas et al., 1995a; Thomas et al., 1995b; Institute of Genetic Medicine et al., 2015). Some of these have a significant enough effect to be classified as lethal mutations, while others are compatible with survival, some with very minor handicaps.

Recently, single nucleotide polymorphisms (SNPs) in ATP7B have been suggested to be associated with an increase in non-ceruloplasmin copper, and with an increased incidence of Alzheimer's disease (Bucossi et al., 2012; Squitti, 2012). Further, different ATP7B SNPs have been shown to be associated with an increased incidence of Alzheimer's disease (Squitti et al., 2013). However, whether the link is causal or not has yet to be demonstrated. Further, in a pilot Phase 2 clinical trial, Kessler et al. (2008) showed that supplementation with copper actually had a positive effect on a marker for Alzheimer's disease, in that levels of serum amyloid peptide Aβ42 decreased by only 10 %, compared to 30 % in the placebo group, suggesting a protective effect of copper. The Panel concludes that more data are required before these findings can be considered when setting DRVs for copper.

3. Dietary sources and intake data

3.1. Dietary sources

Foods differ widely in their natural copper content. Factors such as season (copper concentration is higher in greener portions), soil quality (Ginocchio et al., 2002; Chaignon et al., 2003), geography, water source and use of fertilizers influence the copper content in food. Rich dietary sources of copper are liver, some seafood (oysters), cocoa products, nuts (particularly cashew) and seeds (de Romana et al., 2011).

Besides food, drinking water can be another major source of copper, though the mineral content in drinking water is very variable. Factors such as natural mineral content, pH, and a copper or non-copper plumbing system determine copper concentration in water (National Research Council (US) Committee on Copper in Drinking Water, 2000). Soft acidic water, especially when transported by copper pipelines, has a higher copper concentration. In unflushed samples of drinking water in Malmö and Uppsala (Sweden), the 10^{th} and 90^{th} percentiles of copper concentration were 0.17 and 2.11 mg/L, and median concentration was 0.72 mg/L. In a small percentage of dwellings, however, drinking water had copper concentrations of more than 5 mg/L. Median daily intake of copper from drinking water in children aged 9–21 months was estimated at 0.46 mg in Uppsala and 0.26 mg in Malmö (Pettersson and Rasmussen, 1999). In Berlin (Germany), copper concentration in random daytime samples of tap water ranged between > 0.01 and 3.0 mg/L, with a median of 0.03 mg/L (Zietz et al., 2003). In the EU, the maximum permitted concentration of copper in water intended for human consumption is 2 mg/L.[6]

It has been estimated that foods may account for 90 % or more of copper intake in adults when the copper content in drinking water is low (< 0.1 mg/L). If the copper content is higher (> 1–2 mg/L), water may account for up to 50 % of total intake. In infants, contribution of water to daily copper intake may be higher because they consume proportionally more water than adults (de Romana et al., 2011).

Copper as copper lysine complex, cupric carbonate, cupric citrate, cupric gluconate, and cupric sulphate may be added to both food[7] and food supplements,[8] whereas copper L-aspartate, copper bisglycinate, and copper (II) oxide may only be used in the manufacture of food supplements.[6] The copper content of infant and follow-on formulae[9] and processed cereal-based foods and baby foods for infants and young children[10] is regulated.

3.2. Dietary intake

EFSA estimated dietary intake of copper from food consumption data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011b), classified according to the food classification and description system FoodEx2 (EFSA, 2011a). Data from 13 dietary surveys from nine EU countries were used. These countries included Finland, France, Germany, Ireland, Italy, Latvia, the Netherlands, Sweden and the UK. The data covered all age groups from infants to adults aged 75 years and older (Appendix B).

Nutrient composition data of foods and water-based beverages were derived from the EFSA Nutrient Composition Database (Roe et al., 2013). Food composition information from Finland, France, Germany, Italy, the Netherlands, Sweden and the UK were used to calculate copper intake in these

[6] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, p. 32

[7] Regulation No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods, OJ L 404, 30.12.2006, p. 26.

[8] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements, OJ L 183, 12.7.2002, p. 51.

[9] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, p.1.

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

655 countries, assuming that the best intake estimate would be obtained when both the consumption data
656 and the composition data are from the same country. For nutrient intake estimates of Ireland and
657 Latvia, food composition data from the UK and Germany, respectively, were used, because no specific
658 composition data from these countries were available. In case of missing values in a food composition
659 database, data providers had been allowed to borrow values from another country's database. The
660 amount of borrowed copper values in the seven composition databases used varied between 12.7 and
661 100 %, although in six of the seven databases the percentage of borrowed values was higher than 60 %
662 of the total. Estimates were based on food consumption only (i.e. without dietary supplements).
663 Nutrient intake calculations were performed only on subjects with at least two reporting days.

664 Data on infants were available from Finland, Germany, the UK, and Italy. The contribution of human
665 milk was taken into account if the amounts of human milk consumed (Italian INRAN SCAI survey
666 and the UK DNSIYC survey) or the number of breast milk consumption events (German VELS study)
667 were reported. In case of the Italian INRAN SCAI survey, human milk consumption had been
668 estimated based on the number of eating occasions using standard portions per eating occasion. In the
669 Finnish DIPP study only the information "breast fed infants" was available, but without any indication
670 about the number of breast milk consumption events during one day or the amount of breast milk
671 consumed per event. For the German VELS study, the total amount of breast milk was calculated
672 based on the observations by Paul et al. (1988) on breast milk consumption during one eating occasion
673 at different ages, i.e. the amount of breast milk consumed on one eating occasion was set to
674 135 g/eating occasion for infants aged 6–7 months and to 100 g/eating occasion for infants aged 8–12
675 months. The Panel notes the limitations in the methods used for assessing breast milk consumption in
676 infants (Appendices C and D) and related uncertainties in the intake estimates for infants.

677 Average copper intakes ranged between 0.34 and 0.49 mg/day (0.12–0.22 mg/MJ) in infants (< 1 year,
678 four surveys), between 0.56 and 0.94 mg/day (0.14–0.20 mg/MJ) in children aged 1 to < 3 years (five
679 surveys), between 0.82 and 1.43 mg/day (0.12–0.19 mg/MJ) in children aged 3 to < 10 years (seven
680 surveys), between 0.98 and 1.92 mg/day (0.13–0.20 mg/MJ) in children aged 10 to < 18 years (seven
681 surveys), and between 1.15 and 2.07 mg/day (0.14–0.25 mg/MJ) in adults (≥ 18 years, eight surveys).
682 Average daily intakes were in most cases slightly higher in males (Appendix C) compared to females
683 (Appendix D) mainly due to larger quantities of food consumed per day.

684 The main food group contributing to copper intake was grains and grain-based products, except for
685 infants for whom the main contributor to copper intake was food products for young population.
686 Another important contributor to copper intake was the food group meat and meat products, with an
687 average contribution up to 19 % in males and up to 16 % in females. Although grains and grain-based
688 products do not contain as high concentrations of copper as reported for other food groups, such as
689 offal or nuts, the high consumption of foods in this group (e.g. bread) as well as the big variety of
690 products included makes it the most important contributor to copper intake. The food groups starchy
691 roots or tubers and products thereof, sugar plants; coffee, tea and infusions; fish, seafood, amphibians,
692 reptiles and invertebrates were also important contributors, the latter only in certain European
693 populations. Differences in main contributors to copper intakes between sexes were minor. The
694 contribution of water and water-based beverages to copper intake in various age groups was up to
695 12 %.

696 EFSA's copper intake estimates in mg/day were compared with published intake values, where
697 available, from the same survey and dataset and the same age class using the study in Finnish
698 adolescents (Hoppu et al., 2010), the FINDIET 2012 Survey (Helldán et al., 2013), the French national
699 INCA2 survey (Afssa, 2009), the VELS survey in infants and children (Kersting and Clausen, 2003;
700 Mensink et al., 2007), the Irish National Adult Nutrition Survey (IUNA, 2011), the Dutch National
701 Dietary Survey (van Rossum et al., 2011) and the UK National Diet and Nutrition Survey (Bates et al.,
702 2011). When the EFSA copper intake estimates were compared with published intake estimates from
703 the same survey and same age ranges, the EFSA estimates differed at maximum around 25 % from the
704 published values in all countries and surveys, although in several cases differences were less than 5–
705 10 % (Table 2).

706 **Table 2:** EFSA's average daily copper intake estimates, expressed as percentages of intakes
707 reported in the literature

Country	% of published intake (% range over different age classes in a specific survey)
Finland	103–110 (NWSSP), 108–118 (FINDIET2012)
France	96–112 (INCA2)
Germany	99–103 (VELS children), 75–80 (VELS infants)
Ireland	98–121 (NANS)
NL	115–120 (DNFCS)
UK	107–125 (Rolling Programme 2008–2011)

708

709 In addition to the surveys mentioned above, there was also available data on copper intake from the
710 EsKiMo study (Germany) and the DNSIYC-2011 study (UK). In these two cases the comparison with
711 the EFSA average copper intake estimates is difficult; in the case of the EsKimo study EFSA average
712 copper intake estimates were lower by 13–15 % in children aged 6 to < 10 years and by 42–58 % in
713 children aged 10–11 years, compared to published estimates. This could be partially explained by the
714 fact that the EsKiMo study included copper supplement consumption in their data (Mensink et al.,
715 2007). Regarding the DNSIYC-2011 study, the comparison is also difficult as the intakes for infants
716 and young children are reported by ethnic groups and socio-economic classes. However, the EFSA
717 estimated intakes for infants (0.38–0.41 mg/day) and children aged up to 1.5 years (0.57–0.60 mg/day)
718 do not differ much from those published (0.45–0.52 mg/day for infants, 0.45–0.55 mg/day in children
719 aged up to 1.5 years).

