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SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 16 November 2022

doi: 10.2903/j.efsa.2023.7728

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EFSA Scientific Committee,
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Abstract

Copper is an essential micronutrient and also a regulated product used in organic and in conventional farming pest management. Both deficiency and excessive exposure to copper can have adverse health effects. In this Scientific Opinion, the EFSA 2021 harmonised approach for establishing health-based guidance values (HBGVs) for substances that are regulated products and also nutrients was used to resolve the divergent existing HBGVs for copper. The tightly regulated homeostasis prevents toxicity manifestation in the short term, but the development of chronic copper toxicity is dependent on copper homeostasis and its tissue retention. Evidence from Wilson disease suggests that hepatic retention is indicative of potential future and possibly sudden onset of copper toxicity under conditions of continuous intake. Hence, emphasis was placed on copper retention as an early marker of potential adverse effects. The relationships between (a) chronic copper exposure and its retention in the body, particularly the liver, and (b) hepatic copper concentrations and evidence of toxicity were examined. The Scientific Committee (SC) concludes that no retention of copper is expected to occur with intake of 5 mg/day and established an Acceptable Daily Intake (ADI) of 0.07 mg/kg bw. A refined dietary exposure assessment was performed, assessing contribution from dietary and non-dietary sources. Background copper levels are a significant source of copper. The contribution of copper from its use as plant protection product (PPP), food and feed additives or fertilisers is negligible. The use of copper in fertilisers or PPPs contributes to copper accumulation in soil. Infant formula and follow-on formula are important contributors to dietary exposure of copper in infants and toddlers. Contribution from non-oral sources is negligible. Dietary exposure to total copper does not exceed the HBGV in adolescents, adults, elderly and the very elderly. Neither hepatic copper retention nor adverse effects are expected to occur from the estimated copper exposure in children due to higher nutrient requirements related to growth.

Keywords: copper, exposure assessment, health-based guidance value

Requestor: European Commission

Question number: EFSA-Q-2020-00399

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Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Scientific Committee wishes to thank the following for the support provided to this scientific output: the members of the WG of the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), for the preparation of the first draft version of this Opinion and are not among the current authors: Jacqueline Castenmiller, Stefaan de Henauw, Karen-Ildico Hirsch-Ernst, John Kearney, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti, Peter Aggett and Harry J McArdle. The Scientific Committee also wishes to thank the following EFSA staff for the support provided to this scientific output: Davide Arcella, Arianna Chiusolo and Jean Lou Dorne. The Scientific Committee also wishes to thank the hearing expert Mark J Burkitt.

Suggested citation: EFSA Scientific Committee, More SJ, Bampidis V, Benford D, Bragard C, Halldorsson TI, Hernández-Jerez AF, Bennekou SH, Koutsoumanis K, Lambré C, Machera K, Mullins E, Nielsen SS, Schlatter JR, Schrenk D, Turck D, Younes M, Boon P, Ferns GAA, Lindtner O, Smolders E, Wilks M, Bastaki M, de Sesmaisons-Lecarré A, Ferreira L, Greco L, Kass GEN, Riolo F and Leblanc J-C, 2023. Scientific Opinion on the re-evaluation of the existing health-based guidance values for copper and exposure assessment from all sources. EFSA Journal 2023;21(1):7728, 117 pp. https://doi.org/10.2903/j.efsa.2023.7728

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Summary

The European Commission requested the European Food Safety Authority to mandate the Scientific Committee (SC) to review the existing scientific evidence, including all new relevant studies, with the following two aims:

- To provide a scientific opinion on an acceptable daily intake (ADI) for copper that can be used by the Commission as a reference value in managing copper-containing regulated products.
- To perform a new estimation of copper intake, taking into account all sources of exposure and by integrating different approaches and scenarios and all new data available to EFSA for the estimation of exposure, and to assess the contribution from all major sources of exposure, including pesticide residues, to the overall copper intake.

Copper (Cu) is an essential micronutrient for all living organisms, including humans. It is also a regulated product used in organic and in conventional farming pest management. Copper excessive exposure and deficiency can both lead to adverse health effects. Health-based guidance values (HBGVs) have been established in the context of different sectoral assessments: in 2003, the Scientific Committee on Food (SCF) established a tolerable upper intake level (UL) of 5 mg Cu/day for adults, which was adopted as the UL by EFSA in 2006. In the context of the peer review process of plant protection products (PPPs), in 2008, EFSA established an ADI of 0.15 mg Cu/kg bw per day (corresponding to 10 mg/day for a 70-kg adult). This ADI value was confirmed by EFSA in 2018, under the peer review process for the renewal of the approval of copper as PPP. In the context of copper as an essential nutrient, EFSA established adequate intakes (AIs) in 2015 to prevent copper deficiency.

In this Scientific Opinion, the EFSA Statement from 2021 proposed harmonised approach for establishing HBGVs for substances that are regulated products and also nutrients (henceforth 'EFSA Statement on HBGV') was used to resolve the divergent existing HBGVs for copper. The approach proposed in the EFSA Statement on HBGV for hazard assessment is based on the IPCS/WHO biologically based model for essential trace elements and on the concept of acceptable range of oral intake. This model foresees the use of biological endpoints, such as homeostatic and adaptive responses that are not adverse in themselves, as critical endpoints for the identification of reference points (RPs) on which HBGVs can be derived. Consequently, data obtained from human studies relevant to copper homeostasis as predictor of long-term toxicity were utilised for identifying an RP for human risk assessment. In this respect, the methodology may differ from the approach used for the hazard assessment of compounds in food that are not nutrients.

The development of chronic copper toxicity is dependent on copper homeostasis and its tissue retention. Therefore, copper physiology and homeostasis in humans have been central to this assessment. While hepatic sequestration functions as a protective adaptation to increasing copper levels, hepatic accumulation is part of the copper toxicity pathway, as evidenced from Wilson disease pathology. The selection of sensitive endpoints for copper was based on the understanding that the tightly regulated homeostasis prevents toxicity manifestation within the timeframe of human studies. Hence, emphasis was placed on studies reporting copper retention as an early marker of potential adverse effects. The relationships between (a) chronic copper exposure and its retention in the body, particularly in the liver, and (b) hepatic copper concentrations and evidence of toxicity are directly relevant to the present assessment.

Human data are preferable for establishing an HBGV for copper because of the known species differences in its homeostatic regulation. Pertinent information relevant to copper homeostasis was derived from studies in healthy human volunteers. Results from observational studies, data from animal models and from human studies in subjects with Wilson disease (WD, genetic disease with a direct impact on copper homeostasis) were used to aid the interpretation of the data derived from the studies in healthy subjects, in particular for the understanding of the pathophysiological pathways involved in copper toxicity from high intake. Evidence relevant to copper hepatotoxicity and other copper-related toxicity, including neurotoxicity, Alzheimer disease, genotoxicity and carcinogenicity was reviewed. Data relevant to genetic variability in copper homeostatic mechanisms that may increase susceptibility to hepatic copper retention were relevant for assessing interindividual variability in copper homeostasis.

Initial homeostatic responses involve reduced absorption followed by increased hepatobiliary excretion. All available evidence suggests that at increasing exposures, copper is sequestered in the hepatic 'storage depot' of metallothionein (MT), on which zinc and copper homeostasis converge.

Copper homeostasis is tightly regulated, highly conserved across species and is essential in mammalian biology. Whilst it is assumed that these mechanisms of copper homeostasis are fully developed soon after birth, physiological requirements for copper cannot be readily extrapolated from adults to children. While homeostasis mechanisms may operate in a similar fashion, copper retention in children (positive balance) is not physiologically equivalent to copper retention in adults. Because of the higher needs for growth, the retained copper is therefore likely to be widely dispersed throughout the body rather than be focally sequestered.

Documented copper toxicity in humans has been associated either with high-dose exposures (acute toxicity) or with WD progression. There are no reports of copper toxicity under usual dietary exposure conditions in humans without this genetic disorder. The SC notes that previous risk assessments of copper identified an NOAEL of 10 mg/day (equivalent to 0.15 mg/kg bw per day considering a body weight of 70 kg) from a human study in healthy young males, in which liver enzymes were not affected after 12 weeks of supplementation at this level of intake. This NOAEL was used as an RP to establish the ADI for copper. In addition to other limitations, this study assumed that the administered form of copper was readily excreted through the kidneys and no reference was made to homeostatic sequestration in the liver. The absence of effect in this study is consistent with the central role of copper sequestration in protection against copper toxicity, meaning that manifestation of copper toxicity, other than acute toxicity at very high exposures, may not be observed in studies of relatively short duration but longer term studies might be needed to observe toxicity following hepatic copper retention. However, human toxicity data from long-term exposure at lower intake levels are limited, which represents a critical data gap. The few available long-term observational studies do not provide sufficient information to draw conclusions on an association between high copper intake from drinking water and adverse effects.

In WD patients, hepatic copper retention is associated with progressive hepatic damage and peripheral toxicity. The release of stored hepatic copper results in extrahepatic toxicity (primarily in the central nervous system). While there is no clear cut-off value of retention above which copper release and toxicity are more likely, there are various triggers to initiate these events.

Therefore, the quantitative relationship between hepatic copper retention and toxicity remains elusive and introduces additional uncertainty. The evidence from WD suggests that hepatic retention is indicative of potential future (and possibly sudden) onset of copper toxicity under conditions of continuous intake and can be considered an early predictor of adversity in chronic toxicity assessment.

There are a large number of genetic variants that encode an ATP7B protein (a copper-transporting ATPase defective in WD) with a variety of structural and/or functional defects. Based on frequencies of reported cases, 1 in 70 individuals are predicted to be heterozygous carriers of a high penetrance variant and as high as 1 in 25 are carriers of a variant when variants of probable and possible low penetrance are included. The complexity of the genetic profile in WD indicates at least uncertainty about the potential genetic susceptibility to copper retention if the capacity of heterozygous individuals to maintain homeostasis is exceeded.

Data on copper balance can be an early marker of potential adverse effects because copper retention is an early stage in the pathway of copper toxicity that would occur if intake is not reduced, as described in the EFSA HBGV Statement. The SC recognises that an HBGV based on evidence of retention as predictor of future toxicity is conservative and therefore sufficiently protective for most consumers over long-term intake. No additional uncertainty factor is considered necessary in this case. The available data indicate that potential copper toxicity from copper retention may occur at an uncertain time in adult men at chronic copper intakes of 6–8 mg/day. It is uncertain whether effective adaptation and equilibrium may be reached at intakes of 6 mg/day after 8 weeks (2 months) or at intakes of 8 mg/day after 5 months.

In conclusion, the SC considers that the intake of 10 mg/day that was previously used as an RP for previous HBGVs, can no longer be considered an NOAEL. Based on the weight of evidence, the SC concludes that no retention of copper is expected to occur with a copper intake of 5 mg/day. The SC established an ADI of 0.07 mg/kg bw, equivalent to 5 mg Cu/day for adults.

Uncertainty analysis concluded that it is extremely likely (95–99% probability) to almost certain (99–100% probability) that copper is sequestered in the liver above levels required for physiological functions, and extremely unlikely (1–5% probability) that copper that remains effectively sequestered in the liver causes hepatotoxicity. It is also extremely likely (95–99% probability) that both hepatotoxicity and extrahepatic toxicity are dependent on hepatic copper retention and/or release of retained hepatic copper, but there is higher uncertainty about the conditions leading to local hepatocellular toxicity or conditions triggering copper release from the liver (these conditions are

variable and unpredictable, both in nature and timing). Therefore, it is very unlikely (5–10% probability) that copper exposure up to the ADI of 0.07 mg/kg bw per day (5 mg/day in adults) leads to copper retention in the liver and very unlikely (5–10% probability) that such level of copper exposure leads to copper toxicity.

Copper hazard assessment in children takes into account additional lines of evidence indicating that, due to higher nutrient requirements related to growth, it is extremely unlikely (1–5% probability) that copper exposure up to the ADI of 0.07 mg/kg bw per day leads to hepatic copper retention or that it leads to copper adverse effects.

A refined dietary exposure assessment was performed, accounting for food-related uses of copper in a stepwise approach and assessing contribution from dietary and non-dietary sources of exposure to copper. Food consumption data from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) were used for the dietary exposure assessment of copper. According to the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (2017), exposure assessment for infants below 16 weeks of age (for substances present in food intended for infants) should be carried out separately from older infants.

Copper mean concentrations to total copper from all sources were obtained from the EFSA occurrence database (as of March 2021) and from the EFSA nutrient composition database. The mean middle bound (MB) of occurrence values was used to reduce uncertainty in the comparison with the composition data for which the treatment of left censored data was unknown. The occurrence values, where available, were given priority the composition data and a decision tree was applied per food category for the selecting the most reliable mean concentration of copper. Data retrieved from the literature by France, as rapporteur MS in the context of the assessment for the renewal of copper compounds as a pesticide active substance, were compared.

Additional data on copper concentrations included: concentration data generated in supervised field trials by main authorisation holders of copper used as PPP; dietary exposure data for copper used in two food additives Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)); concentrations of copper compounds authorised to be used as nutritional additives in feed; pesticide maximum residue levels (MRLs) for copper in foods of animal origin; and concentrations of copper in soil from application of fertilisers and minimum and maximum limits of copper authorised in infant and follow-on formula and processed cereal-based foods and baby food.

The main contributing food categories were identified as those representing more than 10% to the total dietary exposure in most surveys. Subcategories at level 3 that contributed more than 5% in at least two surveys were selected for estimating the contribution from regulated uses of copper to the total dietary exposure.

Mean dietary exposure to total copper ranged from 0.014 mg/kg bw per day in the elderly to 0.084 mg/kg bw per day in infants. The 95th percentile of dietary exposure to total copper ranged from 0.024 mg/kg bw per day in adults and elderly to 0.155 mg/kg bw per day in infants. For children, data from three European total diet studies (TDSs) were used for the comparison. Although not directly comparable, the intake estimates were similar and ranged from 0.018 to 0.074 mg/kg bw per day.

The main contributing food categories (at level 1 of the FoodEx2 classification) to the dietary exposure to total copper across the different age groups and all surveys were 'Grains and grain-based products' (2–44%), 'Fruit and fruit products' (2–24%), 'Meat and meat products' (< 1–21%), 'Vegetables and vegetable products' (2–24%), 'Coffee, cocoa, tea and infusions' (< 1–21%), 'Food products for young population' (1–57%) and 'Milk and dairy products' (2–33%). It is noted that there are currently no authorised uses of copper as PPP in grains; hence, copper in this category originates from background copper levels in soil. The contribution to the dietary exposure to total copper from mammal liver was above 5% in nine surveys and seven countries, and up to 13% across age groups. The available monitoring data of copper were in the range of copper concentrations in control (untreated) crops reported in trial studies, rather than the concentrations in treated crops in these trials, indicating that the contribution of copper from its use as a PPP to the overall dietary exposure to copper can be considered negligible. Contributions from food and feed additives are also negligible.

Infant formula and follow-on formula, containing total copper in the range of the regulatory limits and human breast milk levels, are important contributors to dietary exposure of total copper in infants (12–39% across surveys) and toddlers (1–19% across surveys).

The uses of copper in fertilisers or PPPs contribute to copper accumulation in soil. While homeostatic control on copper uptake in plants prevents increased crop uptake from short-term

changes in copper soil concentration, copper uptake by crops may increase based on projected increasing concentrations of copper in the soil over a longer time period (100 years).

Contribution of copper from non-oral sources can be considered negligible for the general population compared to dietary exposure.

The SC notes that dietary exposure to total copper does not exceed the HBGV in adolescents, adults, elderly and the very elderly. However, there is some exceedance of the HBGV especially at the higher end of exposure ranges in the young population (infants at the maximum of the mean range in two dietary surveys, infants and toddlers at the P95 in all dietary surveys and in children at p95 in the majority of the dietary surveys).

It is noted that for these age groups exceeding the HBGV, infant and follow-on formula contributed 12–39% across surveys to dietary exposure of copper in infants and 1–19% in toddlers. No other food categories that were identified as main contributors to dietary exposure to total copper appeared to be impacted by regulated uses of copper. In addition, it is also noted that mammal liver, where copper concentration is related to the use of copper in feed, contributed from 3% to 5% in three surveys for toddlers and one survey for infants.

Uncertainty analysis concluded that it is very unlikely to extremely unlikely (5–10%, to 1–5% probability) that the total dietary exposure to copper has been systematically underestimated for the adult general population in the EU. It is more likely than not (> 50% probability) that the total dietary exposure to copper in children population may be underestimated. However, it is very unlikely (5–10% probability) that the estimated copper exposure in children at the highest end of the P95 range relative to the HBGV of 0.07 mg/kg bw per day leads to copper hepatic retention and toxicity.

Moreover, it was concluded that dietary exposure estimates of copper for specific subpopulations (e.g. regular consumers of crops treated with copper, regular consumers of fortified foods or food supplements containing copper or those using copper cooking cookware and utensils) may be higher than the exposure estimated for the adult general population.

A technical report on the outcome of the public consultation (EFSA-Q-2020-00400) held for this opinion is published separately.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

Copper (Cu) is an essential micronutrient for all living organisms, including humans. However, excessive exposure to this micronutrient can lead to adverse effects on health. In the context of the peer review for the active substance copper compounds in plant protection products (PPPs) under Regulation (EC) No 1107/2009, published on 16 January 2018,[1] EFSA confirmed its earlier position from 2008 as regards a reference point (RP) of 0.15 mg Cu/kg bw per day (i.e. 10 mg/day for a 70-kg adult) to derive an acceptable daily intake (ADI) for exposure to copper of 0.15 mg Cu/kg bw per day (i.e. 10 mg/day for a 70-kg adult). This is in line with the values established by the World Health Organization (WHO) for copper upper level intake (WHO, 1996)[2] based on human data for infants (adults: 0.20 mg Cu/kg bw per day and infants: 0.15 mg Cu/kg bw per day). EFSA considered that this value is supported by animal data (90-day rat study) with a no-observed-adverse-effect level (NOAEL) of 16 mg Cu/kg bw per day; applying a standard uncertainty factor (UF) of 100. In contrast, a different conclusion was reached in 2003 by the Scientific Committee on Food (SCF)[3] who derived a tolerable upper intake level (UL) of 5 mg Cu/day for adults.

1.1.2. Terms of Reference

Considering this apparent divergence in the conclusions and taking into account SANTE's, (the Commission's Directorate-General for Health and Food Safety) need for coherent scientific advice, the European Commission requested the European Food Safety Authority to mandate the Scientific Committee to review the existing scientific evidence, including all new relevant studies, with the following two aims:

1) To provide a scientific opinion on an ADI for copper that can be used by the Commission as a reference value in managing copper-containing regulated products.
2) To perform a new estimation of copper intake, taking into account all sources of exposure and by integrating different approaches and scenarios and all new data available to EFSA for the estimation of exposure, and to assess the contribution from all major sources of exposure, including pesticide residues, to the overall copper intake.

1.2. Interpretation of the Terms of Reference

In this Opinion, the health-based guidance value (HBGV) for chronic copper exposure is re-evaluated according to the EFSA proposed harmonised approach for establishing HBGVs for substances that are regulated products and also nutrients (henceforth 'EFSA Statement on HBGV') (EFSA Scientific Committee, 2021). This re-evaluation is based on existing assessments (WHO, 1996; IPCS, 1998; IOM, 2001; EVM, 2003; SCF, 2003; ATSDR, 2004; France, 2007a, 2017; EFSA, 2008, 2018b; VKM, 2017) and additional relevant literature. The following aspects are outside the scope of this evaluation: a re-evaluation of physiological requirements for copper as an essential nutrient (EFSA NDA Panel, 2015); establishing an acute reference dose; occupational exposure to copper and exposure to copper from medicines.

For the contribution to the overall dietary exposure to copper, the following sources are considered: natural occurrence in food and drinking water (including occurrence as a contaminant), PPPs, nutrient uses, food additives, and feed additives, and fertilisers. The contribution from non-dietary sources for copper, such as personal care products, biocides, medical devices and other sources, is also examined.

Although a full risk assessment has not been requested, the dietary exposure to total copper in different subpopulations will be discussed in comparison to the HBGV.

[1] https://www.efsa.europa.eu/en/efsajournal/pub/5152
[2] WHO (1996) Trace elements in human nutrition and health. Geneva, World Health Organisation.
[3] https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out176_en.pdf

1.3. Consultations

In line with its policy on openness and transparency, EFSA consulted EU Member States and interested parties through an online public consultation held between 25 May and 1 August 2022. The comments received were considered by the working group and incorporated into the current Opinion, where appropriate, before adoption of the opinion by the EFSA Scientific Committee. The technical report of the outcome of the public consultation (EFSA-Q-2020-00400) is published separately.

1.4. Existing evaluations used by the Scientific Committee

Copper (Cu) occurs naturally in the environment and it has applications in regulated products, including food and feed additives and PPPs, and in consumer products as an antimicrobial agent. Different HBGVs have been established for copper in regulated products.

Copper deficiency and excessive exposure can both lead to adverse health effects. Adequate intakes (AIs) have been established for copper as an essential nutrient to prevent copper deficiency based on observed intakes in European Union (EU) countries and on supporting evidence from results of balance studies (EFSA NDA Panel, 2015). The AIs for copper for different age groups are 0.4 mg/day for 7–11 months, 0.7 mg/day for 1–2 years and 1.0 mg/day for 3–9 years. For 10–17 years, the AIs are 1.3 mg/day for males and 1.1 mg/day for females; for adults ≥ 18 years, the AIs are 1.6 mg/day for males and 1.3 mg/day for females, whilst for the special population groups of pregnant and lactating women, the AI is 1.5 mg/day.

Existing risk assessments of copper intake have been conducted by several scientific bodies. Human data have been considered as the most relevant to derive an HBGV for copper in most assessments and animal data as supporting evidence (WHO, 1996; IOM, 2001; SCF, 2003; EFSA, 2008, 2018b; France, 2017), whilst two assessments used human data to support HBGVs derived from animal data (EVM, 2003; France, 2007b). The inputs and outcomes of these assessments are summarised in Appendix A.

In 2003, the SCF established a tolerable upper intake level (UL) of 5 mg Cu/day for adults (SCF, 2003), which was adopted as the UL by EFSA (EFSA, 2006). The UL is based on a supplementation trial in healthy adult volunteers (n = 7) who received 10 mg/day of copper (equivalent to 0.15 mg Cu/kg bw per day for a 70-kg adult) for 12 weeks (Pratt et al., 1985). This dose was considered an NOAEL based on the absence of adverse effects, specifically the absence of changes in liver enzymes. The UL was derived by applying a UF of 2 to this RP to address the 'potential variability within the normal population'.

Copper compounds were evaluated in the context of the peer review for active substances in PPPs. The peer review process is initiated with an assessment by a designated Rapporteur Member State (RMS) followed by an independent review of the draft report by EFSA and Member States experts to produce a final report and conclusions intended to support European Commission legislative decisions. In this context of the peer review process of PPPs, in 2008, EFSA established an ADI of 0.15 mg Cu/kg bw per day (corresponding to 10 mg/day for a 70-kg adult) based on the RMS (France) draft assessment report of copper as PPP (France, 2007a; EFSA, 2008). In the EFSA Conclusion on the pesticide peer review, it was stated that the ADI was:

> 'based on the values established by the WHO for copper intake (1996) [which were] based on human data in children (adults: 0.2 mg Cu/kg bw per day and children: 0.15 mg Cu/kg bw per day), and supported by animal data (1-year dog study; Shanaman et al., 1972) with a NOAEL of 15 mg Cu/kg bw per day, the meeting set an upper limit for copper intake of 0.15 mg Cu/kg bw per day' (EFSA, 2008).

The EFSA Pesticide peer review concluded that an UF was not necessary as the ADI was based on human data that included a potentially sensitive population (EFSA, 2008). This ADI value was confirmed by EFSA in 2018, under the peer review process for the renewal of the approval and was considered to be supported by other animal data (90-day rat study; Hebert, 1993) with an NOAEL of 16 mg Cu/kg bw per day (France, 2017; EFSA, 2018b).

Dietary exposure assessments were performed in 2015 by EFSA's NDA Panel (EFSA NDA Panel, 2015) and in 2018 under the framework of the review of the existing maximum residue levels (MRLs) for copper compounds according to Article 12 of Regulation (EC) No 396/2005 (EFSA, 2018).

As part of the Panel on Nutrition, Novel Foods and Food Allergens (NDA Panel) opinion on dietary reference values for copper (EFSA NDA Panel, 2015), EFSA estimated dietary intake of copper using food consumption data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011) and nutrient composition data of foods and water-based beverages derived from the EFSA Nutrient Composition Database.[4] This database contains food composition data provided by Roe et al. (2013). The assessment was based on individual food consumption data from 13 dietary surveys from nine EU countries and covered all age groups. Mean copper intakes in infants (< 1 year, four surveys) ranged from 0.34 to 0.50 mg/day, in children 1 to < 3 years from 0.60 to 0.86 mg/day in boys and from 0.57 to 0.94 mg/day in girls, in children 3 to < 10 years from 0.92 to 1.44 mg/day in boys and from 0.82 to 1.30 mg/day in girls, in children 10 to < 18 years from 1.16 to 1.59 mg/day in boys and from 0.98 to 1.41 mg/day in girls, and in adults 18 years and older ranged from 1.27 to 1.67 mg/day in men and from 1.15 to 1.44 mg/day in non-pregnant women.

As part of the MRL review, EFSA performed a dietary exposure assessment of copper for consumers considering the levels of copper in raw agricultural commodities from supervised residue trials and from monitoring data from national control programmes of 2009–2015, as well as data on background levels of copper (i.e. in non-copper-treated crops) reported in the open literature as provided by the RMS (France, 2016). Dietary exposure calculations were performed using the Pesticide Residue Intake Model (PRIMo) v.3 (EFSA, 2018c). PRIMo contains summary average consumption data of raw agricultural commodities of different European countries and different age groups, including young children. Copper exposure from consumption of the main contributing commodities was calculated as percentage contribution to the ADI.

The Scientific Committee noted that these two dietary exposure assessments were performed using different methodologies, and different data on copper content of foods and food consumption.

1.5. Application of the EFSA Statement on establishing Health-Based Guidance Values for regulated products that are also nutrients to copper

Generally, the evaluation of nutrients and regulated products at EFSA has been conducted within relevant but separate EFSA Panels and Units under different regulatory frameworks. Evaluation of nutrients is conducted by the NDA Panel, which establishes ULs as HBGVs for nutrients within the framework of the General Food Law (Regulation (EC) No 178/2002). The premarket authorisation of regulated products in food and feed (e.g. food or feed additives and pesticides) requires a scientific risk assessment by the relevant Panels and Units (pesticides), which establish ADIs as HBGVs for these products within the framework of sectoral legislation and/or under the general food law (Regulation (EC) No 178/2002). These separate assessments converge on substances that fall under both regulatory contexts and may result in divergent HBGVs.

To address the inherent inconsistencies embedded in risk assessments conducted for different regulatory purposes, the Scientific Committee proposed an integrated and harmonised approach for establishing HBGVs applicable across different sectoral risk assessments, with the involvement of relevant EFSA Units in the assessment process as described in the 'EFSA Statement on HBGV' (EFSA Scientific Committee, 2021). The scientific principles and approach proposed in this Statement are applicable to copper within the scope of the current European Commission mandate. The methodology and the terminology used in the Statement have been applied to the present assessment for copper to resolve the divergent HBGVs published in previous EFSA assessments.

The EFSA Statement on HBGV draws on the 2002 report by the International Programme on Chemical Safety (IPCS) and WHO, which critically re-examined the principles of an estimated safe and adequate range of intakes for nutrients and outlined the methods for the assessment of risk from intake of essential trace elements (ETE). The IPCS/WHO used the term acceptable range of oral intake (AROI) to represent the range of intakes of an ETE at which a population has a minimal risk of deficiency and of toxicity. Directly pertinent to copper hazard characterisation is the approach proposed in the EFSA Statement on HBGV for hazard assessment according to the IPCS/WHO biologically based model for ETEs. This model includes the use of biological endpoints, such as homeostatic and adaptive responses, as critical endpoints (IPCS, 2002; EFSA Scientific Committee, 2021). Specifically for HBGVs, the IPCS/WHO working group 'Biological Based Model' for establishing HBGVs (ADIs/ULs) for ETEs, and the WHO/FAO working group on nutrient risk assessment

[4] Available at: https://www.efsa.europa.eu/en/data-report/food-composition-data

proposed the identification of critical endpoints among the homeostatic and adaptive responses to excessive intakes of nutrients in nutrient risk assessment (IPCS, 2002; EFSA Scientific Committee, 2021). Another difference from the traditional risk assessment approach is that application of an UF, when needed, should consider the nutritional needs to ensure establishing HBGVs that are not too close or within the range of exposures that may lead to nutrient deficiency (i.e. IPCS, 2002; SCF, 2002; EFSA Scientific Committee, 2021).

How the EFSA Statement on HBGV has been applied to copper to resolve the divergent existing HBGVs for copper is detailed in Problem Formulation.

The EFSA Statement on HBGV (2021) also proposes that exposure from all relevant sources should be assessed for nutrients that are also regulated products. This is in line with the terms of reference of the present mandate.

2. Data and methodologies

2.1. Problem formulation

The establishment of an HBGV for copper should protect the general population, including susceptible subpopulations, against adverse health effects due to copper exposure over a lifetime. As copper is also an essential element, it is appropriate that the hazard characterisation of copper in the context of its use in regulated products, as requested in the European Commission mandate, is conducted according to the principles and methodologies described in the EFSA Statement on HBGV (2021) (see above).

The development of chronic copper toxicity is dependent on copper homeostasis and its tissue retention. Hepatic sequestration of copper is well established (i) as part of its homeostasis (Nevitt et al., 2012); (ii) as a result of excessive intake (O'Donohue et al., 1999); or (iii) under conditions of impaired biliary excretion such as in Wilson's disease (WD) (Gaetke et al., 2014; Członkowska et al., 2018; Poujois and Woimant, 2018; Shribman et al., 2019; Linder, 2020; Lucena-Valera et al., 2021). Hepatic sequestration functions as a protective adaptation to increasing copper levels, but evidence from WD pathology indicates that the resulting hepatic accumulation is part of the pathway leading to copper toxicity. Therefore, studies of short duration can be informative for copper toxicity evaluation in so far as they report data for endpoints that are sensitive enough to detect changes consistent with the timeframe of exposure and observation. For copper, endpoints consistent with short duration of observation period include information on copper homeostasis (body burden) and ideally early biological changes and assessment of their sensitivity to detect early toxicity. Apical endpoints of copper toxicity are not sensitive enough to reveal copper toxicity in studies of short duration. The relationships between (i) chronic copper exposure and its retention in the body, particularly in the liver, and (ii) hepatic copper concentrations and evidence of toxicity are directly relevant to the present assessment.

Human data are preferable for establishing an HBGV for copper because of the known species differences in its homeostatic regulation (see below). However, experimental human data are often derived from studies of short duration and small sample size. Therefore, evidence of changes that are considered predictors of potential future effects, such as data on copper homeostasis, is relevant (EFSA Scientific Committee, 2021). In this context, interpretation of the results of available studies is based on the biological relevance and the consistency between the duration of the study and the sensitivity of the endpoints assessed, such as whether adverse effects reported were consistent with copper homeostatic regulation, exposure duration and observation period and/or the exposure range assessed in the study. Review of available evidence of other potential copper-related toxicities can be included to inform the interpretation of dose–response.

Data relevant to genetic variability in copper homeostatic mechanisms that may increase susceptibility to hepatic copper retention, as in those individuals who are heterozygous for *ATP7B* gene variants and who do not meet the diagnostic criteria of WD, are relevant for assessing interindividual variability in copper homeostasis (toxicokinetic). Hence, the frequency of relevant genetic variants in the population is needed in order to assess the size of potential susceptible subpopulations.

In this Scientific Opinion, a refined dietary exposure assessment is needed, accounting for food-related uses of copper in a step-wise approach. Contribution from other sources of exposure to copper, including the contribution from non-oral routes, should also be considered (Figure 1).

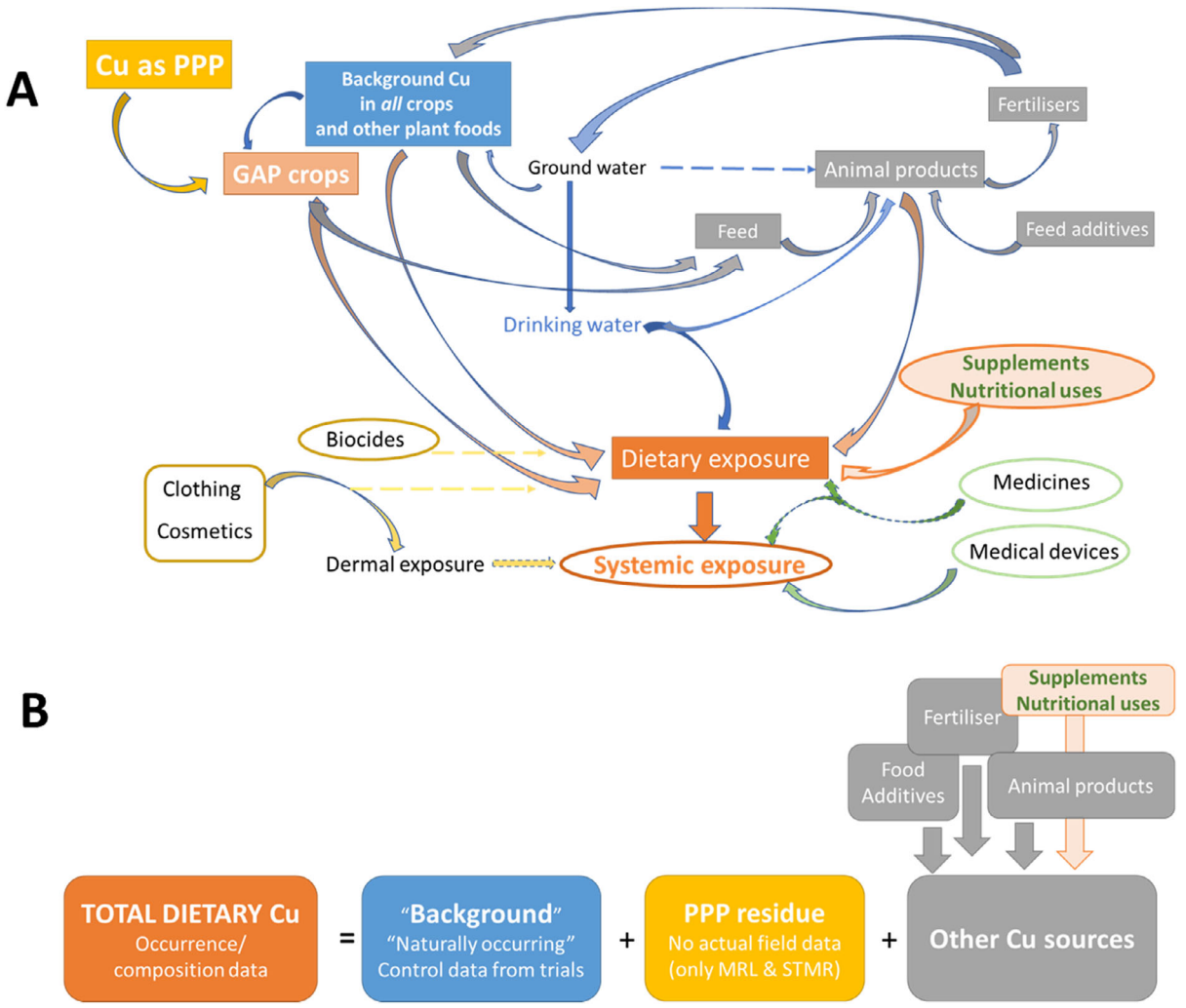


Figure 1: Dietary exposure assessment approach of copper (Cu)(a) Possible relevant sources of copper exposure and (b) modular dietary exposure approach to total copper from all dietary sources. See text in Section 3.2.2 for contribution of non-dietary sources of exposure shown in panel A. GAP: good agricultural practice; MRL: maximum residue level; PPP: plant protection product; STMR: supervised trials median residue

2.2. Data

2.2.1. Hazard identification and characterisation

Data relevant to the hazard identification and hazard characterisation are derived from existing safety assessments of copper (WHO, 1996; IPCS, 1998; IOM, 2001; EVM, 2003; SCF, 2003; ATSDR, 2004; France, 2007a, 2017; EFSA, 2008, 2018b) (summarised in Appendix A). Data obtained from the literature, including any additional relevant publications, and including toxicity studies published after the last safety assessment as well as any publications relevant to copper homeostasis were evaluated (Appendix B.1–B.5). Data on genetic variability related to copper homeostasis were included in light of the large number of genetic variants associated with WD. Literature reporting the relationship between hepatic copper retention and onset of hepatotoxicity was also searched (Appendix C).

Among the animal studies previously reviewed, two studies, including a 90-day study in rats (Hebert, 1993) and a 1-year study in dogs (Shanaman et al., 1972), were used in previous assessments. Although there were methodological limitations in their design (such as small group sizes, short duration, limited extent of histopathological examination), two scientific assessments (EVM, 2003; France, 2007a) used these studies to identify an RP (16 mg/kg bw per day in rat; 15 mg/kg bw per day in dog). Other scientific bodies have noted uncertainties about the relevance of animal

models for humans (such as greater tolerance to high copper intake in rats compared with humans, and the lower affinity of albumin for copper; His replaced by Tyr) and reduced bile excretion of copper in dogs (and pigs) compared with rats and humans (IPCS, 1998; SCF, 2003; ATSDR, 2004; France, 2007a, 2017). As human studies are available and given the limitations of the rat (Hebert, 1993) and dog (Shanaman et al., 1972) studies and other available animal studies (Section 3.2.3), the SC has given priority to the human studies.

So far, all risk assessments based on human data have used an RP of 10 mg/day. In one study, healthy adult volunteers (three men and four women, 42 years average age; no other information was provided) received a supplement of 10 mg Cu/day as copper gluconate (n = 7) or a placebo (n = 7) for 12 weeks (Pratt et al., 1985). No other dose levels were tested. The copper content of the background diet was not characterised. The study reported no changes in copper levels in serum, urine and hair, and no effects on blood parameters (haematocrit, cholesterol, triglycerides), markers of liver function [i.e. liver enzymes including aspartate aminotransferase (AST); referred to as 'serum glutamic-oxaloacetic transaminase (SGOT)' in the original publication], L-γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) or gastrointestinal effects were reported. Samples were collected before the study, in week 6 and in week 12 of supplementation. Copper absorption and faecal excretion were not evaluated. The dose of 10 mg/day was considered an NOAEL and was used as an RP to derive HBGVs for copper or as supportive evidence by most risk assessment bodies (IOM, 2001; SCF, 2003; France, 2007a, 2017; EFSA, 2008, 2018b). Some risk assessments considered supportive evidence from other studies in humans of varying duration (24 days to 12 months) that reported no signs of adverse effects at slightly lower doses (6–8 mg/day) (Turnlund, 1989; O'Connor et al., 2003; Kessler et al., 2008a). Liver enzymes were measured in two out of the three studies, with no changes observed (GGT, ALT in O'Connor et al., 2003; enzymes not specified in Kessler et al., 2008a) (see Appendix B.2).

According to the biologically based model discussed in the Statement on HBGV (EFSA Scientific Committee, 2021), data on copper physiology and homeostasis are appropriate as the basis for copper hazard characterisation. Consequently, data obtained from human studies relevant to copper homeostasis as predictor of long-term toxicity (Turnlund, 1989; Turnlund et al., 1990, 1998, 2004, 2005; Harvey et al., 2003) (Appendix B.1) were utilised for identifying an RP for human risk assessment (EFSA Scientific Committee, 2021). These data were also considered as relevant evidence in previous assessments. Further to previous assessments, data on hepatic copper levels relative to hepatotoxicity and relative to serum levels and urinary excretion, as well as data on interindividual variability and the potential genetic susceptibility to hepatic copper retention have been sought and reviewed; representative reviews are presented (Appendix C). Evidence of other copper-related toxicity, including neurotoxicity, Alzheimer's disease, genotoxicity and carcinogenicity was also reviewed.

