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WP4 –Task 4.3 Rapid Response Mechanism

Response document following DG SANTE's request regarding information needed on Copper compounds.

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Abstract

In October 2018, DG SANTE submitted to HBM4EU an urgent request for information related to Copper (Cu) compounds, via the online Rapid Response Mechanism (RRM). This request relates to the renewal of approval of Copper compounds as active substances for plant protection products. HBM4EU was requested to assess human biomonitoring data and to establish whether all Cu compounds are similarly absorbed and excreted, whether Cu compounds used as plant protection products are more contributing to the burden than other sources and whether a cumulative exposure assessment can realistically bring an added-value to the risk assessment of Cu. In order to address the DG SANTE request, aggregated HBM data on copper from 13 different countries were collected from HBM4EU National Hubs or data owners. From the blood and urinary HBM data available, no strong conclusions could be drawn and almost all values obtained were within the normal ranges as published for children, adults, pregnant women, and women that use intrauterine contraceptives. The parameterisation of the generic PBTK model of the INTEGRA platform has been performed for integrated exposure assessment. This model is able to describe the toxicokinetics associated with the lifelong exposure of the general population and has shown its capacity to capture the Cu homeostasis under real life intake patterns. However, additional toxicokinetics data are needed to cover higher exposure patterns related to occupational exposure, as well as to capture the short exposure regime dynamics and how they may affect copper homeostasis. Moreover, as it seems that long-term copper intake levels are reflected to some extent in the overall body burden, detailed information about the dietary intake or exposure through other routes is needed to explain the observed differences in the blood copper levels

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Abbreviations

ADI	Admissible Daily Intake
ADME	Absoprtion, Distribution, Metabolism, Excretion
ANSES	French Agency for Food, Environmental and Occupational Health & Safety (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail)
AOEL	Acceptable Operator Exposure Level
ATSDR	Agency for Toxic Substances and Disease Registry
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BMI	Body Mass Index
Cu	Copper
DG SANTE	Directorate General for Health and Food Safety
EEA	European Environment Agency
EFSA	European Food Safety Authority
FIOH	Finnish Institute of Occupational Health (Työterveyslaitos)
FISABIO	Foundation for the Promotion of Health and Biomedical Research (Fundación para el Fomento de la Investigación Sanitaria y Biomédica)
HBM	Human Biomonitoring
HBM4EU	Human Biomonitoring for European Union
HEALS	Health and Environment-wide Associations based on Large population Surveys
HSE	Health & Safety Executive
HSL	Health and Safety Laboratory
ICPMS	Inductively Coupled Plasma Mass Spectrometry
ISCIII	Carlos III Health Institute (Instituto de Salud Carlos III)
ISGLOBAL	Barcelona Institute for Global Health (Instituto de Salud Global de Barcelona)
IV	Intraveinous
JSI	Jožef Stefan Institute
LOD/LOQ	Limit of Detection / Limit of Quantification
NIPH	Norwegian Institute of Public Health (Folkehelseinstituttet)
NVKC	Nederlandse Vereniging voor Klinische Chemie en Laboratoriumgeneeskunde
PBTK	Physiologically based toxicokinetics
PIH	Provincial Insitute for Hygiene (Provinciaal Instituut voor Hygiëne)
PPP	Plant Protection Product
RIVM	Netherlands National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu)
ROS	Reactive Oxygen Species
RRM	Rapid Response Mechanism
SEPA	Swedish Environmental Protection Agency (Naturvårdsverket)
SOD	Soperoxide Dismutase
UBA	German Environment Agency (Umweltbundesamt)
UGR	University of Granada
UoM	Unit of Measure
ULPGC	University of Las Palmas de Gran Canaria
VITO	Flemish Institute for Technological Research (Vlaamse Instelling voor Technologisch Onderzoek)
WHO	World Health Organization

Introduction

In October 2018, DG SANTE submitted to HBM4EU an urgent request for information related to Copper (Cu) compounds, via the online Rapid Response Mechanism (RRM).
The main purpose of the request aims at assessing human biomonitoring (HBM) data to confirm or not the absorption and excretion patterns considered in the risk assessment report. The relevance of this request is related to the renewal of approval of Copper compounds as active substances used as plant protection products (PPP), so as to dissipate any concern for human health.

Request of DG SANTE

The risk assessment for Cu coumpounds used as PPP is well substantiated. However human beings are exposed to several sources and various forms of Cu and therefore it would be interesting to establish whether all these forms are similarly absorbed and excreted, whether Cu compounds used as PPP are more contributing to the burden than other sources and whether a cumulative exposure assessment can realistically bring an added-value to the risk assessment of Cu.
It would also be interesting to determine certain focus groups, which are in theory more exposed than others (for instance, professional users, farmers, organic farmers, vineyards and fruityards...).
The dietary exposure resulting from the uses as PPP can be critical for certain sub-groups of the population (young children especially), even if is now proposed to limit the application rate to 4 kg/ha/year.
Non-dietary exposure of residents is considered as critical for vineyards.
The Acceptable Daily Intake (ADI) has been set at 0.15 mg Cu/kg bw/day based on human data (WHO value of 0.15 mg Cu/kg bw/day for children).
The Acceptable Operator Exposure Level (AOEL) has been set at 0.08 mg Cu/kg bw/day.