720 Overall, several sources of uncertainties may contribute to these differences. These include
721 inaccuracies in mapping food consumption data according to food classifications, nutrient content
722 estimates available from the food composition tables, the use of "borrowed" copper values from other
723 countries in the food composition database, and replacing missing copper values by values of similar
724 foods or food groups in the copper intake estimation process. It is not possible to conclude which of
725 these intake estimates (i.e. the EFSA intake estimate or the published one) would be closer to the
726 actual copper intake.

727 ## 4. Overview of Dietary Reference Values and recommendations

728 ### 4.1. Adults

729 The German-speaking countries (D-A-CH, 2015) considered that a copper intake of 1.25 mg/day is
730 needed to replace faecal and urinary losses (Klevay et al., 1980). An AR estimated by WHO of
731 11 µg/kg body weight per day (WHO, 1996) and the PRI set by SCF (1993) were considered as well
732 when setting an Adequate Intake (AI) range of 1.0–1.5 mg/day (Table 3).

733 For the Nordic Nutrition Recommendations (NNR) 2012 it was acknowledged that there are limited
734 data but that the available data demonstrate that a copper intake of 0.7–0.8 mg/day maintains adequate
735 copper status as indicated by plasma copper concentration, SOD activity and ceruloplasmin production
736 (Reiser et al., 1985; Lowy et al., 1986; Lukaski et al., 1988; Turnlund et al., 1990; Turnlund et al.,
737 1997). In addition, it was pointed out that an intake below 0.7 mg/day has been associated with an
738 increase in faecal free radical production, faecal water alkaline phosphatase activity, cytotoxicity, or
739 impaired immune function (Bonham et al., 2002; Davis, 2003). In line with IOM (2001), an AR of
740 0.7 mg/day was set and, using a coefficient of variation (CV) of 15 %, a recommended intake (RI) of
741 0.7 mg/day was derived (Nordic Council of Ministers, 2014).

742 IOM (2001) set an Estimated Average Requirement (EAR) using depletion/repletion studies assessing
743 biochemical indicators of copper status in men and women (Turnlund et al., 1990; Milne and Nielsen,
744 1996; Turnlund et al., 1997). According to IOM (2001), the study by Turnlund et al. (1990) showed
745 that a copper intake in 11 young men above 0.38 mg/day is needed to prevent a decrease in relevant
746 biochemical indicators (SOD activity, serum copper and ceruloplasmin concentration), whereas the

747 study by Turnlund et al. (1997) showed that no decline in these biomarkers was observed in 11 young
748 men at an intake of 0.79 mg/day. A linear model based on the two studies defined an intake of
749 0.55 mg/day at which half of the group of young men would not maintain a satisfactory copper status.
750 IOM (2001) also considered the study of Milne and Nielsen (1996) which showed that a copper intake
751 of 0.57 mg/day in 10 women maintained serum copper and ceruloplasmin concentrations, whereas
752 platelet copper concentration decreased significantly in 8 of 10 women. Based on these studies an
753 EAR of 0.7 mg/day was set. The factorial method was used as supportive. To achieve neutral copper
754 balance, it was considered that an intake of 0.51 mg/day is needed to replace obligatory copper losses
755 via faeces, urine, sweat and other routes (Shike et al., 1981; Milne and Gallagher, 1991; Turnlund et
756 al., 1997; Turnlund, 1998), this value being somewhat lower than the EAR set based on indicators of
757 copper status. A Recommended Dietary Intake (RDA) of 0.9 mg/day was derived applying a CV of
758 15 % to the EAR, to account for the limited data available and the few copper intake levels in
759 depletion/repletion studies.

760 Afssa (2001) set requirements for copper using a factorial approach. Total losses of copper of 400–
761 500 µg/day, consisting of losses via the skin (50–100 µg/day), urinary losses (25–50 µg/day), and
762 faecal losses (300–400 µg/day) were assumed, and the AR was considered to fall within a range of
763 1.35–1.65 mg/day, taking into account 30 % absorption (Sandstead, 1982). A PRI of 2 mg/day for men
764 and 1.5 mg/day for women was set.

765 The SCF (1993) derived an Average Requirement (AR) of 0.8 mg/day and set a PRI of 1.1 mg/day.
766 The AR was largely based on the study by Turnlund et al. (1989) in which men with an intake of
767 0.79 mg/day for 42 days did not show a decrease in copper status. A study with an intake range of 0.7–
768 1.0 mg/day for four weeks, which showed copper-responsive clinical and biochemical defects in some
769 adults, was also considered, but it was stated that these defects could have also resulted from the
770 experimental diet given (Reiser et al., 1985).

771 The Netherlands Food and Nutrition Council (1992) set an adequate range of intake on the basis of a
772 factorial approach and balance studies. In the factorial approach, faecal losses (0.4–1.2 mg/day), sweat
773 losses (0.3 mg/day) and an estimated absorption efficiency of 40 % were taken into account to
774 determine a requirement of 1.75–3.75 mg/day (Bloomer and Lee, 1978; Ting et al., 1984; Turnlund,
775 1987; Turnlund et al., 1988). It was stated that copper balance was positive for an intake above
776 1.4 mg/day (Robinson et al., 1973; Hartley et al., 1974; Turnlund et al., 1981; Turnlund et al., 1983),
777 though these studies did not consider losses via the skin. With some uncertainty, the adequate range of
778 intake was concluded to fall within an intake of 1.5–3.5 mg/day.

779 The UK Committee on Medical Aspects of Food Policy (COMA) (DH, 1991) set a Reference Nutrient
780 Intake (RNI) of 1.2 mg/day, on the basis of data from balance studies (no references given) and
781 biochemical signs of copper deficiency observed at an intake of 0.8–1.0 mg/day, such as a decline in
782 activity of SOD and cytochrome oxidase and altered metabolism of enkephalins. It was stressed that
783 an EAR or a Lower Reference Nutrient Intake could not be set due to the lack of data.

784 The World Health Organization/Food and Agriculture Organization (WHO/FAO, 2004) did not derive
785 DRVs for copper.

Table 3: Overview of Dietary Reference Values for copper for adults

	D-A-CH (2015)	NCM (2014)	Afssa (2001)	IOM (2001)	SCF (1993)	NL (1992)	DH (1991)
Age (years)	≥ 19	≥ 18	20–50	≥ 19	≥ 18	≥ 19	≥ 19
PRI							
Men (mg/day)	1.0–1.5 [(a)]	0.9	2.0	0.9	1.1	1.5–3.5 [(b)]	1.2
Women (mg/day)	1.0–1.5 [(a)]	0.9	1.5	0.9	1.1	1.5–3.5 [(b)]	1.2
Age (years)			≥ 50				
PRI							
Men (mg/day)			1.5				
Women (mg/day)			1.5				

NCM, Nordic Council of Ministers; NL, Netherlands' Food and Nutrition Council
(a): Adequate Intake
(b): Adequate range of intake

4.2. Infants and children

In NNR 2012 it was stated that breast milk copper concentration is about 0.25 mg/L, according to IOM (2001), and that the copper status of fully breast-fed infants is adequate (Nordic Council of Ministers, 2014). For infants from 6 to 11 months and children, copper requirements were extrapolated from adult requirement taking into account an allowance for growth, in line with IOM (2001) (Table 4).

The IOM (2001) set an AI of 0.22 mg/day (or 0.024 mg/kg body weight per day) for infants aged 7–12 months, considering an average human milk copper concentration between 7 and 12 months post partum of 0.2 mg/L, a mean breast milk intake of 0.6 L/day and a median copper intake with complementary foods of 0.1 mg/day, as shown by the Third National Health and Nutrition Examination Survey (n = 45 infants of that age). For children, the IOM (2001) extrapolated from the EAR of adults using allometric scaling (body weight to the power of 0.75), due to the absence of data to set EARs for children. The choice of the scaling approach was justified with the structural and functional role of copper in many enzymes and because it resulted in higher values compared to extrapolation based on isometric scaling (i.e. linear with body weight). In the absence of information about the variability in requirement, a CV of 15 % was applied to the EARs to derive RDAs for various age groups.

For infants and young children, Afssa (2001) assumed that the requirement for copper is between 40 and 80 µg/kg body weight per day.

The SCF (1993) estimated copper requirements for infants factorially, considering endogenous losses according to Zlotkin and Buchanan (1983) and a copper requirement for growth based on a tissue copper content of 1.38 µg/g (Widdowson and Dickerson, 1964). Assuming absorption efficiency to be 50 % resulted in a PRI of 36 µg/kg body weight per day or 0.3 mg/day. For children, the PRI values were interpolated and were given as 30 µg/kg body weight per day at 1–6 years, 24 µg/kg body weight per day at 7–10 years and 18 µg/kg body weight per day at 15–17 years of age.

For infants from 0 to 12 months, the Netherlands Food and Nutrition Council (1992) set an adequate range of intake of 0.3–0.5 mg/day based on a copper concentration of breast milk of about 0.3–0.5 mg/L (Cavell and Widdowson, 1964; Vuori, 1979) and an assumed absorption efficiency of 50 % from breast milk and 40 % from infant formula. For children, adequate ranges of intake were extrapolated from that of adults on the basis of body weight.

The UK COMA (DH, 1991) set an RNI for infants based on the factorial approach. An average tissue copper content of 1.38 µg/g (Widdowson and Dickerson, 1964) was considered as well as losses of endogenous copper (Zlotkin and Buchanan, 1983). An absorption efficiency of 50 % was assumed (Miller, 1987). Thus, RNIs of 47, 39, and 36 µg/kg body weight per day, respectively, were set for successive three months periods of infancy. For children, RNIs were interpolated.