2.2.2. Dietary exposure assessment

In this section, the data used to estimate the dietary exposure to copper are described. This estimation includes exposure to copper from all dietary sources (Figure 1).

2.2.2.1. Food consumption data

Food consumption data from the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) were used for the dietary exposure assessment of copper. This database contains national data on food consumption at the individual level, and are the most complete and detailed data currently available in the EU.

The food consumption data gathered in the Comprehensive Database were collected using repeated 24- or 48-hour dietary recalls or dietary records covering 3–7 days per individual. Owing to the differences in the methods used for data collection, direct country-to-country comparisons of the exposure estimates should be avoided.

Details on how the Comprehensive Database is used to assess the dietary exposure to food chemicals are published in a 2011 EFSA Guidance (EFSA, 2011). The latest version of the Comprehensive Database was updated in 2021 and contains results from 51 dietary surveys carried out in 24 Member States (MS) covering 97,154 individuals. Six surveys (not used within the context of this Opinion) provide information on 'Pregnant women', two on 'Lactating women' and one on Vegetarians. When two different dietary surveys are available for one country and age class, the most recent one is used in the dietary exposure assessment.

Since 2018, all consumption records in the Comprehensive Database have been codified according to the FoodEx2 classification system (EFSA, 2015, 2016). The FoodEx2 classification system consists of a large number of standardised basic food items aggregated into broader food categories in a hierarchical parent–child relationship. Additional descriptors, called facets, are used to provide additional information about the codified foods (e.g. information on food processing and packaging material).

For copper, a chronic dietary exposure assessment is relevant in the context of the terms of reference. For such an assessment, surveys in which food consumption data were collected over only 1 day are not considered appropriate, as described in 2011 EFSA Guidance (EFSA, 2011). Exclusion of these surveys resulted in a total of 41 dietary surveys carried out in 22 MSs covering 83,540 individuals. Table 1 provides an overview of the population groups and countries included in the dietary exposure assessment of copper.

According to the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age, exposure assessment for these infants should be carried out separately from older infants, following the procedure described in the guidance (EFSA Scientific Committee, 2017b). Based on this guidance, infants below 16 weeks of age should be excluded from the dietary exposure estimation of the infants age group. However, for the exposure assessment of copper, due to uncertainty in the reported individual ages of infants in the Comprehensive Database, the cut-off age was set at 12 weeks based on the existing individual age range of this group in this database, with adjustments to include individuals with age around the threshold year. As a result, food consumption data of infants over 12 weeks, i.e. infants between 12 and 16 weeks of age, were included in the exposure assessment. Since the number of children within this age range in the database is limited, it is not expected that this will have affected the exposure estimate of copper for infants of 16 weeks up to 12 months of age.

Table 1: Population groups and countries included in the chronic dietary exposure assessment

Population group	Age range	Countries with food consumption surveys covering more than 1 day
Infants	> 12 weeks to < 12 months old	Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, Slovenia, Spain,
Toddlers	≥ 12 months to < 36 months old	Belgium, Bulgaria, Cyprus, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Netherlands, Portugal, Slovenia, Spain
Other children	≥ 36 months to < 10 years old	Austria, Belgium, Bulgaria, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Spain, Sweden
Adolescents	≥ 10 years to < 18 years old	Austria, Belgium, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Adults	≥ 18 years to < 65 years old	Austria, Belgium, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Elderly	≥ 65 years to < 75 years old	Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Slovenia, Spain, Sweden
Very elderly	≥ 75 years old	Austria, Belgium, Denmark, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Sweden

Table 1 in the Annex provides details on the dietary surveys included in the dietary exposure assessment.

2.2.2.2. Concentration data of copper in food

Two types of concentration data were available to assess the dietary exposure to total copper from all sources. These data sources are described below.

Occurrence data submitted to EFSA

Following a mandate from the European Commission to EFSA, a call for annual collection of chemical contaminant occurrence data in food was issued by the former EFSA Dietary and Chemical

Monitoring Unit (now DATA Unit) in December 2010. Since then, data have been submitted every year with a closing date on 1 October of each year.[5] These data submissions include concentrations of copper in food.

The data submission to EFSA follows the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010b) and the EFSA Guidance on Standard Sample Description 2 (EFSA, 2013). Occurrence data are managed following the EFSA standard operational procedures (SOPs) on 'Data collection and validation' and on 'Data analysis of food consumption and occurrence data'.

For the dietary exposure assessment of copper, analytical results on copper in food were used as stored in the EFSA occurrence database by 10 March 2021. In total, 24,565 analytical results were available, which were provided by national authorities of 19 MS plus Norway and the United Kingdom (UK) between 2004 and 2019. This raw occurrence data set on copper as extracted from the EFSA data warehouse is available at the EFSA Knowledge Junction community on Zenodo.[6] The number of results reported per year and per country is shown in Table 2 in Annex.

Analytical results on copper in food with a reported limit of quantification (LOQ) greater than 40,000 μg/kg (n = 10), and results that were considered as unreliable (n = 7) by the SC, were excluded from the dietary exposure assessment.

The analytical results on copper in fortified biscuits, rusks and cookies for children (n = 78) were excluded (see Section 2.3.2). The Comprehensive Database does not contain specific reference to the consumption of fortified biscuits, rusks and cookies for children.

The final cleaned occurrence value for copper data set contained 24,470 analytical results.

Left-censored data [results below limit of detection (LOD) or below the LOQ] were treated using the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO, 1996). This is the same method as indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010a). The guidance suggests that the lower bound (LB) and upper bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). The LB was obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the LOD (< LOD) or LOQ (< LOQ). The UB was obtained by assigning the numerical value of LOD to values reported as < LOD and LOQ to values reported as < LOQ (maximum possible value), depending on whether LOD or LOQ is reported by the laboratory. In addition, the middle bound (MB) was obtained by assigning the numerical value of LOD/2 to values reported as < LOD and LOQ/2 to values reported as < LOQ (maximum possible value), depending on whether LOD or LOQ was reported by the laboratory.

Based on the analytical results, a mean LB, MB and UB occurrence value was calculated at each level of the FoodEx2 classification.

Details on the occurrence data at the different FoodEx2 classification levels are provided in Table 3 in Annex.

Nutrient composition data submitted to EFSA

The second type of copper concentration data was derived from the EFSA Nutrient Composition Database.[7] This database was compiled as a deliverable of a procurement project to update the food composition database for the estimation of nutrient intake. This project was coordinated by the Institute of Food Research, Norwich, UK and the database was delivered in 2013 (Roe et al., 2013). This database contains concentration data on copper in food from seven MSs.[8] Concentration data included in the national composition databases were derived from various sources such as scientific literature, analytical results, other foods considered to have analogous copper content or calculated from recipes. If no concentration data were available at the national level, the national data compilers of the seven national composition databases used compatible data from other countries.

No information was available about the way left-censored data were treated and if included in the calculation of the mean values available in the nutrient composition database.

Data gaps in the nutrient composition database (e.g. specific FoodEx2 codes for which no concentration value was available for a specific country) were filled by EFSA using the average

[5] Current call: https://www.efsa.europa.eu/en/call/call-continuous-collection-chemical-contaminants-occurrence-data-0
[6] https://zenodo.org/record/7684909
[7] https://www.efsa.europa.eu/en/data/food-composition
[8] Finland, France, Germany, Italy, Sweden, The Netherlands and United Kingdom.

concentration calculated from the concentrations available for the other countries. Details about the composition data of copper concentration at country level at the available FoodEx2 classification levels are provided in Table 4 in the Annex.

For each food, the mean concentration of copper across all countries was calculated. These mean concentrations were then used to calculate a mean concentration at each level of the FoodEx2 classification (available in Table 3 in the Annex).

Selection of concentrations used in dietary exposure assessment of copper from all sources

To estimate the dietary exposure to total copper from all sources, the mean concentrations were selected from the two types of concentration data (occurrence and composition) described above. For this selection, the mean MB occurrence values were used to reduce uncertainty in the comparison with the composition data for which the treatment of left-censored data was unknown. For the food categories for which only one of the two types of data were available, the available mean concentration was used in the dietary exposure assessment. For food categories for which both types of data were available, a selection procedure was applied to select the mean concentration considered most reliable. In general, the occurrence values were given priority, as information was available about the sampling of the occurrence data, which was not available for the composition data. Furthermore, the composition data have not been updated since 2013. To select the most reliable mean concentration of copper, the following decision tree was applied per food category:

- If the number of analytical results in the occurrence data set was less than 20, the mean composition value was selected.
- If the number of analytical results in the occurrence data set was greater or equal to 50, the mean MB occurrence value was selected.
- If the number of analytical results in the occurrence data set was between 20 and 50 and
  - the difference between the mean MB occurrence value and the mean composition value was less than a factor of 2, the mean MB occurrence value was selected;
  - the difference between the mean MB occurrence value and the mean composition value was greater or equal to a factor of 2, but the mean MB occurrence value was in the range of the individual composition values, the mean MB occurrence value was selected;
  - the difference between the mean MB occurrence value and the mean composition value was greater or equal than a factor of 2, and the mean MB occurrence value was outside the range of the individual composition values, the mean composition value was selected.

Table 3 in the Annex contains the mean MB occurrence values and the mean composition values used in the dietary exposure assessment for each of the 1867 FoodEx2 classification levels for which a mean concentration could be calculated from the available data. In order to include all foods likely to contain copper in the dietary exposure assessment, the selected mean concentration values were assigned to additional foods in the same food categories for which the copper concentration was considered to be the same and for which consumption data were available. The mean concentrations were then assigned to the FoodEx2 codes reported in the Comprehensive Database using the most detailed FoodEx2 level available for each food.

As no copper concentrations for dry soups were available, these concentrations were derived from available values for ready-to-eat soups using the recommended drying factor of 10 (EFSA, 2018a).

The available occurrence data for liquid formula were substantially higher than the concentrations estimated from the occurrence data for the powder form, after dilution by the factor of 8 as recommended by manufacturers (EFSA Scientific Committee, 2017b). This indicated high uncertainty in the available concentrations for the liquid form, which was confirmed by the data providers (powder form was reported as liquid form in several instances) and thus, concentrations for infant liquid formula were calculated from occurrence concentrations for the powder form using the dilution factor of 8.

Similarly, concentration values for liquid follow-on formula were calculated from the composition concentrations in the powder form using the recommended dilution factor of 8. The mean concentrations used for each FoodEx2 code reported in the Comprehensive Database (2,427 codes) are provided in Table 5 in the Annex.

It is noted that the highest concentrations used in the dietary exposure assessment were those available for livers of various animals, some molluscs and cocoa products. Concentrations ranged from less than 15 μg/kg in vegetable fats and oils, and water to more than 100,000 μg/kg in veal liver.

Literature data on copper in food from the Renewal Assessment Report

As part of the assessment for the renewal of copper compounds as a pesticide active substance, France, as rapporteur of the active substance, carried out a literature search to estimate the copper concentration in crops in the absence of pesticide application. The sources considered by France and the occurrence data retrieved from the literature search are available in the renewal assessment report prepared by France (France, 2016).

The SC compared the occurrence data of copper from the literature with the copper concentrations used in the exposure assessment of total copper from all dietary sources as described above. The intention was to use these data as representative of the natural level of copper in food commodities in order to estimate the contribution of additional sources of copper to the dietary exposure to total copper. However, the concentrations from the literature were in the same range as those used in the overall dietary exposure assessment. Thus, these data were considered not suitable to be used as background/natural occurrence to assess the specific contribution of different sources of copper to the overall dietary exposure to total copper.

Concentration values extracted from the literature are reported together with the mean copper concentrations used to assess the dietary exposure to total copper for the FoodEx2 codes for which they were available in Table 5 in the Annex.

Additional data on copper concentration

Additional data on copper concentrations were used to assess the contribution of specific sources to the dietary exposure to total copper in the main contributing food categories (see Section 2.3.2.1). These data are described below.

Plant Protection Products

As part of the risk assessment for the renewal of copper as active substance for use in PPPs, specific concentration data were generated in supervised field trials by main authorisation holders to support the review of the existing MRLs (EFSA, 2018). These data were assessed and compiled by France (as RMS) according to legal provisions defined by Regulation (EC) 1107/2009 on the placing of PPPs on the market and in the framework of Article 12 of Regulation 396/2005.

These supervised field trials were performed to simulate the use of copper in PPPs according to the critical agricultural practices (i.e. highest annual rate, highest number of applications, shortest pre-harvest interval, etc.). Therefore, the results of these trials give an indication of the expected concentration of copper residues in crops treated with copper according to a worst-case scenario (field entirely treated according to the critical label). For each crop, the median concentration of copper of the available field trials [supervised trials median residue (STMR)] is usually used to assess the chronic dietary exposure via all crops in a pre-authorisation framework.

In each supervised field trial, control data are generated to check the copper concentration in untreated plots. Whilst concentrations in control samples should always be below the LOQ, this is not the case for copper because copper is naturally present in crops. Therefore, the control data of the supervised field trials can give an indication of the level of copper in crops in the absence of pesticide application whilst the comparison with the STMR values may give an estimate of the contribution to copper concentrations from its use in PPPs.

The levels of copper in foods of animal origin for human consumption resulting from the use of copper-containing PPPs are regulated according to Annex III A Reg. 396/2005 and Reg. (EC) No 149/2008.

Food additives

Copper is used in two food additives, Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)). Data on the dietary exposure to these food additives were obtained from the EFSA Scientific Opinion on the re-evaluation of copper complexes of chlorophylls (E 141(i)) and chlorophyllins (E 141(ii)) as food additives (EFSA ANS Panel, 2015). The exposure reported in this Opinion was assumed to be still relevant for this assessment.

The ANS Panel calculated the dietary exposure to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) as food additives using individual average consumption levels, excluding surveys with only

1 day per subject, and reported use levels as provided by industry. The food categories in which the use of both additives are authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx level 4) and linked to the relevant mean of the typical reported use levels. The refined non-brand-loyal consumer exposure scenario was considered to be the most representative of the dietary exposure to the two food additives by the ANS Panel. This scenario assumes that a consumer is exposed over the long term to the food additives present at the mean reported use levels in food.

The SC converted the dietary exposure estimates of the two food additives of the ANS Panel to the exposure to copper by using a percentage of 8%. This percentage was based on the maximum copper content in mass that may be present in these two additives according to Commission Regulation (EU) No 231/2012.

Subsequently, the exposure to copper from its use in food additives was estimated for the main contributing food categories based on the percentage contribution of these food categories to the total exposure to copper from its use in food additives as reported by the ANS Panel (EFSA ANS Panel, 2015). As not all foods within a food category are likely to contain the two food additives, the SC used information from the Mintel's Global New Products Database (GNPD) to refine the exposure to copper from its use in food additives via the main contributing food categories. The Mintel's GNPD is an online database that contains the compulsory ingredient information present on the label of numerous products.

Nutrient uses

The minimum and maximum limits of copper authorised in infant and follow-on formula and processed cereal-based foods and baby food are set by EC Regulation 609/2013[9] and EC delegated regulation (EU) 2016/127[10]. The regulatory limits for infant formula and follow-on formula set copper content to a minimum of 60 μg/100 kcal and a maximum of 100 μg/100 kcal, whilst for processed cereal-based foods and baby food, it sets copper content to a maximum of 40 μg/100 kcal.

Directive 2002/46/EC[11] and Regulation (EC) 1925/2006[12] provide the forms of copper that are authorised for use in food supplements and addition to foods, respectively. These legislations establish that maximum amounts of vitamins and minerals added to foods and to food supplements shall be set, including for copper. To date, provisions regarding these maximum amounts are not harmonised among EU MS.

Feed additives

Several copper compounds are authorised to be used as nutritional additives in feed for all species. Based on the authorisation, the introduction of copper into the food chain is regulated by setting authorised maximum content of total copper in complete animal feed[13] [e.g. Regulation (EU) 2018/1039]. This maximum content includes copper already present in feed (e.g. due to natural occurrence or use of pesticides) and copper added as feed additive to meet the nutritional requirements of the animals (EFSA FEEDAP Panel, 2016a).

To assess the contribution of nutritional feed additives to the overall dietary exposure of copper, the level of copper in animal-based foods is most relevant to human exposure than the level in feed itself. Therefore, the copper levels in the animal-based foods (muscle meat of mammals, edible offal of mammals and milk) were compared to available data sources on copper levels in these foods, like literature data with background levels from feeding studies as well as MRLs from regulations considering copper levels in the relevant animal based foods.

[9] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, pp. 35–56.

[10] Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding (Text with EEA relevance). OJ L 25, 2.2.2016, pp. 1–29.

[11] 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 (Text with EEA relevance) OJ L 183, 12.7.2002, pp. 51–57.

[12] Regulation (EC) 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, pp. 26–38.

[13] Maximum content of elemental copper in mg/kg of complete feed with moisture content of 12%.

Fertiliser use and the effect of long-term PPP application on food copper concentration via crop uptake from soil

Fertilisers contain copper and their use can gradually increase the concentration of copper in the soil, which can potentially enrich food crops with additional copper. There are various fertiliser regulations that restrict the use of copper for soil, these have been defined to protect the soil organisms and were not defined to protect the food chain because there is a homeostatic control of plants that keeps the concentrations in the plant within rather narrow limits. Nevertheless, small enrichments in crop and food copper concentrations can be found when soil copper concentrations increase (Figure 3). The sewage sludge regulation 86/278/EEC defines maximal concentrations of copper in sewage sludge added to soil, a maximal copper application rate (12 kg Cu/ha per year) and a maximal copper concentration in the soil receiving sewage sludge. The newest EU fertiliser Regulation EU 2019/1009 (into force on 16 July 2022) defines a maximal copper concentration of 300 mg Cu/kg in any organic fertiliser. The EU Regulation 2018/1981 limits the use of copper as PPP on agricultural land to a total application equivalent to a maximum 28 kg of copper per hectare over a period of 7 years.

2.3. Methodologies

2.3.1. Hazard identification and characterisation

As copper is a nutrient (an ETE) and also used in regulated products, the assessment methodology proposed in the EFSA harmonised approach for establishing HBGV across different sectoral risk assessments (EFSA Scientific Committee, 2021) has been adopted in the current Opinion. Only the UB of an acceptable range of intakes is within the scope of the current mandate and data relevant to 'high' or 'excess' copper intake are pertinent in this assessment. Data on the physiological responses to 'high' copper intakes were explored to identify biological endpoints which could be used as critical endpoints for the risk assessment. As noted, for nutrients also used in regulated products, selected endpoints might not be hazards or adverse events in themselves but they must be predictive of adverse events that would occur if intake is not reduced (EFSA Scientific Committee, 2021). In this respect the methodology may differ from the approach used for the hazard assessment of compounds in food that are not nutrients. The selection of sensitive endpoints is important for copper because of the tightly regulated homeostasis that prevents toxicity manifestation within the timeframe of human studies at doses below those associated with acute toxicity. Hence, emphasis was placed on studies reporting copper retention as an early marker of potential adverse effects. Pertinent information relevant to copper homeostasis was derived from studies in healthy human volunteers. Results from observational studies were considered as supportive evidence. Additionally, data from animal models and from human studies in subjects with WD or Menkes Disease (conditions in which genetic variants have a direct impact on copper homeostasis) were used to aid the interpretation of the data derived from the studies in healthy subjects, in particular for the understanding of the pathophysiological pathways involved in copper toxicity from high intake.

Literature searches were conducted in PubMed using search terms related to (i) toxicity studies; (ii) copper homeostasis; (iii) genetic variability, such as genetic polymorphisms associated with WD; (iv) hepatic copper accumulation and hepatotoxicity; (v) neurotoxicity, including Alzheimer's disease. A summary of the search terms is presented in Appendix E. Search filters included publication dates up to 10 years (which includes the period since the most recent EFSA assessment), mammalian species, humans. Topics related to ecotoxicology, non-mammalian species, occupational exposures, nanomaterials and copper-containing medicinal compounds were excluded. Additional relevant articles were identified by checking the citations within the key retrieved full-text research articles and reviews and the citations within the literature that was reviewed in previous assessments, for further studies.

2.3.2. Dietary exposure assessments

A stepwise approach was used to assess the dietary exposure to total copper from all sources and then the contribution of all major dietary sources.

As a first step, the dietary exposure to total copper from all dietary sources was assessed, including copper that is naturally present in food and added to food, using selected concentrations of copper in food (see Section 2.3.2.1). As the concentration data used in this dietary exposure assessment do not allow identification of the source of copper in the diet, as a second step, specific assessments were

conducted for estimating the contribution to exposure from regulated uses of copper, where feasible (PPPs, food additives, feed additives, fertilisers and nutrient use). It should be noted that copper-containing PPPs are used in both conventional and organic farming. Furthermore, the sources for the concentration data (occurrence and composition) used to compute the dietary exposure do not distinguish between foods produced by the different farming practices. Last, the contribution of non-dietary sources of copper to the overall exposure to copper was evaluated, including the contribution of oral and non-oral routes of exposure.

2.3.2.1. Dietary exposure assessment of total copper from all sources

For calculating chronic dietary exposure to total copper, food consumption and body weight data at the individual level were obtained from the Comprehensive Database. The mean daily consumption at the individual level was combined with the mean copper concentration values at the most detailed level of the Foodex2 classification system to calculate individual average daily exposures. On the basis of distributions of individual average daily exposures, the mean and 95th percentile exposure were calculated per survey and per age class.

Also, the contribution of food categories at level 1 of the FoodEx2 classification to the dietary exposure to total copper for each survey and age class for the general population was calculated. Food categories that contributed more than 10% to the total dietary exposure in most surveys were identified as the main contributors. Additionally, main contributing food categories were assessed for consumers exposed to total copper at exposure levels higher than the 75th percentile to verify if the main contributing categories were the same as those for the general population in the relevant age group and survey.

Within these main contributing food categories at level 1 of the FoodeEx2 classification, the subcategories at level 3 that contributed more than 5% in at least two surveys were selected for estimating the contribution from regulated uses of copper (PPPs, food and feed additives, nutrient use and fertiliser) to the dietary exposure to total copper in these main contributing subcategories.

2.3.2.2. Specific assessments of dietary exposure to total copper to estimate the contribution from regulated uses

The relative contribution from regulated uses of copper (PPPs, food and feed additives, nutrient use and fertiliser) to the dietary exposure to total copper was estimated for the main food categories contributing to the dietary exposure to total copper from all sources identified at the level 3 of the Foodex2 classification. The following sections describe the methodology used for each contributing source.

Estimation of the contribution from PPPs

Control and STMR data from supervised field trials (described in Section 2.2.2) were used to estimate the contribution of copper from its use in PPPs to the overall dietary exposure to total copper for the main contributing food categories for which these data were available. For this, the mean concentrations in control samples and treated samples were compared for each food category. In addition, the mean ratio across field trials between the concentrations in untreated and treated crops was calculated.

Estimation of the contribution from food additives

To estimate the contribution of copper used in food additives to the dietary exposure to total copper, dietary exposure estimates to Cu-chlorophylls (E 141(i)) and Cu-chlorophyllins (E 141(ii)) were first converted to estimates of copper intake using a percentage of 8% (see Section 2.2.2.2). Subsequently, the exposure to copper from its use in the two food additives was estimated for the relevant main contributing food categories based on the percentage contribution of these food categories to the total exposure of copper from its use in food additives as reported by the ANS Panel (EFSA ANS Panel, 2015). This resulted in an exposure to copper for each main contributing food category. Percentages of products within the main contributing food categories that were reported to contain the additives according to Mintel's GNPD were used to refine the exposure to copper via these food categories (Table 6 in the Annex). The estimated exposure to copper from its use in food additives was compared to the overall dietary intake of total copper via all dietary sources for the relevant main food categories to ascertain the contribution of copper use in food additives to the overall dietary exposure to total copper.

Estimation of the contribution from nutrient use

The regulatory limits on the use of copper as a nutrient source described in Section 2.2.2.2 were compared to the copper concentrations in infant and follow-on formula used in the dietary exposure assessment to total copper. This comparison was not possible for processed cereal-based foods and baby food considering that the regulatory provisions are expressed in μg/100 kcal and the energy content of processed cereal-based foods and baby food is highly variable.

The contribution of the addition of copper to food as a nutrient source, under the provisions of Regulation (EC) No 1925/2006, to the dietary exposure to total copper could not be assessed as occurrence data on fortified food was only available for 'Biscuits, rusks and cookies for children' and this category was not referenced in the consumption data (see Section 2.2.2.2).

The contribution through the use of copper in food supplements[14] was not assessed as no concentration data were available for food supplements in the EFSA occurrence database and the EFSA Food Composition Database.

In addition, no harmonised maximum limits of copper that can be added to food and food supplements are available in the EU regulation.

Estimation of the contribution from feed

The contribution of the use of copper as a feed additive to the dietary exposure to total copper could not be estimated because (i) monitoring data do not differentiate between feeding practices and (ii) maximum copper content is defined for total copper in feed regardless of the source (e.g. natural, PPP or feed additive).

Of the possible sources of copper in feed, copper-containing PPPs are likely to be a minor one as they are not used on crops consisting the main feed materials (e.g. cereals or protein sources). However, copper PPP uses are authorised on several oilseeds (sunflower, rapeseed, soybean), on potatoes, citrus, apples, and grapes, the by-products of which are possibly used in feed (meals, peels, pomaces, etc.). The highest copper content in feed materials has been found in dried grape pomace and pulp of grapes grown in the EU and possibly associated with the use of copper fungicides in vineyards in the EU [102–124 mg Cu/kg dry matter (DM), for Italian grape pulp and 80–95 mg/kg of 25% DM, for Spanish fermented grape marc, compared with copper content in California grape pulp (23 mg/kg DM)] (EFSA FEEDAP Panel, 2016a).

The possible contribution of use of copper as a feed additive to the dietary exposure to total copper has been assessed previously (EFSA FEEDAP Panel, 2012, 2014, 2015, 2016b, 2019). In addition, literature data were used to compare the background levels from feeding studies with the copper concentrations used in the dietary exposure assessment of total copper for the main contributing foods of animal origin.

Estimation of the contribution from fertiliser and the long-term application of PPP on crop copper concentrations via the soil

To assess the contribution of copper present in the soil due to its use in fertilisers and PPP to the dietary exposure to total copper, the effect of fertilisers and PPP on crop copper concentrations in the soil was estimated as well as the subsequent uptake of copper into the crops. Mass balance modelling on the effect of fertilisers and long-term PPP was performed and compared with measured soil copper accumulation rates.

In addition, the effect of soil copper concentration on crop copper uptake is reviewed for relevant main food categories contributing to the dietary exposure to total copper. For this, estimates were made on the percentage rise in copper concentration in the crop due to long-term use of copper-containing fertilisers and PPP via soil that allows a qualitative assessment to be made of the contribution to the dietary exposure to total copper.

[14] 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, pp. 51–57.

3. Assessment

3.1. Copper physiology, homeostasis and toxicology

3.1.1. Copper chemistry

The biologically active form of copper is the copper ion, which is redox reactive and cycles between its reduced cuprous (Cu^{+}) and oxidised cupric (Cu^{2+}) ionic states (Krężel and Maret, 2021). The cuprous ion (Cu^{+}) is the major intracellular form of copper. Extracellularly, copper is usually in the oxidised Cu^{2+} state under both normal and pathological conditions. Cu^{+} is the main ionic state intracellularly and any Cu^{2+} that may be released from copper enzymes is readily reduced to Cu^{+} due to the reducing intracellular environment, with implications in the redox status of the cell. Cu^{+} is more potent than Fe^{2+} in catalysing Fenton chemistry. However, Fenton chemistry can be prevented because of effective copper sequestered (see below) such that there is essentially no free Cu^{+} intracellularly (less than femtomolar concentrations; 10^{-15} M). Comparative bioavailability studies conducted by industry and presented in the 2017 draft renewal assessment report by RMS (France and Germany) have provided data that different copper salts were bioequivalent, i.e. there were no differences in absorption, copper plasma levels, liver or bile levels, or excretion rates between the different forms of copper, including copper sulfate, copper hydroxide, copper oxychloride, Bordeaux mixture, tribasic copper sulfate and copper (I) oxide (France, 2017). A tightly regulated system of copper-binding protein carriers, metallochaperones, protects the body from the presence of free copper systemically and its participation in the Fenton redox reaction (Kim et al., 2008; Ba et al., 2009; Lutsenko, 2010; Nevitt et al., 2012; Gromadzka et al., 2020).

3.1.2. Copper as an ETE

Copper is an essential element for the structure and function of several cuproproteins, which are ubiquitous in humans and highly conserved across mammalian species. Copper metalloenzymes are involved in the synthesis of hormones, neuropeptides (e.g. enkephalins) and other neurotransmitters; in substrate metabolism and oxidative phosphorylation; in endogenous antioxidant activity (e.g. Cu/Zn superoxide dismutase); in the synthesis of connective tissue and of the organic matrix of bone; in the absorption and distribution of iron; in muscle contractility; and in the formation of melanin. Overall, an adult human body is estimated to contain 1.4–2.1 mg/kg bw or 50–150 mg of copper: at least 40% of this is in muscles. Copper concentrations range from 4.8 to 12 μg/g in liver, brain, heart, kidney and skeleton and from 1 to 2 μg/g in other tissues (lung, spleen, intestine). Approximately 60% of the copper in red blood cells is in superoxide dismutase (SOD) (Linder and Hazegh-Azam, 1996; EFSA NDA Panel, 2015; Gromadzka et al., 2020). Copper is also transported into milk during lactation to ensure adequate and relatively constant transfer of copper to the nursing infant, regulated by prolactin (Lönnerdal, 2007).

3.1.3. Copper transporters

The systemic fate of absorbed copper also involves protein binding and cellular transport via specific membrane-spanning copper-transporting ATPases, ATP7A and/or ATP7B (Nevitt et al., 2012; Lutsenko, 2016; Linder, 2020). ATP7A is expressed in most cells, notably enterocytes, whilst ATP7B is mainly expressed in the liver and mammary epithelial cells. Once released from enterocytes via ATP7A into the portal vein blood stream, copper binds to albumin and possibly to transcuprein (Liu et al., 2007; Linder, 2016, 2020; Lutsenko, 2016). Around 40% of copper is taken up into the liver in the first pass. Once taken up in the liver, the copper is either utilised locally or incorporated into caeruloplasmin, which is released into the systemic circulation carrying copper to peripheral tissues. Excess systemic copper is excreted by the liver into the bile via ATP7B or sequestered in the liver bound to metallothioneins (MTs) as part of its homeostatic regulation (see below) (Calvo et al., 2017; Krężel and Maret, 2017; Członkowska et al., 2018; Krężel and Maret, 2021).

Depending on the cell type or its metabolic state, ATP7A and ATP7B may be found predominantly in the *trans*-Golgi compartment or in intracellular vesicles. In the trans-Golgi compartment, the transporters deliver Cu to Cu-dependent enzymes, such as lysyl oxidase, caeruloplasmin, dopamine-beta-hydroxylase and others. In vesicles, ATP7A and ATP7B sequester cytosolic copper for further export out of the cell.

In the liver, copper is transferred from the cytosol via ATP7B to the transporting protein apo-caeruloplasmin in the lumen of the *trans*-Golgi network (TGN), exits the cells via vesicular exocytosis

from the basolateral (sinusoidal) hepatocyte plasma membrane and is transported in the circulation. ATP7A and ATP7B also mediate intracellular transfer of copper to copper-containing proteins in liver and other cells (Linder, 2020). Mammary epithelia regulate copper influx from the blood and its excretion into milk in response to hormonal control (prolactin) and to breast milk demand (in response to suckling). ATP7B is thought to be responsible for constitutive copper secretion into milk via caeruloplasmin, whereas CTR1 and ATP7A transiently increase copper uptake into the mammary gland and secretion into milk (Lönnerdal, 2007). Copper transfer across the placenta is tightly regulated and increases during gestation. Uptake by the placenta is mediated through the high affinity carrier CTR1 and the mechanisms of subsequent copper transfer are thought to be similar to those described for other cells (e.g. gut cells), involving chaperone bound copper delivery to its target molecules or to membrane transporters ATP7A and ATP7B. Both transporters are expressed in placenta and are thought to mediate copper transfer to the fetal circulation and export of excess copper back to the maternal circulation, respectively (McArdle et al., 2008).

When intracellular copper stores are high, the ATPases 7A and 7B are themselves incorporated into copper-carrying vesicles destined for exocytosis and migrate to the plasma membrane. MTs may also be involved in the copper-dependent trafficking of ATP7A copper transporter from the *trans*-Golgi to the plasma membrane in a transgenic animal model (Krężel and Maret, 2021). Copper exocytosis via ATP7A-containing vesicles operates in all cells and evidence in mice indicates that ATP7A releases copper into the extracellular space and to the circulation not bound on proteins (Ke et al., 2006; Wadwa et al., 2014; as cited in Linder, 2020). In the liver, ATP7B transports excess copper into late endosomal or lysosomal vesicles to the apical (canalicular) plasma membrane where copper enters the bile, whilst, once at the apical plasma membrane, this transporter also pumps copper bound on cytosolic carriers, such as ATOX1, into the bile. When the level of cellular copper is low, ATP7A and ATP7B recycle to the TGN (La Fontaine and Mercer, 2007; Inesi, 2017). ATP7B also functions as a vesicular storage pool of copper in some cells, such as enterocytes and kidney epithelial cells (Linder, 2020). Homeostatic regulation under conditions of excess copper is predominantly dependent on trafficking of ATP7B between the TGN membranes and the apical plasma membrane in hepatocytes. Although there are metal-responsive elements within the *ATP7B* gene promoter, there is limited evidence indicating that the expression of this transporter is induced by excess copper. Evidence for reduced ATP7B protein degradation has also been obtained from animal models, but it is unclear whether these mechanisms apply to humans (Linder, 2020).

Hepatic copper retention is well documented in individuals with genetic mutations of the *ATP7B* gene that result in reduced biliary copper excretion (Chang and Hahn, 2017; Członkowska et al., 2018). It should be noted that over 700 variants in *ATP7B* with different degrees of penetrance (impact on function) have been reported as associated with WD (Chang and Hahn, 2017; Wallace and Dooley, 2020). Only a minority of WD patients are homozygous for a single variant, whereas the majority are compound heterozygotes. Copper toxicity associated with the manifestation of WD is predominantly of a hepatic and/or neurologic nature (and may also have psychiatric and ophthalmological manifestations) and is often accompanied by increased urinary copper levels (see Członkowska et al., 2018 for a review). Individuals carrying *ATP7B* genetic mutations who do not meet the criteria for WD diagnosis and are disease-free may constitute a susceptible population for copper retention and toxicity from high copper intake. Genetic disease associated with mutations in the *ATP7A* gene (Menkes disease) leads to insufficient copper absorption. Hence, individuals carrying *ATP7A* genetic mutations are not a susceptible population for copper retention.

3.1.4. Interaction of copper and zinc homeostasis

The interactions between copper and zinc homeostasis are critical in understanding copper physiology, regulation and toxicity. These two essential elements are maintained at significantly different intracellular concentrations, with $\sim$ 10 times more zinc than copper in the cell compared with an $\sim$ 1:1 copper to zinc ratio extracellularly. Zinc supplementation has been reported to reduce GI uptake of copper, particularly at zinc levels attained with supplementation but modest, if any reduction of copper uptake is seen at physiological levels of Zn intake (Lönnerdal, 1998). Zinc supplementation is used therapeutically to attenuate copper overload in WD (EASL, 2012; Członkowska et al., 2018; Poujois and Woimant, 2018). This practice is based on earlier observations that supplementation with 50 mg/day of zinc (in two daily doses of 25 mg) for up to 6 weeks, resulted in a statistically significant decrease of copper-dependent erythrocyte Cu, Zn-SOD activity, an indicator of decreased Cu status, by week 6 (Fischer et al., 1984). However, high doses of Zn intake can also result in copper deficiency

in infants and children (Lönnerdal, 1998). Conversely, high cellular copper has an impact on zinc homeostasis, implicating high copper intake in altered zinc-mediated gene transcription (Meacham et al., 2018), likely to be modulated via the interactions of these two ETEs with MT (Krężel and Maret, 2021).

A recent comprehensive review of MT structure and physiology (Krężel and Maret, 2021) shows that the complex relationship between zinc and copper converges on their interactions with MTs. MTs are ubiquitous intracellular monomeric polypeptides that bind copper in its reduced cuprous (Cu^{+}) ionic state. They are considered a complex and dynamic biological control of ETE homeostasis, primarily zinc, and as a line of defence against exogenous (non-essential) metal toxicity (e.g. cadmium, lead, mercury). The different mechanisms of MT handling of copper and zinc are critical to their respective and inter-dependent homeostasis. MTs are considered scavengers for copper at high copper levels, sequestering copper effectively in contrast to the dynamically regulated pools of free and MT-bound zinc, regulating zinc functions. This is due to the significantly higher affinity of MT for copper compared with zinc (by several orders of magnitude) and the chaperone-mediated copper transfer to and from MT.

MT1 and MT2 typically carry more zinc than copper, whilst MT3, the main neuron isoform in the brain, is rich in copper and MT4 is classified as copper MT. Copper may bind on MTs either in addition to already bound zinc, i.e. without zinc dissociation if there are enough binding sites available (such as in low or deficient zinc conditions), or it may displace zinc from its binding sites due to copper's higher binding affinity for MT. The latter impacts zinc availability, homeostasis and zinc-mediated functions and downstream events. The ratio of 8:1 copper to MT appears to be the most stable stoichiometry, whereas the theoretically possible 20:1 stoichiometry is not expected to exist in practice. Copper displacement may reversely occur in conditions of high zinc influx. Importantly, MT induction in response to increased copper (and cadmium) levels is effected indirectly through displacement of zinc from its MT binding sites. However, MT can also be induced by a number of stressors (endotoxaemia, infections, inflammation, calorie restriction, exercise, hypothermia, hormones and mediators of oxidative and physiological stress, glucocorticoids, xenobiotics), which can have an impact on MT availability and on copper:zinc ratio (Cousins, 1983; Calvo et al., 2017; Krężel and Maret, 2021).

3.2. Copper kinetics

Copper physiology and metabolism have been extensively addressed in a previous NDA Panel Opinion on dietary reference values (DRVs) for copper published in 2015. This covered copper intestinal absorption, transport, distribution, storage, metabolism and elimination. Aspects relevant to the present Opinion are reiterated below. The reader is referred to the copper DRVs Opinion for more detailed information (EFSA NDA Panel, 2015). Relevant studies taken into consideration in the present assessment are summarised in Appendix B.1.

3.2.1. Copper homeostasis in adults

Copper homeostasis, and the regulation of copper absorption and excretion in particular, have been studied using controlled levels of copper intake in several human metabolic studies using Cu isotopes. The first studies conducted with a stable copper isotope (^{65}Cu) to assess copper absorption, excretion and estimated copper balance (defined as 'intake minus excreted copper' in mg/day), evaluated only one dose level or non-standardised base diets (Turnlund et al., 1982, 1985, 1988; Turnlund, 1983, 1988; Jacob et al., 1987). These studies suggested a trend of dependence of copper excretion on the level of intake. Later studies also distinguished 'apparent absorption' from 'true absorption'; the latter was defined as apparent absorption corrected for faecal losses of endogeneous copper (see below).