The Management Board decided to mobilize expertise within the HBM4EU consortium, to seek and process existing evidence of relevance to DG SANTE's request.
Further to the Management Board's decision, countries as part of the HBM4EU Consortium were asked via the National Hubs to share available HBM data on Cu as well as details of ongoing and/or planned studies (e.g. the descriptors of the population providing the samples, residence, geolocation and distance to farm fields, etc.). The availability of current and/or expected HBM data for specific sub-groups, such as for adults or children living near vineyards, was checked. The idea was that critical data gaps should be identified, and recommendations made for future HBM studies to be carried out on specific sub-groups of population.

1. Human exposure and possible exposure routes

For the general population, most exposure to Copper originates from the ingestion of food (ATSDR, 2004). As stated in the deliverable 4.2 (D4.2) of the HEALS project (2015), Copper content of food is variable and can be affected by application to crops of Cu-containing fertilizers and fungicidal sprays. The use of Cu in rural areas may be a significant source of Cu intake. For example, the potential Cu-containing soil treatments applied in order to prevent Cu deficiency in soils; through feed in livestock; to stimulate the growth of pigs and broiler chickens; or the use of Cu sulphate as fungicide in agriculture (www.copper.org; Snoj Tratnik et al., 2019). In addition, the use of Cu-containing cooking vessels contributes to the total intake.
Additional exposure may result from inhalation of dust particles and ingestion of drinking water (Health Canada 2013).

2. Toxicokinetic data for copper and its compounds

2.1 In animals

A bioequivalence study was performed to demonstrate that toxicological studies on Cu sulphate could be used in the risk assessment for the five forms of Cu (EFSA, 2017). Its aim was to compare the bioavailability of Cu hydroxide, Cu oxychloride, Bordeaux mixture (an aqueous solution of calcium hydroxide and 1-2% of Cu sulfate), tribasic Cu sulphate and Cu oxide with Cu sulphate pentahydrate in rats.
The results show that absorption, distribution and excretion rates were similar between the variants of Cu following ingestion of 20 mg Cu/kg bw in rats. Liver was the principal organ of regulation of Cu and the main excretion route was *via* the bile. Liver Cu levels increased significantly following dosing with Tmax at 12 hours; depuration was rapid, with levels returning to control by 48 hours after dosing. Plasma concentrations in both control and dosed rats remained unchanged.
In conclusion, it was demonstrated that following oral administration to rats the five forms of Cu salts were similarly absorbed to Cu sulphate. The findings are consistent with Cu uptake by the liver and biliary elimination as components of homeostatic bioregulation of copper metabolism (EU RAR, 2008)

2.2 In Humans

Copper is absorbed, distributed, stored, and excreted in the body *via* a complex of homeostatic processes, providing a more or less constant level of this micronutrient and avoid excessive whole-body amounts and toxic levels in blood and tissues.

2.2.1 Absorption

Pulmonary absorption of Cu through inhalation of dust, fumes, and smoke does occur, but the rate and extend of that absorption in humans is poorly known. Deposition of copper in the lungs and liver was observed in workers exposed to Bordeaux mixture during the spraying of vineyards (US AF, 1990). Copper oxide was found in alveolar capillaries 3 hours after rats were exposed to welding dusts generated from pure copper wires (Batsura, 1969).

Approximately 24% to 60% of Cu is absorbed following oral ingestion. Absorption is affected by a number of factors, including age, the amount of Cu in the diet (absorption is around 50 % at low Cu intakes (approximately under 1 mg Cu/day) and 20 % at higher intakes (approximately over 5-6 mg Cu/day)) and the presence of other metals or dietary components. Indeed, absorption is reduced by other metals such as zinc, iron and molybdenum and is increased by amino acids and dietary sodium) (ATSDR, 2004; WHO, 1998).

According to the Environmental Health Criteria 234 (WHO, 2006), approximately 30 % of Cu(II) sulfate ($CuSO_4$) is absorbed from the gastrointestinal tract in humans and increasing luminal pH reduces the metal absorption. This is probably due to decreased Cu^{2+} and to a predominance of Cu(II) hydroxide Cu(OH_2) and basic Cu salts, which tend to precipitate out of aqueous solution.

Following ingestion, Cu absorption occurs mainly in the proximal part of the small intestine. Absorbed Cu is bound to plasma protein carriers and transported to the liver in portal blood, where it is incorporated by hepatocytes into cuproenzymes and other proteins and then is exported in peripheral blood to tissues and organs where it is stored bound to metallothionein and amino acids (ATSDR, 2004). It is also reported in the D4.2 from HEALS (2015) that more than 90 % of Cu exported from liver to blood is in the form of glycoprotein ceruloplasmin (increased during pregnancy) and <10 % is bound to albumin (Burtis et al. 2012).