825 **Table 4:** Overview of Dietary Reference Values for copper for children

	D-A-CH (2015)	NCM (2014)	Afssa (2001)	IOM (2001)	SCF (1993)	NL (1992)	DH (1991)
Age (months)	4–<12	6–11	6–12	7–12	6–11	6–12	4–12
PRI (mg/day)	0.6–0.7 [(a)]	0.3	0.6	0.22 [(a)]	0.3	0.3–0.5 [(b)]	0.3
Age (years)	1–<4	1–<2	1–3	1–3	1–3	1–4	1–3
PRI (mg/day)	0.5–1.0 [(a)]	0.3	0.75	0.34	0.4	0.3–0.7 [(b)]	0.4
Age (years)	4–<7	2–5	4–6	4–8	4–6	4–7	4–6
PRI (mg/day)	0.5–1.0 [(a)]	0.4	1.0	0.44	0.6	0.5–1.0 [(b)]	0.6
Age (years)	≥7	6–9	7–10		7–10	7–10	7–10
PRI (mg/day)	1.0–1.5 [(a)]	0.5	1.2		0.7	0.6–1.4 [(b)]	0.7
Age (years)		10–13	11–20	9–13	11–14	10–13	11–14
PRI (mg/day)		0.7	1.5	0.7	0.8	1.0–2.5 [(b)]	0.8
Age (years)		14–17		14–18	15–17	13–16	15–18
PRI (mg/day)		0.9		0.89	1.0	1.5–3.0 [(b)]	1.0
Age (years)						16–19	
PRI (mg/day)						1.5–3.5 [(b)]	

826 NCM, Nordic Council of Ministers; NL, Netherlands' Food and Nutrition Council
827 (a): Adequate Intake
828 (b): Adequate range of intake

829 ### 4.3. Pregnancy and lactation

830 In NNR 2012 it was noted that there is a small additional requirement for copper in the last trimester
831 of pregnancy which may be met by adaptive mechanisms leading to increased absorption efficiency
832 (Nordic Council of Ministers, 2014). Nevertheless, an additional intake of 0.1 mg/day was
833 recommended during pregnancy (Table 5). For lactating women, an additional intake of 0.4 mg/day
834 was set to compensate for secretion of copper into milk, assuming a copper breast milk concentration
835 of 0.25 mg/L, a daily milk volume of 0.75 L/day and an absorption efficiency of 50 %.

836 In the absence of data on copper requirement in pregnancy, IOM (2001) considered the amount of
837 copper accumulated in the fetus (13.7 mg according to Widdowson and Dickerson (1964)) and the
838 products of conception (estimated at one-third of the value for the fetus, i.e. 4.6 mg) over the course of
839 pregnancy. To support a total accumulation of 18 mg it was estimated that an intake of 0.067 mg/day
840 is needed, taking into account a copper absorption of 65–70 %. Though absorption may be up-
841 regulated to meet this additional requirement without additional copper intake, the available data
842 (Turnlund et al., 1983) were considered too limited and an additional EAR of 0.1 mg/day was derived
843 for pregnancy. A CV of 15 % was applied to set the RDA for pregnancy at 1.0 mg/day. For lactating
844 women, an additional EAR of 0.3 mg/day was considered to replace the amount of copper secreted
845 with breast milk (about 0.2 mg/day). An RDA of 1.3 mg/day was derived considering a CV of 15 %.

846 Afssa (2001) advised an increase in intake of 0.5 mg/day for pregnant women due to the additional
847 copper requirement of the fetus during the last trimester. During lactation, the amount of copper
848 secreted in breast milk was estimated at 0.1–0.3 mg/day. An additional intake of 0.5 mg/day was
849 proposed for lactating women.

850 The SCF (1993) and the UK COMA (DH, 1991) did not set an increment for pregnancy, as it was
851 assumed that the requirement for the products of conception of 0.033, 0.063 and 0.148 mg/day for the
852 first, second and third trimesters, respectively (Shaw, 1980) could be met by metabolic adaptation. For
853 lactating women, considering a milk production of 0.75 L/day, a copper concentration in breast milk
854 of 0.22 mg/L (Casey et al., 1989) and an absorption efficiency of 50 %, SCF (1993) advised on an
855 extra intake of 0.3 mg/day. UK COMA (DH, 1991) considered the same copper concentration of
856 breast milk but a slightly higher breast milk volume (0.85 L/day) and calculated an additional intake of
857 0.38 mg/day for lactating women, though a value of 0.3 mg/day was derived as additional RNI for
858 lactating women.

The Netherlands Food and Nutrition Council (1992) noted a copper deposition in the fetus and placenta of 16 mg (Cavell and Widdowson, 1964) and considered that this amount is deposited mainly in the last trimester, equivalent to a requirement for absorbed copper of 0.2 mg/day. Taking into account an absorption efficiency of 40 % led to a proposed increment in intake of 0.5 mg/day for pregnant women during the last trimester of pregnancy. For lactating women a daily secretion of copper in breast milk of 0.2–0.4 mg/day was assumed. Taking into account an absorption efficiency of 40 %, an additional intake of 0.5–1.0 mg/day was proposed for lactation.

The German-speaking countries (D-A-CH, 2015) did not derive (separate) DRVs for copper for pregnant and lactating women.

Table 5: Overview of Dietary Reference Values for copper for pregnant and lactating women

	D-A-CH (2015)	NCM (2014)	Afssa (2001)	IOM (2001)	SCF (1993)	NL (1992)	DH (1991)
Pregnancy:							
additional intake (mg/day)	–	0.1	0.5	0.1	0	0.5 [(a)]	0
PRI (mg/day)	1.0–1.5 [(b)]	1.0	2.0	1	1.1	2.0–3.5 [(a, c)]	1.2
Lactation:							
additional intake (mg/day)	–	0.4	0.5	0.3	0.3	0.5–1.0	0.3
PRI (mg/day)	1.0–1.5 [(b)]	1.3	2.0	1.3	1.4	2.0–3.5 [(c)]	1.5

NCM, Nordic Council of Ministers; NL, Netherlands' Food and Nutrition Council
(a): Third trimester
(b): Adequate Intake
(c): Adequate range of intake

5. Criteria (endpoints) on which to base Dietary Reference Values

5.1. Indicators of copper requirement in adults

The Panel considers that there are no biomarkers of copper status that are sufficiently robust, sensitive and specific to be used for deriving requirements for copper (see Section 2.4).

5.2. Balance studies in adults

Balance studies are based on the assumption that a healthy subject on an adequate diet maintains an equilibrium or a null balance between nutrient intakes and nutrient losses: at this null balance, the intake matches the requirement determined by the given physiological state of the individual. When intakes exceed losses (positive balance), there is nutrient accretion that may be attributable to growth or to weight gain, anabolism or repletion of stores; when losses exceed intakes (negative balance), nutrient stores are progressively depleted resulting, in the long term, in clinical symptoms of deficiency. In addition to numerous methodological concerns about accuracy and precision in the determination of intakes and losses (Baer et al., 1999), the validity of balance studies for addressing requirements has been questioned: they might possibly reflect only adaptive changes before a new steady state is reached (Young, 1986), or they might reflect only the conditions for maintenance of nutrient stores in the context of a given diet and, consequently, the relevance of the pool size for health still needs to be established for each nutrient (Mertz, 1987).

Several studies report estimations of copper balance in adults.

A series of four copper balance studies were carried out in eight men aged 18–36 years (Milne et al., 1990) in order to evaluate the effects of mild copper depletion. During an equilibration period of up to 43 days diets providing 1.22–1.57 mg copper/day were consumed. This was followed by a depletion period with a copper intake of 0.73–0.99 mg/day for up to 120 days. Depletion was then followed by repletion of up to 39 days with a diet containing 4.34–6.42 mg copper/day. Copper balance was calculated as the difference between dietary intake and faecal and urinary losses. Sweat and dermal losses were measured in only three of the men. When the data from the balances on days 30, 42 and 43

of the equilibration period were combined, a mean daily intake of 1.29 ± 0.44 mg copper resulted in a positive balance of 0.015 ± 0.07 mg/day, but this figure did not take into account sweat and dermal losses, which averaged 0.12–0.15 mg/day. The Panel notes that if these losses are included in the balance calculations, an intake of 1.29 ± 0.44 mg is not sufficient to achieve null balance in men. Mean balance on days 105, 108 and 120 of the depletion period was 0.025 ± 0.08 mg/day, and it was 0.51 ± 0.30 mg/day on days 25, 30 and 39 of the repletion period. Only three of the eight men exhibited slightly negative balance during either equilibration or depletion.

Turnlund et al. (1998) examined the effect of consuming "low" and "very low" copper diets (0.66 mg/day for 24 days followed by 0.38 mg/day for 42 days) compared with higher intake (2.49 mg/day for 24 days following the "very low" copper diet period) on copper retention in 11 young men. Balances were calculated for six-day periods throughout the study and copper retention averaged over each dietary period. Mean copper retention over the dietary periods was -0.13, -0.015, and 0.51 mg/day[11] for the "low", "very low" and "high" copper diets, respectively. The Panel notes that faecal copper excretion changed throughout the dietary periods, presumably a feature of adaptation to the differing levels of intake. Sweat/dermal and urinary losses were not taken into account, and the Panel concludes that copper balance is achieved at levels of intake somewhere between 0.66 and 2.49 mg/day.