The first study using ^{65}Cu and more than one controlled dose level was conducted to characterise copper homeostasis at two dose levels covering the range of existing recommended intakes (based on earlier balance studies) and at one higher intake level (Turnlund, 1989). In this study, 11 young men (22–35 years old) received diets with three levels of controlled amounts of copper for a total of 90 days, in three consecutive periods in this order: 1.7 mg/day (equilibration period for 24 days), 0.8 mg/day ('low-Cu' period for 42 days) and 7.5 mg/day ('high-Cu' period for 24 days). The purpose of the equilibration period was to minimise the differences in copper status among participants that represented copper intake from diets under free-living conditions before the study. In this study, the background level of copper intake was estimated to be about 1–1.5 mg/day (Turnlund, 1989). The controlled diets were reported to contain sufficient intake of essential nutrients, including zinc at a

level previously shown to not interfere with the copper balance (Turnlund, 1988). During the equilibration period, mean (±SEM) copper absorption was 36.3 ± 1.3% (0.61 mg/day) and mean (±SEM) balance was 0.167 ± 0.40 mg/day. Mean (±SEM) copper absorption during the controlled 'low-' and 'high-Cu' diet periods was 55.0 ± 1.5% (0.44 mg/day) and 12.4 ± 0.9% (0.93 mg/day), respectively. During the controlled 'high-Cu' period, faecal copper excretion gradually increased, from an average of 4 mg/day at the beginning of the period to 9 mg/day at the end. Average copper balance was 0.002 ± 0.034 and 0.941 ± 0.16 mg/day over the controlled 'low-' and 'high-Cu' periods, respectively. A linear decline in copper balance was observed (mirroring the increasing copper excretion) during the 'high-Cu' period (the mean balance declined from approximately +3.5 mg/day to −1.5 mg/day) suggesting that the subjects were adapting to that level of copper intake. However, the rate of increased excretion was not sufficient to remove the cumulative amount of copper absorbed over the 24-day period, i.e. it was not sufficient for the subjects to equilibrate to the high dietary intake, leading to a positive retention of copper of 0.94 mg/day on average (0.128–1.97 mg/day) over the period (Turnlund, 1989). Furthermore, whilst the mean balance was negative after 24 days of high copper intake, half of the individuals in the study still had a positive balance, suggesting substantial individual variability in the capacity to adapt to high copper intake. Plasma copper and caeruloplasmin concentrations, erythrocyte SOD and urinary and salivary copper did not differ between the three periods (Turnlund et al., 1990).

A subsequent study was designed to assess the homeostatic responses in 11 young men. True copper absorption and excretion were measured under controlled dietary copper intakes of 0.66 mg/day for 24 days, 0.38 mg/day for 42 days and 2.49 mg/day for 24 days. The mean apparent absorption of copper was 54%, 67% and 44% in the three respective periods. Using ^{65}Cu orally in half of the participants, true absorption was found to be 73%, 77% and 66% in the respective periods (Turnlund et al., 1998). Excretion of endogenous copper was assessed using intravenously infused ^{65}Cu in the other half of the participants. Copper excretion over the first 12 days after infusion increased with increasing intake and was 26%, 12% and 34% of the dose in the three respective periods. Total copper excretion in the final 6 days of each period included recently absorbed (within the last 12 days) dietary copper (fast pool) and endogenous copper (slow pool) not accounting for 'recently absorbed' dietary copper. The rate of copper excretion was faster for the first 18 days after the oral dose increased. Increased biliary excretion of copper at the higher doses accounted for most of the apparent difference in absorption. Copper balance was positive early in the period of higher copper intake (2.49 mg/day), but declined to zero balance by the end of the 24 days, indicating adaptation to the change in copper intake between the two periods.

Harvey et al. (2003) investigated copper absorption and retention in 12 men who received 0.7, 1.6 or 6 mg Cu/day in separate 8-week periods with at least 4-week intervening washout periods. In that study, mean ± SD apparent and true absorption (41 ± 12% and 48 ± 13%, respectively) during the 'low-Cu' period were not significantly different from the 'high-Cu' period (45 ± 13% and 48 ± 11%, respectively). Endogenous losses were higher in the 'high-Cu' diet (2.46 ± 1.11 mg/day) compared with the 'low-CU' (0.45 ± 0.25 mg/day) and 'medium-Cu' (0.81 ± 0.16 mg/day) diets. However, the SC notes that these values do not compare directly with those reported by Turnlund et al.; the endogenous copper losses were calculated based on the time profile of the faecal excretion of orally administered rather than of infused ^{65}Cu label (faecal ^{65}Cu content in the first 14 days after label administration and were attributed to the 'fast pool'). Despite the higher excretion, the average balance between (unlabelled) copper intake and excretion was positive (i.e. copper retention) at the end of 'high' copper intake period (0.75 ± 1.05 mg/day), compared with the 'low' (−0.13 mg/day) and 'medium' (0.00 mg/day) intake periods (–0.13 ± 0.32 mg/day, 0.00 ± 0.31 mg/day, respectively). No differences were observed between the three periods on erythrocyte SOD, serum copper concentration and plasma caeruloplasmin concentration and activity (Harvey et al., 2003).

Longer term adaptation to high copper intake was investigated in a group of nine men who consumed an average of 1.6 mg/day total Cu for 18 days under controlled conditions (first metabolic period), then their usual diets were supplemented with 7 mg Cu/day for 129 days under free-living conditions, and finally 7.8 mg/day total Cu for 18 days under controlled conditions (second metabolic period) (Turnlund et al., 2004, 2005). The copper content in the free-living diets was estimated to be 1.6 mg/day on average, based on 5-day diaries and a nutrition database from the Nutrition Coordinating Center, University of Minnesota, 1998; therefore, the total copper intake during the free-living conditions was on average 8.6 mg/day. Comparing the high copper intake with the usual intake, the fraction of administered copper absorbed was significantly lower (16% vs. 29% of apparent absorption; 29% vs. 40% of true absorption), but the total amount absorbed was significantly higher

(1.2 mg/day vs. 0.48 mg/day). Excretion of copper was significantly higher at the higher copper intake (46% vs. 27% faecal ^{63}Cu excretion during the last 12 days of the metabolic periods). However, total balance was positive at the end of the second metabolic period (0.67 mg/day vs. 0.06 mg/day at the end of the first metabolic period), when a zero balance was expected to have been reached following the high copper free-living period of 4.3 months (Turnlund et al., 2005). This was associated with higher urinary copper excretion and hair copper concentrations during the second metabolic period (0.0256 mg/day and 0.0211 mg/day, respectively) than during the first (0.0203 mg/day and 0.0092 mg/day, respectively), although these routes were small contributors to copper excretion (Turnlund et al., 2004). Further indication of the faster turnover of endogenous copper when oral intake is high was provided from the lower plasma enrichment of infused tracer label in subjects of high oral copper intake compared to the subjects of lower oral intake, after the first 24 hours of infusion. Caeruloplasmin activity, benzylamine oxidase and erythrocyte SOD were significantly higher at the end of the second metabolic period than at the end of the first, whilst plasma copper concentration was not affected (Turnlund et al., 2004). The copper content of intestinal cells was also found to be significantly increased when copper intake was high (mean ± SEM copper content of the cells = 1.65 ± 0.19 mg/g protein vs. 0.78 mg/g ± 0.19 protein) (Turnlund et al., 2003). Assuming no further adaptation took place after 5 months and the rate of retention was not reduced, the authors estimated that the copper body burden was projected to double in 100–150 days with continuous intake of approximately 8 mg/day (Turnlund et al., 2005). The SC recognises that projections beyond the period of observation in the study have higher uncertainty than the observed data.

The SC notes that the absorption/excretion profile in these studies indicates a time-dependent adaptation to changing copper intakes as part of homeostatic responses during the monitoring periods. The rate of absorption and excretion at the start of an intervention period reflects the absorption and excretion profiles of copper intake of the period immediately preceding it. Homeostatic changes in absorption and excretion took place and a net zero balance was reached by the end of the respective observation periods at intakes of 0.8 mg/day (42 days) (Turnlund, 1989), 1.6 mg/day (18 days) (Turnlund et al., 2005), 1.7 mg/day (24 days) (Turnlund, 1989) and 2.49 mg/day (24 days) (Turnlund et al., 1998). Effective adaptation without retention is expected at these intake levels over chronic exposure. At copper intakes of 6–8 mg/day, copper balance was not fully restored in the three studies with controlled intake periods of 8 weeks, 3.5 weeks and 21 weeks, respectively, and subjects were still retaining copper at the end of the intervention periods (Turnlund, 1989; Harvey et al., 2003; Turnlund et al., 2005).

The studies by Turnlund described above indicate that copper homeostasis is partially regulated at the level of absorption, with apparent % Cu absorption decreasing with increasing dose of copper (Turnlund, 1989). Further data indicated that the true % absorption may actually change little (66% vs. 73%) (Turnlund et al., 1998) or be relatively stable (48%) (Harvey et al., 2003) and the decrease in apparent absorption is attributable primarily to biliary excretion of copper (Turnlund et al., 1998; Harvey et al., 2003). In fact, biliary excretion of copper appears to be the main excretion mechanism. Sequestration of copper by the intestinal mucosa has also been reported and appears to act as an additional regulatory mechanism, although the contribution to copper excretion from exfoliation of copper-containing intestinal epithelial cells has not yet been quantified (Turnlund et al., 2003).

Copper absorption in women has not been assessed in the above studies and there are limited data comparing homeostasis in men and women. In an earlier study, average (true) copper absorption in young (non-pregnant) women was 41.2% from animal protein diets containing 1.44 mg copper and 33.8% from plant protein diets containing 2.53 mg copper (Turnlund et al., 1983). The average (true) copper absorption in pregnant women was slightly higher, at 42.2% and 40.7% from these respective diets (Turnlund et al. 1983). A direct comparison between women and men cannot be made since there were acknowledged differences in the diets, but absorption appears to be similar for comparable copper intakes. Furthermore, copper absorption in pregnant women appears to be in a similar range as for non-pregnant women. Because of the need for maternal copper transfer to the foetus during gestation (Turnlund et al., 1983; McArdle et al., 1995, 2008), copper retention in pregnant women is not more likely than for men. Similarly, copper retention is not more likely in lactating women than in men because of the higher copper requirements to maintain copper levels in breast milk (Kelleher and Lonnerdal 2005; Lönnerdal, 2007).

Urinary excretion of copper is a minor route and urinary copper levels show little variation in response to changing dietary intake, typically ranging from 11 μg/day to 60 μg/day (Turnlund et al., 1990, 1998, 2005; Milne, 1998). Skin, sweat and hair are also minor routes of copper loss. Additional sources of copper excreted directly into the GI tract, such as saliva, gastric, pancreatic or

duodenal juices, have been reported to contribute appreciably to the excreted copper (Linder, 2020). It has been suggested that copper in bile is found in complexes that are less re-absorbable from the GI tact and therefore biliary elimination and is more effective than other secretions in the GI tract (e.g. saliva, etc.) from which copper may be reabsorbed once it reaches the GI lumen (Linder, 2020).

In summary, copper homeostasis of the systemic copper content is regulated by hepatobiliary excretion and intestinal absorption. Initial homeostatic responses involve reduced absorption followed by increased hepatobiliary excretion (Turnlund, 1989; Turnlund et al., 1998). Reduced absorption is primarily achieved by downregulation of mucosal uptake carriers and induction of enterocytic MT, which sequesters copper in the enterocyte and reduces transfer of copper to the portal circulation. Dietary zinc has a direct impact at the level of copper uptake as it is known to compete with copper transport and reduce gastrointestinal copper absorption (Fischer et al., 1984). Other dietary factors may also have an impact on copper absorption (Lönnerdal, 1998; Hunt and Vanderpool, 2001). Whilst reduced absorption is the first homeostatic response to increasing oral copper intake, increased excretion contributes to a larger degree towards a net zero balance (Turnlund, 1989; Turnlund et al., 1998). All available evidence suggests that at increasing exposures, copper is sequestered in the hepatic 'storage depot' of MT (Huster et al., 2007; Calvo et al., 2017; Krężel and Maret, 2017; Krężel and Maret, 2021). As noted, zinc and copper homeostasis converge on MT with consequences on zinc-mediated MT induction in response to increased copper status (Krężel and Maret, 2021) and with copper impact on zinc-mediated cellular regulation.

3.2.2. Copper homeostasis in infants and children

The available information of copper homeostasis in infants is limited and is described below. No relevant information has been found for copper homeostasis in children older than 16 weeks of age.

In a study by Olivares et al. (2002), measurements of copper absorption and faecal excretion were conducted using a ^{65}Cu tracer in 39 healthy infants, in two age groups, of 1 month (n = 19) or 3 months (n = 20) of age, following supplementation with 0.08 mg Cu/kg bw per day (administered as 1.2 μmol copper sulfate per kg bw per day) for 15 days. Total copper intake in non-supplemented and supplemented infants was estimated by the authors of the study to be 0.06 ± 0.002 and 0.09 ± 0.002 mg/kg bw per day, respectively, at 1 month and 0.04 ± 0.0003 and 0.06 ± 0.002 mg/kg bw per day, respectively, at 3 months. Copper absorption ranged between 45% and 95% across all infants. In infants at 1 month of age (n = 10), the percentile of apparent copper absorption (mean ± SD) was 83.6 ± 5.8% without supplementation (n = 9) and 74.8 ± 9.1% with supplementation. Apparent copper absorption at 3 months of age was 77.6 ± 15.2% (n = 10) and 77.7 ± 11.3% (n = 10), respectively. Total faecal copper (measured total copper in 3-day sample collections) in non-supplemented and supplemented infants was 0.33 ± 0.11 mg/3 days and 0.74 ± 0.33 mg/3 days, respectively, at 1 month and 0.51 ± 0.3 mg/3 days and 0.71 ± 0.3 mg/3 days, respectively, at 3 months of age. The authors of the study concluded that copper intake was correlated with total faecal copper but not with apparent absorption. Apparent absorption (%) was inversely correlated with total faecal copper but not with age of infants. Copper absorption was higher from human milk compared with infant formula and higher in premature infants compared with full-term infants (Olivares et al., 2002).

Another supplementation study (Dorner et al., 1989) assessed the copper intake and retention in 26 infants, ages 2–16 weeks (20 full term and 6 pre-term) through breast milk (0.833 mg Cu/L) compared to supplemented (0.619 mg Cu/L) and non-supplemented formula milk (0.121 mg Cu/L). Copper intake from breast milk and supplemented cow's milk-based formula resulted in positive balances in five 72-hour collections every 3–4 weeks between 2 and 16 weeks of age. Mean daily copper intake was 0.11 ± 0.02 mg/kg bw in breast-fed, 0.02 ± 0.004 mg/kg bw in the non-supplemented formula-fed and 0.11 ± 0.02 mg/kg bw in the supplemented formula full-term infant group. Mean daily copper balance was 0.09 ± 0.05 mg/kg bw, 0.005 mg/kg bw (range −0.012 to 0.01 mg/kg bw) and 0.06 ± 0.02 mg/kg bw, respectively, corresponding to 74%, 23% and 52% of intake levels, respectively. Copper intake in breast-fed infants decreased from 2 to 12 weeks due to decreasing copper levels in breast milk with advancing lactation. Faecal excretion was the main route of excretion and urinary excretion was estimated to contribute 6.4% in breast-fed and supplemented formula infants, but urinary excretion was higher in non-supplemented formula infants where balance was very low and even negative. No differences were noted in supplemented formula-fed pre-term infants, compared to the corresponding full-term infants but the non-supplemented formula pre-term infants had negative balances (Dorner et al., 1989).

In both studies, the age of the infants was under 16 weeks, a period of infant development where homeostatic and other physiological processes are not fully developed. Positive balances in the postnatal period as reported by Dorner et al. are also consistent with higher nutrient requirements per unit of body weight, including ETEs such as copper, compared with later stages. The observation that copper retention increased linearly with increasing intake in infants ('absorption non-saturable') (Dorner et al., 1989; Lönnerdal, 1998) suggests either that homeostatic control is not fully established during this period (i.e. the reduction of absorption expected with increasing intake exceeding nutritional needs did not take place) or is an indication of the increased nutritional needs. Evidence supporting the higher copper requirements in infants is also provided by the following observations: (i) fetal hepatic pools (mostly built during the third trimester) are reduced rapidly during the postnatal phase: this indicates that the requirement for copper in postnatal life is only partly met by breast milk copper content and is complemented with copper stored in the fetal liver (Lönnerdal, 1998; Olivares et al., 2000); and (ii) exclusive postnatal feeding with non-supplemented infant formulae (for over 6 months) leads to copper deficiency in infants (Lönnerdal, 1998, 2007), thereby indicating that they are insufficient to meet infant needs.

Because tighter homeostatic regulation develops within 4 months after birth and because early life retention of nutrients serves the needs of this stage of rapid growth, data on copper disposition reported in infants cannot be utilised to extrapolate copper homeostasis in older infants (> 16 weeks) and in children. It is noted that for the infant subpopulation under 16 weeks of age, HBGVs such as ADI, tolerable daily intake (TDI) or acute reference dose (ARfD) have traditionally not been considered applicable (EFSA Scientific Committee, 2017b).

There is a lack of data on copper homeostasis in children older than 16 weeks of age. However, copper homeostasis is tightly regulated, highly conserved across species and is essential in mammalian biology. Whilst it is assumed that these mechanisms of copper homeostasis are fully developed soon after birth, copper physiological requirements cannot be similarly extrapolated between adults and children. Whilst homeostasis mechanisms may operate in a similar fashion, copper retention in children (positive balance) is not physiologically equivalent to copper retention in adults. Based on evidence of higher requirements for energy (EFSA NDA Panel, 2013) and protein (EFSA NDA Panel, 2012), children can be expected to have higher requirements for nutrients, including copper. Because of the higher needs for growth, the retained copper is therefore likely to be widely dispersed throughout the body rather than be focally sequestered.

3.2.3. Copper homeostasis in animals

Supporting evidence for hepatic copper retention was obtained from two 90-day studies in rats (Hebert, 1993; Kumar et al., 2015). In the 90-day study in rats that has been used as supportive evidence in previous assessments of copper toxicity (Hebert, 1993), statistically significant increase in copper tissue levels was reported in liver and kidney at all dietary levels (starting below the NOAEL) and in a dose-dependent manner (mean copper of 0.24, 1.83, 6.11, 17.90, 127.31, 372.12 μg/g in the liver, and 0.62, 4.81, 3.45, 7.65, 52.89, 181.30 μg/g in the kidney), at dietary (feed) levels of 0, 500, 1000, 2000, 4000 and 8000 mg/kg feed of copper sulfate pentahydrate (CAS 7758-99-8), respectively (equivalent to 127, 254, 508, 1016 and 2032 mg/kg feed of copper[15]) (Hebert, 1993). The estimated intake of cupric sulfate pentahydrate was 0, 32, 64, 129, 259 and 551 mg/kg bw per day in male rats and 0, 34, 68, 135, 267 and 528 mg/kg bw per day in female rats (equivalent to 0, 8, 16, 33, 66 and 140 mg/kg bw per day of copper, respectively, in male rats and 0, 9, 17, 34, 68 and 134 mg/kg bw per day of copper, respectively, in female rats). Plasma copper was increased at the top two dietary levels. Tissue copper concentrations were significantly increased in the liver and kidney at all dietary copper levels starting at 500 mg/kg of feed equivalent to 8 mg Cu/kg bw per day, a level below the NOAEL of 16 mg Cu/kg bw per day derived from this study (Hebert, 1993).

In the second study, rats treated with 100 and 200 mg/kg bw per day of copper sulfate pentahydrate (equivalent to ~25 and 51 mg/kg bw per day of copper10) for 30, 60 or 90 days, statistically significant increase of copper levels was observed in the liver, kidney and brain at both dose levels and all treatment periods (up to 29-fold, 3-fold and 1.5-fold, in liver, kidney and brain, respectively) (Kumar et al., 2015).

These studies provided evidence that hepatic copper increases with increasing intake, albeit with significant quantitative species differences between intake dose and hepatic copper concentrations.

[15] MW of copper sulfate pentahydrate = 250 g/mol; copper MW = 63.5 g/mol.

Increased tissue copper concentration in rats was observed at dose levels that were ~100-fold higher than copper intake in human experimental studies (0.07–0.09 mg/kg bw per day). The hepatic copper levels in the control animals (< 1 μg/g) were lower than reported hepatic copper concentrations in non-WD humans (usually below 50 μg/g). The range of doses administered in rats was from 18-fold up to 300-fold higher than the human dose, resulting in liver failure, described in a single case report (30–60 mg/day for 12 months, equivalent to up to 0.9 mg/kg bw per day; see below).

Increase of hepatic copper of up to four to five times compared to control animals has also been reported in capuchin monkeys in young animals treated with 5.5 mg Cu/kg bw per day and in adult animals treated with 7.5 mg Cu/kg bw per day for 3 years (Araya et al., 2012).

3.3. Copper toxicity

Previous assessments of copper toxicity reviewed data on liver toxicity, developmental and reproductive toxicity, carcinogenicity and genotoxicity, neurotoxicity (including a potential association between copper intake and the risk of Alzheimer's disease) and immunotoxicity (WHO, 1996; IPCS, 1998; IOM, 2001; EVM, 2003; SCF, 2003; France, 2007a, 2017). These assessments identified the liver (and brain) as the critical target organ(s) of copper toxicity. In accordance with the methodological approach outlined in Section 2.2, this assessment focuses on human data of copper toxicity and only considers data from experimental animal studies only as supportive evidence. For a recent review of copper toxicity in animals, see e.g. Taylor et al. (2020). Human intervention studies and observational studies reporting on copper intake and adverse health effects taken into consideration in the present assessment are summarised in Appendices B.2 and B.3. Literature relevant to a potential association of copper with Alzheimer disease is summarised in Appendix B.4. Previous assessments of copper toxicity also concluded that the available evidence does not raise a concern for genotoxicity or carcinogenicity from copper intake in humans (Scientific Committee on Health and Environmental Risks, 2008; France, 2017). No additional relevant studies were identified since the last EFSA assessment in 2018. An overview of genotoxicity and carcinogenicity studies is presented in Appendix B.5.

Documented copper toxicity in humans has been associated either with high-dose exposures (acute toxicity) or with WD progression and is mediated by free and redox active copper. There are no reports of copper toxicity under usual dietary exposure conditions in humans without this genetic disorder.

Acute toxicity of copper has been described in cases of ingestion of a high dose of copper or self-poisoning (attempted suicide). The doses of copper ingested in these cases were usually not well characterised, but have been reported to range between 1 and 70 g (Mittal, 1972). Common symptoms of acute copper toxicity included gastrointestinal symptoms, haemolysis and damage in the gut, kidney and the liver (Chowdhury et al., 1961; Chuttani et al., 1965; Mittal, 1972; Walsh et al., 1977; Hassan et al., 2010). In other studies of acute copper toxicity, copper was administered as $CuSO_4$ in drinking water at concentrations of up to 5 mg/L (Pizarro et al., 1999), up to 8 mg/L (Araya et al., 2001, 2003) or up to 12 mg/L (Olivares et al., 2001). Nausea and GI disturbance were reported as a result of local effects and are not relevant to chronic toxicity.

One case of severe toxicity has been described in a young man who ingested a high dose of copper supplements (30 mg Cu/day for 30 months followed by 60 mg Cu/day for 12 months) (O'Donohue et al., 1999). The man was admitted to hospital after a 6-week history of malaise, jaundice and abdominal swelling. Acute renal failure was diagnosed, together with severe liver cirrhosis, necessitating liver transplantation. The liver had a copper concentration of 3.23 mg/g dry weight, which is about 100 times higher than typical copper liver contents in adults (Bush et al., 1995; Nuttall et al., 2003).

Human toxicity data from longer term exposure at lower intake levels are limited, which represents a critical data gap. As hepatic sequestration initially functions as a protective mechanism, adverse effects are effectively prevented, consistent with the absence of toxicity (Pratt et al., 1985; Olivares et al., 1998; Mendez et al., 2004; Araya et al., 2005; Rojas-Sobarzo et al., 2013). Therefore, the assessment of potential toxicity associated with chronic exposure to copper can only be inferred from evidence from long-term observational studies with non-controlled dietary exposures (e.g. copper-containing drinking water), evidence of compromised homeostasis obtained from studies of shorter duration such as controlled intervention studies, or evidence from patients with genetic disease related to copper overload.

3.3.1. Observational studies

Two cross-sectional (Dassel de Vergara et al., 1999; Zietz et al., 2003) studies with infants of 956 households and 2,944 households, respectively, and two retrospective cohort studies of childhood liver cirrhosis (Scheinberg and Sternlieb, 1994; Dieter et al., 1999) have investigated the association with copper exposure through drinking water (Appendix B.2). In the cross-sectional studies, copper levels in drinking water ranged from < 0.5 mg/L in 87.4% of the households (background) up to a maximum of 2.6 mg/L (Dassel de Vergara et al., 1999). Only eight infants were examined for liver condition endpoints and no changes were noted. One child had slightly elevated serum copper and C-reactive protein (CRP) levels (Dassel de Vergara et al., 1999). Children (8–10 months of age at enrolment) from households with water copper levels > 0.8 mg/L up to 4.2 mg/L exposed to copper in water for at least 6 weeks underwent medical evaluation (Zietz et al., 2003). No signs of adverse effects on liver function (AST, ALT, GGT, total bilirubin) related to copper intake or changes in markers of copper status (serum copper or caeruloplasmin) were found (Zietz et al., 2003).

Scheinberg and Sternlieb retrospectively investigated children (0–5 years) from three Massachusetts towns over a total of 64,124 child-years of copper exposure (between 1969 and 1991) with water levels 8.5–8.8 mg/L (Scheinberg and Sternlieb, 1994). There was no case of cirrhosis-related deaths, which was the only endpoint reported (Scheinberg and Sternlieb, 1994). This study also reported seven cases of young children (< 2 years) with non-Indian childhood cirrhosis consuming water with 0.05–6.8 mg Cu/L (Scheinberg and Sternlieb, 1994). Another study evaluated 103 cases of early, histologically confirmed, childhood cirrhosis in Germany of which five cases with high hepatic copper were considered as probably related to chronic and excessive exposure to copper of 9–26.4 mg Cu/L in water (Dieter et al., 1999).

These studies did not provide sufficient information to draw conclusions on an association between high copper intake from drinking water and adverse effects in young children.

3.3.2. Controlled intervention studies

Data on copper toxicity in humans other than WD patients are limited, partly due to the absence of sensitive, reliable and specific biomarkers of early adverse effects of copper toxicity (Danzeisen et al., 2007; Bost et al., 2016).

In the study by Turnlund et al. (2004), copper intake of 8.6 mg/day for 129 days under free-living conditions (7 mg/day supplementation plus an estimated 1.6 mg/day dietary content) followed by 7.8 mg/day of controlled copper intake for 24 days (5 months total) was reported to be associated with increased urinary excretion of thiobarbituric acid-reactive substances (TBARS), used as a marker of lipid peroxidation. Urinary measurement of TBARS is neither a sensitive nor a robust biomarker of oxidative stress (Moore and Roberts, 1998; Ito et al., 2019). Plasma malondialdehyde concentration (MDA) measured by HPLC, which is considered a more accurate marker, was not affected in this study. Some changes in indices of immune function were also reported (decreased % neutrophils, increased number of lymphocytes but not of basophils, eosinophils or monocytes, decreased IL-2R and doubling of IL-6 which, however, was not statistically significant, and increased antibody titres for the influenza vaccine) (Turnlund et al., 2004) (Appendix B.2). Increased caeruloplasmin activity, benzylamine oxidase activity and superoxide dismutase activity are not markers of toxicity. Exposure levels in this study were also associated with higher urinary copper excretion and higher hair copper concentrations (Turnlund et al., 2005). Increased urinary and hair concentrations provide corroborating evidence of increased body burden but are not markers of toxicity. Subjects were monitored for symptoms of GI disturbance. One subject reported nausea at the beginning of the supplementation period that was alleviated by taking the supplement with a meal. Liver function markers were not evaluated in this study.

Other copper supplementation studies also reported other biomarkers of oxidative stress with similar limitations (Appendix B.3). No effect was found on plasma, erythrocyte and platelet glutathione peroxidase (GPx) activity upon copper supplementation up to 6 mg/day copper for 8 weeks (Harvey et al., 2003). In the multicentre FOODCUE trial, copper supplementation up to 6 mg/day did not affect markers of DNA damage (6-week supplementation) (O'Connor et al., 2003), low-density lipoprotein (LDL) cholesterol susceptibility to oxidation (4–8 weeks of supplementation) (Turley et al., 2000) or red blood cell oxidisability and plasma antioxidants concentrations (6-week supplementation) (Rock et al., 2000). It is noted that most studies used *ex vivo* assays, which are of limited value for assessing *in vivo* oxidative damage.

An intervention study was conducted in healthy young children (n = 128), randomly assigned at 3 months of age to receive copper in drinking water between the ages of 3 and 12 months: one group (n = 48; 27 weaned formula-fed and 21 breast-fed until 6 months) received water with a low copper level (< 0.1 mg/L; 1.57 μmol/L) and one group (n = 80; 56 weaned formula-fed and 24 breast-fed until 6 months) received water with a high copper level (2 mg/L; 31.48 μmol/L) (Olivares et al., 1998). Estimated average copper intake (estimated for three 3-month periods of 4–6 months, 6–9 months, and 9–12 months) in formula-fed infants ranged between 2.3 and 2.5 mg/day for the high-exposure group, and between 0.8 and 1.2 mg/day for the low exposure group. In breast-fed infants, a higher variability of estimated intake was reported between the 3-month age periods: intake was 0.1, 1.5 and 2 mg/day, respectively, in the high-exposure group and 0.1, 0.8 and 1.6 mg/day, respectively, in the low exposure group. Analyses were conducted at 6, 9 and 12 months of age for serum levels of copper ceruloplasmin, ALT, AST, GGT and total bilirubin. Small, but statistically significant, differences were reported in serum ceruloplasmin between the low- and high-exposure groups. No differences in erythrocyte Cu/Zn superoxide dismutase activity or erythrocyte metallothionein content were associated with copper supplementation. Some differences were noted between formula-fed and breast-fed infants (e.g. erythrocyte MT at 12 months) independent of drinking water copper exposure. No differences were detected in liver function parameters between copper exposure groups.

3.3.3. Individuals with Wilson's disease and related genetic disorders

The symptoms associated with copper retention have been most extensively described in cases of WD patients. Whilst the cause of hepatic copper retention in WD (reduced function of the ATP7B transporter) is not representative of copper homeostasis in the general population, the toxicological consequences of copper retention are informative qualitatively and quantitatively when assessing possible adverse effects resulting from increasing hepatic copper sequestration in the general population. During disease progression, when the hepatocyte copper stores are high, released copper from hepatocytes then progressively accumulates in other organs, causing extrahepatic damage. Hepatocytes have a high storage capacity for copper, presumably bound on MT and saturation of the storage capacity may be observed in severe cases of WD patients (Członkowska et al., 2018). Interestingly, MT is significantly upregulated in a mouse model of WD (Huster and Lutsenko, 2007). However, hepatocyte saturation with copper is not a prerequisite for hepatic copper release and copper toxicity in WD patients, and no threshold level for hepatic copper associated with onset of toxicity has been identified.

Copper retention over time may cause hepatocyte damage associated with hepatic inflammation, steatosis, fatty infiltration, which are, in principle, reversible upon cessation of exposure or copper chelation therapy. In the absence of mitigation interventions, these changes may be followed by necrosis, liver fibrosis and liver failure, which are irreversible. The liver damage leads to the release of stored copper into the systemic circulation. This, in turn, increases circulating amounts of copper bound to low-molecular-weight compounds leading to higher urinary copper excretion and the increased deposition of copper in peripheral tissues. It can accumulate notably in the brain and kidneys and will cause further damage, possibly through oxidative damage. Because liver damage releases stored copper, the amount of copper that becomes available in the systemic circulation can be substantial if hepatic stores are high and is consistent with the late and/or sudden onset of copper toxicity in asymptomatic WD patients (late acute hepatotoxicity described as fulminant WD) reported in the literature (Eisenbach et al., 2007; Ferenci et al., 2007; Weitzman et al., 2014). Liver damage may occur also from other triggers or causation and result in release of stored copper.

Neurotoxicity is associated with both extremes of copper homeostatic imbalance, either copper accumulation or copper deficiency (Lutsenko et al., 2019). In the context of high copper levels, evidence of copper-induced neurotoxicity is primarily obtained from WD patients presenting the neurological profile of the disease with or without hepatic toxicity (EASL, 2012; Członkowska et al., 2018; Gromadzka et al., 2020). In these patients, a neurodegenerative disease is relatively easily diagnosed and is associated with low plasma caeruloplasmin and the presence of Kayser–Fleischer rings reflecting copper deposition in the cornea (Członkowska et al., 2018; Espinós and Ferenci, 2020).

In addition to WD, Indian Childhood Cirrhosis (ICC) and non-Indian or idiopathic copper toxicosis (ICT) disorders are also believed to be primary copper associated liver diseases, whereas copper hepatic accumulation due to chronic cholestasis is secondary to reduced bile secretion independent of copper (Müller et al., 1998). The characteristics of ICC and ICT are informative of copper toxicity in that both are manifesting in early life, present with a slow and mild onset, are rapidly progressing,

they are fatal within a short period and with rarely successful response to treatment, and are associated with very high copper concentrations in the liver (190–3,360 μg/g dry weight; comparable with or even higher than WD). They are partly attributed to high copper intake in susceptible children, through identifiable sources, including the diet or drinking water, originating from contact with copper utensils, copper water pipes or copper contamination of water supply from other sources. The susceptibility factor is a prerequisite for the manifestation of the pathology and appears to be genetic, specifically via an autosomal recessive gene, but is so far unidentified. It has been suggested that it may be related to heterozygosity of some *ATP7B* mutations (Petrukhin and Gilliam, 1994; Thomas et al., 1995) but may involve mutations in other copper-associated proteins. ICT has been identified in many countries and has been characterised as endemic in certain geographic regions, such as in the Tyrol, Austria (Muller et al., 1996). Histology has shown granular deposits of copper or copper-containing proteins in hepatocytes and mesenchymal cells. Unlike in WD, serum copper and ceruloplasmin levels are either normal or slightly increased and provide another line of differential diagnosis from WD. Abnormal levels of liver enzymes have been observed, but without correlation to the stage of pathology.

3.3.4. Supporting evidence from animals and mechanistic studies

Corroborating evidence that the liver is the target organ of copper-induced toxicity has been obtained from animal studies, albeit at higher dose levels compared with intakes reported in human studies [at least 175 times higher[16] in rats than the human intake of 10 mg/day in Pratt et al. (1985)]. The organ susceptibility to copper toxicity has been described using a rat model, although species differences should be noted, as mentioned in Section 2.2.1, e.g. the absence of gall bladder in rodents, that may affect biliary excretion (Hebert, 1993; Kumar et al., 2015; Kumar et al., 2016a,b). In a 90-day NTP study, F344/N rats and B6C3F1 mice received copper sulfate pentahydrate in the diet and were evaluated by histopathology, clinical pathology, reproductive toxicity and target organ toxicity. The estimated intake of cupric sulfate pentahydrate was 0, 32, 64, 129, 259 and 551 mg/kg bw per day in male rats and 0, 34, 68, 135, 267 and 528 mg/kg bw per day in female rats (equivalent to 0, 8, 16, 33, 66 and 140 mg/kg bw per day of copper, respectively, in male rats and 0, 9, 17, 34, 68 and 134 mg/kg bw per day of copper, respectively, in female rats) (Hebert, 1993). Rats were more sensitive than mice, which received diets equivalent to 0, 44, 97, 187, 397 and 813 mg/kg bw per day of copper, respectively, in males and 0, 52, 125, 266, 535 and 1056 mg/kg bw per day of copper, respectively, in females. The liver and kidneys were the primary target organs in the rat. Liver changes included dose-related hepatic inflammation and clinical chemistry changes (increase in serum ALT and SDH) indicative of hepatocellular damage and cholestasis, starting at 33 mg/kg bw per day of copper in male rats, although no clinical chemistry was performed al lower copper intake levels. In the kidneys, dose-related histopathological changes, such as accumulation of cytoplasmic eosinophilic protein droplets in the proximal convoluted tubules and urinalysis parameter changes suggestive of renal tubular epithelial damage, at 33 mg/kg bw per day and above in male and female rats. The NOEL for the hepatic and renal pathology was 1000 mg/kg feed of copper sulfate pentahydrate, equivalent to 16 and 17 mg/kg bw per day of copper, in male and female rats, respectively. Hyperplasia and hyperkeratosis in the forestomach were also reported and attributed to local irritation effect. Other findings included depletion of spleen iron stores and indications of microcytic anaemia in male and female rats, but not in mice. Unlike rats, no hepatic or renal lesions were observed in mice and an NOEL could not be obtained. No adverse effects were noted on any of the reproductive parameters in male or female rats or mice.

In other studies in rats, high daily doses of copper sulfate pentahydrate, i.e. 100 mg/kg bw and 200 mg/kg bw for 90 days (equivalent to ~25 and 51 mg/kg bw per day of copper10), were administered in the feed (Kumar et al., 2015; Kumar et al., 2016a,b). At these two dose levels, the severity of the histopathological findings in liver, kidney and brain were dose and time dependent and included oedema, haemorrhage, necrosis and fibrosis/gliosis. Damage in the liver was reported to be more severe and to occur earlier than in other organs. The histopathological changes were correlated with increased free copper and malondialdehyde (MDA) concentrations and decreased glutathione (GSH) concentration and total antioxidant capacity (TAC) in these organs. The biggest reduction in TAC and GSH was observed in the liver followed by the brain and kidney and the highest increase in

[16] E.g. the lowest dose of 25 mg/kg bw per day where adverse effects were reported in Kumar et al. (2015) compared with 10 mg/day for 70 kg adult body weight (0.143 mg/kg bw per day) in Pratt (1985).

MDA concentration was found in the liver, again followed by the brain and kidney. TAC and GSH levels in the liver were inversely correlated with serum aminotransferases and bilirubin and tissue-free copper, and with MDA levels.

In Beagle dogs (n = 6–8/sex/group), copper gluconate exposure through the diet at concentrations of 0, 0.012%, 0.06% and 0.24% copper gluconate for up to 1 year, equivalent to 0, 3, 15 and 60 mg Cu/kg bw per day, resulted in no adverse effects up to 15 mg/kg bw per day. The study included evaluation of haematological, biochemical and urinalysis parameters, as well as copper concentrations in kidney, liver and spleen. A reversible increase in ALT (serum GPT in the original) was reported at the top dose level of 60 mg/kg bw per day in two of the 12 dogs in this dose group [Shanaman et al. (1972); as reported in France (2007a) and WHO Food Additives Series (WHO FAS 17)]. Two dogs per sex per dose group were sacrificed after 6 months and the remaining at the end of the 12 months treatment. A dose-dependent increase in copper concentrations in the liver, kidney and spleen was reported at 6 and 12 months. Liver biopsies showed evidence of reversibility at 4 and 12 weeks after cessation of treatment.

Additional available data from experimental animal studies report consistent findings, including increased hepatic copper concentrations, and hepatic and renal toxicity following repeated oral intake at high dose levels of copper, with higher sensitivity of the rat compared with the mouse (Haywood, 1980; Fuentealba, 2000 #437; Haywood and Loughran, 1985; Haywood et al., 1985; Fuentealba et al., 1989, 2000; Aburto et al., 2001), as cited in Scientific Committee on Health and Environmental Risks (2008) and Taylor et al. (2020, supplementary information table).

Increased hepatic copper levels without overt clinical or hepatic toxicity have been reported in capuchin monkeys, treated with 5.5 mg Cu/kg bw per day in young animals or 7.5 mg Cu/kg bw per day in adult animals (38-fold and 52-fold, respectively, higher than the human intake of 10 mg/day in Pratt et al. (1985)) (Araya et al., 2012; Latorre et al., 2019). This observation is consistent with the protective homeostatic regulation of copper against manifestation of toxicity seen in human studies.

Cases of inherited copper toxicosis similar to WD have been reported in dogs, particularly in Bedlington Terriers, but also in other breeds (Wu et al., 2016; Blakley, 2021). Chronic active hepatitis in dogs is associated with copper toxicosis, but the direction of the cause–effect relationship is unclear.