As indicated by Bost et al. (2016), it is complicated to measure true fractional absorption of Cu because of important faecal Cu excretion and the existence of only two stable isotopes, ^{63}Cu (69.2% natural abundance) and ^{65}Cu (30.8% natural abundance), which makes it impossible to use a dual labelling technique to correct apparent absorption for biliary and gastro-intestinal re-excretion. In most studies, fractional Cu absorption has been measured using extrinsic meal labelling with ^{65}Cu and faecal Cu monitoring for several days. The addition of the non-absorbable rare earth element Holmium to ^{65}Cu-labeled meal has been used to accurately quantify the amount of absorbed Cu re-excreted in faecal matter (Harvey et al., 2002, 2003 - described in Annex 1). Using this approach, it has been concluded that true fractional absorption of Cu was close to 50 % and remained constant for Cu intake ranging from 0.7 mg/day to 6.0 mg/day. This estimation has also been confirmed by the analysis of faecal Cu excretion and plasma Cu appearance data after the oral or intravenous administration of ^{65}Cu, using a simple compartmental model (Harvey et al., 2005 - described in Annex 1).

2.2.2 Excretion

As mentioned in the D4.2 of HEALS (2015), the major excretory route is the bile with up to 70 % of orally ingested element that may be excreted in the faeces ; normally only 0.5 % to 3.0% of daily intake is excreted in the urine (ATSDR, 2004; Health Canada 2010). Elimination of Cu is biphasic with a biological half-life in the plasma of 2.5 and 69 days for the first and second phases, respectively (ATSDR, 2004, Health Canada 2010, 2013). Other minor or potential routes of excretion includes saliva, sweat, menstrual flow, nails and hair.

After IV injection of radioactive Cu to healthy individuals, it was observed that after 72 hr, only about 10 % of the dose had been excreted in urine and faeces indicating a biological half-time of several weeks. The half-life of injected Cu was found to be about 4 weeks in healthy

subjects, whereas it was much higher in subjects with Wilson's disease[1] (Friberg et al. 1986; Ellenhorn et al. 1997).

2.2.3 Copper homeostasis

Cu belong to the group of essential metals. It is a redox-active metal that can cycle between its oxidized cupric (Cu^{2+}) and reduced cuprous (Cu^{+}) forms. This cycle between transition states is critical for its biological function as a catalytic cofactor for Cu-dependent enzymes such as the cytochrome c oxidase, a component of the mitochondrial respiratory chain, and the Cu,Zn-superoxide dismutase (Cu,Zn-SOD, SOD1) devoted to the cell defence against the excess of reactive oxygen species (ROS) (Puig et al., 2002; Bost et al., 2016). Cu becomes toxic in the case of excessive intracellular accumulation, which plays a role in initiating the generation of ROS and apoptotic processes or other pathologies such as cancer, neurological diseases, and aging (Bocca et al., 2011).

Cu status in the body is regulated by both duodenal absorption and/or biliary excretion. As long as exposure is within the homeostatic range, in healthy adults, high Cu exposure results in down regulation of Cu uptake in the duodenum, and up regulation of biliary excretion. As a result, high Cu exposure or intake does not necessarily cause an equivalent body 'Cu load' (Danzeisen et al., 2007). This is one of the reasons why Cu status cannot be estimated by exposure levels. Currently, the only precise indicator of Cu load is the Cu content in the liver. Plasma Cu is very tightly regulated and does not correspond to Cu status (liver Cu). Its measurement is made more complex by the fact that most Cu in serum is in the protein ceruloplasmin, and its regulation may reflect regulation of the protein, rather than of Cu levels (Danzeisen et al., 2007). Indeed, most of the copper in blood is bound to ceruloplasmin, with values varying from 80 to 95 % (Wirth and Linder, 1985; Hellman and Gitlin, 2002 as cited in EFSA 2015).

However according to Barceloux 1999, the level of Cu in blood that is sequestered by ceruloplasmin may reflect recent exposure to Cu (Bocca et al., 2011).

Relevant biological effects associated with mild to moderate Cu excess are unknown. It is difficult to identify markers of early changes because limits of the homeostatic range are still undefined and early changes may represent adaptive responses that do not imply necessarily risk of damage (Araya et al. 2003).

2.3 Biomarkers of exposure

Exposure to Cu can lead to increased Cu concentration in whole blood, serum, urine, faeces, hair and the liver (ATSDR, 2004).

[1] Wilson disease is a rare inherited disorder, affecting approximately 1 in 30,000 individuals, and in which excessive amounts of copper accumulate in the body, particularly in the liver, brain, and eyes. The signs and symptoms of Wilson disease usually first appear between the ages of 6 and 45, but they most often begin during the teenage years. The features of this condition include a combination of liver disease and neurological and psychiatric problems.