Turnlund et al. (2005) examined the effect of high copper intake on copper metabolism. In this study nine men aged 26–49 years consumed a diet containing 1.6 mg copper/day for 18 days before receiving a copper-supplemented diet containing 7.8 mg copper/day, and copper retention was calculated as the difference between copper intake and faecal and urinary excretion over the last 12 days of each metabolic period. With a copper intake of 1.6 mg/day, the retention of copper was calculated to be 0.06 mg/day, and it was 0.67 mg/day with the high intake. The Panel notes that this study did not make an allowance for sweat and dermal losses.

Harvey et al. (2003) examined the effect of consuming "low" (0.7 mg/day), "medium" (1.6 mg/day) and "high" (6.0 mg/day) copper diets for eight weeks on copper absorption and endogenous losses in 12 men aged 20–59 years. There was a washout period of four weeks between study periods. Copper balance calculated as the difference between intake and faecal excretion was -0.13 ± 0.32, 0.00 ± 0.31, and 0.75 ± 1.05 mg/day on the "low", "medium" and "high" copper diets, respectively. The Panel notes that urinary, sweat and dermal losses were not measured.

Milne et al. (2001) examined the effect of changing zinc status on copper balance in postmenopausal women. Twenty-one women were fed a diet containing 2 mg copper/day for 10 days. They were then divided into two groups, one given 1 mg copper/day and the other 3 mg copper/day. After 10 days of equilibration, they were given a diet either high or low in zinc. The results showed that the women were in positive copper balance only when given 3 mg copper/day and 53 mg zinc/day (high zinc diet). Further, the ratio of immunoreactive ceruloplasmin and serum ferroxidase activity was dependent on the copper/zinc ratio. The authors concluded that low zinc status can amplify the consequences of a low dietary copper intake, and that an intake of 1 mg/day of copper is inadequate for postmenopausal women.

Prystai et al. (1999) studied the effect of drinking tea on the balance of several micronutrients in five men and four women, who underwent four experimental treatments (black, decaffeinated black, green or no tea) of 14 days each. The first seven days of each period were used as dietary adaptation, while the second seven days were used in the calculation of mineral balances. Data from both sexes were combined, as no sex differences were recorded. At copper intakes in the four treatment periods between 1.2 and 1.4 mg/day (least square means), balances were slightly negative and ranged between -0.1 and -0.4 mg/day (least square means). The Panel notes that the number of participants was small and that balance figures are given for combined sexes only, but considers that the data suggest that intakes of about 1.3 mg/day may be marginal for both men and women.

[11] A figure of 511 mg/day is reported in the paper which is assumed to be a reporting error.

The Panel notes that copper intakes of 2.49 mg/day (Turnlund et al., 1998) and of 7.8 mg/day (Harvey et al., 2003) resulted in a positive copper balance in men. The Panel also notes that balance values were negative for copper intakes from 0.38 to 0.7 mg/day in men (Turnlund et al., 1998; Harvey et al., 2003), while zero balance was reached at a copper intake of approximately 1.6 mg/day in men (Harvey et al., 2003; Turnlund et al., 2005). The Panel considers that, although dietary intake was usually carefully controlled, not all potential sources of copper loss were considered (such as losses via urine and sweat and skin), so that the intake at which balance may be zero is likely underestimated. The Panel also considers that some of the balance studies (Prystai et al., 1999; Turnlund et al., 2005) have a rather short duration of the periods during which dietary intake is maintained at a fixed level before balance measurement, which may be insufficient for homeostatic adaptation to occur.

The Panel considers that, although there are significant limitations to the balance studies so that they cannot be used alone, they may be used, together with other data, to inform the setting of DRVs for copper.

5.3. Indicators of copper requirement in children

Few studies are available on copper balance in older infants and children.

Price and colleagues examined copper balance in 15 girls aged 7–9 years (Price et al., 1970). After a six-day adjustment period with a diet containing 1.86 mg copper/day, the girls were randomly allocated to one of four different diets for five balance periods consisting of six consecutive days. Food, faeces and urine were collected and average 24-hour balances derived. Intakes ranged between 1.55 and 1.7 mg/24 hours and net balance (apparent retention) between 0.48 and 0.77 mg/24 hours. The authors compared their data to a previous study in girls (Engel et al., 1967), where balance was (close to) zero (0.0 to -0.06 mg/24 hours) following copper intakes of 1.08–1.33 mg/24 hours and positive (0.66 and 1.38 mg/24 hours) when copper intake was either 2.83 or 3.87 mg/24 hours. The Panel notes that copper balance seems to be zero or positive for copper intakes above about 1 mg/day in girls, though the Panel notes that dermal losses were not measured.

Alexander and colleagues carried out three-day balance studies in eight healthy children aged between about 3 months and 8 years (Alexander et al., 1974). Urine and faeces were collected and duplicate diets analysed. Mean copper intake was 35.0 ± 22.0 µg/kg body weight per day and mean total excretion was 30.4 ± 17.0 µg/kg body weight per day. This gave a mean retention of 4.6 ± 12.3 µg/kg body weight per day. The Panel notes that a copper intake of about 35 µg/kg body weight per day was associated with positive balance in infants and children and also notes that children were heterogeneous with respect to age and that there was large variability around the mean estimate.

The Panel concludes that the information is too limited to be used for deriving DRVs for copper for children.

5.4. Indicators of copper requirement in pregnancy and lactation

McCance and Widdowson (1951) reported that the copper content of a human fetus at term is 4.7 mg/kg fat free mass. A total value of about 16 mg in the placenta and fetus was reported (Cavell and Widdowson, 1964). The Panel considers that the accretion rate is not linear, with most of the accumulation occurring in the last trimester of pregnancy. There are no data on changes in absorption in pregnant women, but in mice, the increase in copper levels in the fetus is not matched by a comparable increase in percentage absorption (McArdle and Erlich, 1991). However, given the total accretion and the time of gestation, an approximate increment of about 0.2 mg/day in the last trimester (16 mg/93 days) can be estimated.

There are very limited data describing how the body adapts to pregnancy and its requirements. Some early studies suggested that maternal copper deficiency does not alter serum copper concentrations in the fetus or placenta, but how this is mediated is not known. In rats, maternal copper deficiency is not

associated with alteration in expression in the genes for copper transport and metabolism (Andersen et al., 2007). There is no information on what changes, if any, occur in humans.

5.5. Copper intake and health consequences

A comprehensive search of the literature published between January 1990 and February 2012 was performed as preparatory work to identify relevant health outcomes on which DRVs for copper may be based (Bost et al., 2012). Additional literature searches were performed until April 2015.

5.5.1. Cardiovascular disease-related outcomes

Copper deficiency diseases such as Menkes, although primarily neurological, are also associated with an increased risk of cardiovascular disease (Tumer and Moller, 2010). An increased occurrence of ventricular premature discharges during copper depletion has been reported in two trials in postmenopausal women (Milne and Nielsen, 1996; Milne et al., 2001). In the first trial, 13 women underwent an equilibration period with 1.37 mg copper/day for 35 days, followed by a deprivation period with 0.57 mg copper/day for 105 days and a repletion period with 2 mg copper/day for 35 days). In three women, a significant increase in the number of ventricular premature discharges was observed after 21, 63 and 91 days, respectively, on the diet providing 0.57 mg copper/day. However, the authors do not provide any information regarding the results of the electrocardiogram or the extent of the increase in premature ventricular discharges. No significant increase was observed for the other women consuming the diet with 0.57 mg copper/day (Milne and Nielsen, 1996). In the 2001 study, three women out of 12 on a diet providing 1 mg copper/day during a 90-day period exhibited abnormal electrocardiographic recording (premature ventricular discharge) requiring copper supplementation before the end of the study. However, two of these women still exhibited an increased number of abnormal premature ventricular discharges after copper supplementation with 3 mg/day (the duration of copper supplementation is not specified). After the study, it was noted that these two women had very high zinc levels from the cement they were using for their dentures. Their data were not included in the final paper. Moreover, none of the women receiving 3 mg copper/day showed significant changes in their electrocardiograms (Milne et al., 2001). The Panel notes the absence of information regarding blinding and randomization, and the enrolment of some subjects with specific health conditions. The Panel considers that no conclusions can be drawn from these studies on whether cardiac arrhythmia may be a result of copper deficiency.

The results of heart rate monitoring are also reported in a third study examining the effect of copper depletion in 11 young men (Turnlund et al., 1997). In this study, no difference was observed in the occurrence of ventricular premature discharges and supraventricular ectopic beats between the adaptation (0.66 mg/day for 24 days), depletion (0.38 mg/day for 42 days) and repletion (2.49 mg/day for 24 days) periods.

In an RCT in 16 women aged 21–28 years, Bugel et al. (2005) examined cardiovascular risk factors following copper supplementation with either 0, 3, or 6 mg/day in a cross-over design of three four-week periods with three weeks of washout in between. The copper content of the habitual diet of the women was not assessed. Supplementation with 3 and 6 mg/day increased serum copper concentration and erythrocyte SOD activity, but some biomarkers associated with cardiovascular disease (LDL-, HDL- and VLDL-cholesterol, triacylglycerol, lipoprotein(a), Apo A-1, Apo B and various haemostatic factors associated with thrombosis) were not altered, though there was a decrease in fibrolytic enzyme activity.

The Panel considers that the evidence on copper intake and cardiac arrhythmia and on copper intake and biomarkers associated with cardiovascular disease cannot be used for setting DRVs for copper.

5.5.2. Cancer

The World Cancer Research Fund (WCRF) considered the role of micronutrients in cancer development. It was suggested that copper status might play a role in the development of cancers with an immune function origin, but that the evidence for such a link was very limited. It was also

considered that there was no evidence for an association between copper intake/status and lung cancer (WCRF/AICR, 2007).