Species differences in copper biliary excretion and MT binding, notably between ruminant (particularly sheep, but also cattle and goats) and non-ruminant species (particularly pig and poultry), resulting in large differences in the capacity of hepatic copper sequestration, have been discussed more extensively previously (EFSA FEEDAP Panel, 2016a). Non-ruminant (monogastric) species can tolerate dietary copper of up to 50 times the requirements, whilst in ruminants copper begins to accumulate in the liver at high concentrations at dietary copper concentrations slightly above the requirements (twofold) (EFSA FEEDAP Panel, 2016a).

Evidence from farm animals indicates pathology similar to that in humans (EFSA FEEDAP Panel, 2016a; Blakley, 2021). Inherited copper toxicosis is common in farm animals, most notably in sheep, that have been exposed to high copper levels through feed or grazing on plants with high copper content for prolonged periods of time (Blakley, 2021). As in WD, the condition may be asymptomatic with significant copper accumulation in the liver, whilst copper may be released from the liver by a range of triggers, including pregnancy, lactation and stress resulting from transport, handling, intense physical activity, weather conditions or attacks from dogs or coyotes (Blakley, 2021). Intakes associated with chronic copper toxicity are reported in the range of 3.5 mg/kg of plants containing 15–20 ppm of copper (sheep) or above 250 mg/kg of feed (pigs). Copper toxicity presents with increases in liver enzyme concentrations preceding a haemolytic crisis and is characterised by extensive hepatic necrosis and sudden death. Serum copper levels may remain low or be increased only transiently and confirmation is obtained only post-mortem, in liver (> 150 μg/g wet weight) and kidney samples (EFSA FEEDAP Panel, 2016a; Blakley, 2021). Prevention is the most efficient intervention of copper toxicosis in sheep and other farm animals and often involves periodic liver biopsies (Blakley, 2021).

It has been reported that oxidative species mediated copper toxicity in the brain in rats after 30 days of dietary administration of copper at dose levels of 0.67 or 3.0 mg/kg bw per day (Arnal et al., 2014). Effects seen in a 90-day study in rats at dose levels of 25 or 51 mg/kg bw per day of copper were reduced grip strength and rotarod coordination, as well as impaired Y maze exploratory behaviour at all evaluation periods at 30, 60 and 90 days (Kumar et al., 2015).

Copper ions bound to proteins generally represent a stabilised pool of Cu^{+} that would otherwise readily participate in Fenton redox reactions. There is evidence suggesting that pro-oxidant species, such as peroxynitrite or reactive oxygen species (ROS), can release copper ions from proteins,

including caeruloplasmin (Gaetke et al., 2014) and MT (Krężel and Maret, 2021). Copper released in this manner from high-molecular weight (MW) pools may be associated with low-MW partners in which the electron flow is not well contained and in which it remains redox active. Redox cycling and oxidative damage are considered to be an important mode of action of copper toxicity. The labile low-MW copper pools increased upon continuous release of copper from high-MW binding partners. The subcellular location of these pools of labile copper, rather than the overall cellular copper content, as well as their extracellular presence, e.g. in the circulation, contribute to the oxidative activity of copper and are toxicologically relevant.

Other proposed modes of copper toxicity include altered (downregulation of) lipid metabolism, regulation of gene expression, protein aggregation (e.g. synuclein) and apoptotic signalling via ceramide release (Huster and Lutsenko, 2007; Gaetke et al., 2014). It has been proposed that changes in subcellular localisation and early biochemical activity of accumulated cellular copper (unrelated to oxidative stress) rather than the overall liver load may explain the onset of copper toxicity (Huster et al., 2006; Huster et al., 2007). Alternatively, based on the evidence of altered zinc homeostasis in a mouse WD model, it has been suggested that copper-induced hepatocyte toxicity may be, at least partly, due to disrupted zinc distribution and zinc-dependent functions, even if copper itself is efficiently sequestered in MT (emphasis added) (Meacham et al., 2018). This is consistent with the interactions in the homeostasis of the two ETEs converging on MT, including induction of MT resulting from high copper levels and MT modulation of zinc-dependent gene expression.

Whilst these studies are useful in characterising copper-related redox activity, adverse effects associated with oxidative stress typically have multiple aetiologies and it is difficult to attribute them to copper exposure in humans. As noted above, oxidative stress markers are neither sensitive nor specific enough to be used as indicators of potential copper toxicity in humans exposed to copper under free-living conditions.

3.3.5. Relationship between copper intake, hepatic copper retention and toxicity

The relationships between copper intake, hepatic copper retention and onset of toxicity are directly relevant to the prediction of chronic copper toxicity in the context of excess copper intake in the general population. Of these, attempts have been made to model the relationship between copper intake and hepatic copper levels based on the results of net positive balance, indicating retention in human volunteers. Considering the complexity of the interactions between copper homeostasis and other nutritional factors, particularly zinc intake, but also antioxidant, high fibre diets, etc., the SC recognises that predictions of hepatic copper levels are highly uncertain because these factors are difficult to include in the modelling.

The quantitative relationship between hepatic copper retention and toxicity remains elusive and introduces additional uncertainty. However, hepatic copper retention leads to progressive hepatic disease; hence, the time of onset, time of diagnosis and clinical profile may vary substantially in reported WD cases in the literature (Ferenci et al., 2007).

The relationship between hepatic copper content and hepatic toxicity has been assessed in a few studies, but no strong correlation has been identified (Ferenci, 2005; Yang et al., 2015). A pragmatic threshold of hepatic copper content exists for diagnostic purposes only (250 μg/g dry weight) which, however, captures only 83.3% (95% CI, 75.2–89.6%) of the patients diagnosed with the disease (Ferenci, 2005). Liver content of over 1,000 μg/g dw has been reported in asymptomatic patients (Ferenci, 2005) and maximum liver copper content has been reported in young animals of a WD mouse model with minimal liver pathology (Huster et al., 2006). However, the determination of the hepatic copper levels in humans is invasive and subject to sampling limitations, such as insufficient and unrepresentative sample collection (Yang et al., 2015). It has been proposed that the subcellular localisation of copper accumulation rather than total hepatic copper content may be a more consistent predictor of toxicity (Huster and Lutsenko, 2007; Ralle et al., 2010; Chang and Hahn, 2017) and because copper distribution in the liver is heterogeneous, tissue sample size of the biopsy has an impact on the accuracy of the measurements (Yang et al., 2015). However, data from farm animals, particularly sheep, provide supporting evidence of the relationship between hepatic copper levels and the severity of the toxicity that follows, including the asymptomatic early period that may culminate in a sudden and severe onset of toxicity. Additionally, copper release from hepatic stores may occur as a result of various triggers and may not be simply a function of saturated storage capacity (Ferenci et al., 2007; Ferenci, 2020, expert contribution to the working group), consistent with the high hepatic copper content in asymptomatic patients, as noted above. Other causes of liver damage may release

stored copper, partly explaining the lack of correlation with hepatic levels. However, copper overload itself is a major contributor to progressive hepatic damage in WD. Evidence from farm animals again provides supporting evidence on the variety of triggers that may release copper from hepatic pools and result in sudden toxicity.

The variety of factors that may trigger release of sequestered copper is consistent with the complexity of MT regulation, the main known sequestration pool of copper. MTs interact dynamically with cellular conditions, such as pH changes within the physiological pH range, redox status and other factors. Changing cellular conditions result in changing thiol cluster coordination, protein folding, 3D protein coordination and MT redox buffering activity. In turn, these result in significant changes in metal-binding affinities, metal composition, exchange and metal occupancy, and dynamically impact the affinities of additional metal binding on remaining thiol sites (Krężel and Maret, 2021). These dynamic changes have implications for metal homeostasis at different levels of cellular metal load, on the cross-talk between zinc and copper. As an example, MT reactivity with hydroxyl radicals has been reported to be substantially higher (340-fold higher rate constant) than glutathione and oxidative stress conditions may result in copper release from MT, in addition to zinc mobilisation (Krężel and Maret, 2021).

In summary, with few exceptions of asymptomatic WD patients with very high hepatic copper levels, hepatic copper retention is associated with progressive hepatic damage and peripheral toxicity. The release of stored hepatic copper results in extrahepatic toxicity (primarily in the central nervous system) and, whilst there is no clear cut-off value of retention, above which copper release and toxicity are more likely, there are various triggers to initiate these events, including other hepatobiliary disease, indirect impact on the liver from other disease states and non-specific stressors (Ferenci et al., 2007; Burkhead et al., 2011; Ferenci, 2020; expert contribution to the working group).

Therefore, evidence from WD suggests that hepatic retention is indicative of potential future (and possibly sudden) onset of copper toxicity under conditions of continuous intake and can be considered an early predictor of adversity in chronic toxicity assessment. The triggers or causation of liver damage in the population are heterogeneous, subject to variability in lifestyle, presence of disease or other risk factors, and introduce variability and uncertainty in the assessment of risk associated with hepatic copper retention. Released copper as a result of liver damage may cause injury to neighbouring liver tissue or to peripheral tissues, particularly to the nervous system where injury may not be reversible, but cumulative over time.

3.3.6. Alzheimer's disease

The possible involvement of copper in the development and progression of Alzheimer's disease (AD), independently of WD, has been postulated based on the presence of a copper binding site on the extracellular domain (ECD) of amyloid-beta precursor protein (APP) (Reinhard et al., 2005) and the presence of copper in amyloid plaques (Lovell et al., 1998; Atwood et al., 2002). The APP ECD has close structural homology with copper chaperones (Barnham et al., 2003) and binds copper with a nanomolar affinity (Hesse et al., 1994).

There is conflicting evidence in the literature on the role of copper in the formation of amyloid plaques and it has been a subject of debate in the scientific community. Transgenic animal models of AD and human studies have been used to investigate the effects of copper deficiency and copper supplementation on the progression of AD.

Overall, a causal relationship of Cu intake with AD remains speculative and inconclusive. The SC considers that the scientific evidence is not sufficient to conclude that increased copper intake contributes to the development and/or exacerbation of AD and that additional dysregulation of copper homeostatic mechanisms in the brain may be involved.

For an overview of literature related to copper association with AD, see Appendix B.4.

3.3.7. Genetic susceptibility

Subpopulations that may be at higher risk for copper toxicity include those at the highest percentiles of exposure and/or those with genetic susceptibility to copper toxicity. Individuals who are heterozygous for *ATP7B* variants are free of disease, but evidence that the gene is 'not completely recessive' and associated with partially compromised copper homeostasis has been reported, based on intermediate values of serum caeruloplasmin (10–20% of carriers), and elevated urinary copper (53% of carriers) and hepatic copper levels (Brewer, 2000; Yang et al., 2015). Data on the hepatic copper content and urinary copper concentration in heterozygote siblings of WD patients have shown a shift

in these distributions to the right (Figure 2) (Brewer, 2000; Yang et al., 2015). The size of genetically susceptible subpopulations depends not only on the prevalence of heterozygosity but also on the penetrance of the genetic variant, as recently shown (Wallace and Dooley, 2020). The global prevalence of the disease is estimated to range from 1 in 400,000 to 1 in 17,000 (0.25–5.87 per 100,000) based on epidemiological studies (Gao et al., 2019). Recent estimates based on identified genetic variants indicate that these figures have been underestimated and that 'silent' conditions remain undiagnosed. Of the 732 variants predicted to be causative of WD, 231 were identified in a multi-ethnic population of 120,000, where 24% are predicted to be loss-of-function variants. Based on the frequencies of reported cases, 1 in 70 individuals are predicted to be heterozygous carriers of a high penetrance variant and as high as 1 in 25 are carriers of a variant when those of probable and possible low penetrance are included (Wallace and Dooley, 2020).

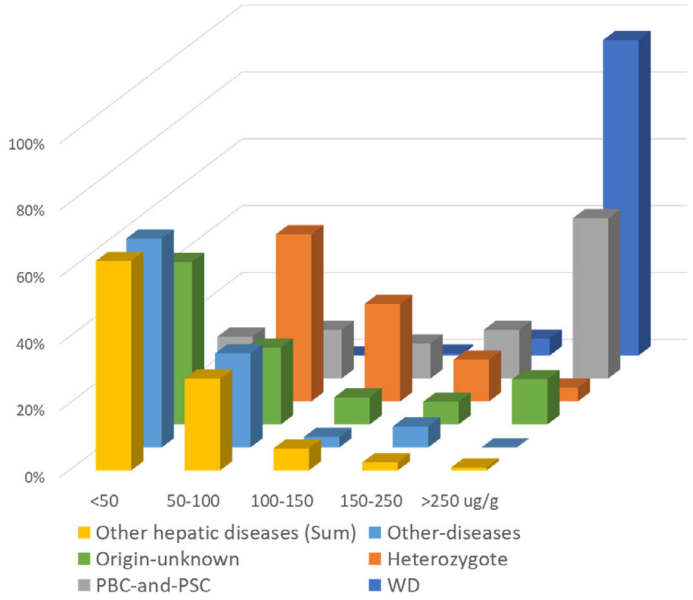


Figure 2: Distribution of hepatic copper concentration in WD patients (n = 178). ATP7B gene heterozygotes (n = 24), individuals with cholestatic liver disease, other liver conditions and non-liver disease. Based on data presented in Yang et al. (2015). PBC: primary biliary cirrhosis; PSC: primary sclerosing cholangitis (PBC and PSC 48); other hepatic diseases: viral hepatitis (198), autoimmune hepatitis (50), non-alcoholic steatohepatitis (40), hemochromatosis (9), Idiopathic portal hypertension (10), Gilbert's syndrome and Dubin–Johnson syndrome (28); other diseases, including neurologic diseases other than WD (8), haemolytic jaundice (5), polymyositis (5), venous occlusive disease (4), Budd–Chiari syndrome (3), alcoholic hepatitis (3), lymphoma (1), glycogen storage disease (1), congenital hepatic fibrosis (1) and schistosomiasis Japonica (1)

There are large numbers of genetic variants that encode an ATP7B protein with a variety of structural and/or functional defects, such as absent protein, unstable or misfolded protein, or defective copper binding or transport. Considering also that most pathogenic genotypes are compound heterozygotes, the variety of genotypes seems to be consistent with the complex and quite variable phenotypic pathophysiology that ranges from asymptomatic to severe disease states, although no clear correlation has been identified between genotype and phenotype. It is therefore difficult to assess the impact of heterozygosity on copper handling at high intake levels. The complexity of the genetic profile in WD indicates at least uncertainty about the potential genetic susceptibility to copper retention if the capacity of heterozygous individuals to maintain homeostasis is exceeded. Evidence of interactions between gene variants that alter their subcellular localisation and trafficking pattern has recently been reported in human populations (Roy et al., 2020). The role of heterozygosity for the *ATP7B* gene on copper homeostasis requires further investigation (Yi et al., 2020).

3.3.8. Weight of evidence assessment for copper kinetics and dynamics

Based on the data collected in Section 3.1 and Appendix B, an assessment of the weight of evidence for copper kinetics and dynamics is presented in Tables 2 and 3, following the EFSA Guidance on the use of the weight of evidence approach in scientific assessments (EFSA Scientific Committee, 2017a). This is a process in which evidence is integrated to determine the relative support for the identification of a relevant RP upon which to establish the HBGV: (1) assembling the evidence into lines of evidence (LOE) of similar type, (2) weighing the evidence, (3) integrating the evidence.

Table 2: Weight of evidence assessment for copper kinetics

Question		Copper kinetics
Assemble evidence	Select evidence	Human studies on copper kinetics after copper intake
	Lines of evidence	**LOE1:** Human metabolic studies addressing copper homeostasis, retention and variability in kinetics in healthy adults and infants **LOE2:** Hepatic levels of copper in WD patient population and ATP7B gene heterozygotes **LOE3:** Tissue levels of copper in animals
Weigh the evidence	Methods	Determination of copper kinetics, including interindividual variability, at different levels of intake. Comparison of copper retention indices between healthy adults and WD patients.
	Results	**LOE1:** • In 11 young men, % Cu absorption (mean ± SEM) was of 36 ± 1.3 with a copper intake of 1.7 mg/day (equilibration period) and 12.4 ± 0.9 with an intake of 7.5 mg/day (Turnlund, 1989). Faecal excretion was on average 0.61 mg/day and 0.97 mg/day, respectively, and balance of 0.167 ± 0.4 mg/day and 0.941 ± 0.16 mg/day, respectively. • In 11 young men, estimated true Cu absorption was 77%, 73% and 66% at doses 0.38, 0.66 and 2.49 mg/day, respectively, with increased biliary excretion of copper of 34% at the higher dose compared to 12% and 26%, at 0.38 and 0.66 mg/day, respectively, accounting for most of the apparent difference in true Cu absorption (Turnlund et al., 1998). Lag time in adaptive excretion in the early part of the third period (2.49 mg/day) following low intake resulted in positive balance of 0.511 mg/day which however reached equilibrium after 24 days. • In 12 men, true Cu absorption was relatively stable (45–48%) at copper intakes between 0.7 and 6 mg/day, whilst endogenous losses increased with the copper dose (from 0.45 ± 0.25 to 2.46 ± 1.11 mg/day; 30% to 40% of dose, respectively) (Harvey et al., 2003). Positive net balance of 0.75 ± 1.05 mg/day at 6 mg/day over 56 days. • In nine men, estimated true absorption decreased from 40% to 29% after increasing Cu intake from 1.6 mg/day to 7.8 mg/day. Endogenous losses increased from 0.58 mg/day to 1.56 mg/day (Turnlund et al., 2005). Positive net balance of 0.67 mg/day after a total of 147 days of the high intake (129 days with intake of 7 mg/day and 18 days of 7.8 mg/day). • Significant copper retention was observed in the three metabolic studies with copper intake of 6–8 mg/day (Turnlund, 1989; Harvey et al., 2003; Turnlund et al., 2005). Although Turnlund (1989) observed a gradual decrease in copper retention over the 24-day high-Cu period, Turnlund et al. (2005) reported an average retention of 0.67 mg/day after 5 months (147 days) of a copper intake of ~ 8 mg/day, whilst Harvey et al. (2003) reported (mean ± SD) 0.75 ± 1.05 mg retention after 8 weeks of a copper intake of ~ 6 mg/day. In the study of Turnlund et al. (2005), increases in urinary copper excretion and hair copper concentrations were observed with copper intake of ~8 mg/day compared with baseline intake, suggesting an increase in the copper body burden (Turnlund et al., 2005).

Question		Copper kinetics
		• In 39 healthy infants, % apparent copper absorption (mean ± SD) and faecal copper were evaluated at 1 month and 3 months of age, either without copper supplementation or with 0.08 mg Cu/kg bw per day supplemented for 15 days. Copper absorption ranged between 45% and 95% across all infants. Copper intake was correlated with total faecal copper but not with apparent absorption (Olivares et al., 2002). • Copper intake in 26 infants (20 full term and 6 premature) resulted in positive balances in five 72-hour collections between 2 and 16 weeks of age when fed with breast milk or supplemented infant formula, but not with non-supplemented formula. Mean daily copper intake was 0.11 ± 0.02 mg/kg bw in breast-fed, and 0.11 ± 0.02 mg/kg bw in the supplemented formula full-term infants, compared with 0.02 ± 0.004 mg/kg bw in the non-supplemented formula-fed infants. Mean daily copper balance was 0.09 ± 0.05 mg/kg bw and 0.06 ± 0.02 mg/kg bw, respectively, compared with negligible or negative retention (0.005 mg/kg bw; range −0.012 to 0.01 mg/kg bw) in the non-supplemented formula group (Dorner et al., 1989). • Copper intake in breast-fed infants decreased from 2 to 12 weeks due to decreasing copper levels in breast milk with advancing lactation (Dorner et al., 1989). This is in contrast to the increasing absorption with increasing copper intake in the same study, thereby indicating that reduced intake was due to decreased supply rather than decreased absorption, and further suggests that fetal hepatic copper complements infant copper needs as copper in breast milk decreases. **LOE2:** • In WD patients, hepatic copper levels typically exceed 250 μg/g dry weight, significantly higher than hepatic copper of < 50 μg/g in non-WD individuals (Członkowska et al., 2018). • Total serum copper is decreased due to decreased serum caeruloplasmin but unbound copper in serum is increased. Elevated 24-hour urine copper (> 100 μg/24 h; or > 40 μg/24 h, depending on the laboratory) is diagnostic of WD (Członkowska et al., 2018). • Heterozygotes for ATP7B gene mutations have a distribution of hepatic copper levels intermediate between that in the general population and WD patients (Brewer, 2000; Yang et al., 2015). **LOE3:** • In the 90-day study in rats (Hebert, 1993), statistically significant increase in copper tissue levels was reported in liver and kidney at all dietary levels (starting below the NOAEL) and in a dose-dependent manner (mean copper of 0.24 (control), 1.83, 6.11, 17.90, 127.31, 372.12 μg/g in the liver, and 0.62 (control), 4.81, 3.45, 7.65, 52.89, 181.30 μg/g) in the kidney (Hebert, 1993). Plasma copper was increased at the top two dietary levels. • In rats treated with 25 and 51 mg/kg bw per day of copper sulfate pentahydrate for 30, 60 or 90 days, statistically significant dose and time dependent increases of tissue copper levels were observed in the liver, kidney, and brain (up to 29-fold, 3-fold and 1.5-fold, in liver, kidney and brain, respectively) (Kumar et al., 2015). • Evidence in farm animals, particularly sheep, reports high levels of copper sequestration in the liver under conditions of chronic intake of food material with high copper content (Blakley, 2021).
Integrate the evidence	Methods	Integration of **LOE1**, **LOE2** and **LOE3** based on expert judgement
	Results	• Homeostatic mechanisms tightly regulate copper absorption, distribution, sequestration and excretion, preventing the appearance of copper in chemical forms that are able to promote oxidative damage to cellular components. • When copper balance is positive (i.e. copper retention), evidence suggests that additional copper is sequestered in the liver and MT acts

Question		Copper kinetics
		as a sink for hepatic copper (Huster et al., 2007; Calvo et al., 2017; Krężel and Maret, 2017). • The capacity of the liver to sequester copper and the duration over which copper retention could be sustained is, however, unknown. • Copper retention in humans is extrapolated from data of retention from studies with controlled intake and there is evidence of retention or positive copper balance at levels at 8 mg/day and possibly even 6 mg/day. It is unclear whether the positive retention observed in these studies would reach zero balance over longer exposure and observation periods. • Supporting evidence for hepatic copper retention is obtained from 90-day studies in rats (Hebert, 1993; Kumar et al., 2015) • Evidence on human interindividual variability in copper kinetics in the general population is limited. • The reports of increased copper retention in the liver in the population of ATP7B gene heterozygotes compared with the general population, suggest potential increased susceptibility to copper retention. • Specific data on copper homeostasis and copper kinetics in infants are limited to ages below 16 weeks when homeostatic regulation is not fully developed. The evidence of positive copper balance in breast-fed and supplemented formula-fed infants up to 16 weeks of age must be interpreted within the context of incomplete homeostasis regulation and different nutrient needs during this developmental stage. The data related to homeostasis cannot be extrapolated to infants older than 16 weeks and older children, when homeostasis is fully functional and approaches that of adults. The nutrient needs, however, can be assumed to continue to be higher during all stages of physical growth compared to adults. • Whilst plasma, urinary or hair copper levels have been used as biomarkers of copper body burden in controlled intervention studies and in biomonitoring studies (HBM4EU Report on copper[17]), they are not sufficiently specific biomarkers to be useful for population biomonitoring of exposure to copper and copper retention in the body (Danzeisen et al., 2007; Bost et al., 2016). • Evidence of sustained retention in adults is indicative of challenged homeostasis and therefore can be used as a marker of potential redistribution into pools of copper able to promote oxidative damage.

LOE: line of evidence; MT: metallothionein; SD: standard deviation; SEM: standard error of the mean.

Table 3: Weight of evidence assessment for copper toxicodynamics

Question		Copper toxicodynamics
Assemble evidence	Select evidence	Critical studies identified in previous RAs (IPCS, 1998; IOM, 2001; EVM, 2003; SCF, 2003; France, 2017; EFSA, 2018b). Available *in vivo* animal studies, controlled intervention human studies, and observational evidence in healthy humans and in WD patients and *ATP7B* gene heterozygotes.
	Lines of evidence	**LOE1:** Human trials reporting biomarkers of copper toxicity. **LOE2:** Human observational studies reporting biomarkers of copper toxicity. **LOE3:** Human observational studies in WD patients and *ATP7B* gene heterozygotes. **LOE4:** *In vivo* animal studies.

[17] HBM4EU Report on copper https://www.hbm4eu.eu/wp-content/uploads/2022/01/HBM4EU_4.3_Rapid-respose-Mechanism_Response-Document_Copper-compounds.pdf; available at https://www.hbm4eu.eu/result/policy-briefs-and-reports/

Question		Copper toxicodynamics
Weigh the evidence	Methods	Comparison of human data from different population groups (LOE1 and LOE2) and animal data (LOE4). Comparison of human data in the general population to data in the WD population.
	Results	**LOE1:** • After 8 weeks of a total dietary intake of 6 mg Cu/day, plasma, erythrocyte and platelet GPx not affected in 12 men (Harvey et al., 2003). • After 6-week supplementation periods, no effect of 3 and 6 mg/day supplemental copper on liver enzymes (GGT, ALT) and no alteration in mononuclear leucocyte DNA damage as marker of DNA oxidative damage in 24 men and women (O'Connor et al., 2003). • After 6-week supplementation periods, no adverse effect of 3 and 6 mg/day supplemental copper on the resistance of red blood cell to oxidation (*ex vivo* test) in 26 men and women (Rock et al., 2000). • After 6- to 8-week periods with up to 6 mg/day supplemental copper, no effect on LDL susceptibility to oxidation (*ex vivo* test) in 79 adults from four different centres (Turley et al., 2000). • After 2-week total copper intake of 7.94 ± 2.69 mg/day, no effect on liver enzymes (GGT, ALT, AST) in 60 women (Pizarro et al., 1999). • In a 12-month trial, no effect of 8 mg/day supplemental copper on liver enzymes (not specified) in 35 patients with mild Alzheimer's disease (Kessler et al., 2008a). • After 5 months total copper intake of ~8 mg/day compared with that of 1.6 mg Cu/day, a significant increase in urinary TBARS excretion was observed, whilst plasma MDA concentration and ascorbate and dehydroascorbate were unaffected. Plasma SOD and benzylamine oxidase activity significantly increased. Lymphocyte count increased significantly, whilst plasma concentration of IL-2R, % of circulating neutrophils and the antibody response to a viral strain of influenza (immunisation challenge) decreased significantly (Turnlund et al., 2004). • In a 12-week trial in 14 men, no effect of 10 mg/day supplemental copper (0.15 mg/kg bw per day) on liver enzymes (AST, GGT and ALP) (Pratt et al., 1985). • No adverse effects were reported in other studies of copper supplementation with 10 mg/day in 41 adults (men and women) for 2 months (Araya et al., 2005; Mendez et al., 2004) or 8 mg/day in 30 healthy men for 6 months (Rojas-Sobarzo et al., 2013). • In a 9-month trial, no effect of 2.3–2.5 mg/day total copper intake on liver enzymes (bilirubin, AST, GGT, ALT) in 80 infants aged 3 months at baseline (Olivares et al., 1998). **LOE2:** • Liver cirrhosis and acute liver failure in one subject who took copper supplementation of 30 mg/day for 30 months, followed by 60 mg/day for 1 year (O'Donohue et al., 1993; O'Donohue et al., 1999). • No indication of liver damage in infants (8–10 months of age at enrolment) consuming water containing ≥ 0.8 mg Cu/L (≥ 200 mL tap water/day) for at least 6 weeks (max. water concentration: 4.2 mg Cu/L) (Zietz et al., 2003) and up to 12 months (max. water concentration: 2.6 mg Cu/L) (Dassel de Vergara et al., 1999). • Deaths from cirrhosis was the only outcome evaluated. No increased incidence of death from cirrhosis or any form of liver disease in children (0–5 years) consuming drinking water containing 8.5–8.8 mg Cu/L (64,124 child-years of exposure) (Scheinberg and Sternlieb, 1994). • Five cases among a total of 103 cases of early childhood cirrhosis considered as probably related to repeated and excessive intake of copper in children from households consuming water containing 9–26.4 mg Cu/L (Dieter et al., 1999).

Question		Copper toxicodynamics
		LOE3: • Copper is sequestered in the liver of WD patients for many years with no overt toxicity, at levels significantly higher than those expected from estimated daily intake in non-WD individuals (Espinós and Ferenci, 2020). • Hepatic copper levels do not correlate with toxicity in studies with WD patients and no threshold of hepatic content has been identified relative to toxicity. Toxicity results from release of copper from its hepatic stores by so far undefined triggers, through the lifetime of the patients (Ferenci et al., 2007) (Ferenci, 2020, personal communication). • The time and triggers upon which stored hepatic copper exerts toxicity in hepatocytes of WD patients are variable and are not directly related to the hepatic copper concentration (Ferenci, 2005; Yang et al., 2015; Ferenci et al., 2019). • Once diagnosed, WD toxicity is managed with copper chelation therapy ('decoppering') to maintain hepatic copper levels within a lower range (Członkowska et al., 2018). • Heterozygotes for *ATP7B* show evidence of intermediate hepatic copper distribution indicating susceptibility to copper retention, but no data are available on copper toxicity in this population (Brewer, 2000; Yang et al., 2015). **LOE4:** • In rats exposed to high copper intakes (25 mg/kg bw and 51 mg/kg bw for 90 days), hepatotoxicity occurs (Kumar et al., 2015). • In rats exposed to copper sulfate in the diet, changes were reported at intakes of 33 mg/kg bw per day of copper and above (2000 ppm copper sulfate in the diet and above) in males (and at higher doses in females), including dose-related increase in the incidence and severity of chronic inflammation in the liver, characterised by multiple foci of a mixture of mononuclear inflammatory cells, haematological changes, increase in the size and number of cytoplasmic protein droplets present in the epithelium of proximal convoluted tubules, and hyperplasia and hyperkeratosis in the forestomach mucosa (Hebert, 1993). The NOAEL was 16 mg/kg bw per day of copper (dietary level of 1000 mg/kg). • In Beagle dogs, copper gluconate exposure in the diet for 1 year was reported to result in no adverse effects up to 15 mg/kg bw per day; increase in ALT (referred to as 'SGPT' in the original) was reported at the next higher level of 60 mg/kg bw per day (Shanaman et al., 1972). • Severe copper toxicosis is often observed in farm animals, particularly sheep, as a result of high copper content of feed materials and high copper sequestration in the liver (Blakley, 2021).
Integrate the evidence	Methods	Integration of LOEs based on expert judgement
	Results	• Copper toxicity in non-WD individuals has been documented under conditions of acute poisoning, or cases of unusually high dose supplement intake. Acute exposure conditions manifest with symptoms of local GI irritation, whilst repeated excessive intake (O'Donohue et al., 1999) exceeds copper homeostasis mechanisms such that severe liver damage is manifested within months of observation time. • Hepatic sequestration is part of homeostasis and functions as a protective mechanism against the adverse effects of this transition metal in the general population. • In WD patients, neither the measured amount of hepatic copper nor the timeframe (latency) after which copper toxicity may be manifested following hepatic retention are clearly established. • Whilst hepatic sequestration is initially protective, subsequent retention under conditions of continuous exposure is an early stage on the pathway of copper toxicity. Although there is no cut-off value of hepatic retention that results in copper release, the nature of copper complexes

Question		Copper toxicodynamics
		in subcellular pools and a variety of possible triggers of hepatic copper release unrelated to copper exposure impact the onset of toxicity. • Although there are no toxicity data available for the population of *ATP7B* gene heterozygotes, the propensity of increased copper retention in the liver compared with the general population indicates potential increased susceptibility of this subpopulation to future copper-mediated toxicity. • The absence of toxicity in human studies of up to several months (LOE1 and LOE2) is consistent with the tight homeostatic regulation and protective hepatic sequestration of copper. The duration of studies that reported no adverse effects from copper supplementation (Pratt et al., 1985; Mendez et al., 2004; Araya et al., 2005; Rojas-Sobarzo et al., 2013) may not have been sufficiently long as to capture copper toxicity following hepatic retention; hence whilst the absence of adverse effects in adults and young children in these studies within the timeframe of the observation periods is consistent with effective copper sequestration, they are not sufficient to alleviate concerns for later manifestation of toxicity resulting from these exposure conditions. • The toxicological relevance of the changes in biomarkers of lipid peroxidation reported by Turnlund et al. at a copper intake level of ~8 mg/day (Turnlund et al., 2004, 2005) remains unclear in light of the above; additionally, the SC considers that the reported indicators of oxidative stress and immune function changes cannot currently be considered validated biomarkers of copper toxicity and are therefore not appropriate as the basis for establishing a HBGV. • As none of the available studies was designed to capture the possible latent toxicity of accumulated copper and as hepatotoxicity can be triggered by factors unrelated to copper intake, evidence of copper retention can be used as an early and sensitive predictor of potential toxicity if intake is not reduced, according to the principles outlined in the HBGV Statement (EFSA Scientific Committee, 2021). Consequently, the doses where retention is observed can be the basis upon which to establish HBGV. • Although the three metabolic studies each have their limitations (Turnlund, 1989; Harvey et al., 2003; Turnlund et al., 2005) that introduce uncertainty about the specific values of the homeostasis parameters (i.e. the figures of absorption, excretion or retention) or their extrapolation to conditions of chronic exposure, they provide the most direct available evidence of copper retention with intakes of copper of 6–8 mg/day. • The rather limited data in specific population subgroups, such as older people (Kessler et al., 2008a) and infants (Olivares et al., 1998), where the absence of changes in liver enzymes in response to copper supplementation is reported, do not suggest higher sensitivity to Cu toxicity in these groups compared with the (younger) adult population used in the metabolic studies. • Although there has been no study addressing biomarkers of copper toxicity in pregnant and lactating women, circulating levels of copper are regulated by the same homeostatic systems, with additional specific mechanisms operating at the level of the placenta and the mammary gland (Turnlund, 1983; Moser et al., 1988; McArdle, 1995; Kelleher and Lonnerdal, 2005). The SC considers that, given maternal homeostatic mechanisms, pregnant and lactating women are at no greater risk of toxicity from copper than other women.

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; DNA: deoxyribonucleic acid; GGT: l-γ-glutamyl transferase; GPx: glutathione peroxidase; LDL: low-density lipoprotein; LOE: line of evidence; MDA: malondialdehyde; RA: risk assessment; RP: reference point; SOD: superoxide dismutase.

3.3.9. Identification of a reference point for establishing HBGVs

Based on the weight of evidence, the SC concludes that no retention of copper is expected to occur with a copper intake of 5 mg/day and identifies 5 mg Cu/day as an RP, therefore replacing the previous RP based on an NOAEL of 10 mg/day. The SC considers that the application of UFs to this RP is not necessary as it is based on copper retention, an early and sensitive indicator of potential future toxicity, and therefore, sufficiently conservative to account for the identified uncertainties (see Section 4.1).

3.4. Total copper exposure

3.4.1. Results of the exposure assessment to total copper from all sources

Tables 4 and 5 show the summary statistics of the estimated chronic dietary exposure to total copper (in units of 'mg/kg bw per day' and 'mg per day', respectively) from all sources for each age group using the selected mean concentrations from the composition and occurrence database (Table 4 in the Annex and Section 2.2.2.2). Detailed mean and 95th percentile (P95) dietary exposure estimates for all age groups and dietary surveys are presented in Table 7 in the Annex.

Table 4: Summary statistics of the chronic dietary exposure to total copper (mg/kg bw per day) across European dietary surveys by age group

Age group (Age range)	Range across surveys of chronic dietary exposure (mg/kg bw per day)					
	Mean			P95[(a)]		
	N surveys	Minimum	Maximum	N Surveys	Minimum	Maximum
Infants (> 12 weeks to 12 months)	11	0.029	0.084	9	0.072	0.155
Toddlers (1–3 years)	15	0.054	0.068	13	0.078	0.108
Other Children (3–10 years)	19	0.039	0.053	19	0.059	0.090
Adolescents (10–18 years)	21	0.017	0.032	20	0.033	0.052
Adults (18–65 years)	22	0.015	0.022	22	0.024	0.037
Elderly (65–75 years)	19	0.014	0.022	19	0.024	0.037
Very elderly (> 75 years)	14	0.015	0.022	10	0.027	0.055

(a): 95th percentile range from surveys with more than 60 subjects.

Mean dietary exposure to total copper ranged from 0.014 mg/kg bw per day in older people to 0.084 mg/kg bw per day in infants. The 95th percentile of dietary exposure ranged from 0.024 mg/kg bw per day in adults and elderly to 0.155 mg/kg bw per day in infants.

Table 5: Summary statistics of the chronic dietary exposure to total copper (mg per day) across European dietary surveys by age group

Age group (Age range)	Range across surveys of chronic dietary exposure (mg per day)					
	Mean			P95[(a)]		
	N surveys	Minimum	Maximum	N Surveys	Minimum	Maximum
Infants (> 12 weeks to 12 months)	11	0.25	0.68	9	0.62	0.99
Toddlers (1–3 years)	15	0.54	0.93	13	0.83	1.29
Other Children (3–10 years)	19	0.77	1.29	19	1.13	1.94
Adolescents (10–18 years)	21	0.94	1.42	20	1.53	2.44
Adults (18–65 years)	22	1.04	1.62	22	1.65	2.66
Elderly (65–75 years)	19	1.01	1.67	19	1.65	2.57
Very elderly (> 75 years)	14	1.12	1.55	10	1.75	3.22

(a): 95th percentile range from surveys with more than 60 subjects.

3.4.1.1. Main contributing food categories

The main contributing food categories (at level 1 of the FoodEx2 classification) to the dietary exposure to total copper across the different age groups were 'Grains and grain-based products', 'Fruit and fruit products', 'Meat and meat products', 'Vegetables and vegetable products', 'Coffee, cocoa, tea and infusions', 'Food products for young population' and 'Milk and dairy products'. A summary on the contribution of these categories across age groups and surveys is given in Table 8 in the Annex, whilst details are given in Table 9 in the Annex. These categories contributed individually more than 10% to the dietary exposure in most of the surveys (Table 10 in the Annex) and together they contributed more than 50% to the overall dietary exposure to total copper in all surveys, except in one survey with a high reporting rate on 'composite dishes'.

Across the different age groups, the main contributing food categories to the mean dietary exposure to total copper were the same for the population of consumers with a higher dietary exposure (above the 75th percentile) compared to the general population.

All other food categories reported in the Comprehensive Database and included in the dietary exposure assessment and for which there are authorised uses (PPPs, food and feed additives, nutrient use and fertiliser), resulted in a contribution lower than 10% to the dietary exposure to total copper across surveys at the level 1 of the FoodEx2 classification.

Within the food category 'Grains and grain-based products', 'Bread and similar products' was the main contributor at FoodEx2 level 2 and 'Infant and follow-on formulae' was the main contributor at FoodEx2 level 2 for 'Food products for young population'.

Table 6 shows the food categories at FoodEx2 level 3 within the identified main contributing food categories at FoodEx2 level 1 that contributed more than 5% in at least two surveys. The level 3 food categories were selected to estimate the contribution of regulated uses of copper (PPPs, food and feed additives, nutrient use and fertiliser) to the dietary exposure to total copper. Details of the contribution of food categories at FoodEx2 level 3 are given in Table 11 of the Annex.