2.3.1 Blood, plasma and serum copper

Determination of serum Cu level is one of the most common means of establishing the Cu status. Data included in the review conducted by Harvey et al. (2009) suggest that serum Cu responds to Cu supplementation in a Cu status–dependent manner, i.e. serum Cu appears to reflect changes in Cu status in both Cu-depleted and Cu-replete individuals, although with smaller increases in Cu-replete individuals. Nevertheless, while it can reflect severely depleted Cu stores, it may not be sensitive to minimal changes in Cu status. Serum Cu values are also affected by other non-nutritional factors, mainly sex and age. Serum ionic copper rapidly diminishes to normal levels following an acute bolus dose, indicating that it may reflect only recent exposures. (Chuttani et al., 1965 as cited in ATSDR, 2004). In addition, pregnancy and the use of oral contraceptives can influence the blood levels of total Cu significantly (Dutch Society for Clinical Chemistry - NVKC; Ellingsen et al., 2015).
Overall plasma Cu does not seem to respond significantly to Cu supplementation, according to the meta-analysis conducted by Harvey et al., 2009. According to the authors, plasma Cu may be useful in showing Cu repletion in depleted individuals after supplementation. However, only limited evidence is available indicating that this biomarker works with severe depletion at baseline and even less evidence that it works in repleted individuals. The conclusion that neither plasma Cu (nor plasma cuproenzymes) reflects Cu status is also reached by Danzeisen et al. (2007) based on e.g. results from Araya et al., 2003a and Linder et al., 1998 publications.

2.3.2 Copper in urine

The urinary Cu concentration appears to be suitable to only a limited extent as indicator of internal exposure (WHO 1998). In healthy individuals, concentrations in urine are by a factor of 50–60 less than the corresponding concentrations in blood (Krause et al. 1996 as cited by the Letzel et al. 2018). Moreover, because of the described homeostatic regulation of Cu (see section 2.2.3), its concentration in urine (as for blood), should not be related to the Cu supply (Letzel et al. 2018).

2.3.3 Copper in hair

Copper content of scalp hair is 10–100 time higher compared to serum, with values ranging from 7 to 95 µg/g (Bost et al., 2016). Few studies have investigated the relationship between Cu intake and Cu content in hair. In one long-term study in healthy young men fed controlled diet, Cu content in scalp hair increased from 9.2 ± 3.1 to 21.1 ± 5.9 µg/g when intake shifted from 1.6 to 7.8 mg/day. In contrast, no correlation was observed between Cu content in scalp hair and Cu intake assessed with three 24 h dietary recalls in a cohort of 70 menstruating women (Suliburska, 2011). Besides Cu intake, factors affecting the Cu content of scalp hair are still largely unknown and may include sex, use of hormonal contraception, cancer and other pathological situations associated with change in Cu distribution between plasma and tissues as well as environmental pollution (Kempson et al., 2007). Hair matrix presents advantages, with easy collection and conservation, and with retrospective characteristics in addition. However, these advantages are still hindered by the problems of analytical difficulties with this matrix for metal determination due to the the lack of internal and external quality controls and external contamination. Indeed, there is a considerable uptake of Cu from exogenous sources (e.g. hair washing procedures, swimming pool) and once adsorbed Cu binds very tight to the hair structure (Wilhelm et al. 1989; 1994). For all these reasons, hair Cu is not regarded as a suitable biomarker of Cu status.

2.3.4 Conclusion on biomarkers

While it is possible to detect Cu deficiency or excess in their extremes, due to ensuing tissue damage (for example, an increase in liver enzymes), it is currently not possible to detect minor but biologically significant variations of Cu status (Danzeisen et al., 2007). A good biomarker of Cu is needed, to monitor and avoid chronic health effects in large populations, and to give an 'early warning' in sensitive populations (infants, pregnant or lactating women, individuals with idiopathic or genetic changes in metabolism, the elderly, disease), before any tissue damage occurs.

Rather than testing each of the cuproenzymes, Cu-binding proteins or Cu chaperones for potential use as a Cu biomarker, the advent of high-throughput technologies has made it possible to screen for potential biomarkers in the whole proteome of a cell. Since Cu is involved in so many biological processes, a good biomarker may be a downstream product, with no immediate role in Cu metabolism (Danzeisen et al., 2007).

3. Biological monitoring results for Copper in Europe

3.1 Biomonitoring results for copper in EU countries

3.1.1 Data received from data owners via request submitted to HBM4EU National Hubs

In order to address DG SANTE's request, HBM aggregated data on copper from 13 different countries were collected from HBM4EU National Hubs or data owners. These aggregated data are presented here in table 1.

Table 1: Data received from data owners via request submitted to HBM4EU National Hubs

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Adipose Tissue											
Granada-Motril cohort	GraMo	Spain	Hospital-based adult cohort	18 to 90	2003	2004	228	mg/Kg	1.03	0.87	1.18
Blood – plasma											
German Environmental Specimen Bank	ESB	Germany	Student volunteers	20 to 29	2014	2018	2518	µg/L	1097	1081	1112
Cross-Mediterranean Study	SLO-CROME - mothers	Slovenia	General population mothers	30 to 49	2016	2016	170	µg/L	912	875	948
Exposure of children and adolescents to selected chemicals through their habitat environment	SLO-CRP	Slovenia	General population	6 to 15	2018	2018	228	µg/L	1033	1006	1060
Cross-Mediterranean Study	SLO-CROME - children	Slovenia	General population	7 to 8	2016	2016	139	µg/L	872	852	893