Two prospective cohort studies and one case-control study assessed the association between copper intake/status and risk for several types of cancer. Mahabir et al. (2010) showed no association between total (dietary and supplemental) copper intake and lung cancer risk in a cohort study with a mean follow-up of seven years and including 482 875 subjects (7 052 cases) in the USA. Subjects were mostly white (91 %), 60 % were men, 49 % of subjects were former smokers, 12 % were current smokers and 36 % never smoked (smoking status was unknown for 3 % of the subjects). Thompson et al. (2010) did not observe an association between total copper intake or that from diet or supplements only and the risk for Non-Hodgkin's lymphoma, diffuse large B-cell lymphoma or follicular lymphoma in a cohort study with a follow-up of 20 years and including 35 159 women (415 cases).

Senesse et al. (2004) studied the association between copper intake and colorectal cancer. A total of 171 colorectal cancer cases (109 males, 62 females) were compared to 309 (159 males, 150 females) controls and there was an increased risk of colorectal cancer in the fourth quartile of copper intake (odds ratio 2.4, 95 % CI 1.3–4.6, $P < 0.01$) compared to the first quartile.

The Panel considers that there is very limited evidence for an association between copper intake and cancer incidence and that the data cannot be used for setting DRVs for copper.

6. Data on which to base Dietary Reference Values

6.1. Adults

The Panel considers that there are no biomarkers of copper status which are sufficiently robust to be used to derive requirements for copper (see Section 5.1). The Panel also considers that there are significant limitations to copper balance studies (Section 5.2), but that they may be used in conjunction with intake data to inform the setting of DRVs for copper for adults. The Panel proposes to set an AI, using both observed intakes and the results from balance studies, despite their limitations.

The range of average copper intakes in eight EU countries is 1.47–1.67 mg/day (midpoint 1.57 mg/day) for men and 1.20–2.07 mg/day (midpoint 1.63 mg/day) for women aged 18 to < 65 years (see Section 3.2 and Appendices C and D). The Panel notes, though, that the Latvian survey, for which the average intake was 2.07 mg/day, comprised pregnant women only. Excluding this survey, the range of average intakes is 1.19–1.44 mg/day (midpoint 1.32 mg/day). For older adults (65 to < 75 years), the ranges are 1.33–1.67 mg/day (midpoint 1.50 mg/day) for men and 1.20–1.41 mg/day (midpoint 1.30 mg/day) for women. For adults from 75 years, the ranges are 1.27–1.50 mg/day (midpoint 1.38 mg/day) for men and 1.15–1.37 mg/day (midpoint 1.26 mg/day) for women. The Panel notes that midpoints of ranges for intake estimates in these age and sex groups are in good agreement with medians, for the respective sex and age groups, of the average intakes estimated per survey.

The Panel notes that there is at present insufficient evidence for considering different DRVs according to age in adults, and decided to merge the ranges for all men aged 18 years and older (observed mean copper intakes of 1.27–1.67 mg/day), for which the midpoint is 1.47 mg/day. Similarly, for women, the merged range for all women aged 18 years and older is 1.15–1.44 mg/day (excluding Latvian pregnant women), with a midpoint at 1.30 mg/day. The median of average intakes of adult women (≥ 18 years, excluding Latvian pregnant women) is 1.29 mg/day, and the median of average intakes of adult men (≥ 18 years) is 1.52 mg/day.

Given these differences in intake, the Panel proposes to set AIs for men and women separately. For men, based on observed intakes and taking into account that zero copper balance was reported at a copper intake of approximately 1.6 mg/day in men (Harvey et al., 2003; Turnlund et al., 2005), the Panel proposes an AI of 1.6 mg/day. For women, based on observed intakes, the Panel proposes an AI of 1.3 mg/day.

6.2. Infants aged 7–11 months

Considering a mean copper concentration in breast milk of 0.35 mg/L and a volume of milk intake during the first six months of life in exclusively breast-fed infants of 0.8 L/day (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009), it can be estimated that the copper intake of infants aged 0–6 months is 0.2 mg/day. In order to estimate the copper intake of infants aged 7–11 months from the calculated copper intake in infants from birth to six months, allometric scaling may be applied on the assumption that copper requirement is related to metabolically active body mass. Using averages of the median weight-for-age of male and female infants aged three months (6.1 kg) and nine months (8.6 kg) according to the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006), a value of 0.36 mg/day was calculated.

Average copper intakes of infants in four surveys in the EU ranged between 0.34 and 0.49 mg/day (Section 3.2 and Appendices C and D). Taking into account the results of upward extrapolation of copper intakes in exclusively breast-fed infants, the Panel proposes an AI for infants aged 7–11 months of 0.4 mg/day.

6.3. Children

As for adults, the Panel proposes to set AIs based on observed intakes in EU countries.

In young children (1 to < 3 years), mean observed copper intakes from five surveys in four EU countries range from 0.60–0.86 mg/day in boys and from 0.57–0.94 mg/day in girls (Appendices C and D). The Panel notes, though, that for both sexes the upper value is derived from the Italian survey with very few children (20 boys and 16 girls). Excluding this survey, the ranges of mean intakes are 0.60–0.79 mg/day (midpoint 0.69 mg/day) in boys and 0.57–0.73 mg/day (midpoint 0.65 mg/day) in girls. For boys and girls aged 1 to < 3 years, considering the absence of a strong basis for a distinct value according to sex and the distribution of observed mean intakes, the Panel selects the midpoint of average intakes and sets an AI of 0.7 mg/day for boys and girls.

In children aged 3 to < 10 years, mean observed copper intakes from seven surveys in six EU countries range from 0.92–1.44 mg/day (midpoint 1.18 mg/day) in boys and from 0.82–1.30 mg/day (midpoint 1.06 mg/day) in girls (Appendices C and D). It was considered unnecessary to derive a distinct value according to sex. The median of average intakes of both sexes combined is 0.98 mg/day. For boys and girls aged 3 to < 10 years, considering the distribution of the observed mean intakes, the Panel sets an AI of 1.0 mg/day.

In children aged 10 to < 18 years, mean observed copper intakes from seven surveys in seven EU countries range from 1.16–1.59 mg/day (midpoint 1.38 mg/day) in boys and from 0.98–1.92 mg/day in girls (Appendices C and D). However, the Panel notes that the data provided for Latvia include pregnant girls below 18 years of age and are rather high compared to other datasets; excluding Latvian pregnant girls provides a narrower range of 0.98–1.41 mg/day (midpoint 1.20 mg/day). The median of average intakes of girls aged 10 to < 18 years (excluding Latvian pregnant girls) is 1.12 mg/day, and the median of average intakes of boys aged 10 to < 18 years is 1.32 mg/day.

Considering the rather large differences in copper intakes between boys and girls aged 10 to < 18 years, the Panel proposes to set AIs according to sex. For boys aged 10 to < 18 years, considering the distribution of the observed average intakes, the Panel sets an AI of 1.3 mg/day. For girls aged 10 to < 18 years, considering the distribution of the observed average intakes, the Panel sets an AI of 1.1 mg/day.

6.4. Pregnancy

The Panel accepts that a total quantity of 16 mg of copper is accumulated in the fetus and the placenta over the course of pregnancy (Section 5.3). In order to allow for the extra need related to the growth of maternal tissues and fetal and placental requirements, and given the limited information on what

adaptive changes may occur during pregnancy, the Panel proposes an additional requirement of 0.06 mg/day (16 mg/280 days) to the whole period of pregnancy.

Considering a fractional copper absorption of 50 %, and in anticipation of copper requirements for lactation, the Panel proposes that the AI of non-pregnant women is increased by 0.2 mg/day during pregnancy.

6.5. Lactation

During the first six months of lactation, the Panel notes that available data indicate that copper concentrations in mature breast milk of population from Western countries are variable and range between 0.1 and 1.0 mg/L, decreasing as lactation proceeds. Based on a mean milk transfer of 0.8 L/day (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009) during the first six months of lactation in exclusively breastfeeding women and a concentration of copper in breast milk of 0.35 mg/L (mean value over time), a loss of 0.28 mg/day of copper in breast milk is estimated during the first six months of lactation.

Taking into account a fractional absorption of copper of about 50 %, an increment of 0.56 mg/day would be required to compensate for these losses. The Panel assumes that this can be mitigated in part by the increased AI in pregnancy. Thus, the Panel proposes that the AI of non-pregnant women is increased by 0.2 mg/day during lactation.

CONCLUSIONS

The Panel concludes that Average Requirements (ARs) and Population Reference Intakes (PRIs) for copper cannot be derived for adults, infants and children, and proposes Adequate Intakes (AIs). For adults, this approach considers the range of average copper intakes estimated from dietary surveys in eight EU countries and results of some balance studies. For infants aged 7–11 months and children, the Panel proposes AIs after consideration of observed intakes and taking into account, for infants aged 7-11 months, upwards extrapolation from the estimated copper intakes of breast-fed infants aged 0-6 months. For pregnant and lactating women, the Panel considers it appropriate to increase the AI to cover the amount of copper deposited in the fetus and placenta over the course of pregnancy and in anticipation of the needs for lactation and the amount of copper secreted with breast milk, respectively.

Table 6: Summary of Adequate Intakes (AIs) for copper

Age	AI (mg/day)	
	Males	Females
7 to 11 months	0.4	0.4
1 to < 3 years	0.7	0.7
3 to < 10 years	1.0	1.0
10 to < 18 years	1.3	1.1
≥ 18 years	1.6	1.3
Pregnancy		1.5
Lactation		1.5

RECOMMENDATIONS FOR RESEARCH

The Panel suggests that there is a need for research to identify sensitive and specific markers of copper status.

The Panel suggests that there is a need to investigate copper status in vulnerable populations, especially older adults and women of reproductive age.