Table 6: Main contributors at FoodEx2 level 3 (number of surveys per age group with a contribution greater than 5%, in brackets the range of percentage contribution across surveys)

foodex2_L1	foodex2_L3	Infants	Toddlers	Children	Adolescents	Adults	Elderly	Very elderly	Total
Coffee, cocoa, tea and infusions	**Coffee beverages**					12 (5.1–12.6)	8 (5.6–15.7)	7 (6.2–18.2)	27
Coffee, cocoa, tea and infusions	**Herbal and other non-tea infusions**		3 (5.1–8.6)	4 (6.2–10.6)	1 (9.4)				8
Coffee, cocoa, tea and infusions	**Tea beverages**					1 (5.5)	1 (7.6)	1 (8.2)	3
Food products for young population	**Cereals with an added high protein food reconstituted**	5 (7.5–15.3)	2 (6.5–6.7)						7
Food products for young population	**Follow-on formulae**	6 (10.9–15.3)	4 (5.4–16.9)						10
Food products for young population	**Infant formulae**	10 (8.5–35)	1 (15.6–15.6)						11
Food products for young population	**Ready-to-eat meal for infants and young children**	5 (7.9–16.4)	1 (9.3)						6
Food products for young population	**Simple cereals for infants or children, reconstituted**	2 (5.3–7.1)	1 (5.8)						3

foodex2_L1	foodex2_L3	Infants	Toddlers	Children	Adolescents	Adults	Elderly	Very elderly	Total
Fruit and fruit products	Berries and small fruits	1 (13.4)	1 (6.7)						2
Fruit and fruit products	Miscellaneous fruits with inedible peel, large	5 (6–8.1)	7 (5–7.3)						12
Fruit and fruit products	Pome fruits	3 (5.6–12.6)	3 (6.4–7.3)	1 (8.4)	2 (5.9–8.2)	2 (5.1–6.7)	7 (5.2–8.5)	3 (5.7–8.2)	21
Fruit and vegetable juices and nectars (including concentrates)	Fruit juices (100% from named source)		1 (8.3)	2 (5.5–7.6)	3 (5–8.1)				6
Fruit and vegetable juices and nectars (including concentrates)	Mixed juices with added ingredients								0
Grains and grain-based products	Biscuits		1 (5.9)	1 (5.1)					2
Grains and grain-based products	Breakfast cereals, plain	4 (5.6–15)	5 (7–13.8)			2 (5.2–11.2)	2 (10.7–17.2)	1 (12.9)	14
Grains and grain-based products	Cereal and cereal-like flours	2 (5.3–5.8)	1 (9)	1 (16.6–16.6)	1 (21.4)				5
Grains and grain-based products	Cereal grains (and cereal-like grains)		1 (5.6)		1 (6.4)				2
Grains and grain-based products	Leavened bread and similar	2 (6.4–10.1)	11 (6.1–21)	18 (8.7–21.6)	20 (9.1–21.8)	22 (8.3–21.5)	19 (9.2–22.8)	14 (8.8–27)	106
Grains and grain-based products	Pasta and similar products		2 (5.2–9.4)	5 (5.2–8.2)	4 (5.1–6.8)	1 (6.2)	1 (6.4)	1 (6.7)	14
Grains and grain-based products	Processed and mixed breakfast cereals			1 (5.8)	1 (5.7)				2
Meat and meat products	Mammals liver		1 (5.5)			2 (6.7–9.2)	5 (5.1–13.5)	1 (13.6)	9
Meat and meat products	Mammals meat				2 (5.1–5.5)	2 (5.5–5.6)			4
Milk and dairy products	Milk	9 (5.4–29.1)	3 (6.1–7.5)	6 (5.3–10.1)	2 (5.2–7.1)			1 (6)	21
Vegetables and vegetable products	Processed tomato products		1 (8.6–8.6)	1 (7.7)	2 (5.5–7.3)	2 (5.4–6.1)	1 (6.1–6.1)	1 (5.9)	8
Vegetables and vegetable products	Solanacea				1 (5.2)	1 (5.2)	1 (6)	1 (9.5)	4
Vegetables and vegetable products	Vegetable puree or paste	1 (21.6)	2 (5.8–8.4)						3

3.4.2. Results of the specific assessments of the contribution of regulated uses to the dietary exposure to total copper

The results of the assessment of the contribution of regulated uses of copper (PPPs, food and feed additives, nutrient use and fertiliser) to the dietary exposure to total copper are described in the following sections.

3.4.2.1. Contribution from regulated PPPs uses

PPPs containing copper are authorised on two of the main contributing food categories at FoodEx2 level 1 (Table 6): Vegetables and vegetable products (Solanacea, Processed tomato products, Vegetable purée or paste) and fruits and fruit products (pome fruits and berries and small fruits). Therefore, the potential contributions from the use of copper in PPPs were investigated for apples and pears (corresponding to 'pome fruits'), currants, grapes, raspberries, strawberries (corresponding to 'berries and small fruits'), sweet peppers and tomatoes (corresponding to 'Solanacea') using results of supervised field trials (as described in Section 2.2.2.2) that were available for these crops. For the other possible relevant crops belonging to these food categories (at foodex2 level 3), no data were available for this purpose.

Table 7 shows the comparison of the copper concentration for these crops in untreated and treated crops from supervised field trials (mg/kg) described in Section 2.2.2.2 as well as the mean ratio of the copper concentration in untreated and treated crops across field trials.

Table 7: Mean copper concentration in untreated (control) and treated crops from supervised field trials and as used in the overall dietary exposure assessment to total copper from all sources (mg/kg)

Food category	Control samples		Treated samples		Ratio		Occurrence data[a]	
	N	Mean ± STDEV (mg/kg)	N	Mean ± STDEV (mg/kg)	N[c]	Mean Ratio (Control/ Treated)	N	Mean ± STDEV (mg/kg)
Apples	13	0.51 ± 0.08	62	1.9 ± 0.83	8	0.33	52	0.72 ± 1.1
Pears							13	0.87 ± 0.45
Currants (black, red and white)	2	0.77 ± 0.21	2	0.91 ± 0.19	1	0.85	–	1.13 (0.71–1.40)[b]
Grapes and similar fruits	21	1.37 ± 0.61	62	14.73 ± 11.81	12	0.16	19	1.09 (0.39–2.70)[b]
Raspberries and similar-	2	0.96 ± 0.37	2	1.02 ± 0.09	2	0.97	4	1.07 (0.89–1.2)[b]
Strawberries	35	0.35 ± 0.18	164	1.93 ± 1.67	23	0.23	43	0.57 ± 0.54
Strawberries (washed)	1	0.53 ± 0	2	2.21 ± 0.07	1	0.24		
Sweet peppers	29	0.76 ± 0.71	135	3.14 ± 2.7	20	0.28	27	0.57 ± 0.52
Sweet peppers (washed)	6	0.61 ± 0.1	21	1.68 ± 0.86	3	0.48		
Tomatoes	5	0.88 ± 0.16	37	1.92 ± 0.57	2	0.42	42	0.85 ± 1.79

(a): Copper concentration used in the overall dietary exposure assessment to total copper from all sources.
(b): Concentration derived from the composition database. Minimum and Maximum value in parenthesis. All other concentration values derived from the occurrence database.
(c): N, the number of studies in which a mean ratio was calculated; the mean ratio was calculated from the individual study averages.

The mean concentration values of the control samples give an indication of the copper content in crops when PPPs containing copper are not used. In the control samples, the standard deviations of the mean concentrations are small (except for sweet peppers), which indicates that the natural copper concentration in plants is in general quite stable.

The mean concentration values from treated samples reflect the levels of copper expected in crops treated with PPPs containing copper according to the critical good agricultural practices (GAP) currently authorised. The standard deviations in treated crops suggest a large variability in copper concentrations when copper is used as PPP. As results of the supervised residue trials are used to

derive MRL that should cover all uses authorised in the different geographical zones in Europe, the variability observed among results is totally justified. Although the trials are performed under standardised conditions (same number of applications, same application rate, same growth stage at application), in line with the current guidance on MRL setting, they are carried out in different regions of the EU, on different types of soils and under different climatic conditions. Furthermore, samples taken at different time intervals after applications (preharvest intervals (PHI)) were considered to calculate the mean concentration, which might impact on the variability of the results.

Despite the variability among results, the ratio 'control/treated' can be a good indicator of the impact of the use of copper as PPP on the final copper concentration in crops. For example, the ratio in apples (0.33) suggests that PPPs contribute significantly to the overall concentration of copper in this commodity. The same is observed in grapes, strawberries, sweet peppers and tomatoes, where PPPs' applications seem to contribute significantly to the final concentration. On the contrary, in berries (currants and raspberries), the ratio 'control/treated' is high (0.85–0.97), showing that most of the copper observed in samples from treated crops is related to the natural background and suggesting that the effect of PPPs on the copper concentration in these crops is limited. These results are consistent with the critical GAPs currently authorised on these crops. Actually, for apples, grapes, strawberries, sweet peppers, copper PPPs are used by broadcast applications, carried out close to harvest and applied directly on the edible part of the crop, whilst for currants and raspberries according to the authorised critical GAPs, the application is carried out an early growth stage (before flowering).

Furthermore, a comparison between the concentration values observed in samples of supervised field trials (control and treated) with the actual concentration values (from occurrence/monitoring data and composition data) considered in the exposure calculations, is useful to better understand the copper levels found during monitoring and in the composition database. For apples, the mean occurrence data (0.72 mg/kg) are closer to the mean of control samples (0.51 mg/kg) than to the mean of treated samples (1.9 mg/kg). This implies that, on average, crops analysed during monitoring were not treated according to the critical GAP parameters. Most samples from the monitoring may come from either crops not treated with copper as a PPP, or from crops treated with copper as a PPP but according to a less critical GAP. A similar situation is observed in strawberries, sweet peppers and tomatoes. For these crops, the average of the occurrence data is in the range of the control samples of the residue trials. It should be noted that higher results are observed in the occurrence data, up to levels expected after critical PPP uses. However, those levels are diluted when considering the overall average.

For grapes, as the number of samples from the monitoring data was insufficient, the values from the composition database (ranging between 0.71 and 1.40 mg/kg) were considered for the exposure assessment. It is noted that the average value from the composition database (1.13 mg/kg) is in the same range as the control samples from the trials (1.37 mg/kg). This result confirms that the composition data are representative of the natural copper content in crops. Furthermore, additional data from the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) monitoring report of 2018[18] indicate that the average copper concentration in grapes samples taken from the market was 1.11 mg/kg (n = 104) and therefore in the same range as the value considered in the exposure. The P90 (2.04 mg/kg) and maximum (7.29 mg/kg) values of these monitoring data indicate much higher concentrations, probably due to samples from vineyards treated with copper as PPP. However, on average, considering as well the monitoring data from the BVL, copper used as a PPP does not seem to have an effect on the overall concentration in grapes, and therefore does not significantly contribute to the overall dietary exposure to copper.

Overall, among the main contributors identified in Section 3.4.1.1, although a contribution from regulated PPPs could in theory be expected for apples, grapes, strawberries, sweet peppers and tomatoes, it does not appear to be the case in practice.

Considering that the available monitoring data used in the dietary exposure to total copper from all sources are in line with control data from trial studies measured on untreated crops, the contribution of copper used as a PPP to the overall dietary exposure to total copper, can be considered negligible.

It should be noted that copper applied as a PPP also contributes to copper accumulation in soil, which consequently may increase the copper concentration in food via uptake from soil. The indirect contribution of copper as a PPP via soil on the overall dietary exposure is assessed in Section 3.4.2.5.

[18] Harms (2016) BVL-Report • 12.4 Berichte zur Lebensmittelsicherheit.

3.4.2.2. Contribution from regulated food additives uses

Based on the refined dietary exposure assessment of the ANS Panel, it was noted that the main food commodities contributing to the non-brand loyal scenario dietary exposure estimates of the two copper-containing food additives [E141(i) and E141(ii)] and which are included in the main food categories listed in Table 6 were 06.3 Breakfast cereals and 07.2 Fine bakery wares. Additionally, it was noted that 2.6% of the foods belonging to fine bakery wares are labelled in the EU market with both food additives according to Mintel's GNPD (Table 6 in the Annex, data extracted in January 2021).

Based on this information, the contribution of copper from food additives present in breakfast cereals and fine bakery wares to the overall dietary exposure to total copper was documented by the SC. Table 12 in the Annex provides the summary results.

These results show that the dietary exposure to total copper from its use as food additives Cu-chlorophylls [E141(i)] and Cu-chlorophyllins [E141(ii)] in breakfast cereals and fine bakery wares contributed on average less than 0.7% to the dietary exposure to total copper from all sources across all population groups.

3.4.2.3. Contribution from regulated nutrient uses

As indicated in Section 3.4.1.1, infant formula and follow-on formula are the main contributors to the dietary exposure to copper in infants (12–39% across surveys) and toddlers (1–19% across surveys) (see Table 11 of the Annex). This contribution includes natural occurrence of copper in infant and follow-on formula's ingredients and copper added in order to achieve a total copper content which is compliant with the regulatory limits.

The concentrations in infant and follow-on formulae used in the dietary exposure assessment to total copper from all sources derived from the occurrence and nutritional composition databases (Section 2.2.2.2) are displayed in Table 8 and compared with the regulatory range.

Table 8: Copper concentrations (μg/kg) in infant and follow-on formulae used in the overall dietary exposure and regulatory limits

Food category at Foodex2 level 3	Conc. used in exp. assess.	Regulatory min.–max.
Infant formulae, powder (milk, soya and protein hydrolysates based)	3,605–3,726	2,880–5,600[(a)]
Infant formulae, liquid (milk, soya and protein hydrolysates based)	451–494	360–700[(b)]
Follow-on formulae, powder liquid (milk, soya and protein hydrolysates based)	3,000	2,880–5,600[(a)]
Follow-on formulae, liquid (milk, soya and protein hydrolysates based)	375	360–700[(b)]

(a): A dilution factor of 8 was applied to the regulatory limits for the liquid formulae.
(b): EC delegated regulation (EU) 2016/127 sets a minimum content for copper of 60 μg/100 kcal and a maximum content for copper of 100 μg/100 kcal in infant and follow-on formulae. This corresponds to 360–600 μg/L considering an energy content of 60 kcal/100 mL and 420–700 μg/L considering an energy content of 70 kcal/100 mL.

It is noted that concentrations used in the dietary exposure assessment to total copper were in the range of the regulatory limits and also in the range of the copper concentration in mature breast milk of populations from Western countries (range from 100 to 1000 μg/L according to EFSA NDA Panel, 2015).

It was not possible to assess the contribution to the dietary exposure to total copper from nutrient use in food fortification and food supplements as described in Section 2.2.2.2.

3.4.2.4. Contribution from regulated feed additives uses

Table 6 shows that mammal liver, mammal meat and milk are the foods of animal origin that are among the main contributors to the dietary exposure to total copper. Concentrations in these foods as used in the dietary exposure assessment are compared with the MRLs set in these foods in the pesticide regulation. These MRLs can be interpreted as total concentrations resulting from all uses of copper including feed additives. All mean occurrence values for milk and muscle meat from different species as used in the exposure assessment, except for reindeer meat (5,637 μg/kg; Table 5 of the Annex), were below the MRL of 2,000 μg/kg in milk and the MRL of 5,000 μg/kg for muscle meat

(Reg. 149/2008). The SC noted that the mean copper concentrations in liver of most food animals obtained from the market substantially exceeded the MRLs (e.g. for liver of mammals 30,000 μg/kg). This confirms, as already stated in 2016 by the EFSA FEEDAP Panel (EFSA FEEDAP Panel, 2016a) and in the EFSA Opinion on MRL review in 2018 (EFSA, 2018) that (assuming feed was compliant with maximum copper content) the MRLs for liver considerably underestimate the occurrence of copper in liver from ruminants. The data available for this Opinion do not allow evaluation of whether mean copper concentrations in food of animal origin reflected only the natural occurrence of copper in feed or also the use of copper as a pesticide or as a feed additive. Of the possible copper sources in feed, copper-containing PPPs are likely a minor one (see Section 2.2.2.2) (EFSA FEEDAP Panel, 2016a). Information on the contribution of copper from feed supplementation to copper content in animal tissues has been previously reviewed (EFSA FEEDAP Panel, 2015).

Engle and Spears (2000a,b) showed increased copper concentrations in liver from steers fed with feed that was supplemented with 20 mg Cu/kg DM feed or higher (290,000–380,000 μg/kg DM, corresponding to ~116,000–152,000 μg/kg wet weight) compared with controls with background copper levels in feed (63,000 μg/kg DM, corresponding to ~25,000 μg/kg wet weight) (EFSA FEEDAP Panel, 2012). It was not documented whether the background levels for the feed used in this study resulted only from natural occurrence or also from pesticide use. However, the contribution from the use of copper as a feed additive to the copper concentration in liver was obvious. In comparison, copper levels in liver from animals that were not given feed high in copper as reported in Engle and Spears (2000a,b) were lower than the copper concentrations used in the dietary exposure assessment but for steers fed feed that was supplemented with copper, the levels in beef, bovine or mammal liver were higher and in the same range as occurrence data for veal liver used in the dietary exposure assessment.

In 2015, the FEEDAP Panel pointed out that copper residues found in milk are low and are not influenced by dietary copper supplements (EFSA FEEDAP Panel, 2015). In addition, Schwarz and Kirchgessner (1978) did not find significant influence of copper supplementation on the copper contents of milk. On average, copper concentrations of 150–200 μg/kg milk were reported for the various experimental groups across all experimental weeks. The copper concentration in milk used in the dietary exposure assessment was slightly below the concentrations reported in the feeding study of Schwarz and Kirchgessner (1978), indicating that there is only a marginal or low contribution of feed supplementation to the copper exposure via milk.

Bradley et al. (1983) reported a pig feeding study in which pigs were fed feed that was supplemented with copper at 7.5–120 mg Cu/kg. For the lowest supplementation level, which was far below the current maximum copper content for feed, the respective copper concentration in pig's liver was 10,000 μg/kg wet weight equal to 22,500 μg/kg DM (EFSA FEEDAP Panel, 2012). This concentration was below the concentration used for pigmeat in the exposure assessment and indicated that there was a relevant contribution of copper supplementation or other sources such as pesticide use above the levels used for feed in this study. It was also shown that copper begins to accumulate markedly in pig liver from 60 mg Cu/kg in feed (Bradley et al., 1983). The same study also looked at copper concentrations in pig muscle, but no increase was observed with increasing copper feed supplementation. The copper levels in pig muscle ranged from 1700 to 1900 μg/kg (DM) between all supplementation groups (Bradley et al., 1983).

Overall, comparing the concentrations used in the dietary exposure assessment to total copper with the concentrations reported in studies from the literature, it is obvious that the administration of copper as a feed additive may influence the levels of copper in mammal livers but not in muscle and in milk. However, it should be noted that the contribution of copper as a feed additive to the levels of copper in mammal livers was mostly evident at copper concentrations in feed exceeding the maximum total copper authorised in feed in the EU (EFSA FEEDAP Panel, 2015). Since then, the currently authorised maximum contents for total copper in feed have been reduced further (Commission Implementing Regulation (EU) 2018/1039) following EFSA's revised recommendations (EFSA FEEDAP Panel, 2016a). Although it was not possible to distinguish between the individual sources of contribution of copper to feed, the fact is that legislation regulates the maximum total copper content in feed in the EU. Therefore, when in compliance with the regulatory maximum limits, copper in feed additives does not contribute significantly to copper content in animal tissues and hence to consumer exposure from consumption of animal products.

3.4.2.5. Contribution from fertilisers uses and from long-term PPP application via the soil

Fertilisers contain copper and fertiliser and addition may gradually enrich soil with copper that potentially enriches food crops with additional copper. This section first describes to what extent fertilisers affect the soil copper concentrations in European agriculture and subsequently describes how that affects food copper concentrations. The long-term use of PPPs containing copper may also enhance soil copper concentrations and, indirectly, copper concentrations in food. This pathway is also addressed in this section. It is important to distinguish between this indirect effect of PPPs via the soil on copper concentrations in crops (this section: only important after decades of PPP use) and the direct effect of PPPs via treatment of crops with copper-containing PPPs as discussed in Section 3.4.2.1 and Table 7. As noted in the EFSA PPR Panel Statement (2021), '*... it is not always possible to determine the concentrations of transition metals in soil, ... that originate from use of the compounds as a PPP*' (EFSA PPR Panel 2021). In addition, this section discusses the role of soil concentrations on copper concentrations in crops related to the food categories that were identified as main contributors to the dietary exposure to total copper.

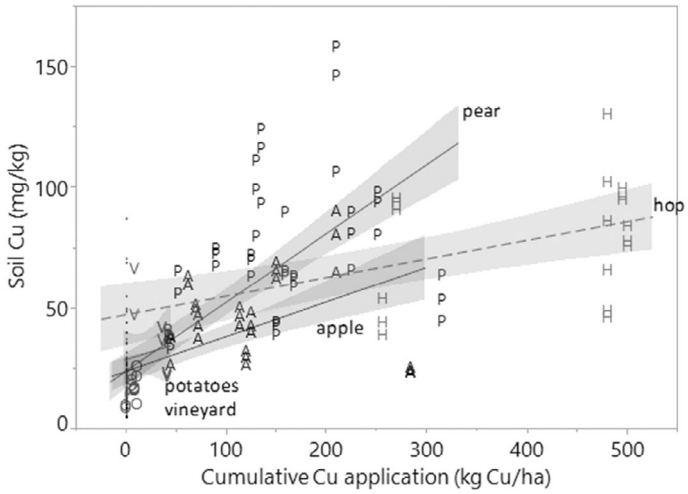
Role of fertilisers and PPP on soil copper concentrations

The median total copper concentration in European agricultural soils is 15 mg/kg (Albanese et al., 2015) while it is 12 mg Cu/kg for all types of land cover on Europeans soils (Ballabio et al. 2018). Soil copper concentrations are higher in southern Europe than in northern Europe. Most of that copper is natural, but it is logical that the addition of fertiliser and PPP may gradually enrich soil with copper. The main sources of copper input to agricultural soils via fertilisers are manure, sewage sludge and mineral fertilisers (Oorts, 2013). Historical use of sewage sludge, compost or manure clearly enriched agricultural soils with copper (Smolders et al., 2012) (Cambier et al., 2014) and the same is true for soils where copper-containing PPPs have been used, e.g. in established vineyards (Michaud et al., 2007; Ruyters et al., 2013). A mean total concentration of 49 mg/kg was reported in vineyards, with a very high variability between countries, climatic, geological and pedological factors (Ballabio et al., 2018; EFSA PPR Panel 2021). Within different types of fertilisers, the annual rate of copper addition via manure, expressed per surface area of land (kg/ha per year) is much lower than via sewage sludge (or PPP). For example, more than 100 years of fertilising a field with animal manure in one of the oldest field trials in Denmark has enriched soil copper from 6 mg/kg to 12 mg Cu/kg. In contrast, only 25 years of fertilising with sewage sludge in Sweden enhanced soil copper concentrations up to 15 mg Cu/kg (Smolders et al., 2012). There are numerous examples of much larger copper enrichments on farms where sewage sludge was added experimentally and outside regulatory limits (Hooda et al., 1997; Smolders et al., 2012) and the same is true for the long-term use of copper-containing PPPs resulting in local enrichments of more than 100 mg Cu/kg in the topsoil of vineyards (Michaud et al., 2007).

The enrichment of soil copper with fertilisers is gradual, but steady (Smolders et al., 2012). Enrichments of Dutch soils with copper, depending on soil and fertiliser strategies, was estimated to increase soil copper concentrations from 18 mg Cu/kg currently to 25 mg Cu/kg in 100 years from now (Groenenberger et al., 2006). Similar conclusions arise in the study by Monteiro et al. (2010) that had been commissioned by EFSA (Monteiro et al., 2010).

Regulated use of copper via PPP is 28 kg Cu/ha in 7 years (between 2019 and 2025) according to the latest regulation (EU 2018/1981). A survey was made in Belgium on soil copper accumulation in fields and orchards in 2013 as affected by PPP application (Figure 3).

The total copper concentrations in soil increase with about 30 mg Cu/kg (= 2 to 3-fold increase above background) for a cumulative application of 100 kg Cu/ha in pears, and only with 30 mg Cu/kg in soil at a cumulative dose of 400 kg Cu/ha because of the much deeper ploughing depth in hop than in pear orchards. That latter scenario can be seen as a scenario for a sustained maximal dose of 4 kg Cu/ha per year (as allowed now between 2019 and 2025 in the EU 2018/1981 regulation) over the next 100 years, here termed the PPP scenario.



A = apple; H = hop; P = pear; V = vineyards; O = potatoes and small points are the reference soils. Regression lines and their confidence intervals of fit are shown. (Smolders, personal communication; unpublished figure.)

Figure 3: Soil copper concentrations in fields and orchards surveyed in Belgium in 2013 in relation to the cumulative copper fungicides application on the field estimated from farmer's recall

Role of soil copper concentrations on copper concentrations in crops

It is well established that crop copper concentrations are very constant, irrespective of changes in external supply due to homeostatic control on copper uptake (e.g. Degryse et al., 2008). Even the metal hyperaccumulating plants show only a small rise in leaf copper concentrations despite large increases in soil copper concentrations (Song et al., 2004). Table 9 shows data for selected food crops that illustrate that the crop copper concentrations change much less than those in soil copper because of the homeostatic control of biological copper uptake.

Homeostatic control can also be seen for grains and grain-based products, which contributed largely to the dietary exposure to total copper, for which enrichment is likely to be small and can be considered negligible (see Section 3.4.2.1).

Table 9: Selected examples of changes in crop copper concentrations (mg Cu/kg product) due to long-term application of copper-containing fertilisers, CuSO4 salt or PPPs.

	Soil copper concentration mg Cu/kg soil		Plant copper mg Cu/kg dry weight			Reference
	Untreated	**Highest treatment**	**Crop**	**Untreated**	**Highest treatment**	
Sewage sludge (different soil samples)	33 33 33	182 182 182	Wheat grain Carrot root Spinach leaves	4.2 5.2 9.5	3.9 7.2 16.9	(Hooda et al., 1997)
Sewage sludge	3–23	29–164	Wheat grain	2.7–4.1	4.9–7.9	(Heemsbergen et al., 2010)
CuSO4	5–41	56–183	Wheat grain	2.5–4.0	3.9–5.6	(Heemsbergen et al., 2010)
PPP (vineyard soils)	25	200	Durum wheat leaves	8	8–25	(Michaud et al., 2007)

In addition, an estimate of long-term effects of fertiliser and PPP application in Europe on copper concentrations in grain was made. The prediction of copper concentrations in grains (wheat, millet, sorghum, triticale and canola) was derived from a published model (Heemsbergen et al., 2010) calibrated to data of soil and grain copper concentrations in field trials where soil copper had been amended up to about 150 mg Cu/kg soil by the use of biosolids or of $CuSO_4$ in 12 locations and

monitored for 3 years; the biosolid data were used for the manure scenario and the $CuSO_4$ data were used for the PPP scenario. Table 10 shows how copper concentrations in grains may rise in response to long-term fertiliser addition in the following scenarios: a 'manure scenario' in The Netherlands where animal manure is a major source of copper addition and, in the 'PPP scenario' where a regulated use of 4 kg Cu/ha per year was used. Both estimate the soil copper concentration over a long-term period of 100 years. Data show that the percentage rise in grain copper concentrations is 2% in the manure scenario and up to 12% in the PPP scenario after 100 years.

These estimates should be taken as an upper value as it is most likely that Cu derived from manure and from PPP is, on the long term, less available than the soluble CuSO4 that had been added in the field experiment for which the model was made.

Table 10: Prediction of changes in grain copper concentrations due to long-term use of fertiliser (manure) and/or PPPs that enrich the soil copper (based on Heemsbergen et al., 2010, modelling)

Scenario	Predicted soil copper mg Cu/kg topsoil		Predicted grain copper mg Cu/kg grain	
	Untreated	Highest treatment	Untreated	Highest treatment
Manure scenario in EU (100 years from now	18	25	7.55	7.72 (2% increase)
PPP scenario 100 years from now)	15	45	7.04	7.86 (12% increase)

The results in Table 10 might suggest that copper uptake by crops can be affected by increasing concentrations of copper in the soil over a long time period although at a lower rate compared to the increase in the concentration in the soil.

Coffee is a product of attention because the dietary exposure estimates suggest that coffee beverages are a main contributor to the dietary exposure to total copper (Table 6). Coffee beans imported in the EU are subject to European PPP Regulation 149/2008 (MRL of 50 mg/kg; reduction to 20 mg/kg was proposed in EFSA, 2018).

During the MRL review, no EU uses as PPP nor import tolerances on coffee beans were reported; therefore, no direct contribution of PPP is expected for this crop. Although extensive use of PPPs is made to control coffee berry disease and coffee leaf rust (Loland and Singh, 2004) estimated an annual Cu dose in eastern Africa of 6–15 kg Cu per ha per year, copper concentrations in coffee beans exhibit a markedly narrow range due to the homeostatic control of copper uptake in coffee plants. This is suggested from the rather constant concentrations in coffee beans in published data (13–15 mg Cu/kg; Impellitteri et al., 2000; Stelmach et al., 2013) as well as from the EFSA database (8.2–15.5 mg Cu/kg, mean 14 mg Cu/kg). This indirectly suggests that the impact of copper concentration soil due to the use of PPP on the copper concentration in coffee beans is considered to be negligible.

Pome fruits are also a significant contributor to the dietary exposure to total copper. Apple and pear orchards are treated with copper-containing fungicides. The growing position of the apple in tree relative to the exposure to the PPP spray affects the copper in the peel and the flesh of apples, with the higher copper levels in the fruits in the outside position of a canopy compared with in those in the inside position (Wang et al., 2014). In contrast to the impact of short-term application of copper-containing PPP to the soil and the copper content of pome fruits (Table 7), the long-term impact of application of PPP enriches the soil with copper, resulting in a positive correlation between copper concentrations in apples and in soil (Li et al., 2005); however, no study has shown raw data to identify how strong is that association, therefore no calculation can be made to make a forecast on how long-term PPP application may enhance the copper exposure via consumption of pome fruits.

3.4.3. Other sources of copper exposure

Although dietary exposure is the primary source of exposure to copper in humans, assessment of other sources of copper exposure that may result in systemically available copper are relevant for understanding the overall exposure to copper in the general population and subpopulations. Critical for assessing the contribution of other sources to total copper body burden is the copper bioavailability from non-oral routes of exposure. However, such data are limited.

Possible sources of exposure other than dietary, include:

1) Dermal exposure can occur from use of copper in cosmetics and personal care products (PCPs) and from copper-treated clothing or skin treatment dressings (antibacterial, antifungal, antiviral dressings) used for prevention or treatment of bacterial or other microbiological infections associated with various skin conditions. Of the cosmetics, lip products may also result in possible oral exposure of the product.
2) Several studies have assessed copper content in cosmetics and PCPs (Sani et al., 2016; Massadeh et al., 2017; Lim et al., 2018; Ghaderpoori et al., 2020; Shomar and Rashkeev, 2021); however, copper systemic exposure has been calculated only based on default dermal absorption values (also referred to as 'retention'), such as those established by the Scientific Committee on Consumer Safety (Scientific Committee on Consumer Safety, 2012). Indicatively, the doses estimated to be systemically available for copper from dermal application of cosmetics range between 1.47×10^{-11} and 3.73×10^{-7} mg/kg bw per day, assuming 100% retention (Ghaderpoori et al., 2020); between 1.8×10^{-10} and 1×10^{-8} mg/kg bw per day, assuming 0.1% absorption (Shomar and Rashkeev, 2021); and between 4.85×10^{-10} and 2.78×10^{-8} mg/kg bw per day, assuming 0.3% absorption (Lim et al., 2018). In the latter study, oral exposure to copper from lip products was estimated to be $< 0.006\%$ of the California EPA RfD of 0.01 mg/kg bw per day (Lim et al., 2018). Therefore, copper intake from cosmetics is negligible relative to dietary intake.
3) Use of copper in textiles has been reviewed with focus on the beneficial effects as a biocidal, with no data on its systemic availability from this application (Borkow and Gabbay, 2005; Borkow et al., 2009; Borkow et al., 2010a; Borkow et al., 2010b; Borkow and Mellibovsky, 2012; Borkow, 2014; Borkow et al., 2021). In other studies, content of copper in the fabric has been reported (Rovira et al., 2015; Nguyen and Saleh, 2017; Rovira et al., 2017a; Rovira et al., 2017b; Herrero et al., 2019; Rovira and Domingo, 2019; Herrero et al., 2020). In some studies, the systemic availability of copper from copper-treated clothing has been modelled using default parameter values and the dose from dermal exposure was estimated to be approximately $1.5–1.6 \times 10^{-4}$ mg/kg bw per day from sleep wear, assuming 0.5% migration from fabric to skin and 1% skin penetration (parameters reference: US EPA 2015); and similar range from denim clothing, of up to 1.23×10^{-4} or up to 2.26×10^{-4} mg/kg bw per day, based on simulated extraction in acidic and basic sweat, respectively (Herrero et al., 2019; Herrero et al., 2020); whilst others estimated $2.5–5.5 \times 10^{-6}$ mg/kg bw per day from T-shirts and underwear (2×10^{-7} mg/kg bw per day for children) (Rovira et al., 2015; Rovira et al., 2017a; Rovira et al., 2017b; Rovira and Domingo, 2019). Therefore, copper exposure from contact with clothing is negligible relative to dietary intake. Biocidal products may also come into contact with food and contribute indirectly to dietary exposure to copper. Potential contribution from this source has been captured in the dietary exposure assessment to total copper from all sources.
4) A time-dependent passive diffusion of copper from the outer skin to the deeper layers has been suggested following dermal application of 25 mg copper metal as powder on a skin surface of 12 mm diameter (1.15 cm^2) with and without occlusion for up to 72 hours, in three Caucasian volunteers (AUC for 72 hours up to 83 $\mu g/cm^2$, with semi-occlusion) (Hostýnek et al., 2006). In an earlier study, minimal absorption of copper through human skin over 72 hours was reported regardless of the form or vehicle used (Pirot et al., 1996). Cutaneous treatment with ointments containing 0.4% CuO or 20% elementary copper for 4 weeks followed by a 4-week washout period was reported to be associated with elevated serum and hair concentrations, and decreased urine concentration for the CuO treatment and unchanged hair and decreased urine concentration for the elemental copper (Gorter et al., 2004). These exposure conditions are not considered to contribute significantly to copper exposure in the general population.
5) Direct internal exposure may result from the use of copper in medical devices and implants, such as intrauterine devices (IUDs), orthopaedic prosthetic components, other such implants.
6) Copper use in biomaterials has been increasing (Jacobs et al., 2020). However, little information is available about the release of copper from medical devices and other implant applications *in vivo*. In an *in vitro* model of copper release from Ti–Cu–O coating containing 20%, 40% or 80% copper, used in prosthetics to protect against infection, it was reported

that copper release was highest in the first 24 h (269.4 μmol/L) and dissipated over time (days 1,2, 3, 7, 14 and 28) (Norambuena et al., 2017).

7) Copper release from IUDs *in vivo* has been reported in a few studies since the technology was first developed, with an estimated release rate in the range of 0.02–0.0736 mg/day (Hagenfeldt, 1972; Timonen, 1976; Chantler et al., 1977; Thiery and Kosonen, 1987). It correlated with the surface area of the IUD and decreased with time after IUD insertion, up to 50 months (Timonen, 1976; Chantler et al., 1977). In these studies, copper was detected in the local tissues (e.g. endometrium, cervical mucus), but there was no increase in serum copper levels from IUD use (Hagenfeldt, 1972; Timonen, 1976; Chantler et al., 1977; Anteby et al., 1978; Prema et al., 1980; Arowojolu et al., 1989). In one study, serum copper concentration increased only in women with low starting copper serum levels (Anteby et al., 1978), and in another study, serum copper was reported to be higher in 86 IUD users (0.21663 mg/dL) compared with eight non-users (0.10747 mg/dL) (De la Cruz et al., 2005). The data on systemic copper levels must be interpreted in the context of study design and analytical limitations. For a review, see Crandell and Mohler (2021).
8) In a more recent *in vitro* simulation study, nine types of Cu-IUDs were selected and incubated in simulated uterine fluid. They were paired for comparison according to the device properties and the release of cupric ion was determined by flame atomic absorption spectrometer for about 160 days. Consistent with the *in vivo* studies, copper release was higher during the first month, followed by a slower steady rate (Zhou et al., 2010; Zhang et al., 2015). Another recent study reporting on copper IUD corrosion (Cu_2O), measured by X-ray diffraction was focused on the benefit of intended use but neither copper release nor systemic exposure was considered (Wildemeersch et al., 2014).
9) Inhalation exposure (and dermal to some extent), resulting from ambient levels of copper originating from industrial facilities, whether air-borne copper and in soil/dust may serve to define subpopulations at higher risk, such as those living near or working in related industries (Fry et al., 2020).
10) Inhalation is a minor route of exposure for the general population, as documented in a copper multimedia exposure assessment in a United States population (Georgopoulos et al., 2006). In this assessment, diet was found to be the major exposure pathway and drinking water a potentially significant contributor, depending on local drinking water supply sources (Georgopoulos et al., 2006).

Copper exposure from all sources has been assessed and reported in an industry voluntary risk assessment (by the European Copper Institute, ECI) that was submitted to ECHA (Scientific Committee on Health and Environmental Risks, 2008). The estimated 'reasonable' typical and worst-case exposures (defined as the median and 90th percentile values) for consumers in general from different sources of the regional environment were reported as follows: by inhalation, negligible to up to 0.0005 mg/day in worst case; by the dermal route (from face cream, copper jewellery, handling of coins and hair products) up to 0.38 mg/day and up to 2.13 mg/day, respectively (excluding rare events such as handling of metallic paint). The typical and reasonable worst-case exposures from the local environment were estimated to be as follows: by inhalation 0.057 and 0.093 mg/day, respectively; from dust ingestion in children, it was estimated to range from 0.0034 to 0.034 mg/day and from 0.0135 to 0.135 mg/day, respectively (highest intake in the 1- to 4-year-old group and lowest in children over 12 years). Internal exposure by inhalation from consumer and local environment was estimated to be 9.5×10^{-4} mg/kg bw per day in a typical scenario and up to 0.0016 mg/kg bw per day in the reasonable worst-case scenario. Internal exposure via the dermal route was estimated for typical and worst-case scenario to be 1.9×10^{-5} mg/kg bw per day and 0.0003 mg/kg bw per day, respectively.

Based on all available evidence on the uses and sources of copper exposure, the contribution of copper from non-oral sources can be considered negligible for the general population compared with dietary exposure.

4. Discussion

The present Opinion evaluates the evidence for establishing a harmonised HBGV for copper by implementing the principles for establishing HBGVs for substances added to food as regulated products that are also nutrients, as outlined in the SC Statement aimed at harmonising approaches for this purpose (EFSA Scientific Committee, 2021). In that publication, the SC noted that the overall risk from

all exposure sources must be assessed using the 'total' and 'added risk' concepts. The harmonised approach to establish a HBGV for ETEs such as copper is based on the biological model for nutrients and the concept of 'acceptable range of oral intake' as described by the WHO/IPCS (IPCS, 2002). Specifically, it is appropriate to identify markers of excess nutrient, or of potential toxicity rather than observed ('actual') toxicity, using the sequence of accumulating events as line of evidence. Candidate endpoints would not be expected to be adverse in themselves but are predictive of adverse effects if intake is not reduced. It was also noted that HBGVs are established to protect the general population, and do not apply to infants under 16 weeks of age, or population groups under medical supervision for certain diseases (e.g. genetic diseases). Therefore, in the context of copper, the HBGV does not apply to individuals with WD.