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Blood – plasma											
UK_HSL_GenPop_Plasma_Cu	UK_HSL_GenPop_Plasma_Cu	United Kingdom	General population	18 to 65	2018	2018	81	µg/L	826.10	781.62	876.93
Blood – serum											
Body burden of toxic metals and rare earth elements in non-smokers. cigarette smokers and electronic cigarette users.	Smok_ Metals	Romania	General population	18 to 60	2017	2018	150	µg/L	864.93	-	-
Blood - whole blood											
FLemish Environment and Health Study 2 Reference Newborns	FLEHS 2 Ref Nb	Belgium	General population mothers	18 to 43	2008	2009	243	µg/L	1341.54	1307.31	1375.76
FLemish Environment and Health Study 2 Reference Adolescents	FLEHS 2 Ref Ado	Belgium	General population	14 to 15	2008	2009	210	µg/L	799.92	782.84	817.00
FLemish Environment and Health Study 2 Hotspot Genk-Zuid	FLEHS 2 Hotspot Genk-Zuid	Belgium	Inhabitants of Hotspot area	14 to 15	2010	2010	197	µg/L	853.92	828.78	879.05
FLemish Environment and Health Study 2 Hotspot Menen	FLEHS 2 Hotspot Menen	Belgium	Inhabitants of Hotspot area	14 to 15	2010	2011	199	µg/L	842.28	826.43	858.12
FLemish Environment and Health Study 3 Reference Adolescents	FLEHS 3 Ref Ado	Belgium	General population	14 to 15	2013	2013	207	µg/L	905.18	880.94	929.42

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Blood - whole blood											
FLemish Environment and Health Study 3 Hotspot Gentse Kanaalzone	FLEHS 3 Hotspot GKZ	Belgium	Inhabitants of Hotspot area	14 to 15	2013	2014	199	µg/L	900.35	875.92	924.77
Human Early Life Exposome (HELIX) mothers: Study of Prenatal and Postnatal Determinants of Child Development and Health (EDEN)	HELIX mothers: EDEN	France	Pregnant women	20 to 43	2003	2005	56	µg/L	1414.04	1355.70	1472.37
Human Early Life Exposome (HELIX) children: Study of Prenatal and Postnatal Determinants of Child Development and Health (EDEN)	HELIX children: EDEN	France	General population	9 to 12	2014	2015	226	µg/L	939.43	904.06	974.80
Human Early Life Exposome (HELIX) children: The Mother-Child Cohort in Crete, Greece (RHEA)	HELIX children: RHEA	Greece	General population	6 to 7	2014	2015	228	µg/L	991.64	973.77	1009.50

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Blood - whole blood											
Human Early Life Exposome (HELIX) mothers: The Mother-Child Cohort in Crete, Greece (RHEA)	HELIX mothers: RHEA	Greece	Pregnant women	17 to 42	2007	2008	198	µg/L	1485.69	1446.75	1524.63
Human Early Life Exposome (HELIX) mothers: KANus Cohort	HELIX mothers: KANC	Lithuania	Pregnant women	19 to 43	2007	2009	203	µg/L	1456.33	1422.18	1490.49
Human Early Life Exposome (HELIX) children: KANus Cohort	HELIX children: KANC	Lithuania	General population	5 to 7	2014	2015	233	µg/L	909.98	893.00	926.95
Health Study Firework Disaster Enschede	GGVE	Netherlands	Victims and relief workers involved in Firework Disaster Enschede May 2000	-	2000	2000	3846	µg/L	P50: 783	-	-
Mother and child cohort study (MoBa)/Norwegian Environmental Biobank Part I	MoBa/NEB I	Norway	Pregnant woman (gestational week 18)	18 to 45	2002	2008	2982	µg/L	1554.6	1545.7	1563.6
Human Early Life Exposome (HELIX) mothers: Mother and child cohort study (MOBA)	HELIX mothers: MOBA	Norway	Pregnant women	23 to 43	2004	2007	259	µg/L	1337.39	1310.43	1364.35

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Blood - whole blood											
Human Early Life Exposome (HELIX) children: Mother and child cohort study (MOBA)	HELIX children: MOBA	Norway	General population	6 to 9	2014	2015	270	µg/L	837.69	824.49	850.89
National HBM Survey	SLO-HBM	Slovenia	General population	18 to 49	2008	2014	1084	µg/L	966	955	976
DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale (DEMOCOPHES)	SLO-DEMOCOPHES-parents	Slovenia	General population	20 to 49	2011	2012	198	µg/L	811	788	833
Cross-Mediterranean Study	SLO-CROME - mothers	Slovenia	General population mothers	30 to 49	2016	2016	170	µg/L	636	615	657
Public Health Impact of Long-Term. Low-Level Mixed Element Exposure in Susceptible Population Strata	SLO-PHIME-3-1	Slovenia	General population	6 to 11	2007	2007	123	µg/L	862	834	890
DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale (DEMOCOPHES)	SLO-DEMOCOPHES - children	Slovenia	General population	6 to 11	2011	2012	95	µg/L	872	846	899