1167 The Panel suggests more research is needed to understand copper metabolism, especially in relation to
1168 homeostatic adaptations in pregnancy and lactation.

1169

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APPENDICES

Appendix A. COPPER CONCENTRATION IN HUMAN MILK OF HEALTHY MOTHERS OF TERM INFANTS PUBLISHED AFTER THE REVIEW OF DOREA (2000)

Reference	Number of women (number of samples)	Country	Stage of lactation (time post partum)	Copper concentration (µg/L)			Analytical method
				Mean ± SD	Median	Range	
Bjorklund et al. (2012)	60 (840)	Sweden	14–21 days	471 ± 75	471	327–670	Samples collected by manual breast milk pump and/or passive breast milk sampler, collected at the beginning and end of a breastfeeding session. Samples analysed by ICP-MS.
Domellof et al. (2004)	105 (105) 86 (86)	Central America Sweden	3 months	160 ± 21 120 ± 22			Milk samples collected by hand or manual pump in the morning ≥ 1 hour after the previous breastfeeding and analysed by AAS.
Gulson et al. (2001)	17 (78)	Australia	0–6 months	370 ± 87 [(b)]	340 [(b)]		Copper analysed by ICP-MS
Hannan et al. (2005)	25 (75)	Libya	0–4 days 5–9 days 10–20 days	840 ± 60 660 ± 60 390 ± 50			Milk samples (5-7 mL) expressed manually and freeze-dried.
Leotsinidis et al. (2005)	180 (180) 180 (95)	Greece	Day 3 Day 14	381 ± 132 390 ± 108	368 408	97–690 120–614	Milk samples (10–20 mL) collected 2 hours after the previous breastfeeding. Copper analyzed by AAS.
Nakamori et al. (2009)	95 (Not reported)	Vietnam	6–12 months	190 ± 50			Breast milk samples (20 mL) manually collected in the morning and frozen at -20°C until analysis. Copper analysed by ICP-AES.
Orun et al. (2012)	125	Turkey	52–60 days		241	200–296 [(a)]	Milk samples collected by manual expression 2 hours after the last feeding session in the morning. Copper

Reference	Number of women (number of samples)	Country	Stage of lactation (time post partum)	Copper concentration (µg/L)			Analytical method
				Mean ± SD	Median	Range	
							determined by ICP-MS.
Turan et al. (2001)	Not reported (30)	Turkey	48 hours	278 ± 58		179–454	Milk samples (8 mL) expressed by a breast pump. Samples analysed by AAS.
Ustundag et al. (2005)	20	Turkey	0–7 days 7–14 days 21 days 60 days	1 120 ± 138 1030 ± 98 1 090 ± 107 970 ± 88			Manual expression of milk within 2 hours of first feeding in the morning (defined as between 8 and 11 AM), analysis by AAS.
Wasowicz et al. (2001)	131 (43) 131 (46) 131 (41)	Poland	0–4 days 5–9 days 10–30 days	450 ± 110 390 ± 91 270 ± 90			Milk samples (5–7 mL) expressed by hand and determined by ICP-AES.
Winiarska-Mieczan (2014)	323 (323)	Poland	1–12 months	137 ± 92	106	25–455	Milk samples (25 mL) collected by manual expression and analysed by FAAS.
Wünschmann et al. (2003)	23	Germany, Poland, Czech Republic	Mature milk (age of child 0.4–16.4 months)	189 (b)		49–485 (b)	Milk samples collected from both breasts (30–50 mL per breast, eventually by a breast pump) and analysed by ICP-MS.

AAS, atomic absorption spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; ICP-AES, inductively coupled plasma atomic emission spectrometry; FAAS, flame atomic absorption spectrometry

(a): 25^{th}–75^{th} percentile

(b): After conversion from mg/g or µg/kg into mg/L using a conversion factor of 1.03 kg/L of breast milk, as reported in Brown et al. (2009).

Appendix B. DIETARY SURVEYS IN THE EFSA COMPREHENSIVE EUROPEAN FOOD CONSUMPTION DATABASE INCLUDED IN THE NUTRIENT INTAKE CALCULATION AND NUMBER IN THE DIFFERENT AGE CLASSES

Country	Dietary survey	Year	Method	Days	Age (years)	Number of subjects						
						Infants 1–11 mo	Children 1–< 3 y	Children 3–< 10 y	Children 10–< 18 y	Adults 18–< 65 y	Adults 65–< 75 y	Adults ≥ 75 y
Finland/1	DIPP	2000–2010	Dietary record	3	0.5–6	499	500	750				
Finland/2	NWSSP	2007–2008	48-hour dietary recall [(a)]	2 × 2 [(a)]	13–15				306			
Finland/3	FINDIET2012	2012	48-hour dietary recall [(a)]	2 [(a)]	25–74					1 295	413	
France	INCA2	2006–2007	Dietary record	7	3–79			482	973	2 276	264	84
Germany/1	EsKiMo	2006	Dietary record	3	6–11			835	393			
Germany/2	VELS	2001–2002	Dietary record	6	<1–4	158	347	299				
Ireland	NANS	2008–2010	Dietary record	4	18–90					1 274	149	77
Italy	INRAN-SCAI	2005–2006	Dietary record	3	<1–98	16 [(b)]	36 [(b)]	193	247	2 313	290	228
Latvia	FC_PREGNANTWOMEN	2011	24-hour dietary recall	2	15–45				12 [(b)]	991 [(c)]		
Netherlands	DNFCS	2007–2010	24-hour dietary recall	2	7–69			447	1 142	2 057	173	
Sweden	RISKMATEN	2010–2011	Dietary records (Web)	4	18–80					1 430	295	72
UK/1	DNSIYC	2011	Dietary record	4	0.3–1.5	1 369	1 314					
UK/2	NDNS-Rolling Programme (1–3 y)	2008–2011	Dietary record	4	1–94		185	651	666	1 266	166	139

mo, months; y, years; DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungsstudie als KIGGS-Modul; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale de Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione - Studio sui Consumi Alimentari in Italia; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): A 48-hour dietary recall comprises two consecutive days.

(b): 5^{th} or 95^{th} percentile intakes calculated from fewer than 60 subjects require cautious interpretations as the results may not be statistically robust (EFSA, 2011a) and, therefore, for these dietary surveys/age classes, the 5^{th} and 95^{th} percentile estimates will not be presented in the intake results.

(c): One subject with only one 24-hour dietary recall day was excluded from the dataset, i.e. the final n = 990.

Appendix C. COPPER INTAKE IN MALES IN DIFFERENT SURVEYS ACCORDING TO AGE CLASSES AND COUNTRY

Age class	Country	Survey	Intake expressed in mg/day					Intake expressed in mg/MJ				
			n [(a)]	Average	Median	P5	P95	n	Average	Median	P5	P95
Infants [(b)]	Germany	VELS	84	0.50	0.49	0.25	0.79	84	0.15	0.15	0.09	0.23
	Finland	DIPP_2001_2009	247	0.37	0.39	0.07	0.63	245	0.21	0.18	0.13	0.38
	United Kingdom	DNSIYC_2011	699	0.41	0.38	0.13	0.73	699	0.12	0.11	0.05	0.19
	Italy	INRAN_SCAI_2005_06	9	0.44	0.40	–[(c)]	–[(c)]	9	0.14	0.14	–[(c)]	–[(c)]
1 to < 3	Germany	VELS	174	0.79	0.75	0.46	1.20	174	0.17	0.17	0.13	0.22
	Finland	DIPP_2001_2009	245	0.64	0.62	0.39	0.94	245	0.18	0.17	0.12	0.28
	United Kingdom	NDNS–RollingProgrammeYears1–3	107	0.72	0.67	0.48	1.12	107	0.15	0.14	0.10	0.20
	United Kingdom	DNSIYC_2011	663	0.60	0.57	0.29	0.96	663	0.14	0.14	0.09	0.20
	Italy	INRAN_SCAI_2005_06	20	0.86	0.84	–[(c)]	–[(c)]	20	0.18	0.16	–[(c)]	–[(c)]
3 to < 10	Germany	EsKiMo	426	1.44	1.41	0.89	2.05	426	0.19	0.18	0.14	0.26
	Germany	VELS	146	0.97	0.95	0.66	1.37	146	0.17	0.17	0.13	0.22
	Finland	DIPP_2001_2009	381	0.92	0.88	0.58	1.33	381	0.16	0.15	0.11	0.22
	France	INCA2	239	0.98	0.95	0.52	1.55	239	0.16	0.15	0.11	0.23
	United Kingdom	NDNS–RollingProgrammeYears1–3	326	0.94	0.91	0.57	1.35	326	0.15	0.14	0.11	0.20
	Italy	INRAN_SCAI_2005_06	94	1.31	1.18	0.75	2.68	94	0.18	0.15	0.11	0.34
	Netherlands	DNFCS 2007–2010	231	1.09	1.07	0.65	1.58	231	0.13	0.13	0.08	0.17
10 to < 18	Germany	EsKiMo	197	1.50	1.48	0.92	2.17	197	0.19	0.18	0.13	0.25
	Finland	NWSSP07_08	136	1.32	1.25	0.76	1.96	136	0.16	0.16	0.12	0.21
	France	INCA2	449	1.28	1.20	0.70	2.06	449	0.16	0.16	0.11	0.24
	United Kingdom	NDNS–RollingProgrammeYears1–3	340	1.16	1.12	0.69	1.78	340	0.14	0.14	0.11	0.18
	Italy	INRAN_SCAI_2005_06	108	1.59	1.51	0.85	2.74	108	0.17	0.15	0.11	0.28
	Netherlands	DNFCS 2007–2010	566	1.33	1.27	0.78	2.04	566	0.13	0.12	0.08	0.17
18 to < 65	Finland	FINDIET2012	585	1.67	1.56	0.90	2.77	585	0.19	0.17	0.11	0.28
	France	INCA2	936	1.52	1.44	0.83	2.46	936	0.18	0.17	0.12	0.26
	United Kingdom	NDNS–RollingProgrammeYears1–3	560	1.47	1.37	0.76	2.50	560	0.17	0.16	0.11	0.25
	Ireland	NANS_2012	634	1.67	1.59	0.85	2.74	634	0.17	0.16	0.11	0.25
	Italy	INRAN_SCAI_2005_06	1 068	1.52	1.43	0.81	2.55	1 068	0.17	0.16	0.11	0.28
	Netherlands	DNFCS 2007–2010	1 023	1.57	1.46	0.85	2.55	1 023	0.14	0.13	0.09	0.20
	Sweden	Riksmaten 2010	623	1.65	1.59	0.83	2.58	623	0.17	0.16	0.12	0.24
65 to < 75	Finland	FINDIET2012	210	1.52	1.46	0.82	2.48	210	0.19	0.18	0.12	0.28
	France	INCA2	111	1.67	1.62	0.89	2.81	111	0.20	0.18	0.13	0.30
	United Kingdom	NDNS–RollingProgrammeYears1–3	75	1.55	1.45	0.76	2.61	75	0.19	0.18	0.12	0.26
	Ireland	NANS_2012	72	1.54	1.43	0.66	2.79	72	0.18	0.17	0.10	0.26
	Italy	INRAN_SCAI_2005_06	133	1.55	1.47	0.82	2.42	133	0.18	0.17	0.12	0.27
	Netherlands	DNFCS 2007–2010	91	1.33	1.29	0.78	1.90	91	0.15	0.14	0.10	0.21
	Sweden	Riksmaten 2010	127	1.53	1.48	0.84	2.24	127	0.18	0.17	0.13	0.23