4.1. Hazard assessment and establishment of a HBGV

The SC notes that previous risk assessments of copper were based on evaluations of adverse effects in human studies and supportive evidence from animal studies. These assessments identified an NOAEL of 10 mg/day (equivalent to 0.15 mg/kg bw per day considering a body weight of 70 kg) from a human study in healthy young males, in which liver enzymes were not affected after 12 weeks of supplementation at this level of intake (Pratt et al., 1985) (Section 2.1). This NOAEL was used as an RP to establish HBGVs for copper. The SC notes that the study by Pratt et al. (1985), upon which previous assessments were based, had significant limitations, such as small sample size (7 adults), evaluation of a single dose level, minimal documentation, no justification that the endpoints assessed were appropriate indicators of copper toxicity in the timeframe of observation and absence of characterisation of copper exposure before the study. This study also assumed that the administered form of copper was readily excreted through the kidneys and no reference was made to homeostatic sequestration in the liver. As in the study in healthy young adults (Pratt et al., 1985), no effect was observed on liver enzymes in elderly patients with mild Alzheimer's disease who received a supplemental dose of 8 mg Cu/day for 1 year in addition to their background intake (Kessler et al., 2008a). Also, no evidence of adverse effects was found, including on liver enzymes, in infants who consumed ~2.5 mg Cu/day from drinking water between 4 and 12 months of age (corresponding to 0.37 mg/kg bw per day[19]) (Olivares et al., 1998). These three studies did not detect evidence of hepatic injury, during treatment periods up to a year.

However, copper toxicity associated with copper dietary exposure is a function of both dose and time. As noted, tight homeostatic regulation provides effective protection against copper toxicity. Whilst the endpoints evaluated in these three studies are appropriate to detect apical adverse effects (e.g. hepatotoxicity), these effects may not be manifested within the timeframe of exposure and observation periods, either in adults (Pratt et al., 1985; Mendez et al., 2004; Araya et al., 2005; Rojas-Sobarzo et al., 2013) or in children (> 16 weeks of age) (Olivares et al., 1998). Thus, in adults and children, including infants older than 16 weeks, the evaluated endpoints were not sensitive enough to detect early biological changes predictive of copper toxicity following continuous chronic intake, and the duration of the studies may not have been sufficiently long for copper toxicity to become manifest following hepatic retention.

Copper toxicity must be considered within the context of continuous chronic exposure, the homeostatic adaptive responses to increasing exposures (such as the reported hepatic sequestration, potential retention and pattern of total body burden over time) and the timeframe of the onset of adverse effects. According to the Statement on establishing HBGVs for regulated products that are also nutrients (EFSA Scientific Committee, 2021):

'the endpoints identified would not be expected to be hazards or adverse events, i.e. they are predictive of adverse events that would occur if intake is not reduced. The identification and characterisation of critical endpoints depends on a sound understanding of the nutrient kinetics and dynamics of the nutrient of interest' (EFSA Scientific Committee, 2021).

For copper, hepatic sequestration is initially protective against adverse effects caused by the reactivity of free copper, but subsequent hepatic retention from continuous exposure at elevated levels is a prerequisite for copper toxicity and constitutes an early biological process within the pathway to toxicity. Copper homeostasis is assumed to be fully functional in children older than 4 months of age.

The present assessment has adopted an approach guided by the principles presented in the Statement on HBGVs on regulated products that are also nutrients, as noted above. The central role of

[19] Considering a mean body weight of 6.7 kg in the age range 3–6 months (EFSA Scientific Committee, 2012).

copper sequestration in protection against copper toxicity means that manifestation of copper toxicity, other than acute toxicity at very high exposures, may not be observed in studies of relatively short duration. However, manifestation of toxicity *following* hepatic copper retention might be observed in longer term studies.

The SC considers that data on copper balance can be an early marker of potential adverse effects because copper retention is an early stage in the pathway of copper toxicity. Hence, data on copper balance are relevant for the identification of an RP for copper toxicity, based on the interpretation of the weight of evidence. Copper homeostasis data were also taken into account in previous assessments but were not used as the basis for the ADI. The pivotal study identified by the SC is a metabolic study by Turnlund et al. (2005), where significant copper retention (0.67 mg/day on average) was observed in healthy males, following a daily intake of about 8 mg total copper for almost 5 months (Turnlund et al., 2005). Despite decreased copper absorption and increased faecal copper excretion, an equilibrium between intake and losses (i.e. null balance) could not be restored at this level of intake within the observation period of 5 months. This was associated with increased urinary copper excretion and hair copper concentrations (Turnlund et al., 2004). The copper body burden was projected to increase with continuous intake assuming no further adaptation took place (Turnlund et al., 2005). The SC recognised the additional uncertainty associated with a projection beyond the period of observation compared to the uncertainties associated with observed changes. Also, the copper content of intestinal epithelial cells was significantly increased at 7 mg/day of copper supplementation over 129 days (Turnlund et al., 2003). In another metabolic study, Harvey et al. reported a copper retention of 0.75 mg/day in subjects consuming 6 mg/day total copper from a controlled diet for 8 weeks (Harvey et al., 2003). Because the monitoring timeframe of this latter study was shorter than the Turnlund et al. (2005), and this intake level was not assessed in Turnlund et al. (2005), the possibility that equilibrium could be reached over longer observation periods (beyond 8 weeks) with 6 mg/day of copper intake has not been assessed. Therefore, the longest observation period for which there are data on copper retention is 5 months with a daily intake of approximately 8 mg/day (Turnlund et al., 2005). Therefore, the intake of 10 mg/day that was previously used as a RP can no longer be considered an NOAEL.

The SC considers that the data of both of these studies (Harvey et al., 2003; Turnlund et al., 2005) indicate a concern that the body's regulatory homeostatic capacity may be exceeded, leading to copper retention, at levels of copper intake around 6–8 mg/day in adult men. The SC notes that most of the copper retained in the body is likely to be deposited in the liver, bound to metallothionein (MT), which is a protective mechanism against toxicity of this transition metal (Huster and Lutsenko, 2007; Calvo et al., 2017; Krężel and Maret, 2017, 2021). In addition, copper homeostasis is also dependent on the individual's zinc status partly via MT regulation (Krężel and Maret, 2021). The capacity of the liver to store copper until any overt toxic effect occurs is, however, unknown and a correlation between hepatic copper levels and onset of hepatotoxicity (or other copper-related toxicity) has been elusive. The upper level of the 'normal' liver copper range of 50 μg/g dry weight is equivalent to approximately 14 μg/g wet weight (28% DM content). Based on an average adult liver weight of 1500 g, the upper level of total hepatic copper content is estimated to be 21 mg. The level of retention at intake of 8 mg/day (0.67 mg/day on average) presents concern of potential hepatic retention in adults in the context of chronic exposure. Individuals with low zinc status, and consequently lower hepatic MT levels may be more susceptible to copper retention. The SC recognises the challenges in designing a robust study that could address the data gaps related to copper toxicity in the general population. The absence of reported presymptomatic copper retention and toxicity in humans without WD owes to the lack of reliable non-invasive biomarkers for the detection and measurement of increased copper body burden, such that only rare cases of severe liver damage directly attributed to excessive copper intake have been documented (O'Donohue et al., 1999). Cases of severe overt toxicity such as this, or studies reporting only acute gastrointestinal effects are not considered appropriate for the evaluation of chronic copper toxicity and are not within the scope of this mandate. They are, however, informative on the qualitative manifestation of toxicity when copper homeostasis is overwhelmed or under conditions that compromise the effective sequestration of copper. The studies examining copper homeostasis (Turnlund, 1989; Turnlund et al., 1998, 2005; Harvey et al., 2003) have some design limitations such as the small group size, relatively short durations, male adult subjects and limited dose range. The SC recognises that, despite these limitations, these studies have major strengths such as controlled dose administration, controlled prepared diets with documented nutrition panels, participant housing within the research facility, complete faecal and urinary sample collections and administration of ^{63}Cu or ^{65}Cu labels for a more accurate estimation of copper excretion profile. In addition, unlike

the endpoints that were the basis of previous HBGVs, these studies assess early biological changes in the pathway of copper toxicity that are possible to capture within the duration of the studies. Therefore, the SC concluded that the studies examining copper homeostasis provide evidence of changes in homeostasis that may be predictive of adverse events that would occur if intake is not reduced, as described in the SC HBGV Statement (EFSA Scientific Committee, 2021).

In its assessment of copper intake, the SC also considered evidence of disease progression in WD, a genetic disorder leading to copper retention. Whilst WD pathology is complex, it provides evidence of progressive copper-related hepatotoxicity and copper release with tissue toxicity, notably in the brain and kidneys. Stored copper may be released from hepatic damage of other causation and possibly from other triggers (e.g. of stress and inflammation). Therefore, the amount of time over which these levels of intake could be sustained without toxic effects cannot be determined.

Liver biopsy is still to date the only reliable, albeit invasive method to measure hepatic copper (and is used in diagnosis of WD, ICT cases and in monitoring copper toxicosis in farm animals) and there are no useful, non-invasive biomarkers for monitoring of increased copper body burden in the general population. The SC recognises that an HBGV based on evidence of retention as predictor of future toxicity is conservative and therefore sufficiently protective for most consumers over long-term intake. No additional uncertainty factor is considered necessary in this case.

The available data indicate that potential copper toxicity from copper retention may occur at an uncertain time in adult men at chronic copper intakes of 6–8 mg/day. It is uncertain whether effective adaptation and equilibrium may be reached at intakes of 6 mg/day after 8 weeks (2 months) (Harvey et al., 2003), or at intakes of 8 mg/day after 5 months (Turnlund et al., 2005). Therefore, this evidence suggests that the HBGV should remain below 8 and possibly below 6 mg/day of copper intake, pending new evidence on copper homeostasis at these levels of intake over longer periods of time.

As noted, the intake of 10 mg/day that was previously used as an RP, can no longer be considered a NOAEL since future toxicity following hepatic copper retention might be expected to occur at this intake level over chronic exposure.

Based on the weight of evidence, the SC concludes that no retention of copper is expected to occur with a copper intake of 5 mg/day. The SC established an ADI of 0.07 mg/kg bw, equivalent to 5 mg Cu/day for adults.

Considering the ADI of 0.07 mg/kg bw per day established for the general population, ULs for all age groups will be established by the NDA Panel in line with the NDA Panel Guidance on establishing and applying tolerable upper intake levels for vitamins and essential minerals (EFSA NDA Panel, 2022).

4.2. Exposure to total copper from all sources

In the present Opinion, a step-wise approach was followed to assess the dietary exposure to total copper from all sources and then the contribution of each specific source. Mean dietary exposure to total copper ranged from 0.014 mg/kg bw per day in older people to 0.084 mg/kg bw per day in infants. The 95th percentile of dietary exposure to total copper ranged from 0.024 mg/kg bw per day in adults and elderly to 0.155 mg/kg bw per day in infants.

The dietary exposure to total copper was compared with other exposure estimates from the past 10 years in Europe based on total diet studies (TDS). For adults TDS from Europe (Kalonji et al., 2015; Perelló et al., 2015; Marín et al., 2017; Kolbaum et al., 2019), the copper intakes ranged between 0.015[20] mg/kg bw per day (Perelló et al., 2015) and 0.02820 mg/kg bw per day (Kalonji et al., 2015) and were in the same order of magnitude as the exposure estimates presented in this Opinion.

For children, data from three European TDSs (Kalonji et al., 2015; Perelló et al., 2015; Boon et al., 2022)[21] were used for the comparison. Perelló et al. (2015) determined intake values of 0.055 mg/kg bw per day for 4- to 9-year-old children in Catalonia. Children aged 3–6 years in France have an average intake of 0.030 mg/kg bw per day, children aged 7–10 years of 0.040 mg/kg bw per day, children from 11 to 14 years of 0.024 mg/kg bw per day and adolescents from 14 to 17 years of 0.018 mg/kg bw per day (Kalonji et al., 2015). At the P95 3- to 17-year-old children in France achieve an intake value of 0.074 mg/kg bw per day (Kalonji et al., 2015). In a recent TDS among Dutch children aged 1 and 2 years, the average intake of copper was estimated at 0.04 mg/kg bw

[20] Values reported as mg/day were divided by the EFSA default body weight for adults of 70 kg body weight (EFSA, 2012).
[21] All values for children are calculated by using EFSA default body weights for the respective age groups, for the age group 3–17 years the lowest of the defaults was used as conservative approach (EFSA, 2012).

per day, and the P95 at 0.07 mg/kg bw per day (Boon et al., 2022). Due to the different age groups, the intake estimates are not directly comparable. However, they indicate similar orders of magnitude.

The main contributing food categories (at level 1 of the FoodEx2 classification) to the dietary exposure to total copper across the different age groups and all surveys were 'Grains and grain-based products' (2–44%), 'Fruit and fruit products' (2–24%), 'Meat and meat products' (< 1–21%), 'Vegetables and vegetable products' (2–24%), 'Coffee, cocoa, tea and infusions' (< 1–21%), 'Food products for young population' (1–57%) and 'Milk and dairy products' (2–33%). Within these food categories, the food subcategories at FoodEx2 level 3 that contributed more than 5% in at least two dietary surveys were selected to estimate, when feasible, the contribution of regulated uses of copper (PPPs, food additive, nutrient use, feed additive and fertiliser) to the dietary exposure to total copper.

On the contribution of copper use as a PPP to the dietary exposure to total copper, note that the available monitoring data of copper that were used to estimate the dietary exposure to total copper from all sources are in the range of copper concentrations in control (untreated) crops reported in trial studies, rather than the concentrations in treated crops in these trials. This provides evidence that the contribution of copper from its use as a PPP to the overall dietary exposure to copper can be considered negligible.

Exposure to copper through food additives [Cu-chlorophylls E141(i) and Cu-chlorophyllins E141(ii)] used in fine bakery wares and breakfast cereals contributed on average less than 0.7% to the total dietary copper exposure from all sources, across all population groups, and can thus also be considered negligible.

It was found that infant formula and follow-on formula are important contributors to dietary exposure of total copper in infants (12–39% across surveys) and toddlers (1–19% across surveys). This contribution includes all sources of copper in these two formulae, such as its natural occurrence in the main formula ingredients (e.g. cow milk) and copper added in order to achieve a total copper content in formula which is in the range of the regulatory limits and human breast milk levels.

Assuming a brand loyalty consumer exposure scenario with the maximum concentration of regulatory limits for added copper in infant formula and follow-on formula (up to 700 μg/L) resulted in an increase of 10% of the maximum of the mean (up to 0.1 mg/kg bw per day) and of 20% of the maximum of the P95 (up to 0.18 mg/kg bw per day).

It was not possible to assess the contribution of copper exposure from its use as a nutrient source in food fortification and food supplements in the EU due to lack of sufficient data. In national food consumption surveys in Germany (NVS II report 2012[22]), Finland (FinDiet report 2018[23]) and Poland (Stoś et al., 2021), mean/median copper intakes from food supplements were between 0.4 and 0.5 mg/day among supplement users, representing 30% of total copper intake. The Dutch national food consumption survey (RIVM, 2020[24]) reported a mean contribution of dietary supplements to total copper intake of 5% among whole population. Data on the contribution of fortified foods to copper intake in EU populations are lacking.

It was not possible to evaluate the contribution of copper from animal feed. Although the use of copper as feed additive can have an impact on the copper levels in mammal liver, this was mostly evident at copper concentrations in feed exceeding the maximum total copper content authorised in feed in the EU. EU legislation regulates the maximum total copper amount in feed, and therefore, the contribution of copper from feed additives will not lead to a higher dietary exposure to total copper of the consumer from consuming products of farm animals, provided they comply with the EU regulatory maximum total copper levels. The contribution to the dietary exposure to total copper from mammal liver was above 5% in nine surveys and seven countries, and up to 13% across age groups.

For the main food categories reviewed that could be affected by the presence of copper in the soil derived from the use of copper in fertilisers or PPPs, namely grains and grain-based products, pome fruits and coffee, it is noted that copper concentrations are not affected by short-term changes in copper soil concentration due to homeostatic control on copper uptake. However, over a longer time period (100 years), results suggest that copper uptake by crops can be affected by increasing concentrations of copper in the soil although at a lower rate compared to the increase in soil concentrations.

The contribution of copper from use of copper pipes through drinking water has not been evaluated in this assessment. Use of copper pipes is country and region specific. A study in the Netherlands has shown that exposure to copper may be significantly higher if the water pipes in old

[22] Available at https://www.dge.de/wissenschaft/ernaehrungsberichte/ernaehrungsbericht-2012/?L=0
[23] Available at https://urn.fi/URN:ISBN:978-952-343-238-3
[24] Available at https://www.rivm.nl/bibliotheek/rapporten/2020-0083.pdf

houses have not been replaced (built before the 1960s). The public is advised to replace the pipes as soon as possible.

Based on all available information on the uses and sources of total copper exposure, the contribution of copper from non-oral sources can be considered negligible for the general population compared to dietary exposure.

Following the EFSA Statement on establishing HBGVs for nutrients that are also regulated products, the critical endpoint selected to establish the HBGV for copper is retention of copper which is expected to result in potential toxicity in adults after prolonged exposure. The SC notes that dietary exposure to total copper does not exceed the HBGV in adolescents, adults, elderly and the very elderly.

The SC also notes that there is some exceedance of the HBGV especially at the higher end of exposure ranges in the young population (infants at the maximum of the mean range in two dietary surveys, infants and toddlers at the P95 in all dietary surveys and in children at p95 in most the dietary surveys).

It is noted that for these age groups exceeding the HBGV, infant and follow-on formula contributed 12–39% across surveys to dietary exposure of copper in infants and 1–19% in toddlers. In addition, it is also noted that mammal liver, whose copper concentration is affected by the use of copper in feed, contributed from 3% to 5 % in three surveys for toddlers and one survey for infants. No other food categories that were identified as main contributors to dietary exposure to total copper seemed affected by regulated use of copper.

The contribution of infant formula and follow-on formula to dietary exposure to total copper in infants and toddlers has been examined closely in this assessment. The regulatory limits of copper in infant and follow-on formulae are set to match the range of copper concentrations reported in breast milk (EFSA NDA Panel, 2014). Breast milk copper level is a reliable standard against which to regulate infant and follow-on formulae products. This is based on evidence that copper levels in breast milk are tightly regulated and are independent of the mother's dietary exposure to copper (even of WD status) as long as there is sufficient copper supply.

Evidence for higher copper requirements in infants is consistent with the following observations: (i) copper absorption increases linearly with increasing intake in infants (Dorner et al., 1989; Lönnerdal, 1998); (ii) fetal hepatic pools (mostly built during the third trimester) are reduced rapidly during the postnatal phase, indicating that the requirement for copper in postnatal life is only partly met by breast milk copper content and is complemented with copper stored in the fetal liver (Lönnerdal, 1998; Olivares et al., 2000) and (iii) exclusive postnatal feeding with non-supplemented infant formulae (for over 6 months) leads to copper deficiency in infants (Lönnerdal, 1998, 2007).

Similarly, copper utilisation in children is also higher than adults to meet the higher nutrient demands during the age range of physical growth. There is uncertainty about the levels of copper intake that begin to exceed the higher requirements for growth in children. However, considering that dietary copper intake is variable, it is not expected to be maintained at levels that continuously exceed nutritional requirements throughout childhood.

As a consequence, the SC concluded that the exceedance of the HBGV in younger age groups would not pose a lifetime risk for copper toxicity and therefore, are not considered to be of concern.

4.3. Uncertainty analysis

This Opinion includes 'case-specific' assessment methods with elements of standardised uncertainties, according to the categorisation detailed in the EFSA Guidance on Uncertainty (EFSA Scientific Committee, 2018b). The procedure described in Figure 4 for case-specific uncertainty assessments in Section 4 of the Guidance was followed. The uncertainty analysis is divided into the two main parts of the assessment (Step D, in Figure 4), namely copper hazard assessment (Table D.1, Appendix D) and copper dietary exposure assessment (Table D.2, Appendix D). The quantities of interest in the hazard and exposure assessment are the HBGV and the dietary exposure in the general population, respectively. Of those, the HBGV is a non-variable quantity, by virtue of the process by which it has been established, and the exposure is a variable quantity characterised by a mean and 95th percentile of exposure in different population age groups (Step F, in Figure 4).

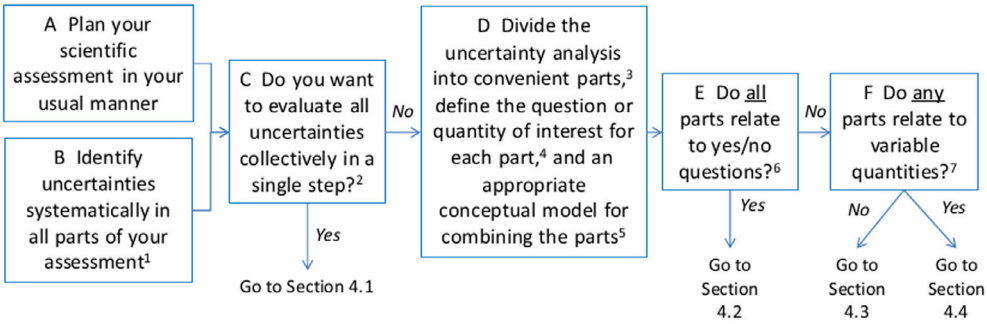


Figure 4: Deciding how to handle uncertainty in a case-specific assessment. Numbered superscripts refer to text notes following the figure in the original document (Guidance on Uncertainty Analysis in Scientific Assessments, EFSA Scientific Committee, 2018b). Letters (A,B, C, etc.) are to facilitate reference to specific steps in the figure. See original document for additional details

The procedures described in sections 7, 8 and 9 of the EFSA 2018 Guidance on Uncertainty analysis were followed to (i) identify uncertainties in each of the two main parts, (ii) further group uncertainties, when possible, according to their source and (iii) prioritise uncertainties according to their impact on the respective assessment (hazard or exposure). Specifically, uncertainties in hazard assessment (Table D.1, Appendix D) were identified and prioritised in two parts: (A) relationship between dietary exposure and hepatic retention and (B) relationship between hepatic copper content and toxicity. Uncertainties in exposure assessment (Table D.2, Appendix D) were also identified and prioritised in two parts: (A) uncertainties related to the total dietary exposure in the general population and (B) uncertainties related to the assessment of the contribution of each source of copper to the total dietary exposure. Prioritisation was based on the combined level of the magnitude of each identified uncertainty and its potential impact on the quantity of interest (HBGV or exposure).

The major uncertainties, i.e. those of highest priority, were reformulated into assessment questions and were further analysed semi-quantitatively based on the contributing LOE following the procedures described in sections 10, 11 and 12 of the EFSA 2018 Guidance on Uncertainly analysis. The magnitude of each uncertainty was evaluated semi-quantitatively using expert judgement following the procedure presented in Tables B.12 and B.13 of the EFSA 2018 Scientific Opinion on the principles and methods for uncertainty analysis (EFSA Scientific Committee, 2018a). The likelihood of each uncertainty question to be true was assessed based on the expert judgement of the members of the WG according to their area of expertise using semi-quantitative probability bounds according to the approximate probability scale recommended for harmonised use by EFSA (Table 2 in EFSA Scientific Committee, 2018b).

4.3.1. Uncertainty characterisation for copper hazard assessment in adults

Uncertainties that were assigned high priority in the prioritisation stage (Table D.1, A and B, Appendix D) are further assessed for their direction and impact on specific assessment questions and on the HBGV. Other uncertainties assigned with low/moderate priority would not substantially impact the HBGV if the latter is established on the basis of the high priority uncertainties.

Uncertainties associated with copper hazard assessment at the established HBGV for copper of 0.07 mg/kg bw per day (5 mg/day) in the general population were assessed for adults separately than for children. This separation was considered necessary for a nutrient such as copper that has physiological functions because of differences in metabolic activity and growth in children.

The following sequence of assessment questions was included in the semi-quantitative uncertainty analysis of high priority uncertainties (see Appendix D), based on the probability scale shown in Table 2 in EFSA Scientific Committee (2018b):

1) Is copper sequestered in the liver at levels of exposure above those that meet physiological functions (i.e. above the adequate intake)?
2) Does copper that remains effectively sequestered in the liver cause hepatotoxicity?
3) Is copper hepatic toxicity dependent on hepatic copper retention?
4) Is copper extrahepatic toxicity dependent on release of retained hepatic copper?

5) Does copper exposure up to 0.07 mg/kg bw per day (5 mg/day in adults) lead to hepatic copper retention?
6) Does copper exposure up to 0.07 mg/kg bw per day (5 mg/day in adults) lead to copper toxicity?

4.3.2. Uncertainty characterisation for copper hazard assessment in children

The following sequence of assessment questions were included in the semi-quantitative uncertainty analysis for children (see Appendix D):

1) Does copper exposure up to 0.07 mg/kg bw per day lead to hepatic copper retention in children?
2) Does copper exposure up to 0.07 mg/kg bw per day lead to copper toxicity in children?
3) Does the estimated copper exposure in children at the highest end of the P95 range relative to the HBGV of 0.07 mg/kg bw per day lead to hepatic copper retention?
4) Does the estimated copper exposure in children at the highest end of the P95 range relative to the HBGV of 0.07 mg/kg bw per day lead to copper toxicity?

4.3.3. Uncertainty characterisation for copper exposure assessment

Most uncertainties in the exposure assessment were assigned low priority and no uncertainty was assigned a high priority in the prioritisation stage (Table D.2, A and B, Appendix D). Hence, uncertainties assigned moderate priority are further assessed for their impact on the overall exposure assessment question. The uncertainties assigned low priority would not substantially impact the exposure.

The following assessment question was included in the semi-quantitative uncertainty analysis (see Appendix D), based on the moderate priority uncertainty:

1) Is copper exposure underestimated for the general EU population, including all age groups?

4.3.4. Summary of uncertainty analysis

In summary, there is little uncertainty on whether copper is sequestered in the liver above levels required for physiological functions or whether high copper exposure causes hepatic and/or extrahepatic toxicity: It is extremely likely (95–99% probability) to almost certain (99–100% probability) that copper is sequestered in the liver above levels required for physiological functions, and extremely unlikely (1–5% probability) that copper that remains effectively sequestered in the liver causes hepatotoxicity. It is also extremely likely (95–99% probability) that both hepatotoxicity and extrahepatic toxicity are dependent on hepatic copper retention and/or release of retained hepatic copper, but there is higher uncertainty about the conditions leading to local hepatocellular toxicity or conditions triggering copper release from the liver (triggers of copper release from the liver are variable and unpredictable, both in nature and timing). Based on these uncertainties, the WG considered that hepatic copper retention is a conservative biological basis upon which to establish the HBGV for copper. Hence, the available evidence obtained from copper balance studies in humans that copper retention is observed at 6 mg/day for up to 2 months of observation period and at 8 mg/day for up to 5 months of observation period, was weighed against the two previously established HBGVs (5 mg/day and 10 mg/day). In conclusion, it is very unlikely (5–10% probability) that copper exposure up to the ADI of 0.07 mg/kg bw per day (5 mg/day in adults) leads to copper retention in the liver and very unlikely (5–10% probability) that such level of copper exposure leads to copper toxicity.

Copper hazard assessment in children takes into account additional LOE that have an impact on the level of uncertainty associated with the application of the HBGV. Specifically, due to higher nutrient requirements related to growth in children, it is extremely unlikely (1–5% probability) that copper exposure up to the ADI of 0.07 mg/kg bw per day leads to hepatic copper retention or that it leads to copper adverse effects. It is also very unlikely (5–10% probability) that the estimated copper exposure in children at the highest end of the P95 range relative to the HBGV of 0.07 mg/kg bw per day leads to copper hepatic retention and toxicity.

Last, it is very unlikely to extremely unlikely (5–10%, to 1–5% probability) that the total dietary exposure to copper has been systematically underestimated for the adult general population in the EU. On the other hand, it is more likely than not (> 50% probability) that the total dietary exposure to copper in children population may be underestimated. Moreover, it was concluded that dietary

exposure estimates of copper for specific subpopulations (e.g. regular consumers of crops treated with copper, regular consumers of fortified foods or food supplements containing copper or those using copper cooking cookware and utensils) may be higher than the exposure estimated for the adult general population.

5. Conclusion

In line with the SC Statement on the derivation of HBGVs for regulated products that are also nutrients (EFSA Scientific Committee, 2021), the SC considers that the RP (10 mg/day) previously used to establish an UL or an ADI (SCF, 2003; EFSA, 2018b), can no longer be considered an NOAEL. Consistent with the biological model of a sequence of effects leading to adversity for substances such as copper, evidence of positive retention is indicative of potential future toxicity resulting from copper retention. Potential copper toxicity is likely subject to multifactorial triggers of copper release or localised injury (e.g. redox status), even in the absence of direct liver injury reported in the available human studies reviewed here. Therefore, the absence of hepatic accumulation is considered protective against copper toxicity regardless of the triggers. The SC considers that the studies by Turnlund et al. (2005) and Harvey et al. (2003) provide critical evidence for the establishment of HBGV for copper. Based on the weight of evidence and the uncertainties identified, the SC considers that the previously established UL of 5 mg/day for adults is sufficiently protective as it remains below the levels where copper retention has been observed. Accordingly, the SC established an ADI of 0.07 mg/kg bw, which is considered conservative and sufficiently protective for all age groups. The SC notes that the newly established ADI replaces the previous ADI of 0.15 mg/kg bw (EFSA, 2008, 2018b).

The SC concluded that the dietary exposure to total copper in the general population does not exceed this ADI. However, for specific subpopulations of regular consumers of foods with higher copper content, the exposure may have been underestimated. The SC also concluded that the exceedance of the ADI in younger age groups would not pose a lifetime risk for copper toxicity and therefore is not considered to be of concern.

6. Recommendations

The SC recommends further research on markers of homeostatic and adaptive responses to high copper intakes for all population subgroups.

The SC recommends to generate more data for the dietary exposure assessment of copper for regular consumers of crops treated with copper, fortified food or food supplements containing copper or using copper cooking utensils in order to allow for a refined exposure assessment for these specific population groups and to verify if there is any safety concern arising from their current use and consumption patterns.

The SC noted that the copper concentration in mammal liver, a main food contributing to the dietary exposure to total copper, substantially exceeds the current MRLs. This exceedance was also highlighted by the 2016 FEEDAP Panel Scientific Opinion and the 2018 EFSA Opinion on MRL review for copper as PPP.

The SC noted that the use of copper as a PPP and copper in fertilisers will lead to long-term increases of copper in the soil with potential impact on copper concentrations in certain crops. Therefore, continued monitoring of copper in soil is recommended.

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Abbreviations

ADI	Acceptable Daily Intake
AI	Adequate Intake
AROI	Acceptable Range of Oral Intake
Cu	copper
DRV	Dietary Reference Value
ETE	essential trace elements
GSH	glutathione
HBGV	Health-Based Guidance Value
IPCS	International Programme on Chemical Safety (World Health Organization)
LOAEL	Lowest Observed Adverse Effect Levels

LOE	line of evidence
MDA	malondialdehyde
MT	metallothionein
NDA Panel	Panel on Nutrition, Novel Foods and Food Allergens
NOAEL	no-observed-adverse-effect level
POD	Point of Departure
RP	reference point
SCF	Scientific Committee on Food
SOD	superoxide dismutase
TAC	total antioxidant capacity
UF	uncertainty factor
UL	tolerable upper intake level
WHO	World Health Organization
Zn	zinc

Appendix A – Overview of previous assessments

A.1. Health-based guidance values for adults

Ref. HBGV type	HBGV		Critical endpoint	Based on	NOAEL	UF and rationale	Critical studies
	mg/day	mg/kg bw per day					
(WHO, 1996) Upper limit to the safe range of intake	M: 12[(a)] F: 10[(a)] Including pregnant & lactating	0.18 (65 kg) 0.18 (55 kg)	NR	Human data	10 mg/day	1	No references cited
(IPCS, 1998) Upper limit to the Adequate Range of Oral Intake (AROI)	Uncertain > 2 or 3	–	–	Human data	Data inadequate to establish effect levels	NA	H: (Wyllie, 1957; Spitalny et al., 1984; O'Donohue et al., 1993; Knobeloch et al., 1994)
(IOM, 2001) Tolerable Upper Intake Level (UL)	10 Including pregnant & lactating	–	Liver damage	Human clinical studies	10 mg/day (Pratt et al., 1985)	1 NOAEL protective of the general population; 'larger UF considered unnecessary as the large international database in humans indicated no adverse effects from daily consumption of 10–12 mg/day of Cu in foods *[No refs cited]* and due to the rarity of observed liver damage from Cu exposure in human population with normal Cu homeostasis'	H: (Pratt et al., 1985; O'Donohue et al., 1993)
(SCF, 2003) Tolerable Upper Intake Level (UL)	5 Excluding pregnant & lactating (lack of data)	–	Liver damage	Human clinical studies	10 mg/day (Pratt et al., 1985) 7 mg/day (O'Connor et al., 2003)	2 Decrease in copper absorption as copper intake increases; putative indices of status (plasma Cu, erythrocyte SOD, caeruloplasmin and urinary Cu excretion) resistant to change except under extreme	H: (Pratt et al., 1985; O'Donohue et al., 1993; Baker et al., 1999; Rock et al., 2000; Turley et al., 2000; O'Connor et al., 2003)

Ref. HBGV type	HBGV		Critical endpoint	Based on	NOAEL	UF and rationale	Critical studies
	mg/day	mg/kg bw per day					
						dietary conditions (Turnlund et al., 1990, 1991) 'UF of 2 adequate to allow for potential variability within the normal population'	
(EVM, 2003) Safe upper level	10	0.16	R: Damage to the forestomach, kidney and liver H: Liver damage	90-day study in rats; Supported by human clinical studies	R: 16 mg/kg bw per day (Hebert, 1993) H: 10 mg/day (Pratt et al., 1985) 7.5 mg/day (Turnlund, 1989)	R: 10 (interspecies) x 10 (intraindividual) H: 1	R: (Hebert, 1993) H: (Pratt et al., 1985; Turnlund, 1989; Olivares et al., 1998; Pizarro et al., 1999)
(ATSDR, 2004) Chronic oral Minimal Risk Level	–	–	–	Animal and human data	database inadequate for the derivation of a chronic oral minimal risk level	NA	Mice: (Massie and Aiello, 1984)
(France, 2007a) Acceptable Daily Intake (ADI)	–	0.15	D: elevation of SGPT (= ALT) R: liver and kidney damage H: liver damage	1-year study in dogs Supported by 90-day study in rats and human clinical studies	D: 15 mg/kg per day (Shanaman et al., 1972) R: 16 mg/kg bw per day (Hebert, 1993) H: 10 mg/day (Pratt et al., 1985)	D: 100 R: 100 H: 1 'the population has a uniform genetic profile for copper metabolism, in that the proteins for copper transport are very uniform, not only in the human population, but also in most life-forms, and in that genetic abnormalities which result in diseases of copper metabolism are both extremely rare and (if untreated) fatal. It is logical to conclude that as there is regular exposure and that as defects in sensitivity to copper result in	D: (Shanaman et al., 1972) R: (Harrisson et al., 1954; Hebert, 1993; Mylchreest, 2005) H: (Pratt et al., 1985)

Ref. HBGV type	HBGV		Critical endpoint	Based on	NOAEL	UF and rationale	Critical studies
	mg/day	mg/kg bw per day					
						dramatic (lethal) illness, the vast majority of the population are similar, and the traditional safety factor of 10 for individual variation could be considered as too high'	
(EFSA, 2008) Upper limit[(b)]	–	0.15	NS	WHO (1996) based on human data Supported by 1-year dog study	H: 0.2 mg/kg per day in adults and 0.15 mg/kg per day in children D: 15 mg/kg bw per day in a 1-year dog (Shanaman et al., 1972)	H: 1 D: 100	H: (WHO, 1996) D: (Shanaman et al., 1972)
(Nordic Council of Ministers, 2012)	5	–	NA	(SCF, 2003)	NA	NA	NA
(VKM, 2017) Tolerable Upper Intake Level (UL)	5	–	NA	(SCF, 2003)	NA	2 'UF suitable because human data are limited, the uncertainty of the copper content of drinking water and the potential severe and irreversible adverse effects'	NA
(France, 2017) Acceptable Daily Intake (ADI)	–	0.15	Liver damage	WHO (1996) based on human data; Consistent with ULs from other bodies based on human clinical studies	H: 0.2 mg/kg per day in adults and 0.15 mg/kg per day in infants	H: 1	H: (WHO, 1996; IPCS, 1998; IOM, 2001; EVM, 2003)
(EFSA, 2018b) Acceptable Daily Intake (ADI)[(c)]	–	0.15	Liver damage	WHO (1996) based on human data; Supported by 90-day rat study	H: 0.2 mg/kg per day in adults and 0.15 mg/kg day in infants R: 16 mg/kg per day	H:1 'It was noted that an upper limit for copper as a nutrient had been established in an opinion of the EU Scientific Committee for Food (SCF, 2003), based on a NOAEL of 10 mg Cu/day value but adding	H: (WHO, 1996) R: (Hebert, 1993)

Ref. HBGV type	HBGV		Critical endpoint	Based on	NOAEL	UF and rationale	Critical studies
	mg/day	mg/kg bw per day					
						an uncertainty factor of 2, resulting in a proposed tolerable upper intake level of 5 mg Cu/day for adults (corresponding to half of the ADI currently set in the PPPs area). This approach was considered by the Peer Review in 2008 as inadequate for setting an ADI in the area of pesticides (France, 2007b,c). It was considered at that moment that there is greater risk of health effects from deficiency of copper intake than from excess of copper intake (WHO, 1998), there is no evidence of significant variability within humans and the additional uncertainty factor does not take into consideration the natural homeostatic mechanisms that regulate copper. During the current Peer Review, the experts have confirmed the previous assessment, and no changes in the ADI are proposed.' R: 100	

ADI: acceptable daily intake; ALT: alanine aminotransferase; D: dog; F: females; GI: gastrointestinal effects; H: humans; HBGV: health-based guidance value; M: males; NA: not applicable; NOAEL: no-observed-adverse-effect level; NR: not reported; NS: not specified; R: rats; SGPT: serum glutamic-pyruvic transaminase SOD: superoxide dismutase; UF: uncertainty factor; UL: tolerable upper intake level; WHO: World Health Organization.

(a): 'This will take account of the quantity likely to be consumed from the usual diet (< 10 mg/day) and will limit both the amount of copper that can be introduced by dietary fortification and the quantity of contaminating copper that can be regarded as tolerable.'

(b): 'It was felt by the meeting of experts that the term 'ADI' was not adequate to copper as an essential micronutrient essential for life; the term 'upper limit' was considered as more appropriate.'

(c): 'It should be noted that it was felt by the experts that the term 'ADI' was not fully adequate to copper as a micronutrient essential for life; the term 'upper limit' used in the nutrient area would be more appropriate; therefore in the specific case of copper, the ADI is considered equivalent to an UL.'