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Blood - whole blood											
Exposure of children and adolescents to selected chemicals through their habitat environment	SLO-CRP	Slovenia	General population	6 to 15	2018	2018	229	µg/L	844	828	861
Cross-Mediterranean Study	SLO-CROME - children	Slovenia	General population	7 to 8	2016	2016	139	µg/L	682	665	699
Pattern of blood concentrations of 47 elements in two populations from the same geographical area but with different geological origin and lifestyles: Canary Islands (Spain) vs. Morocco.	Canary vs. Morocco	Spain	General population	20 to 40	2017	2017	120	µg/L	649.96	-	-
Human Early Life Exposome (HELIX) children: INfancia y Medio Ambiente (INMA)-Sabadell	HELIX children: INMA-SAB	Spain	General population	7 to 10	2014	2015	263	µg/L	922.33	904.84	939.82
Gusum-study	Gusum-study	Sweden	People living at or close to a contaminated site	23 to 90	2008	2008	95	µg/L	820	-	-

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Blood - whole blood											
Human Early Life Exposome (HELIX) mothers: Born in Bradford	HELIX mothers: BIB	United Kingdom	Pregnant women	16 to 42	2007	2008	129	µg/L	1582.24	1533.23	1631.25
Human Early Life Exposome (HELIX) children: Born in Bradford	HELIX children: BIB	United Kingdom	General population	6 to 7	2014	2015	232	µg/L	951.05	930.93	971.16
Cord blood - whole blood											
FLemish Environment and Health Study 3 Reference Newborns	FLEHS 3 Ref Nb	Belgium	newborns	0	2013	2014	281	µg/L	565.80	554.15	577.46
FLemish Environment and Health Study 1 Reference Newborns	FLEHS 1 Ref Nb	Belgium	newborns	0	2002	2004	1092	µg/L	574.6	564.49	584.7
FLemish Environment and Health Study 2 Reference Newborns	FLEHS 2 Ref Nb	Belgium	newborns	0	2008	2009	249	µg/L	611.46	597.00	625.92
3xG Study	3xG	Belgium	newborns	0	2010	2015	279	µg/L	571.30	561.35	581.24
Public Health Impact of Long-Term. Low-Level Mixed Element Exposure in Susceptible Population Strata	SLO-PHIME	Slovenia	newborns	0	2008	2010	407	µg/L	589	578	600

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Cord blood - whole blood											
Occurrence of 44 elements in human cord blood and their association with growth indicators in newborns.	Newb_Blood	Spain	newborns	0	2015	2016	471	µg/L	402.04	-	-
Breast milk											
National HBM Survey	SLO-HBM	Slovenia	mothers and their newborns	18 to 49	2008	2014	470	µg/L	372	362	382
Public Health Impact of Long-Term. Low-Level Mixed Element Exposure in Susceptible Population Strata	SLO-PHIME	Slovenia	mothers and their newborns	22 to 44	2008	2010	287	µg/L	555	538	571
Metals and trace element concentrations in breast milk of first time healthy mothers: a biological monitoring study	SEPA_Trace elements in breast milk	Sweden	mothers and their newborns	25 to 34	1990	1999	60	µg/L	471	-	-
Hair											
Cross-Mediterranean Study	SLO-CROME - mothers	Slovenia	General population. mothers	30 to 49	2016	2016	178	ng/g	12485	11572	13399
Exposure of children and adolescents to selected chemicals through their habitat environment	SLO-CRP	Slovenia	General population	6 to 15	2018	2018	246	ng/g	11799	10986	12611

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Hair											
Cross-Mediterranean Study	SLO-CROME - children	Slovenia	General population	7 to 8	2016	2016	179	ng/g	13894	12063	15725
Urine - 24h											
German Environmental Specimen Bank	ESB	Germany	Student volunteers	20 to 29	2014	2018	2513	µg/L	7.66	7.396	7.915
Urine-Morning urine											
FLemish Environment and Health Study 2 Reference Adolescents	FLEHS 2 Ref Ado	Belgium	General population	14 to 15	2008	2009	136	µg/L	11.45	10.32	12.58
FLemish Environment and Health Study 2 Hotspot Genk-Zuid	FLEHS 2 Hotspot Genk-Zuid	Belgium	Inhabitants of Hotspot area	14 to 15	2010	2010	196	µg/L	13.80	12.88	14.71
FLemish Environment and Health Study 2 Hotspot Menen	FLEHS 2 Hotspot Menen	Belgium	Inhabitants of Hotspot area	14 to 15	2010	2011	199	µg/L	13.35	11.44	15.27
DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale (DEMOCOPHES)	SLO-DEMOCOPHES-parents	Slovenia	General population	20 to 49	2011	2012	221	µg/L	11.6	10.6	12.5
DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale (DEMOCOPHES)	SLO-DEMOCOPHES - children	Slovenia	General population	6 to 11	2011	2012	155	µg/L	16.6	15.3	17.9

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Urine-Morning urine											
Exposure of children and adolescents to selected chemicals through their habitat environment	SLO-CRP	Slovenia	General population	6 to 15	2018	2018	246	µg/L	12.6	11.9	13.3
Biovigilance program of food contaminants in the Valencian Community	BIOVAL	Spain	General population	6 to 11	2016	2016	611	µg/L	11.65	6.86	16.45
Bettermilk	Bettermilk	Spain	General population	20 to 45	2015	2015	119	µg/L	43.59	36.04	51.15
Gusum-study	Gusum-study	Sweden	People living at or close to a contaminated site	24 to 90	2008	2008	95	µg/L	23.1	-	-
Urine-spot											
3xG Study	3xG	Belgium	Pregnant women	22 to 43	2010	2015	301	µg/L	13.95	13.17	14.72
FLemish Environment and Health Study 3 Reference Adults	FLEHS 3 Ref Adults	Belgium	General population	50 to 65	2014	2014	208	µg/L	8.61	7.49	9.72