Age class	Country	Survey	Intake expressed in mg/day					Intake expressed in mg/MJ				
			n [(a)]	Average	Median	P5	P95	n	Average	Median	P5	P95
≥ 75	France	INCA2	40	1.34	1.30	–[(c)]	–[(c)]	40	0.17	0.17	–[(c)]	–[(c)]
	United Kingdom	NDNS–RollingProgrammeYears1–3	56	1.40	1.07	–[(c)]	–[(c)]	56	0.20	0.16	–[(c)]	–[(c)]
	Ireland	NANS_2012	34	1.27	1.21	–[(c)]	–[(c)]	34	0.17	0.16	–[(c)]	–[(c)]
	Italy	INRAN_SCAI_2005_06	69	1.46	1.43	0.81	2.49	69	0.17	0.16	0.11	0.27
	Sweden	Riksmaten 2010	42	1.50	1.40	–[(c)]	–[(c)]	42	0.18	0.17	–[(c)]	–[(c)]

P5, 5th percentile; P95, 95th percentile; DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungsstudie als KIGGS-Modul; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale de Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione - Studio sui Consumi Alimentari in Italia; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): Number of individuals in the population group.

(b): The proportions of breastfed infants were 58 % in the Finnish survey, 40 % in the German survey, 44 % in the Italian survey, and 21 % in the UK survey. Most infants were partially breastfed. For the Italian and German surveys, breast milk intake estimates were derived from the number of breastfeeding events recorded per day multiplied by standard breast milk amounts consumed on an eating occasion at different age. For the UK survey, the amount of breast milk consumed was either directly quantified by the mother (expressed breast milk) or extrapolated from the duration of each breastfeeding event. As no information on the breastfeeding events were reported in the Finnish survey, breast milk intake was not taken into consideration in the intake estimates of Finnish infants.

(c): 5th or 95th percentile intakes calculated from fewer than 60 subjects require cautious interpretation as the results may not be statistically robust (EFSA, 2011a) and, therefore, for these dietary surveys/age classes, the 5th and 95th percentile estimates will not be presented in the intake results.

Appendix D. COPPER INTAKE IN FEMALES IN DIFFERENT SURVEYS ACCORDING TO AGE CLASSES AND COUNTRY

Age class	Country	Survey	Intake expressed in mg/day					Intake expressed in mg/MJ				
			n [(a)]	Average	Median	P5	P95	n	Average	Median	P5	P95
Infants [(b)]	Germany	VELS	75	0.43	0.42	0.22	0.69	75	0.15	0.14	0.09	0.22
	Finland	DIPP_2001_2009	253	0.34	0.34	0.06	0.67	251	0.22	0.18	0.13	0.46
	United Kingdom	DNSIYC_2011	670	0.38	0.35	0.12	0.70	670	0.12	0.12	0.05	0.19
	Italy	INRAN_SCAI_2005_06	7	0.48	0.49	–[(c)]	–[(c)]	7	0.17	0.15	–[(c)]	–[(c)]
1 to < 3	Germany	VELS	174	0.73	0.71	0.37	1.13	174	0.17	0.16	0.11	0.24
	Finland	DIPP_2001_2009	255	0.58	0.56	0.35	0.96	255	0.17	0.16	0.12	0.25
	United Kingdom	NDNS–RollingProgrammeYears1–3	78	0.67	0.63	0.37	1.15	78	0.15	0.15	0.10	0.19
	United Kingdom	DNSIYC_2011	651	0.57	0.54	0.28	0.94	651	0.14	0.14	0.09	0.20
	Italy	INRAN_SCAI_2005_06	16	0.94	0.81	–[(c)]	–[(c)]	16	0.20	0.17	–[(c)]	–[(c)]
3 to < 10	Germany	EsKiMo	409	1.30	1.27	0.82	1.86	409	0.19	0.19	0.14	0.26
	Germany	VELS	147	0.90	0.89	0.61	1.27	147	0.18	0.17	0.13	0.23
	Finland	DIPP_2001_2009	369	0.82	0.78	0.48	1.28	369	0.16	0.15	0.11	0.21
	France	INCA2	243	0.90	0.86	0.50	1.39	243	0.16	0.15	0.11	0.22
	United Kingdom	NDNS–RollingProgrammeYears1–3	325	0.89	0.87	0.53	1.29	325	0.15	0.14	0.11	0.19
	Italy	INRAN_SCAI_2005_06	99	1.17	1.16	0.65	1.76	99	0.16	0.15	0.11	0.25
	Netherlands	DNFCS 2007–2010	216	1.00	0.95	0.66	1.45	216	0.12	0.12	0.09	0.16
10 to < 18	Germany	EsKiMo	196	1.41	1.39	0.93	1.97	196	0.19	0.19	0.14	0.26
	Finland	NWSSP07_08	170	1.13	1.08	0.70	1.86	170	0.17	0.17	0.13	0.23
	France	INCA2	524	1.06	1.02	0.59	1.63	524	0.17	0.16	0.12	0.25
	United Kingdom	NDNS–RollingProgrammeYears1–3	326	0.98	0.96	0.56	1.49	326	0.15	0.14	0.11	0.20
	Italy	INRAN_SCAI_2005_06	139	1.38	1.22	0.67	2.77	139	0.17	0.15	0.10	0.35
	Latvia [(d)]	FC_PREGNANTWOMEN_2011	12	1.92	1.96	–[(c)]	–[(c)]	12	0.20	0.22	–[(c)]	–[(c)]
	Netherlands	DNFCS 2007–2010	576	1.12	1.10	0.69	1.65	576	0.13	0.13	0.08	0.18
18 to < 65	Finland	FINDIET2012	710	1.42	1.36	0.77	2.28	710	0.20	0.19	0.13	0.31
	France	INCA2	1 340	1.25	1.17	0.65	2.09	1 340	0.19	0.18	0.13	0.32
	United Kingdom	NDNS–RollingProgrammeYears1–3	706	1.20	1.11	0.64	1.90	706	0.18	0.17	0.12	0.29
	Ireland	NANS_2012	640	1.28	1.22	0.68	1.94	640	0.17	0.17	0.12	0.26
	Italy	INRAN_SCAI_2005_06	1 245	1.33	1.26	0.70	2.17	1 245	0.18	0.17	0.11	0.30
	Latvia [(d)]	FC_PREGNANTWOMEN_2011	990	2.07	1.91	1.12	3.51	990	0.25	0.22	0.14	0.41
	Netherlands	DNFCS 2007–2010	1 034	1.31	1.25	0.75	2.05	1 034	0.16	0.15	0.10	0.25
	Sweden	Riksmaten 2010	807	1.44	1.37	0.78	2.35	807	0.20	0.18	0.12	0.28
65 to < 75	Finland	FINDIET2012	203	1.34	1.20	0.74	2.22	203	0.22	0.20	0.13	0.34
	France	INCA2	153	1.29	1.27	0.62	2.10	153	0.21	0.19	0.14	0.38
	United Kingdom	NDNS–RollingProgrammeYears1–3	91	1.21	1.12	0.64	2.04	91	0.20	0.18	0.13	0.36
	Ireland	NANS_2012	77	1.25	1.26	0.72	1.96	77	0.19	0.18	0.13	0.26
	Italy	INRAN_SCAI_2005_06	157	1.30	1.23	0.74	2.07	157	0.19	0.18	0.12	0.31
	Netherlands	DNFCS 2007–2010	82	1.20	1.17	0.73	1.72	82	0.17	0.17	0.12	0.23