A.2. Health-based guidance values for infants and children

Ref. HBGV type	HBGV			Based on	Critical studies
	Age	mg/day	mg/kg bw per day		
(WHO, 1996) Upper limit to the safe range of intake	12–15 years 10–12 years 6–10 years 1–6 years 0–1 year	8 6 3 1.5 –	0.16 (48 kg) 0.16 (37 kg) 0.12 (25 kg) 0.09 (16 kg) 0.15	Extrapolating adult upper limit based on relative body weight. 'Lower population maximum mean intakes have been suggested for young children (1–10 years) because of their potentially higher exposure to non-dietary sources of copper and because of suspicions that some contaminants of food or drinking water may promote hepatic copper accumulation in this age group'	No refs cited
(IOM, 2001) Tolerable upper intake level (UL)	14–18 years 9–13 years 4–8 years 1–3 years Infants	8 5 3 1 No UL (lack of data)	–	Extrapolating adult UL values for children based on relative body weight. 'Liver damage in children having defects in copper homeostasis'	
(SCF, 2003) Tolerable upper intake level (UL)	15–17 years 11–14 years 7–10 years 4–6 years 1–3 years Infants	4 4 3 2 1 No UL	–	Extrapolating adult UL values for children based on relative body weight. Values consistent with Scheinberg & Sternlieb (1994) and Dieter et al. (1999) 'Liver damage in children appears to be restricted to children with a predisposition for enhanced copper toxicity.'	(Scheinberg and Sternlieb, 1994; Dieter et al., 1999)
(EFSA, 2008) Upper limit	All	–	0.15	'Based on the values established by the WHO for copper intake (1996) based on human data in children (adults: 0.2 mg Cu/kg bw per day and children: 0.15 mg Cu/kg bw per day), and supported by animal data (1-year dog study, 1982) with a NOAEL of 15 mg Cu/kg bw per day'	(WHO, 1996)
(France, 2017) Acceptable Daily Intake (ADI)	All	–	0.15	Same as adults. 'Considered as sufficiently protective for children and is supported by epidemiological studies (Scheinberg and Sternlieb, 1994) and (Zietz et al., 2003) which demonstrated the absence of effects on children (0–5 years) up to 8 mg Cu/L in water. This value corresponds to 0.23 mg Cu/kg bw per day taking into account a body weight of 15 kg and a mean water consumption for young children of 400 ml/day (derived from the 1988–1994 Third	(Scheinberg and Sternlieb, 1994; Zietz et al., 2003)

Ref. HBGV type	HBGV			Based on	Critical studies
	Age	mg/day	mg/kg bw per day		
				National Health and Nutrition Examination Survey (NHANES III) water consumption data)'	
(EFSA, 2018b) Acceptable Daily Intake (ADI)	All	–	0.15	'Values established by the WHO for copper upper level intake (WHO, 1996) based on human data for infants (adults: 0.2 mg Cu/kg bw per day and infants: 0.15 mg Cu/kg bw per day)'	(WHO, 1996)

Appendix B – Human studies considered in the assessment

B.1. Human intervention studies addressing copper kinetics

Reference	Study population	Duration of study	Form of copper doses	Method	Copper absorption/excretion/ retention	Biochemical markers of copper status
(Turnlund, 1989; Turnlund et al., 1990)	11 young M (22–35 years)	P1: 24 days P2: 42 days P3: 24 days	P1: 1.7 mg/day total Cu (^{65}Cu fed on day 13) P2: 0.8 mg/day total Cu (^{65}Cu fed on days 7 and 8 and days 31 and 32) P3: 7.5 mg/day total Cu (^{65}Cu fed on day 13) Food and formula in the diet contained 0.4 mg Cu; additional Cu provided as $CuSO_4$ (liquid formula)	Complete urine and stool collections were made throughout the study Cu absorption and retention (using ^{65}Cu)	Mean ± SEM (range) % ^{65}Cu absorption P1: 36.3 ± 1.3 (30.2, 42.1) P2 (early; late): 56.2 ± 1.1 (48.9, 61.3); 55.0 ± 1.5 (46.0, 60.3) P3: 12.4 ± 0.9 (7.4, 16.4) Cu absorption (mg/day) P1: 0.61 ± 0.022 P2 (early; late): 0.441 ± 0.009; 0.43 ± 0.012 P3: 0.93 ± 0.068 Endogenous faecal loss (mg/day) P1: 0.61 (average day 7 to day 24 of P1) P2 (early; late): 0.36 (average day 7 to day 24 of P2); 0.33 (average day 25 to day 42 of P2) P3: 0.97 (average day 7 to day 24 of P3) Retention (mg/day) (average over each metabolic period) P1: 0.167 ± 0.40 (–0.046, 0.308) P2: 0.002 ± 0.034 (–0.255, 0.125) P3: 0.941 ± 0.160 (0.128, 1.97) P3: balance was strongly positive at first and decreased linearly with time (became negative at the end of the period); the 24-day period was insufficient for men to equilibrate.	Plasma Cu, CP and eSOD: no effect Salivary Cu: no effect

Reference	Study population	Duration of study	Form of copper doses	Method	Copper absorption/excretion/retention	Biochemical markers of copper status
(Harvey et al., 2003)	12 M (20–59 years)	P1: 8 weeks P2: 8 weeks P3: 8 weeks ≥ 4 weeks washout between periods	P1: 1.6 mg/day total Cu (^{65}Cu administered orally on day 42) P2: 0.7 mg/day total Cu (^{65}Cu administered orally on day 42) P3: 6.0 mg/day total Cu (^{65}Cu administered orally on day 42) During all periods, diet contained 0.7 mg/day Cu; additional Cu provided as $CuSO_4$ (liquid formula)	Cu absorption and retention (using ^{65}Cu) Apparent Cu absorption: (labelled Cu dose–labelled Cu in faeces)/labelled Cu dose True absorption = Apparent Cu absorption corrected for endogenous Cu loss Retention = unlabelled dose, i.e. unlabelled Cu in faeces	Mean ± SD % ^{65}Cu apparent/true absorption (%) P1: 42 ± 15/45 ± 14 P2: 41 ± 12/48 ± 13 P3: 45 ± 13/48 ± 11 Cu apparent/true absorption (mg) P1: 1.27 ± 0.44/1.36 ± 0.41 P2: 1.23 ± 0.35/1.45 ± 0.39 P3: 1.34 ± 0.38/1.45 ± 0.33 Faecal Cu (mg/day) (unlabelled Cu recovered 14 days after label administration) P1: 1.6 ± 0.31 P2: 0.83 ± 0.32 P3: 5.25 ± 1.05 ^{65}Cu endogenous loss (%) P1: 38 ± 20 P2: 30 ± 15 P3: 40 ± 11 Total endogenous loss (slow and fast pools) (mg/day) P1: 0.81 ± 0.16 P2: 0.45 ± 0.25 P3: 2.46 ± 1.11 Retention (mg/day) (at the end of the period) P1: 0.00 ± 0.31 P2: −0.13 ± 0.32 P3: 0.75 ± 1.05	Plasma Cu, CP and eSOD: no effect
(Turnlund et al., 1998)	11 M (mean 26 ± 4 years)	P1: 24 days P2: 42 days P3: 24 days	P1: 0.66 mg/day (diet copper was replaced with ^{65}Cu in six subjects on days 13 and 14 and 0.34 mg was administered intravenously to five subjects on day 13)	Cu absorption, excretion and retention (using ^{65}Cu) Apparent Cu absorption = amount of ^{65}Cu fed – amount of	Mean % Cu apparent/true absorption (%) P1: 54/73 P2: 67/77 P3: 44/66	None

Reference	Study population	Duration of study	Form of copper doses	Method	Copper absorption/excretion/retention	Biochemical markers of copper status
			P2: 0.38 mg/day, basal dietary content with no added copper (0.2 mg of ^{65}Cu was added to the diet of six subjects on days 31 and 32 and days 55 and 56, and 0.34 mg was administered intravenously to five subjects on day 55) P3: 2.49 mg/day (copper was replaced with ^{65}Cu in the diet given to six subjects on days 79–80 and 0.34 mg was administered intravenously to five subjects on day 79)	^{65}Cu recovered in the stools in a 12-d period after the feeding True absorption = Apparent Cu absorption corrected by the fraction of infused ^{65}Cu excreted over the same time period Retention = average Cu intake – average faecal Cu (averages of entire metabolic period)	Cu apparent/true absorption (mg/day) P1: 0.35/0.48 P2: 0.26/0.29 P3: 1.08/1.64 Faecal ^{65}Cu (% of dose of infused ^{65}Cu excreted in the 12 days after infusion) P1: 26 P2: 12 P3: 34 Faecal Cu (mg/day) P1: 0.65 P2: 0.33 P3: 2.17 Endogenous loss (mg/day) P1: 0.47 P2: 0.24 P3: 1.33 Retention (mg/day) (average over entire metabolic period) P1: −0.13 P2: −0.015 P3: 0.511 (positive balance in first 6 days decreased rapidly afterwards; equilibrium was reached at the end of the period; see text)	
(Turnlund et al., 2005)	9 M (26–49 years)	P1: 18 days P2: 129 days P3: 18 days	P1: 1.6 mg/day (^{63}Cu administered orally to three subjects and intravenously to six subjects on day 7) P2: usual diet + 7 mg/day Cu supplement P3: 7.8 mg/day (^{63}Cu administered orally to three	Complete urine and stool collections were made throughout P1 and P3 Apparent Cu absorption = amount of ^{63}Cu fed – amount of ^{63}Cu recovered in the	Mean % Cu apparent/true absorption (%) P1: 29/40 P3: 16/29 Cu apparent/true absorption (mg/day) P1: 0.48/0.65	eSOD: ↑ P3 vs P1 (P1: 1,065 r ± 47; P3: 1,206 r ± 24 U/g Hb) Plasma benzylamine oxidase activity:

Reference	Study population	Duration of study	Form of copper doses	Method	Copper absorption/excretion/retention	Biochemical markers of copper status
			subjects and intravenously to six subjects on day 7) During periods 1 and 3, diet contained 1.6 mg/day Cu; additional Cu provided as $CuSO_4$ (liquid formula) During P2, subjects consumed supplements that provided 7 mg Cu/day as copper sulfate	stools in a 12-d period after the feeding True Cu absorption = apparent Cu absorption corrected by the fraction of infused ^{63}Cu excreted over the same time period. Total endogenous gastrointestinal losses = total faecal Cu – unabsorbed dietary Cu Cu retention = average Cu intake – average faecal and urinary Cu (averages of the last 12 days of each metabolic period)	P3: 1.2/2.2 Faecal ^{63}Cu (% of dose of infused ^{63}Cu excreted in the 12 days after infusion) P1: 27 P3: 46 Faecal Cu (mg/day) P1: 1.6 P3: 7.1 Endogenous loss (mg/day) P1: 0.58 P3: 1.56 Urinary ^{63}Cu (% of dose of infused ^{63}Cu excreted in the 12 days after infusion) P1: 2.1 P3: 1.3 Urinary Cu (mg/day) P1: 0.0203 ± 0.0008 P3: 0.0256 ± 0.0011 Hair Cu (mg/day) P1: 0.0092 ± 0.0031 P3: 0.0211 ± 0.0029 Retention (mg/day) (average for the last 12 days of each metabolic period) P1: 0.06 P3: 0.67	↑ P3 vs P1 (P1: 283 ± 25; P3: 377 ± 31 U/L) CP activity: ↑ P3 vs P1 (P1: 107 ± 3; P3: 116 ± 5 U/L) Plasma Cu: no effect

↑: significant increase; CP: caeruloplasmin; eSOD: erythrocyte superoxide dismutase; Hb: haemoglobin; M: males; SD: standard deviation; SEM: standard error of the mean.

B.2. Human intervention studies addressing safety endpoints

Reference	Study design and population	Duration of study	Form of copper doses	Safety-related parameters investigated	Potential markers of Cu status	Liver enzymes	Markers of oxidative damage	Other adverse effects
(Olivares et al., 1998)	Double-blinded randomised placebo-controlled trial 128 healthy infants	From 3 to 12 months of age	G1 (N = 48): < 0.1 mg/L water (27 FF; 21 BF) G2 (N = 80): 2 mg/L water (56 FF; 24 BF) FF infants in G1: 0.8 ± 0.5 mg/day Cu at months 4 to 6; 1.2 ± 0.7 mg/day Cu at months 6 to 9; 1.2 ± 0.7 mg/day Cu at months 9 to 12. FF infants in G2: 2.3 ± 0.8 mg/day Cu at months 4 to 6; 2.5 ± 0.7 mg/day Cu at months 6 to 9; 2.4 ± 0.7 mg/day Cu at months 9 to 12. Formula was fortified with Fe, Cu (7.87 μmol/L) and Zn	Serum Cu, CP eSOD, eMT Bilirubin; AST, GGT, SGPT (= ALT) at 6, 9 and 12 months of age Monthly examination for clinical and anthropometric evaluations	Serum Cu, CP: no effect eSOD, eMT: no effect	Bilirubin; AST, GGT, SGPT (= ALT): no effect	–	Growth and morbidity: no effect between groups
(Harvey et al., 2003)	Metabolic study 12 M (20–59 years)	P1: 8 weeks P2: 8 weeks P3: 8 weeks ≥ 4 weeks washout between periods	P1: 1.6 mg/day total Cu P2: 0.7 mg/day total Cu P3: 6.0 mg/day total Cu During all periods, diet contained 0.7 mg/day Cu; additional Cu	Plasma Cu, CP and eSOD Platelet aggregation, packed cell volume, Hb, mean cell Hb concentration Plasma lipoproteins and	Plasma Cu, CP, eSOD: no effect	–	Plasma, erythrocyte, platelet GPx: no effect	Platelet aggregation, plasma lipoproteins (total, HDL-, LDL-cholesterol), triacylglycerols, apo A1 and B: no effect Hb, mean cell haemoglobin concentration and

Reference	Study design and population	Duration of study	Form of copper doses	Safety-related parameters investigated	Potential markers of Cu status	Liver enzymes	Markers of oxidative damage	Other adverse effects
			provided as $CuSO_4$ (liquid formula)	triacylglycerols, Apo A1 and B Plasma, erythrocyte and platelet GPx Plasma ferritin				packed cell volume: no effect
(Baker et al., 1999) (FOODCUE England)	Longitudinal intervention trial 11 healthy M (20–59 years)	8 weeks/period 4 weeks washout between periods	P1: 1.6 mg/day total Cu P2: 0.7 mg/day total Cu P3: 6 mg/day total Cu Controlled diet contained 0.7 mg/day; additional Cu provided as $CuSO_4$ (solution)	Serum Cu, CP Serum osteocalcin (bone formation) Urinary pyridinoline and deoxypyridinoline (bone resorption) Urinary creatinine Blood count (WBC, RBC, Hb, HTC, platelet) Clinical chemistry (Na, K, bicarbonate, urea, creatinine, total bilirubin, total protein, albumin, globulin, LDH, AST, ALT, ALP, GGT, Ca, P, total cholesterol, glucose)	Serum Cu, CP: no effect	NR	–	Biochemical markers of bone metabolism: no effect Urinary creatinine: no effect
(O'Connor et al., 2003) (FOODCUE Northern Ireland)	Double-blind ed crossover trials 24 healthy M and F (22 completed, 11 M and 11 F) (22–45 years)	6 weeks/period	P1: 3 mg/day as $CuSO_4$ P2: placebo P3: 3 mg/day as CuGC P4: placebo P5: 6 mg/day as CuGC P6: placebo	eSOD, WBC SOD Platelet and WBC Cyt *c* oxidase activities Plasma CP Serum DO Liver enzymes (GGT, ALT)	CP, eSOD: no effect Serum DO: ↑ following all three suppl. periods WBC Cyt c oxidase: ↑ following suppl.	GGT, ALT: no effect	DNA damage: no effect (samples lost for 4 to 6 subjects in each period)	–

Reference	Study design and population	Duration of study	Form of copper doses	Safety-related parameters investigated	Potential markers of Cu status	Liver enzymes	Markers of oxidative damage	Other adverse effects
			In addition to background diet; diet contained 1.43 ± 0.1 mg/day Cu in M and 1.03 ± 0.1 mg/day in F	DNA damage (Comet assay, with and without endonuclease)	with 6 mg CuGC/day (data not shown)			
(Rock et al., 2000) (FOODCUE France)	Double-blinded crossover trials 13 healthy M and 13 healthy F (50–72 years)	6 weeks/period 6 weeks washout between periods	P1: 3 mg/day as $CuSO_4$ P2: placebo P3: 3 mg/day as CuGC P4: placebo P5: 6 mg/day as CuGC P6: placebo In addition to background diet; mean Cu intake was ~1.4 mg/day Cu in M and ~1.2 mg/day Cu in F	RBC resistance to oxidation (AAPH-induced oxidation *in vitro* test) Plasma fat-soluble vitamins eSOD	eSOD: no effect	–	RBC oxidation: ↑ half-time (LT50) haemolysis after 3 mg/day $CuSO_4$ and 6 mg/day CuG Plasma tocopherols, retinol, lutein: no effect Plasma lycopene and plasma α and β carotene: ↓ after 3 mg/day CuGC	–
(Turley et al., 2000) (FOODCUE)	Double-blinded crossover trials (4 centres, diff. doses) 24 healthy M and F (22–45 years) [Northern Ireland]	6 weeks/period	Up to 3 mg/day as $CuSO_4$ and up to 6 mg/day as CuGC, in addition to background diet Background diet contained 1.43 mg/day Cu in M and ~1.03 mg/day in F	Serum/plasma Cu (Denmark, France) Plasma CP (all centres) Susceptibility of LDL to *in vitro* induced oxidation (all centres)	Serum/plasma Cu: ↑ after 3 mg/day $CuSO_4$ and 6 mg/day $CuSO_4$ (Denmark); no effect (France) Plasma CP: no effect observed in any centre	–	LDL susceptibility to Cu- or peroxynitrite-induced oxidation: no effect in any centre	–
	Same study 11 healthy M (20–59 years) [England]	8 weeks/period	Up to 6 mg/day total Cu controlled diet contained 0.7 mg/day;					

Reference	Study design and population	Duration of study	Form of copper doses	Safety-related parameters investigated	Potential markers of Cu status	Liver enzymes	Markers of oxidative damage	Other adverse effects
			additional Cu provided as $CuSO_4$ (solution)					
	Same study 16 healthy F (18–30 years) [Denmark]	4 weeks/period	Up to 6 mg/day as $CuSO_4$ In addition to background diet (Cu content ND)					
	Same study 14 M and 14 F (50–72 years) [France]	6 weeks/period	Up to 6 mg/day as CuGC In addition to background diet; mean Cu intake was ~1.4 mg/day Cu in M and ~1.2 mg/day Cu in F (Rock et al., 2000)					
(Turnlund et al., 2004)	Metabolic study 9 M (26–49 years)	P1: 18 days P2: 129 days P3: 18 days	P1: 1.6 mg/day total Cu P2: usual diet + 7 mg/day Cu supplement P3: 7.8 mg/day total Cu During periods 1 & 3, diet contained 1.6 mg/day Cu; additional Cu provided as $CuSO_4$ (liquid formula) During P2, subjects consumed supplements that provided 7 mg Cu/day as $CuSO_4$	Plasma Cu, CP and eSOD, plasma benzylamine oxidase Plasma MDA, urinary TBARS Plasma ascorbate and dehydroascorbate after 1 g vitamin C dose given on day 14 of each period Complete and differential blood cell count Serum IL-2R, IL-6, IgG and C3	eSOD: ↑ P3 vs P1 Plasma benzylamine oxidase activity: ↑ P3 vs. P1 CP activity: ↑ P3 vs. P1 Urinary Cu: ↑ P3 vs. P1 Hair copper ↑ P3 vs P1 Plasma Cu: no effect	–	Plasma MDA: no effect Urinary TBARS: ↑ P3 vs. P1 Plasma ascorbate and dehydroascorbate: no effect	Polymorphonuclear cells: ↓ P3 vs. P1 (total count, % WBC) Lymphocytes: ↑ P3 vs. P1 (total count) IL-2R: ↓ P3 vs. P1 Serum influenza antibody titres: ↓ experimental vs control subjects WBC count, IL-6, IgG and C3: no effect Delayed hypersensitivity skin response test: no effect

Reference	Study design and population	Duration of study	Form of copper doses	Safety-related parameters investigated	Potential markers of Cu status	Liver enzymes	Markers of oxidative damage	Other adverse effects
				Delayed hypersensitivity skin response test (battery of seven recall antigens) All study subjects, along with a control group of 10 subjects, were immunised with a trivalent influenza vaccine 2 weeks before the end of the high copper intake period and antibody titres were measured 14 days after immunisation				
(Pizarro et al., 1999)	Randomised controlled crossover trial (Latin-square design) 60 healthy adult women	2 weeks/period 1 week washout with tap water between periods	Mean ± SD Cu intake from water: P1: 0.04 ± 0.02 (0 mg/L water) P2: 1.74 ± 0.66 (1 mg/L water) P3: 4.68 ± 2.24 (3 mg/L water) P4: 7.94 ± 2.69 mg/day (5 mg/L water) As $CuSO_4$ in water in addition to background diet; average background intake of Cu: 1.7 mg/day	GI symptoms Serum Cu, CP and GGT, ALT, AST Hb	Serum Cu, CP: no effect	GGT, ALT, AST: no effect	–	Hb: no effect Nausea, vomiting, abdominal pain: ↑ for Cu ≥ 3 mg/L Headache, salivation: non-significant ↑

Reference	Study design and population	Duration of study	Form of copper doses	Safety-related parameters investigated	Potential markers of Cu status	Liver enzymes	Markers of oxidative damage	Other adverse effects
(Kessler et al., 2008a)	Prospective, double-blinded phase 2 clinical trial 68 patients with mild Alzheimer's disease; n = 33 in G1 (age 69.5 ± 1.4); n = 35 in G2 (age 70.4 ± 1.1)	12 months	G1: placebo G2: 8 mg/day as Cu-(II)-orotate-dihydrate In addition to background diet (Cu content ND)	Plasma Cu, Zn, CP, liver enzymes. Aβ42, Tau and Phospho-Tau in CSF Cognition test Adverse event recorded	Plasma Cu, CP: no effect	Liver enzymes: no effect	–	Haematological parameters: no effect Alzheimer's disease Assessment Scale (Cognitive subscale) or the Mini Mental Status Examination: no diff between groups
(Pratt et al., 1985)	Double-blinded placebo-controlled trial G1: 7 adults (3 M and 4 F) (mean age 42 years) G2: 7 adults	12 weeks	G1: 10 mg/day CuGC G2: Placebo In addition to background diet (Cu content ND)	Serum Cu; hair Cu HTC, MCV and serum chemistry (triglyceride, cholesterol, AST, GGT, LDH, ALP, K, mg, Zn) Side effects reported	Serum Cu: no effect Hair Cu: no effect	AST, GGT, LDH, ALP: no effect	–	HTC, MCV and serum chemistry: no effect Nausea, diarrhoea, heartburn: no diff between groups

↑: significant increase; ↓: significant decrease; AAPH: 2,29-azo-bis(2-amidinopropane) hydrochloride; ALP: alkaline phosphatase; ALT: alanine transaminase; Apo: apolipoprotein; BF: breast-fed; Ca: calcium; CP: caeruloplasmin; CSF: cerebrospinal fluid; Cyt c: cytochrome c; CuGC: copper glycine chelate; DNA: deoxyribonucleic acid; DO: diamine oxidase; eMT: erythrocyte metallothionein; eSOD: erythrocyte superoxide dismutase; F: females; FF: formula fed; GGT: γ-glutamyl transferase; Hb: haemoglobin; GPx: glutathione peroxidase; HDL: high density lipoprotein; HTC: haematocrit; IgG: Immunoglobulin G; IL: interleukin; K: potassium; LDH: lactate dehydrogenase; LDL: low-density lipoprotein; M: males; MCV: mean corpuscular volume; MDA: malondialdehyde; Na: sodium; ND: not determined; NR: not reported; P: potassium; RBC: red blood cell; SD: standard deviation; SGOT: serum glutamic-oxaloacetic transaminase (= AST: aspartate aminotransferase); SGPT: serum glutamic-pyruvic transaminase (ALT: alanine aminotransferase); SOD: superoxide dismutase; suppl.: supplementation; TBARS: thiobarbituric acid-reactive substances; WBC: white blood cell; Zn: zinc.

B.3. Observational studies

Reference	Study design and population	Duration of study	Form of copper doses	Safety-related parameters investigated	Summary of results
(Dassel de Vergara et al., 1999)	Cross-sectional 956 households with infants and drinking water delivered in Cu pipes	NA	836 households (87.4%): water samples < 0.5 mg/L Cu 83 households: water samples ≥ 0.5 mg/L (including 38 households: ≥ 0.8 mg/L; max 2.6 mg/L)	Subsample of eight infants who were breast-fed for up to 12 weeks or received ≥ 200 mL tap water/day with ≥ 0.8 mg Cu/L during their first 12 months: Examination of the liver by palpation and ultrasound Blood samples (serum Cu, caeruloplasmin, immunoglobulins (IgG, IgM, IgA), transaminases (GOT, GPT), GGT, total bilirubin and CRP)	Liver disease: liver palpation and ultrasound revealed no sign in any child Serum copper values above normal, CRP slightly above normal range: one infant at 8 months; other parameters in the norm
(Dieter et al., 1999)	Retrospective study 103 cases of early childhood cirrhosis in Germany	Between 1984 and 1994	Cu content of drinking water	Histologically confirmed early childhood cirrhosis	5 cases considered as probably related to chronic and excessive intake of copper (coincided with high hepatic copper contents and copper plumbing/ acid well water); 9–26.4 mg Cu/L in water
(Scheinberg and Sternlieb, 1994)	Case reports 7 children (< 2 years) with non-Indian childhood cirrhosis	NA	Cu content of drinking water: 0.05 to 6.8 mg Cu/L		Cirrhosis; evidence of a genetic aetiology in three of the seven infants
	Retrospective study Children (0–5 years) from three Massachusetts towns	64,124 child-years of exposure (between 1969 and 1991)	Cu content of drinking water: 8.5–8.8 mg Cu/L	Records from Massachusetts Department of Public Health	No deaths from cirrhosis or any form of liver disease

Reference	Study design and population	Duration of study	Form of copper doses	Safety-related parameters investigated	Summary of results
(Zietz et al., 2003)	Cross-sectional 2,944 households with infants (Berlin area)	NA	Cu content of drinking water: Composite sample type 1 (aliquots of 100 ml of tap water collected each time it was used in the household): mean 0.44 mg/L; max 3.5 mg/L Composite sample type 2: collection of 250 ml of tap water in the morning, at noon, in the evening and before going to bed: mean 0.56 mg/L; max. 4.2 mg/L 0.8–4.2 mg Cu/L	Infants from families having a Cu concentration ≥ 0.8 mg/L in water samples (29.9% of all sampled households) and who had ingested ≥ 200 mL tap water/day for at least 6 weeks were recommended to undergo a paediatric examination (541 infants eligible): 517 infants were inspected and examined by a physician 183 received a paediatric examination (liver palpation and ultrasound imaging) and blood serum analysis (serum Cu; caeruloplasmin; IgG, IgM, IgA; GOT; GPT; total bilirubin; CRP)	No sign of liver dysfunction (serum GOT, GPT, GGT and serum copper outside the reference range in eight cases of which six had clinically diagnosed infection and one had a liver haemangioma and a ureteric obstruction; abdominal ultrasound imaging slightly unusual in five cases considered likely caused by infection) No signs of a negative health effect found in dose–response analyses of daily and total copper intakes of the infants from tap water and serum GOT, GPT, GGT, total bilirubin, serum copper or caeruloplasmin
(O'Donohue et al., 1999)	Case report 1 subject (26 years)	42 months	30 mg Cu/day for 30 months followed by 60 mg Cu/day for 1 year	Blood and urine samples Physical symptoms	Admitted to hospital after 6-week history of malaise, jaundice and abdominal swelling Acute renal failure Severe liver cirrhosis, necessitating liver transplantation The explanted liver had a copper conc. of 3.230 mg/g dry weight; zinc conc. was normal

CRP: C-reactive protein; GGT: γ-glutamyl transferase; GOT: glutamic-oxaloacetic transaminase; GPT: glutamic-pyruvic transaminase; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; NA: not available.

B.4. Evidence on copper association with Alzheimer's disease

Copper involvement in the development and progression of Alzheimer's disease, independently of WD, has been postulated based on the presence of a copper binding site on the extracellular domain (ECD) of amyloid-beta precursor protein (APP) (Reinhard et al., 2005) and the presence of copper in amyloid plaques (Lovell et al., 1998; Atwood et al., 2002). The APP ECD has close structural homology to copper chaperones (Barnham et al., 2003) and binds copper with nanomolar affinity (Hesse et al., 1994). Release of the amyloid-beta (Aβ) peptide, which spans the APP transmembrane domain (TMD), occurs following proteolytic cleavage of APP through one of two proteolytic pathways, each involving two sequential enzymatic cleavage steps that release soluble ECD peptides and sections of the TMD (Chen et al., 2017). Whilst it is not clear whether copper binding on the ECD is associated with conformational changes that favour proteolytic cleavage via α- or β-secretases (non-amyloidogenic or amyloidogenic cleavage, respectively), one *in vitro* study suggests that copper is associated with the non-amyloidogenic pathway and suppressed the formation of Aβ (Borchardt et al., 1999).

There is conflicting evidence in the literature on the role of copper in the formation of amyloid plaques and it has been a subject of debate in the scientific community. Transgenic animal models of AD and human studies have been used to investigate the effects of copper deficiency and copper supplementation on the progression of AD.

In transgenic animals, copper is reported to either protect against (Bayer et al., 2003; Phinney et al., 2003; Schäfer et al., 2007) or contribute to (Sparks and Schreurs, 2003; Sparks et al., 2006; Sparks, 2007; Quinn et al., 2010; Bourassa et al., 2013; Singh et al., 2013; Harris et al., 2014; Lim et al., 2020; Pilozzi et al., 2020) amyloid plaque formation. Some evidence suggests that copper deposition in Aβ aggregates was the cause rather than the consequence of aggregation (Atwood et al., 1998; Cherny et al., 1999; Atwood et al., 2000; Cherny et al., 2001). Results of the same study (Cherny et al., 2001) were subject to different and conflicting interpretations by opposite sides of the debate: reduced plaques were interpreted as evidence that excess copper contributes to AD and plaque formation (Coelho et al., 2020), whilst increased intracellular copper and soluble copper levels in CNS tissue homogenates were interpreted as evidence that correction of copper deficiency protects from the disease (Kessler et al., 2006, 2008a,b).

In humans, several authors consider the evidence that has been presented as supportive of a protective role of copper against AD (Kessler et al., 2006, 2008a,b; Strozyk et al., 2009). In a single-centre, prospective, double-blinded, placebo-controlled study of AD patients, with parallel-group randomised design, Cu deficiency has been associated with AD progression, where the AD status was assessed using biomarkers (levels of Aβ42, Tau and Phospho-Tau) in cerebrospinal fluid (CSF) (Kessler et al., 2006). In these patients, aberrant CSF biomarker concentrations were associated with significantly reduced plasma copper ($p = 0.014$) and caeruloplasmin ($p = 0.005$) levels. However, only AD patients were included in this study and, as already noted, blood copper levels are not a sensitive indicator of copper status. In another study in AD patients, CSF Cu levels negatively correlated with Aβ1–42 levels (Strozyk et al., 2009). In a Phase II clinical trial, oral supplementation of AD patients with 8 mg Cu for 12 months neither exacerbated the disease, assessed based on CSF biomarker analysis, nor improved cognitive abilities in AD patients (Kessler et al., 2008b). However, neither the timing of copper administration after AD is established nor the timeframe of the study relative to copper homeostasis would be expected to result in a tangible change of AD status under the conditions of this study.

Other authors consider that the available evidence in humans suggests copper contribution to plaque formation and AD progression, such as studies with AD patients reporting an association between elevated levels of free (non-Cp-bound) Cu in blood and declined cognitive performance, reduced CSF amyloid-beta (Aβ), increased CSF tau and accelerated progression of cognitive impairment from mild to clinically diagnosed AD stage (Squitti et al., 2005, 2006, 2011, 2014; Vural et al., 2010; Ventriglia et al., 2012; Wang et al., 2015; see also Brewer, 2008, 2015, 2016). In non-AD elderly, an association has been suggested between copper supplementation and reduced memory and reduced volume of hippocampus (Morris et al., 2006; Salustri et al., 2010; Silbert et al., 2018). Human intervention trials conducted on the basis of copper contribution to AD progression have been inconclusive (Ritchie et al., 2003; Lannfelt et al., 2008) (including erratum). A Cochrane review of the Ritchie et al. (2003) and Lannfelt et al. (2008) human trials concluded that larger studies are needed

to demonstrate the validity of the hypothesis that copper chelation may result in significant improvement of cognitive function and/or delayed disease progression (Sampson et al., 2014).

Overall, a causal relationship of Cu intake with AD remains speculative and inconclusive. Other scientists propose that the body of evidence indicates more subtle mechanisms of potential copper involvement in APP proteolytic processing and amyloid plaque formation, such as intra-/extracellular transport, CNS homeostasis between bound and labile copper pools and possible contribution of APP binding to the export of copper from cells into the extracellular matrix (Treiber et al., 2004; James et al., 2012; Leary and Ralle, 2020). The SC considers that the scientific evidence is not sufficient to conclude that increased copper intake contributes to the development and/or exacerbation of AD and that additional dysregulation of copper homeostatic mechanisms in the brain may be involved. The absence of either improvement or decline in AD patients receiving copper supplementation at 8 mg/day for 1 year (Kessler et al., 2008a) supports this interpretation.

B.5. Genotoxicity and carcinogenicity

B.5.1. Genotoxicity

Previous assessments of copper toxicity concluded that the available evidence does not raise a concern for genotoxicity or carcinogenicity from copper intake in humans (Scientific Committee on Health and Environmental Risks, 2008; France, 2017). No additional relevant studies were identified since the last EFSA assessment in 2018.

Documentation of a direct role of copper in the nucleus, such as copper-dependent nuclear enzymes or transcription factors has not been found, whilst copper binding to DNA is reported *in vitro* in the context of stabilisation of its tertiary structure (Dijkwel and Wenink, 1986; Geierstanger et al., 1991; Burkitt, 1994). Translocation to the nucleus of the cytosolic Cu/Zn-SOD1 has been reported with evidence of gene transcription regulatory activity of oxidative response related genes and Cu/Fe homeostasis genes (Tsang et al., 2014). Increased presence of copper MT in the nucleus has been reported to be correlated with a decrease in cytoplasmic zinc-MT, indicating that metal exchange occurs in the cytoplasm and then Cu(I)-MT translocates to the nucleus. However, the function of MT in the nucleus is currently unknown. It has been hypothesised that, through its redox capacity, labile copper may cause ROS generation in the nucleus and DNA damage based on evidence in cell-free and cell-based *in vitro* assays and *in vivo* (e.g. Scientific Committee on Health and Environmental Risks, 2008; Fragou et al., 2011; Linder, 2012). In addition, despite significant copper entry in the nucleus reported in the mouse WD model, there is no increased HCC pathology in these animals (Huster et al., 2006).

Reverse mutation assays with copper sulfate (Moriya et al., 1983; Singh, 1983; Marzin and Phi, 1985), copper sulfate pentahydrate (Ballantyne, 1994), copper chloride (Tso and Fung, 1981; Wong, 1988), copper oxychloride (Dillon and Riach, 1994a), Bordeaux mix (Dillon and Riach, 1994b) and Nordox copper formulation (Bossotto et al., 2000) have been negative in *Salmonella typhimurium*, *Saccharomyces cerevisiae* and *Escherichia coli*. Induction of DNA strand breaks in rat hepatocytes with copper sulfate (Sina et al., 1983) and Chinese hamster V79 cells with copper nitrate (Sideris et al., 1988), induction of sister chromatid exchange in Chinese hamster V79 cells with copper nitrate (Sideris et al., 1988) and *in vitro* unscheduled DNA synthesis (UDS) in rat hepatocytes with copper sulfate (Denizeau and Marion, 1989) have been reported. Of the *in vitro* genotoxicity assays, three reverse mutation assays were GLP-compliant and OECD 471-compliant (Ballantyne, 1994; Dillon and Riach, 1994a, 1994b); the rest were non-GLP studies.

Negative results were reported with copper sulfate in three *in vivo* studies: a GLP-compliant and OECD 474-compliant micronucleus assay in CD-1 mice following oral administration (114 mg Cu/kg bw) (Riley, 1994); a non-GLP micronucleus assay in CBA mice following intraperitoneal administration (up to 20 mg/kg bw of copper sulfate) (Tinwell and Ashby, 1990); and a non-GLP UDS assay in hepatocytes of Wistar rats after oral administration (up to 509 mg Cu/kg bw) (Ward, 1994). Positive genotoxicity has been reported for copper sulfate in non-GLP studies *in vivo*, including bone marrow chromosomal aberrations (chromatid gaps) and micronucleus assays, erythrocyte micronucleus assay and sperm abnormalities in Swiss mice following intraperitoneal and subcutaneous administrations (up to 20 mg/kg bw of copper sulfate) (Bhunya and Patti, 1987) and intraperitoneal injection of up to 6.6 mg/kg (Agarwal et al., 1990), and in white Leghorn chick following intraperitoneal and oral administration (Bhunya and Jena, 1996) (as described in the voluntary risk assessment by the industry group European Copper Institute, ECI (2007), which was reviewed by rapporteur MS Italy, reported in

ECHA's Voluntary Risk Assessment Reports for copper compounds and evaluated by the European Commission's Scientific Committee on Health and Environmental Risks (Scientific Committee on Health and Environmental Risks, 2008)). All the above studies were previously reviewed and no additional relevant data have been located since the last evaluation (EFSA, 2018b). The results of the studies by Bhunya 1986 and 1987 are not considered reliable due to deviations from acceptable protocols and significant methodological limitations (Scientific Committee on Health and Environmental Risks, 2008). The results of the study by Agarwal et al. (1990) were not reproduced by Tinwell and Ashby (1990).

B.5.2. Carcinogenicity

No evidence for carcinogenicity has been reported in 2-year carcinogenicity and chronic toxicity animal studies at dose levels of copper that resulted in significant hepatic and renal toxicity. These studies included dietary administration of copper sulfate, copper gluconate and potassium sodium copper chlorophyllin (~80 mg Cu/kg bw per day) for 2 years (Harrisson et al., 1954) and 250–300 mg/kg bw per day for 52 weeks in rats (Haywood, 1985; Haywood and Loughran, 1985). Additional evidence for absence of carcinogenicity is suggested from a study in which copper co-administered with a known liver carcinogen to two strains of rats for up to 19 months resulted in reduced tumour incidence and delayed tumour onset (Howell, 1958).

The most reported liver malignancy in WD patients is hepatocellular carcinoma (HCC), which however is associated with cirrhosis in general, regardless of underlying causation (Xu and Hajdu, 2008; van Meer et al., 2015). Whilst the most reported cancer in WD, HCC is still a rare complication of WD progression (Pfeiffenberger et al., 2015), after liver damage (including cirrhosis) and its associated risk factors, including severity of disease, and/or age at onset of disease (Walshe et al., 2003; Harada, 2004; Iwadate et al., 2004; Reyes, 2008; Thattil and Dufour, 2013; Shiani et al., 2017; Brandi et al., 2020) Copper retention in the liver is considered a risk factor for development of HCC as a result of liver toxicity and cirrhosis (Iwadate et al., 2004; Reyes, 2008; van Meer et al., 2015).

Copper retention in the liver is considered a risk factor for development of HCC only in so far as it results in liver toxicity and cirrhosis (Iwadate et al., 2004; Reyes, 2008; van Meer et al., 2015). Lack of direct association between copper and HCC is also corroborated by evidence in animal models, where HCC is reported in the rat WD model but not the WD mouse model (Huster et al., 2006).

Whilst markers of copper homeostasis dysregulation have been reported in HCC cells (Davis et al., 2020), they may indicate a dysregulated state of tumour cells, rather than HCC causation by copper (Li et al., 2019). Similarly, increased blood levels in cancer patients relative to healthy individuals have been interpreted as evidence of copper involvement in carcinogenesis, although they are measured in individuals after cancer is present. Lack of a direct association between copper and HCC is also corroborated by evidence in animal models, where HCC is reported in the rat WD model but not the WD mouse model (Huster et al., 2006).

Absence of copper-induced hepatocarcinogenicity in the general population is supported by the lower incidence of hepatic tumours in WD patients compared to other chronic liver diseases and it is not explained by copper chelation therapy in these patients (Reyes, 2008; El-Serag, 2011; Pfeiffenberger et al., 2015; van Meer et al., 2015; Shiani et al., 2017). Extrahepatic malignancies in WD are even rarer, with only one reported case of breast cancer (Li et al., 2019), one reported case of colon cancer (Lee et al., 2013) and two reported cases of lymphoblastic leukaemia (Yüce et al., 2000; Maeda et al., 2014).

An association of copper status with non-hepatic human cancers in the general population has been proposed based on redox activity of increased cellular levels of labile copper and the resulting oxidative stress (Michalczyk and Cymbaluk-Płoska, 2020).