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Urine-spot											
FIOH biomonitoring database on exposure to Copper	FIOH_occup_copper	Finland	Occupationally exposed population *(incl. workers especially from smelters and metal surface treatment and from the use of copper based wood impregnation agents)*	19 to 65	2000	2015	480	µg/L	13.92	-	-
Update of the Reference Limits for the Non-Occupationally Exposed Population in Finland	FIOH_RefLim2011	Finland	General population	22 to 67	2011	2011	153	µg/L	27.26	-	-
National HBM Survey	SLO-HBM	Slovenia	General population	18 to 49	2008	2014	812	µg/L	8.15	7.5	8.81
Cross-Mediterranean Study	SLO-CROME - mothers	Slovenia	General population mothers	30 to 49	2016	2016	177	µg/L	5.72	3.89	7.55
Cross-Mediterranean Study	SLO-CROME - children	Slovenia	General population	7 to 8	2016	2016	176	µg/L	6.67	5.35	7.99

Name Study	Acronym study	Country	Type of study population	Age range	Start year	End year	N° of subjects	UoM	Arythmetic mean (AM) value	AM Lower limit 95% CI	AM Upper limit 95% CI
Urine-spot											
UK_HSL_GenPop_Urine_Cu	UK_HSL_GenPop_Urine_Cu	United Kingdom	General population	18 to 67	2004	2017	398	µg/L	9.1	8.5	9.6
UK_HSL_Occupational_Urine_Cu	UK_HSL_Occupational_Urine_Cu	United Kingdom	Occupationally exposed population	18 to 72	1997	2019	1397	µg/L	15.3	14.5	16.1

3.1.2 Additionnal data identified from a scientific literature review

Before the aggregated data above were gathered, a scientific litterature review was carried out, altough not exhaustively, from the perspective of having a first overview of HBM data available regarding Cu compounds within European countries. This search was performed from the Scopus database with the following equation:

(TITLE-ABS-KEY (copper) AND TITLE-ABS-KEY (biomonitoring) OR TITLE-ABS-KEY (biological AND monitoring) AND TITLE-ABS-KEY (human OR female OR man OR child*)) AND (EXCLUDE (EXACTKEYWORD , "Environmental Monitoring")) AND (EXCLUDE (SUBJAREA , "AGRI") OR EXCLUDE (SUBJAREA , "ENGI") OR EXCLUDE (SUBJAREA , "EART") OR EXCLUDE (SUBJAREA , "CENG") OR EXCLUDE (SUBJAREA , "MATE")).*

Moreover, only publications in English language were reviewed. Additional data of interest retrieved (excluding the data as provided by the HBM4EU National Hubs or data owners as shown in table 1) are presented in table 2, for any purpose it may serve.

Table 2: Additionnal data identified from a scientific literature review

Country	Population	Biological matrix	Mean	P5	P25	P50	P75	P95	Reference / Study name
Urine									
Spain	6-11y N=125	Urine (µg/L) (first-spot)	Geometric Mean (CI): 35.3 (29.1-41.7) Arithmetic Mean (SD): 37.5 (11.9)	-	29.06 (23.86-34.26)	Median 37.6 (30.9-44.4)	-	57.7 (47.4-68.1)	Roca et al., 2016 Biomonitoring of 20 elements in urine of children. Levels and predictors of exposure.
		Urine – creatinine adjusted (µg/g Cre)	Geometric Mean (CI): 35.0 (28.7-41.3) Arithmetic Mean (SD) 36.7 (12.3)	-	28.5 (23.4-33.6)	Median 34.4 (28.3-40.6)	-	56.0 (46.0-66.0)	
Blood/serum									
Italy	18->60y N=215 (104M +111F)	Blood (µg/L) ICP-MS after microwave-assisted acid digestion of blood	M: 957 (932-983) F: 1127 (1080-1175) M+F: 1036	M: 769 F: 805 M+F: 776	M: 878 F: 959	M: 944 F: 1148	M: 1070 F: 1291	M: 1200 F: 1574 M+F: 1495	Bocca et al. 2011 Assessment of reference ranges for blood Cu, Mn, Se and Zn in a selected Italian population

Country	Population	Biological matrix	Mean	P5	P25	P50	P75	P95	Reference / Study name
Blood/serum									
	N=110	Blood (µg/L)	-	686	-	935	-	1157	Alimonti et al. 2005 Assessment of reference values for selected elements in a health urban population.
Italy	Mean age 48y N=65 (exposed) subjects living and working within 4 km of the incinerator 103 (unexposed) subjects living and working outside this area	Serum (µg/L) GF-AAS	-	-	-	-	-	600-1600	Ranzi et al., 2013 Biomonitoring of the general population living near a modern solid waste incinerator: a pilot study in Modena, Italy.
Czech Rep	N=1216 (896M + 320 F) Average age 33y	Blood (µg/L)	812	-	730	-	-	1131	Benes et al. 2000 The concentration levels of Cd, Pb, Hg, Cu, Zn and Se in blood of the population in the Czech Republic.
	N=758 (397 boys + 361 girls) Average age 9.9y	Blood (µg/L)	-	-	1047	-	-	-	
	N=3207	Blood (µg/L)	999	-	840	-	-	1510	Benes et al. 2005 Effects of age, body mass index (BMI), smoking and contraception on levels of Cu, Se and Zn in the blood of the population in the Czech Republic