Age class	Country	Survey	Intake expressed in mg/day					Intake expressed in mg/MJ				
			n [(a)]	Average	Median	P5	P95	n	Average	Median	P5	P95
	Sweden	Riksmaten 2010	168	1.41	1.34	0.83	2.12	168	0.20	0.19	0.15	0.26
≥ 75	France	INCA2	44	1.29	1.14	–[(c)]	–[(c)]	44	0.22	0.18	–[(c)]	–[(c)]
	United Kingdom	NDNS–RollingProgrammeYears1–3	83	1.15	1.02	0.61	1.77	83	0.19	0.17	0.13	0.32
	Ireland	NANS_2012	43	1.17	1.16	–[(c)]	–[(c)]	43	0.19	0.18	–[(c)]	–[(c)]
	Italy	INRAN_SCAI_2005_06	159	1.17	1.13	0.69	1.77	159	0.18	0.17	0.12	0.26
	Sweden	Riksmaten 2010	30	1.37	1.27	–[(c)]	–[(c)]	30	0.20	0.19	–[(c)]	–[(c)]

P5, 5th percentile; P95, 95th percentile; DIPP, type 1 Diabetes Prediction and Prevention survey; DNFCS, Dutch National Food Consumption Survey; DNSIYC, Diet and Nutrition Survey of Infants and Young Children; EsKiMo, Ernährungsstudie als KIGGS-Modul; FINDIET, the national dietary survey of Finland; INCA, étude Individuelle Nationale de Consommations Alimentaires; INRAN-SCAI, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione - Studio sui Consumi Alimentari in Italia; FC_PREGNANTWOMEN, food consumption of pregnant women in Latvia; NANS, National Adult Nutrition Survey; NDNS, National Diet and Nutrition Survey; NWSSP, Nutrition and Wellbeing of Secondary School Pupils; VELS, Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): Number of individuals in the population group.

(b): The proportions of breastfed infants were 58 % in the Finnish survey, 40 % in the German survey, 44 % in the Italian survey, and 21 % in the UK survey. Most infants were partially breastfed. For the Italian and German surveys, breast milk intake estimates were derived from the number of breastfeeding events recorded per day multiplied by standard breast milk amounts consumed on an eating occasion at different age. For the UK survey, the amount of breast milk consumed was either directly quantified by the mother (expressed breast milk) or extrapolated from the duration of each breastfeeding event. As no information on the breastfeeding events were reported in the Finnish survey, breast milk intake was not taken into consideration in the intake estimates of Finnish infants.

(c): 5th or 95th percentile intakes calculated from fewer than 60 subjects require cautious interpretation as the results may not be statistically robust (EFSA, 2011a) and, therefore, for these dietary surveys/age classes, the 5th and 95th percentile estimates will not be presented in the intake results.

(d): Pregnant women only.

Appendix E. MINIMUM AND MAXIMUM % CONTRIBUTION OF DIFFERENT FOOD GROUPS TO COPPER INTAKE IN MALES

Food groups	Age (years)						
	< 1	1 to < 3	3 to < 10	10 to < 18	18 to < 65	65 to < 75	≥ 75
Additives, flavours, baking and processing aids	< 1	< 1	0	0	0	0	0
Alcoholic beverages	< 1	< 1	< 1	< 1–1	1–11	1–13	1–6
Animal and vegetable fats and oils	< 1	< 1	< 1	< 1–1	< 1	< 1	< 1
Coffee, cocoa, tea and infusions	< 1–2	< 1–3	< 1–3	1–2	3–13	4–13	3–11
Composite dishes	< 1–4	< 1–9	< 1–8	< 1–11	1–11	1–10	< 1–10
Eggs and egg products	< 1	< 1–1	< 1–1	< 1–1	< 1–11	< 1–1	< 1–1
Fish, seafood, amphibians, reptiles and invertebrates	< 1–1	< 1–10	< 1–16	< 1–16	1–15	1–15	3–10
Food products for young population	26–31	1–13	< 1	< 1	< 1	–	–
Fruit and fruit products	3–26	11–17	5–10	3–7	3–7	5–10	5–10
Fruit and vegetable juices and nectars	< 1–2	1–7	2–9	2–8	1–3	< 1–3	< 1–2
Grains and grain-based products	7–21	26–34	30–39	32–38	27–36	24–38	26–44
Human milk	< 1–34	< 1–2	–	–	–	–	–
Legumes, nuts, oilseeds and spices	< 1–5	2–7	2–8	2–6	4–7	2–7	1–5
Meat and meat products	< 1–3	3–7	4–13	6–12	7–14	7–18	7–19
Milk and dairy products	2–13	6–19	6–11	3–9	2–8	2–8	3–10
Products for non-standard diets, food imitates and food supplements or fortifying agents	0–1	0–1	0–1	< 1–1	< 1–4	< 1	0–1
Seasoning, sauces and condiments	< 1–1	1	< 1–2	< 1–2	< 1–2	< 1–1	< 1–1
Starchy roots or tubers and products thereof, sugar plants	1–12	3–13	5–10	6–11	5–10	5–11	7–11
Sugar, confectionery and water-based sweet desserts	< 1–1	< 1–7	3–11	2–9	1–5	< 1–4	< 1–2
Vegetables and vegetable products	1–6	3–5	3–7	3–9	3–11	3–12	4–12
Water and water-based beverages	3–8	2–10	2–8	2–9	2–10	2–5	2–6

"–" means that there was no consumption event of the food group for the age and sex group considered, whereas "0" means that there were some consumption events, but that the food group does not contribute to the intake of the nutrient considered, for the age and sex group considered.

Appendix F. MINIMUM AND MAXIMUM % CONTRIBUTION OF DIFFERENT FOOD GROUPS TO COPPER INTAKE IN FEMALES

Food groups	Age (years)						
	< 1	1 to < 3	3 to < 10	10 to < 18	18 to < 65	65 to < 75	≥ 75
Additives, flavours, baking and processing aids	0	0	0	0	0	0	0
Alcoholic beverages	< 1	< 1	< 1	< 1	< 1–3	< 1–2	< 1–2
Animal and vegetable fats and oils	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coffee, cocoa, tea and infusions	< 1–10	< 1–17	1–3	1–10	4–12	4–14	4–12
Composite dishes	< 1–3	< 1–9	< 1–8	< 1–12	1–11	< 1–9	1–10
Eggs and egg products	< 1	< 1–1	< 1–1	< 1–1	< 1–1	< 1–1	< 1–1
Fish, seafood, amphibians, reptiles and invertebrates	0	< 1–8	< 1–12	< 1–19	1–15	1–12	1–7
Food products for young population	19–37	1–11	< 1	< 1	< 1	–	< 1
Fruit and fruit products	8–26	5–16	5–10	4–12	5–10	8–13	7–13
Fruit and vegetable juices and nectars	< 1–2	1–7	2–8	2–8	1–3	1–2	1–2
Grains and grain-based products	10–21	24–37	31–38	29–38	24–42	20–38	19–40
Human milk	< 1–15	1–2	–	–	–	–	–
Legumes, nuts, oilseeds and spices	1–6	2–7	2–7	3–6	4–8	4–8	3–6
Meat and meat products	1–3	3–7	4–10	5–11	6–13	6–17	6–16
Milk and dairy products	1–15	7–15	6–11	3–9	2–8	3–8	3–9
Products for non-standard diets, food imitates and food supplements or fortifying agents	0–1	0–1	0–1	< 1–1	< 1–4	< 1–1	0–2
Seasoning, sauces and condiments	< 1–1	< 1–1	< 1–2	< 1–2	< 1–2	< 1–1	1
Starchy roots or tubers and products thereof, sugar plants	3–12	5–12	5–10	6–12	4–9	4–9	6–8
Sugar, confectionery and water-based sweet desserts	< 1–2	< 1–6	3–11	3–10	1–10	1–3	1–3
Vegetables and vegetable products	5–7	3–5	3–8	4–8	4–12	5–13	5–12
Water and water-based beverages	3–9	2–10	2–9	2–10	2–12	3–10	3–8

"–" means that there was no consumption event of the food group for the age and sex group considered, whereas "0" means that there were some consumption events, but that the food group does not contribute to the intake of the nutrient considered, for the age and sex group considered.

ABBREVIATIONS

Afssa	Agence française de sécurité sanitaire des aliments
AI	Adequate Intake
AR	Average Requirement
COMA	Committee on Medical Aspects of Food Policy
CV	coefficient of variation
D–A–CH	Deutschland–Austria–Confoederatio Helvetica
DAO	diamine oxidase
DH	UK Department of Health
DIPP	Type 1 Diabetes Prediction and Prevention survey
DNFCS	Dutch National Food Consumption Survey
DNSIYC	Diet and Nutrition Survey of Infants and Young Children
DRV	Dietary Reference Values
EAR	Estimated Average Requirement
EsKiMo	Ernährungsstudie als KIGGS-Modul
EU	European Union
FAO	Food and Agriculture Organization
GPI	glycosylphosphatidylinositol
INCA	Etude Individuelle Nationale des Consommations Alimentaires
INRAN-SCAI	Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione – Studio sui Consumi Alimentari in Italia
IOM	U.S. Institute of Medicine of the National Academy of Sciences
MT	metallothionein
NANS	National Adult Nutrition Survey
NDNS	National Diet and Nutrition Survey
NNR	Nordic Nutrition Recommendations
NOAEL	No Observed Adverse Effect Level
NWSSP	Nutrition and Wellbeing of Secondary School Pupils

RCT	randomised controlled trial
RDA	Recommended Dietary Allowance
RNI	Reference Nutrient Intake
SCF	Scientific Committee for Food
SD	standard deviation
SE	standard error
SNP	single nucleotide polymorphism
SOD	Superoxide dismutase
UL	Tolerable Upper Intake Level
UNU	United Nations University
VELS	Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln
WHO	World Health Organization

1692