Association of copper with increased carcinogenicity has been suggested based on, for example, potential regulation of signal transduction pathways through binding and activation of MEK-1-mediated signal transduction (with subsequent ERK1/2 kinases phosphorylation) (Brady et al., 2014), displacement of zinc from its binding sites on p53 with subsequent disruption of p53 protein folding (Formigari et al., 2013), hypothesised pro-angiogenic activity as a means of tumorigenicity (Finney et al., 2009; Antoniades et al., 2013) and indirectly from reports suggesting efficacy of copper chelation as antitumour treatment (for a review, see Finney et al. (2009)). Whilst it has been reported that copper homeostasis is altered in tumour tissues or in cancer patients, and they do not consist evidence supporting a causative role of copper in carcinogenesis.

The weight of evidence in humans and animals indicates that copper is not genotoxic or carcinogenic in humans.

B.5.3. Comparative carcinogenicity between WD and general population

The HCC prevalence and incidence in WD patients is very low; in a retrospective cohort study of 130 WD patients (van Meer et al., 2015), the estimated annual risk for HCC was 0.09%; 95% CI: 0.01–0.28% (0.04%; 95% CI: 0.01–0.10% in a meta-analysis of five studies), even in patients with liver cirrhosis (estimated annual risk of 0.14%; range 0.02–0.46%) (Harada, 2004; Reyes, 2008; Thattil and Dufour, 2013; Pfeiffenberger et al., 2015; van Meer et al., 2015; Gunjan et al., 2017). In a multicentre study, eight out of 1,186 WD patients developed HCC and six developed intrahepatic cholangiocellular carcinomas, with total hepatobiliary malignancy prevalence of 1.2% and incidence of 0.28 per 1,000 person years (Pfeiffenberger et al., 2015), corresponding to a risk of 0.028% and an estimated annual incidence of 28 per 100,000. This estimate is comparable to the age-standardised liver cancer incidence for the general population in Europe (EU-27) in 2020: 19.8 per 100,000 in males and 7 per 100,000 in females, with highest incidence of 28.5 per 100,000 in males and 11.3 per 100.000 in females (Romania) and lowest incidence of 11.2 per 100,000 in males (Poland) and 4.6 per 100,000 in females (Malta) (European Cancer Information System, ECIS, https://ecis.jrc.ec.europa.eu/). The cumulative risk of liver cancer in the general population in the EU ranged between 0.9% and 2.1% in 2020.

Appendix C – Relationship between copper retention and liver and neurotoxicity in Wilson's disease (WD) patients vs WD heterozygotes[25]

Reference	Study design; Population	Serum copper and/or CP	Urinary copper	Hepatic copper	Liver toxicity markers	Neurotoxicity markers
(Brewer, 2000)	WD patients Heterozygotes	CP-Cu levels in heterozygotes were intermediate between those of normal individuals (higher) and those homozygote for WD (lower)	Elevated Cu levels in 24-h urine of siblings of WD patients, presumed heterozygotes	Evidence of higher levels in suspected heterozygotes		
(Gromadzka et al., 2010)	WD heterozygotes (Hzg) (parents of WD patients) (n = 68) Age-matched controls (n = 31) 24-h urine collection	Reduced serum caeruloplasmin in Hzg ($p < 0.001$) Carriers of H1069Q mutation had higher CP than other Hzg ($p = 0.03$) No difference in serum copper	No difference	Not measured	Reduced ALT ($p = 0.014$) and AST ($p = 0.07$) in Hzg No difference in their ratio and on platelets	No related difference
(Ferenci et al., 2007)	WD patients (n = 46) late onset (> 40 years) Hepatic WD: 15 of 46 Neurologic WD: 31 of 46 Asymptomatic: 2 of 46	CP < 20 mg/dL in 28 of 31 neurologic patients and in 13 of 15 hepatic patients	> 100 μg/L in all	> 250 μg/g in 13 of 17 patients who were sampled Levels similar to early onset patients	Histology evidence in 13 of 15 hepatic patients and in 14 of 31 neurologic patients	In 2 of 5 hepatic patients (and all the neurologic patients)
(Ferenci, 2005)	WD patients (n = 114) Hepatic WD (n = 90) Neurologic WD (n = 39) Non-cholestatic liver disease (NCLD) (n = 219) No evidence of liver disease (n = 26)	Not measured	All WD patients	> 250 μg/g in 95 of 114 WD patients (83.3%) > 250 μg/g in 35 of 39 neurologic WD patients > 250 μg/g in 18 of 20 hepatic WD patients (only 20 were measured) > 250 μg/g in 3 of 219 NCLD patients	Histopathology No correlation of severity of histological findings with copper content	Clinical diagnosis

[25] Relevant details from review articles are summarised; the Appendix is not intended to provide a comprehensive review of the literature.

Reference	Study design; Population	Serum copper and/or CP	Urinary copper	Hepatic copper	Liver toxicity markers	Neurotoxicity markers
(Yang et al., 2015)	Patients with various liver disease (n = 3,350) of whom 714 had two passes of liver biopsies and copper measurement, WD patients (n = 178) Hepatic WD (n = 105) Neurological WD (n = 31) Hepatoneurological WD (n = 25) Presymptomatic WD (n = 17) Heterozygote WD (n = 24)	Not reported	Not reported	> 250 μg/g in 168 of 178 WD patients > 250 μg/g in 105 of 105 hepatic WD patients > 250 μg/g in 60 of 60 neurologic WD patients > 250 μg/g in 1 of 24 heterozygotes; 4 of 24 had > 150 μg/g and 19 of 24 had > 75 μg/g	Clinical diagnosis Hepatic copper was higher in hepatic WD than other phenotypes	Clinical diagnosis

Appendix D – Uncertainty analysis

Table D.1: Identification and prioritisation of uncertainties in copper hazard assessment

Uncertainty	Reasons for uncertainty	Direction of impact	Magnitude of uncertainty	Impact on HBGV	Priority
Relationship between dietary exposure and copper hepatic retention					
Level of copper exposure at which retention occurs in the adult general population (i.e. excluding individuals with genetic disease)	Short duration of human intervention studies of copper kinetics	Unknown effect on direction. Longer studies may help to elucidate at what level and duration of exposure equilibrium is reached	Large[(a)]	Large	High
	Small sample size of human intervention studies of copper kinetics	Unknown effect on direction. Larger sample size may increase or decrease the level of exposure resulting in retention			
	Male subjects only, in human intervention studies of copper kinetics	Unknown effect on direction. Evidence in females may increase or decrease the level of exposure resulting in retention if sex differences in homeostasis exist			
	Absence of direct hepatic copper measurements in human intervention studies of copper kinetics	Unknown effect on direction			
	Lack of non-invasive biomarkers of copper retention	Unknown effect on direction			
Susceptible subpopulation for copper retention: genetic susceptibility - individuals heterozygous for WD genetic variants	Studies in humans did not characterise genetic background of participants	Inclusion of heterozygotes for WD gene variants may decrease the level of exposure reaching liver's capacity for copper retention, i.e. lower tolerance for copper exposure and therefore push the HBGV to lower value	Moderate	Moderate	Moderate
	Limited evidence in heterozygotes for WD gene variants who may retain higher levels of hepatic copper than the general population				
Impact of other nutrients, particularly zinc status on copper homeostasis	Zinc mediates upregulation of hepatic MT needed to sequester increasing copper levels	Low zinc may decrease liver's capacity for copper retention and increase toxicity - impact dependent on level of retained copper	Moderate	Moderate	Moderate
Uncertainty about the copper requirements relative to copper toxicity in younger age groups	Copper is utilised at higher rate relative to body weight compared to adults to meet higher growth needs	Higher copper utilisation in children indicates higher tolerance for copper exposure that would result in hepatic retention and therefore push the HBGV to a higher value	Large	Moderate	High

Uncertainty	Reasons for uncertainty	Direction of impact	Magnitude of uncertainty	Impact on HBGV	Priority
	Copper content is high in muscle during growth and less likely to be stored locally	Higher copper utilisation in muscle drives copper away from liver retention indicating higher tolerance for copper and therefore pushes the HBGV to a higher value			
Relationship between hepatic copper retention and copper toxicity					
Liver's capacity for sequestering copper (i.e. ceiling of hepatic concentration) and duration of copper sequestration before toxicity is observed	Lack of appropriate non-invasive biomarkers of copper retention and biomarkers of effect	Unknown effect on direction	Large	Large	High
	Short duration of intervention studies on copper toxicity in humans	Longer studies may decrease level of exposure reaching liver's capacity for copper retention and onset of toxicity and this would push the HBGV to a lower value			
	Wide range of hepatic copper levels in symptomatic and asymptomatic WD patients	Unknown effect on direction			
Triggers of release of hepatic retained copper	limited empirical evidence from farm animals; observations of possible triggers in WD patients: presence of other comorbidities; liver injury from other exposures; other conditions, including stress and infections	Presence of trigger will increase retained copper release from liver and increase toxicity but at unknown time – impact dependent on level of retained copper	Large	Large	High
Relationship between hepatic copper release and copper toxicity	Lack of data on the form of copper released from liver, e.g. if bound or unbound, or carrier involved if any	Unknown effect on direction	Large	Large	High
	Lack of data on copper levels in circulation or extrahepatic tissues, following hepatic release, that are associated with toxicity	Unknown effect on direction			
	Lack of data on whether release is continued once it occurs, or if it occurs intermittently and at what frequency to lead to toxicity	Unknown effect on direction			

Uncertainty	Reasons for uncertainty	Direction of impact	Magnitude of uncertainty	Impact on HBGV	Priority
	Lack of data on copper scavenging and sequestration by neighbouring hepatocytes or by extrahepatic tissues	If such scavenging occurs, it would increase the tolerance for copper exposure and therefore push the HBGV to a higher value			
	Empirical evidence from WD patients presenting sudden onset of disease following long periods of asymptomatic disease	If released, toxicity may range from mild to severe depending on the retained levels of copper and it would push the HBGV to a lower value			
	Absence of copper-specific biomarkers of effect for detection of toxicity	Absence of biomarkers results would push the HBGV to a higher value			
	Copper release is a stochastic event dependent on triggers of release (see below)	If it occurs it would push the HBGV to a lower value			
Susceptible subpopulations for copper toxicity: non-genetic susceptibility to copper toxicity	Presence of comorbidities, other exposures (e.g. medications, other environmental exposures, oxidative stress), lifestyle factors (e.g. alcohol consumption, diet/nutrition)	These conditions would decrease the tolerance for copper exposure indicating higher likelihood for hepatic retention and would therefore push the HBGV to a lower value	Moderate	Moderate	Low/ Moderate

(a): Measures agreed by consensus among experts in the working group r.

Uncertainty characterisation for copper hazard assessment in adults

The following sequence of assessment questions was included in the semi-quantitative uncertainty analysis of high priority uncertainties based on the probability scale shown in Table 2 in the EFSA 2018 Guidance on Uncertainty Analysis:

1) Is copper sequestered in the liver at levels of exposure above those that meet physiological functions (i.e. above the adequate intake)?
2) Does copper that remains effectively sequestered in the liver cause hepatotoxicity?
3) Is copper hepatic toxicity dependent on hepatic copper retention?
4) Is copper extrahepatic toxicity dependent on release of retained hepatic copper?
5) Does copper exposure up to 0.07 mg/kg bw per day (5 mg/day in adults) lead to hepatic copper retention?
6) Does copper exposure up to 0.07 mg/kg bw per day (5 mg/day in adults) lead to copper toxicity?

Assessment of evidence of uncertainty for each question:

1. Is copper sequestered in the liver at levels of exposure above those that meet physiological functions (i.e. above the adequate intake)?

Lines of Evidence	Influence on conclusion
Line of Evidence 1 - Homeostatic mechanisms tightly regulate copper absorption, distribution, sequestration and excretion, preventing the appearance of copper in chemical forms that are able to promote oxidative damage to cellular components.	↑↑↑
Line of Evidence 2 – When copper balance is positive (i.e. copper retention), evidence suggests that additional copper is sequestered in the liver and MT acts as a sink for hepatic copper.	↑↑↑
Line of Evidence 3 – Copper is increasing in the liver when physiological needs are met, when homeostasis is overwhelmed, such as when exposure is very high, or when excretion is compromised by genetic disease (as in WD) or cholestasis.	↑↑↑
Line of Evidence 4 – Supporting evidence for hepatic copper retention is obtained from 90-day studies in rats.	↑↑
Line of Evidence 5 – Liver copper levels increase in farm animals consuming feed with high copper levels.	↑↑
Line of Evidence 6 – There is absence of direct measurements of hepatic copper in the general population.	?
Conclusion – based on a process of weighing the influence of the different lines of evidence, their consistency and semi-formal expert judgement, the overall evidence supports the premise that copper is sequestered in the liver at levels of exposure above those that meet physiological functions.	Extremely likely (95–99% probability) to almost certain (99–100% probability)

2. Does copper that remains effectively sequestered in the liver cause hepatotoxicity?

Lines of Evidence	Influence on conclusion
Line of Evidence 1 - Hepatic sequestration is part of homeostasis and functions as a high capacity protective mechanism against the adverse effects of this transition metal in the general population	↓↓↓
Line of Evidence 2 – Hepatic sequestration may lead to redistribution of copper complexes into subcellular pools able to promote oxidative damage	↑
Line of Evidence 3 - Evidence of hepatic toxicity from locally sequestered copper is limited and is based on models indicating that protein-bound copper may still participate in redox activity	↑
Line of Evidence 4 - Copper is sequestered in the liver of WD patients for many years with no overt toxicity (long duration), at significantly higher hepatic concentrations than those expected from estimated daily exposure in non-WD individuals	↓↓

Lines of Evidence	Influence on conclusion
Line of Evidence 5 - Absence of evidence of hepatic toxicity in human intervention studies of short duration at relatively low copper doses and in observational studies at relatively low copper levels in drinking water, where copper can be assumed to be effectively sequestered as of LoE 1 and 4	↓↓↓
Conclusion – based on a process of weighing the influence of the different lines of evidence, their consistency and semi-formal expert judgement, the overall evidence does not support the premise that copper effectively sequestered in the liver causes hepatotoxicity.	Extremely unlikely (1–5% probability)

3. Is copper hepatic toxicity dependent on hepatic copper retention?

Lines of Evidence	Influence on conclusion
Line of Evidence 1 - There is no evidence of copper toxicity (hepatic or extrahepatic) in the absence of long-term (chronic) copper hepatic retention; therefore, hepatic retention is a prerequisite for copper toxicity.	↑↑↑
Line of Evidence 2 – A threshold concentration of hepatic copper associated with hepatotoxicity is unclear: Hepatic copper levels do not correlate with onset or severity of toxicity in WD patients.	?
Line of Evidence 3 - The timeframe (latency) after which copper toxicity is manifested *following* hepatic retention is variable.	?
Line of Evidence 4 – There is lack of appropriate non-invasive biomarkers of early hepatic toxicity.	●
Line of Evidence 5 – evidence of cellular and tissue changes??	↑↑
Conclusion – based on a process of weighing the influence of the different lines of evidence, their consistency and semi-formal expert judgement, the overall evidence supports the premise that hepatotoxicity is dependent on hepatic copper retention, under permissive conditions.	Extremely likely (95–99% probability) *under permissive conditions*

4. Is copper extrahepatic toxicity dependent on release of retained hepatic copper?

Lines of Evidence	Influence on conclusion
Line of Evidence 1 – Triggers of copper release from the liver in WD patients are variable and unpredictable, both in nature and timing	?
Line of Evidence 2 – Copper can be sequestered in the liver of some WD patients for many years with no overt extrahepatic (or hepatic) toxicity, at hepatic levels significantly higher than the hepatic levels expected from estimated daily exposure in non-WD individuals	↓↓↓
Line of Evidence 3 – In other WD patients, copper toxicity is observed in extrahepatic organs, notably the brain. However, neither the measured amount of hepatic copper nor the timeframe (latency) after which copper toxicity is manifested *following* hepatic retention are clearly established.	↑↑↑
Line of Evidence 4 – In human studies, no adverse effects were detected at levels of copper supplementation of 8 mg/day for 12 months used as a treatment for Alzheimer disease (at this exposure level copper retention has been reported for up to 5 months)	↓↓↓
Line of Evidence 5 – Evidence of sudden severe copper toxicity in WD patients and in farm animals associated with conditions of stress or altered physiology, or unknown	↑↑↑
Conclusion – based on a process of weighing the influence of the different lines of evidence, their consistency of and semi-formal expert judgement, the overall evidence supports the premise that extrahepatic toxicity is dependent on hepatic copper retention, under permissive conditions, but such conditions cannot be predicted (LoE 1).	Extremely likely (95–99% probability) *under permissive conditions*

5. Does copper exposure up to 0.07 mg/kg bw per day (5 mg/day in adults) lead to hepatic copper retention?

Lines of Evidence	Influence on conclusion
Line of Evidence 1 – Absence of direct hepatic copper measurements	?
Line of Evidence 2 – Lack of appropriate non-invasive biomarkers of copper status	?
Line of Evidence 3 – Evidence of retention or positive copper balance from studies with controlled exposure at levels of 8 mg/day up to 5 months and at 6 mg/day up to 2 months	↓↓
Line of Evidence 4 – It is unclear whether the positive retention observed at 6 mg/day and 8 mg/day would reach zero balance over longer exposure and observation periods	?
Line of Evidence 5 – Interindividual variability of copper kinetics, absorption and excretion, in the population	?
Line of Evidence 6 – The population of *ATP7B* gene variant heterozygotes, may be susceptible to increased copper retention in the liver compared with the general population	↑
Line of Evidence 7 – In pregnant and lactating women, circulating levels of copper are regulated by the same homeostatic systems, with additional specific mechanisms operating at the level of the placenta and the mammary gland	●
Conclusion – based on a process of weighing the influence of the different lines of evidence, their consistency and semi-formal expert judgement, the overall evidence does not support the premise that copper exposure up to 0.07 mg/kg bw per day leads to hepatic copper retention.	Very unlikely (5–10% probability)

6. Does copper exposure up to 0.07 mg/kg bw per day (5 mg/day in adults) lead to copper toxicity?

Lines of Evidence	Influence on conclusion
Line of Evidence 1 – Hepatic copper sequestration at this exposure level is very unlikely as per previous assessment question	↓↓
Line of Evidence 2 – There is no evidence of copper toxicity in the absence of long-term (chronic) hepatic copper accumulation	↓↓
Line of Evidence 3 – Triggers of copper release from the liver in WD patients are variable and unpredictable, both in nature and timing	?
Line of Evidence 4 – Interindividual variability of copper kinetics, absorption and excretion, in the population	?
Line of Evidence 5 – Although there are no toxicity data available for the population of *ATP7B* gene variant heterozygotes, the propensity of increased copper retention in the liver compared to the general population, indicates potential increased susceptibility of this subpopulation to future copper-mediated toxicity	↑
Line of Evidence 6 – Given maternal homeostatic mechanisms, pregnant and lactating women are at no greater risk of toxicity from copper than other women	●
Conclusion – based on a process of weighing the influence of the different lines of evidence, their consistency and semi-formal expert judgement, the overall evidence does not support the premise that copper exposure up to 0.07 mg/kg bw per day leads to copper toxicity.	Very unlikely (5–10% probability)

Uncertainty characterisation for copper hazard assessment in children

7) Does copper exposure up to 0.07 mg/kg bw per day lead to hepatic copper retention in children?
8) Does copper exposure up to 0.07 mg/kg bw per day lead to copper toxicity in children?
9) Does the estimated copper exposure in children at the highest end of the P95 range relative to the HBGV of 0.07 mg/kg bw per day lead to hepatic copper retention?
10) Does the estimated copper exposure in children at the highest end of the P95 range relative to the HBGV of 0.07 mg/kg bw per day lead to copper toxicity?

Assessment of the evidence of uncertainty for each question:

7. Does copper exposure up to 0.07 mg/kg bw per day lead to hepatic copper retention in children?

Lines of Evidence	Influence on conclusion
Line of Evidence 1 – The HBGV established for adults is conservative and is based on absence of retention	↓↓↓
Line of Evidence 2 – Copper homeostasis in children older than 16 weeks is assumed to be fully functional and approaches that of adults based on the essentiality of copper and the tightly regulated homeostatic pathway	↓↓
Line of Evidence 3 – In addition to effective homeostasis, there are recognised higher nutritional requirements during life stages of physical growth compared to adults	↓↓↓
Conclusion – based on a process of weighing the influence of the different lines of evidence, their consistency and semi-formal expert judgement, the overall evidence does not support the premise that copper exposure up to 0.07 mg/kg bw per day leads to hepatic copper retention in children.	Extremely unlikely (1–5% probability)

8. Does copper exposure up to 0.07 mg/kg bw per day lead to copper toxicity?

Lines of Evidence	Influence on conclusion
Line of Evidence 1 – The HBGV is conservative and is based on absence of retention	↓↓↓
Line of Evidence 2 – It is extremely unlikely that copper exposure in children leads to hepatic copper retention (see question 1)	↓↓↓
Line of Evidence 4 – The rather limited data in infants, where absence of changes in liver enzymes in response to copper supplementation is reported, do not suggest higher sensitivity to Cu toxicity in these groups as compared to the adult population used in the metabolic studies	↓↓
Conclusion – based on a process of weighing the influence of the different lines of evidence, their consistency and semi-formal expert judgement, the overall evidence does not support the premise that copper exposure up to 0.07 mg/kg bw per day leads to copper toxicity in children.	Extremely unlikely (1–5% probability)

9. Does the estimated copper exposure in children at the highest end of the P95 range relative to the HBGV of 0.07 mg/kg bw per day lead to hepatic copper retention?

Lines of Evidence	Influence on conclusion
Line of Evidence 1 – Evidence of non-Indian childhood cirrhosis, or idiopathic childhood cirrhosis, from high copper exposure, far exceeding the UL (up to 6.8 mg Cu/L, equivalent to 6.8 mg/day), combined with unidentified genetic component	↑
Line of Evidence 2 – High copper levels in drinking water in Germany, far exceeding the UL (9–26.4 mg Cu/L, equivalent to 9–26.4 mg/day), leading to childhood cirrhosis	↑
Line of Evidence 3 – Susceptible subpopulation of children carrying unidentified genetic component	↑
Line of Evidence 4 – The HBGV is established based on conservative criteria, as described above, related strictly to retention in adults without accounting for higher needs in children	↓↓↓
Line of Evidence 5 – There are recognised higher nutritional requirements during life stages of physical growth compared to adults	↓↓↓
Line of Evidence 6 – The exceedance of the HBGV in children is primarily associated with the maximum estimated exposure of the 95th percentile which is not considered to be maintained for extended periods of time during childhood to maintain hepatic retention	↓↓↓
Conclusion – based on a process of weighing the influence of the different lines of evidence, their consistency and semi-formal expert judgement, the overall evidence does not support the premise that the estimated copper exposure in children at the highest end of the P95 range relative to the HBGV of 0.07 mg/kg bw per day leads to hepatic copper retention.	Very unlikely (5–10% probability)

10. Does the estimated copper exposure in children at the highest end of the P95 range relative to the HBGV of 0.07 mg/kg bw per day lead to copper toxicity?

Lines of Evidence	Influence on conclusion
Line of Evidence 1 – Evidence of non-Indian childhood cirrhosis, or of idiopathic childhood cirrhosis, from high copper exposure, far exceeding the UL (up to 6.8 mg Cu/L, equivalent to 6.8 mg/day), combined with unidentified genetic component	↑
Line of Evidence 2 – It is very unlikely that copper exposure in children leads to hepatic copper retention (see question 3)	↓↓
Line of Evidence 3 – High copper levels in drinking water in Germany, far exceeding the UL (9–26.4 mg Cu/L, equivalent to 9–26.4 mg/day), leading to childhood cirrhosis	↑
Line of Evidence 4 – Susceptible subpopulation of children carrying unidentified genetic component	↑
Line of Evidence 5 – The HBGV is established based on conservative criteria, as described above, related strictly to retention in adults without accounting for higher needs in children	↓↓↓
Line of Evidence 6 – There are recognised higher nutritional requirements during life stages of physical growth compared to adults	↓↓↓
Line of Evidence 7 – The rather limited data in infants do not suggest higher sensitivity to Cu toxicity as compared to the adult population	↓↓
Line of Evidence 8 – The exceedance of the HBGV in children is primarily associated with the maximum estimated exposure of the 95th percentile which is unlikely to result in sustained hepatic retention (see question 3)	↓
Conclusion – based on a process of weighing the influence of the different lines of evidence, their consistency and semi-formal expert judgement, the overall evidence does not support the premise that the estimated copper exposure in children at the highest end of the P95 range relative to the HBGV of 0.07 mg/kg bw per day leads to copper toxicity.	Very unlikely (5–10% probability)

Table D.2: Identification and prioritisation of uncertainties in dietary exposure assessment of copper

Uncertainty	Reasons for uncertainty	Direction of impact	Magnitude of uncertainty	Impact on exposure	Priority
Uncertainties related to the total dietary exposure assessment of copper in the general population					
Food consumption data from the EFSA Comprehensive Food Consumption Database	Use of different dietary survey methodologies	Unknown effect on direction	Low[(a)]	Low	Low
	Use of standard portion sizes for some of the surveys/ foods	Unknown effect on direction			
	Representativeness of participants included in surveys	Unknown effect on direction	Low	Low	
	Estimating high percentiles of chronic exposure based on consumption surveys covering only a few days	This approach is generally acknowledged to lead mostly to an overestimation of high percentiles of exposure (P95)	Low	Low	
	Different details of the food description and therefore different levels of food aggregation	Unknown effect on direction	Low	Low	
Representativeness and completeness of the available composition data in food and drinking water	Composition data were available from a limited number of MS and may not be representative for all EU countries	Unknown effect on direction	Moderate	Low	Low
	Concentration data derived from the composition database provided no indication of the analytical method or left censorship treatment	Unknown effect on direction	Low	Low	
	Concentration data are not all measured values, but can also be calculated using standard factors or borrowing data from other countries	Unknown effect on direction	Low	Low	
Representativeness and completeness of the available occurrence data in food and drinking water	Part of the data were reported as below the limit of detection or quantification – the substitution method for those values was used	Unknown effect on direction	Low	Low	Low
	Data may not be representative of the entire EU market (e.g. limited number of samples per food category and data were provided by limited number of MSs)	Unknown effect on direction	Low	Low	
Copper concentrations in foods without data	Extrapolation of the mean copper concentration of foods to similar foods in the same food category and across classification levels	Unknown effect on direction	Low	Low	Low

Uncertainty	Reasons for uncertainty	Direction of impact	Magnitude of uncertainty	Impact on exposure	Priority
Copper concentration in coffee as consumed	Copper concentrations in coffee beans were used without accounting for the loss of copper due to processing resulting in lower copper in the coffee drink	Overestimation of copper exposure via coffee consumption	Moderate	Low	Low
Occurrence of copper and consumption data for fortified foods and food supplements	Lack of data to understand if the contribution from fortified foods was covered by the exposure assessment (fortification captured by generic concentration data). Food supplements were not included for lack of concentration data	Underestimation of total exposure for the general population	Moderate	Moderate	Moderate
	Consumption of fortified foods is assumed to be more regular in younger age groups than in older age groups (e.g. specific fortified foods are listed in foods categories dedicated to the young population)	Possibly larger underestimation of total exposure in younger age groups	Moderate	Moderate	High
Possible use of copper kitchen cookware and utensils could result in higher copper concentrations in foods	Personal choice of cookware; traditionally used copper-containing cookware in remote areas. Copper concentration in foods prepared with this cookware was not accounted for in the assessment. It is more plausible than not that this cookware is not commonly used by the general population	Underestimation of total exposure	Low	Low	Low
Uncertainties related to the assessment of the contribution of each source of copper to the total dietary exposure of the general population					
Contribution of copper-containing PPP to the dietary exposure	Concentration data may not be representative of the entire EU market, but the impact of this uncertainty is low (see part A above)	Unknown effect on direction	Low	Low	Low
	Lack of information on the relative market share (availability) of treated and untreated crops. The share of treated vs. untreated crops is not considered underrepresented in the exposure assessment for the general population	Minimal, if any, underestimation of contribution to exposure	Low	Low	
	Contribution was assessed based on food categories identified as major contributors to the dietary exposure to total copper	Possible underestimation of contribution to exposure	Low	Low	
Contribution of copper-containing food additives	There is a limited number of food additives containing copper and have limited applications	No effect on direction	Low	Low	Low

Uncertainty	Reasons for uncertainty	Direction of impact	Magnitude of uncertainty	Impact on exposure	Priority
Contribution of copper-containing feed additives	Difficulties to differentiate whether copper in feed results from treatment with pesticides, natural occurrence or use of copper-containing feed additives	Possible overestimation of contribution of feed additives to exposure	Large	Low	Low
Contribution of copper-containing fertilisers	Fertilisers may increase the copper content in the soil over time. However, homeostatic control of copper in plants prevents excessive copper concentrations in plants from long-term use of copper in fertilisers or PPPs	No effect on direction	Low	Low	Low
Contribution from some authorised nutrient uses (e.g. fortified foods and food supplements)	It was not possible to assess the contribution for the general population due to lack of consumption patterns for these products and lack of specific concentration data	Possible underestimation of contribution to exposure	Moderate	Moderate	Moderate /High
	The contribution of these products may be higher in younger age groups	Possible underestimation of contribution to exposure	Moderate	Moderate	

(a): Measures agreed by consensus among experts in the working group.

Uncertainty characterisation for dietary exposure assessment of copper

Most uncertainties in the exposure assessment were assigned low priority and no uncertainty was assigned a high priority in the prioritisation stage (Table D.2, A and B). Hence uncertainties assigned moderate priority are further assessed for their impact on the overall exposure assessment question. The uncertainties assigned low priority would not substantially impact the exposure.

The followingassessment question was included in the semi-quantitative uncertainty analysis based on the moderate priority uncertainty:

11. Is copper exposure underestimated for the general EU population, including all age groups?

Assessment of evidence of uncertainty:

11. Is copper exposure underestimated for the general EU population, including all age groups?

Lines of Evidence	Influence on conclusion
Line of Evidence 1 – Sufficiently reliable and representative food consumption data	↓↓↓
Line of Evidence 2 – Sufficiently reliable and representative concentration data on copper	↓↓↓
Line of Evidence 3 – The diet of the general population includes foods from both copper-treated and non-copper-treated crops, thus exposure of copper from the use of copper-containing PPPs can be assumed already to be covered	↓↓↓
Line of Evidence 4 – The major foods contributing to the dietary exposure to copper are covered in the assessment	↓↓↓
Line of Evidence 5 – Homeostatic control of copper in plants prevents excessive copper concentrations in plants from long-term use of copper in fertilisers or PPPs	↓↓↓
Line of Evidence 6 – The consumption of fortified foods was not fully captured in the current exposure assessment. Food supplements were not included for lack of data. The contribution of these products is assumed to be impact mainly the younger populations	↑↑↑
Conclusion - based on a process of weighing the influence of the different lines of evidence, their consistency and semi-formal expert judgement, the overall evidence does not support the premise that the dietary exposure to copper in the general adult EU population has been systematically underestimated	Unlikely/extremely unlikely (5–10%, 1–5% probability)
Conclusion - based on a process of weighing the influence of the different lines of evidence, their consistency and semi-formal expert judgement, the overall evidence suggests that the dietary exposure to copper in children of the EU population may be underestimated	More likely than not (> 50% probability)

Appendix E – Literature search

Literature search for copper hazard identification and characterisation

Literature search was conducted in PubMed using search terms related to (i) copper toxicity; (ii) copper homeostasis; (iii) genetic variability, such as genetic polymorphisms associated with WD; (iv) hepatic copper accumulation and hepatotoxicity; (v) neurotoxicity, including Alzheimer disease.

Search filters included publication dates up to 10 years, mammalian species, humans. Topics related to ecotoxicology, non-mammalian species, occupational exposures, nanomaterials and copper-containing medicinal compounds were excluded.

A first screening for relevance was based on article title, followed by abstract screening of the short-listed hits. All the selected potentially relevant abstracts of the search results were saved and directly relevant articles were retrieved. In initial comprehensive searches that returned too large a number of hits, review articles were searched first and relevant citations within them were identified.

Additional relevant articles were identified by checking the citations within the key retrieved full-text research articles and review articles and from citations within the literature that was reviewed in previous assessments, for further studies.

A summary of the search terms, filters and number of hits is shown below.

Query	Filters	Results
(Copper) AND ((Heterozyg*) OR (polymorph*) OR (mutat*)) AND ((transgen*) OR (rodent) OR (mouse) OR (mice) OR (rat) OR (in vivo))	in the last 10 years	405
(Copper) AND ((Heterozyg*) OR (polymorph*) OR (mutat*)) AND ((Kinetic*) OR (TK) OR (toxicokinetics) OR (ADME))	in the last 10 years	135
(Copper) AND (toxicity) AND ((Heterozyg*) OR (polymorph*) OR (mutat*))	in the last 10 years	377
((Heterozyg*) OR (polymorph*) OR (mutat*)) AND (copper) AND (frequency) AND (y_10[Filter])	in the last 10 years	170
((Heterozyg*) OR (polymorph*) OR (mutat*)) AND (ATP7*)	in the last 10 years	571
((copper[Title/Abstract]) AND ((Heterozyg*)[Title/Abstract] OR (polymorph*)[Title/Abstract] OR (mutat*)[Title/Abstract])) AND ((transgen*)[Title/Abstract] OR (rodent)[Title/Abstract] OR (mouse)[Title/Abstract] OR (mice)[Title/Abstract] OR (rat)[Title/Abstract] OR (in vivo)[Title/Abstract])	in the last 10 years	364
((copper[Title/Abstract]) AND ((Heterozyg*)[Title/Abstract] OR (polymorph*)[Title/Abstract] OR (mutat*)[Title/Abstract])) AND ((Kinetic*)[Title/Abstract] OR (TK)[Title/Abstract] OR (toxicokinetics)[Title/Abstract] OR (ADME)[Title/Abstract])	in the last 10 years	116
((copper[Title/Abstract]) AND (toxicity[Title/Abstract])) AND ((Heterozyg*)[Title/Abstract] OR (polymorph*)[Title/Abstract] OR (mutat*)[Title/Abstract])	in the last 10 years	139
((copper[Title/Abstract]) AND (toxicity[Title/Abstract])) AND ((Kinetic*)[Title/Abstract] OR (TK)[Title/Abstract] OR (toxicokinetics)[Title/Abstract] OR (ADME)[Title/Abstract])	in the last 10 years	351
((Copper[Title/Abstract]) AND (((Heterozyg*)[Title/Abstract] OR (polymorph*)[Title/Abstract] OR (mutat*))[Title/Abstract])) AND (Frequency[Title/Abstract])	in the last 10 years	67
((Heterozyg*)[Title/Abstract] OR (polymorph*)[Title/Abstract] OR (mutat*)[Title/Abstract]) AND (ATP7*[Title/Abstract])	in the last 10 years	557
((Copper[Title/Abstract]) AND ((Heterozyg*)[Title/Abstract] OR (polymorph*)[Title/Abstract] OR (mutat*)[Title/Abstract])) AND (Transport*[Title/Abstract])	in the last 10 years	461
("copper"[MeSH Terms] OR "copper"[All Fields] OR "coppers"[All Fields] OR "copper s"[All Fields]) AND ("heterozyg*"[All Fields] OR "polymorph*"[All Fields] OR "mutat*"[All Fields]) AND "transport*"[All Fields]	in the last 10 years	779
(((liver)[Title/Abstract] OR (hepat*)[Title/Abstract]) AND (copper[Title/Abstract])) AND (toxicity[Title/Abstract])	in the last 10 years	419
((liver) OR (hepat*)) AND (copper) AND (toxicity) AND (y_10[Filter])	in the last 10 years	932
(Copper) AND (toxicity) AND ((Kinetic*) OR (TK) OR (toxicokinetics) OR (ADME))	in the last 10 years	834
((Copper) AND (toxicity) AND ((Kinetic*) OR (TK) OR (toxicokinetics) OR (ADME)) AND (y_10[Filter])) NOT (Nano*)	in the last 10 years	639

Query	Filters	Results
((copper[Title/Abstract]) AND (bioavailability[Title/Abstract]) AND (y_10 [Filter])) AND (human[MeSH Terms])	in the last 10 years	87
((copper[Title/Abstract]) AND (bioavailability[Title/Abstract]) AND (y_10 [Filter])) AND (medical device)	in the last 10 years	15
((copper[Title/Abstract]) AND (bioavailability[Title/Abstract]) AND (y_10 [Filter])) AND (dermal)	in the last 10 years	2
((copper[Title/Abstract]) AND (bioavailability[Title/Abstract]) AND (y_10 [Filter])) AND (IUD)	in the last 10 years	0
(copper[Title/Abstract]) AND (bioavailability[Title/Abstract])	in the last 10 years	633
(copper[Title/Abstract]) AND (bioavailability[Title/Abstract])	Humans	251
(copper[Title/Abstract]) AND (bioavailability[Title/Abstract])	in the last 10 years, Humans	88
(copper[Title]) AND (bioavailability[Title])	in the last 10 years	79
((copper[Title/Abstract]) AND (bioavailability[Title/Abstract])) AND (route [Title/Abstract])	in the last 10 years	12
((copper[Title/Abstract]) AND (cosmetics[Title/Abstract])) AND (exposure [Title/Abstract])	in the last 10 years	11
((copper[Title/Abstract]) AND (cosmetics[Title/Abstract])) AND (absorption [Title/Abstract])	in the last 10 years	4
((copper[Title/Abstract]) AND (exposure[Title/Abstract]) AND (y_10[Filter])) AND (dermal)	in the last 10 years	40
((copper[Title/Abstract]) AND (absorption[Title/Abstract]) AND (y_10[Filter])) AND (dermal)	in the last 10 years	11
(copper[Title/Abstract]) AND (absorption[Title/Abstract])	in the last 10 years	3,101
(Copper[Title/Abstract]) AND (IUD[Title/Abstract] OR intra-uterine[Title/ Abstract]) AND ("blood copper"[Title/Abstract] OR "serum copper"[Title/ Abstract] OR "plasma copper"[Title/Abstract])		14
(Copper[Title/Abstract]) AND (IUD[Title/Abstract] OR intra-uterine[Title/ Abstract]) AND (blood[Title/Abstract] OR serum[Title/Abstract] OR plasma [Title/Abstract])	in the last 10 years	32
(("Copper/pharmacokinetics"[MAJR]) NOT (ecolog*)) NOT (plant[MeSH Terms])		441
"Copper/pharmacokinetics"[MAJR]		575
("Copper/blood"[Mesh] OR "Copper/pharmacokinetics"[Mesh] OR "Copper/ urine"[Mesh])		7,474
(carcinoma, hepatocellular[MeSH Terms]) AND (diseases, hepato neurologic wilson[MeSH Terms]) "carcinoma, hepatocellular"[MeSH Terms] AND "hepatolenticular degeneration"[MeSH Terms]	none	72
(carcinoma, hepatocellular[MeSH Terms]) AND (diseases, hepato neurologic wilson[MeSH Terms]) "carcinoma, hepatocellular"[MeSH Terms] AND "hepatolenticular degeneration"[MeSH Terms]	in the last 10 years	22
((Alzheimer[Title/Abstract]) AND (Copper[Title/Abstract]))	2000–2021	1,272
((Alzheimer[Title/Abstract]) AND (Copper[Title/Abstract]))	1995–2000	68
((copper[Title/Abstract]) AND (toxicity[Title/Abstract])) AND ((copper[Title/ Abstract]) AND (liver[Title/Abstract] OR hepatic[Title/Abstract]))	2000–2021	165
(copper[Title/Abstract]) AND (genotoxic*[Title/Abstract])	in the last 5 years	145
("wilson disease"[Title/Abstract]) AND (cancer[Title/Abstract])	from 2005 to 2021	34
(Wilson[Title/Abstract]) AND (fulminant[Title/Abstract])	from 2000 to 2021, Review	32