Country	Population	Biological matrix	Mean	P5	P25	P50	P75	P95	Reference / Study name
Blood/serum									
Spain	N=82	Whole blood (µg/L)	Mean M:1050 F: 1110	-	-	-	-	-	Moreno et al. 1999 Trace element levels in whole blood samples from residents of the city Badajoz, Spain
Spain	N=84 (33M + 51F)	Serum (mg/L)	Mean ±SD All: 1.10 ±0.32 (95%CI 1.05-1.15) M:1.11±0.25 (95%CI 1.03-1.19) F: 1.09±0.36 (95%CI 1.03-1.16)	-	-		-	-	Terrés-Martos et al., 1997 Determination of copper levels in serum of healthy subjects by atomic absorption spectrometry
Germany	N=130 unexposed subjects living in northern Germany	Blood (µg/L) (ICP–MS)	Geometrical Mean: 1020 AM: 1042	804	-	-	-	1620	Heitland and Köster, 2006 Biomonitoring of 37 trace elements in blood samples from inhabitants of northern Germany by ICP–MS
		Plasma (mg/L)	1.07	-	-	-	-	-	Choi et al. 2015 (EFSA supporting publication)

Country	Population	Biological matrix	Mean	P5	P25	P50	P75	P95	Reference / Study name
Hair									
Spain	0-18y N=648 (253M +395F) Healthy child and adolescents	Hair (µg/g) ICP-MS LOD: 0.04 µg/g	mean: 50.8	<0.3	0.3	0.4	0.5	1.0	Llorente Ballesteros et al. 2017 Reference levels of trace elements in hair samples from children and adolescents in Madrid, Spain, calculated ref range : 1.4-186.8 µg/g
	6-9y N=117	Hair (µg/g) ICP-OES LOD: 0.01	mean ± SD: 19.24 ±26.02	-	-	-	-	-	Pena-Fernández, et al. 2016 Evaluating the effect of age and area of residence in the metal and metalloid contents in human hair and urban topsoils
	13-16y N=117	Hair (µg/g) ICP-OES LOD: 0.01	Mean ± SD: 11.99 ±6.85	-	-	-	-	-	
GerES IIb	6-14y N=736	Hair (µg/g)	-	-	-	12	-	-	Seifert et al. 2000 GerES II: reference concentrations of selected environmental pollutants in blood, urine, hair, house dust, drinkingwater and indoor air

Country	Population	Biological matrix	Mean	P5	P25	P50	P75	P95	Reference / Study name
Hair									
Czech Rep	8-11y N=3556	Hair (µg/g)	-	-	-	12	-	-	Benes et al. 2003 Determination of normal concentration levels of Cd Cr, Cu, Hg, Pb, Se and Zn in hair of the child population in the Czech Republic
Italy	11-13y N=137	Hair (µg/g) ICP-MS	-	-	-	19.95	-	-	Senofonte et al. 2000 Assessment of reference values for elements in human hair of urban schoolboys
	3-15y N=412	Hair (µg/g) ICP-MS	-	-	-	10.1	-	-	Dongarra et al. 2011a; 2011b Concentration and reference interval of trace elements in human hair from students living in Palermo Trace elements in scalp hair of children living in differing environmental contexts in Sicily

Country	Population	Biological matrix	Mean	P5	P25	P50	P75	P95	Reference / Study name
Hair									
Sweden	1-76y N=114 (calculated 48M + 66F) subjects without known occupational exposure	Hair (µg/g) Mean ±SD: 25 ±21	Mean ±SD: 25 ±21	-	-	18	-	-	Rodushkin et al. 2000 Application of double focusing sector field ICP-MS for multielemental characterization of human hair and nails: Part II. A study of the inhabitants of northern Sweden Range: 8.5-96
Nail									
Sweden	N=96	Fingernail (µg/g) ICP-MS	Mean ±SD: 8.4 ±3.5	-	-	7.6	-	-	Rodushkin et al. 2000 Application of double focusing sector field ICP-MS for multielemental characterization of human hair and nails: Part II. A study of the inhabitants of northern Sweden Range: 4.2-17

3.2 Interpretation of provided HBM data on total copper

Aggregated data have been obtained from HBM data collections across Europe. The data collections vary with respect to the matrix in which Cu was determined, from whole blood, plasma, serum, and urine to hair, nails, breast milk, and adipose tissue. Within the urine samples, there were differences with respect to sampling (morning spot urine, unspecified spot urine or 24 h urine). In all cases, Cu was determined as total Cu, usually with Inductively Coupled Plasma Mass Spectrometry (ICPMS), albeit no in-depth information was provided on most data collections with respect to the analytical-chemical workup and quantification method used.

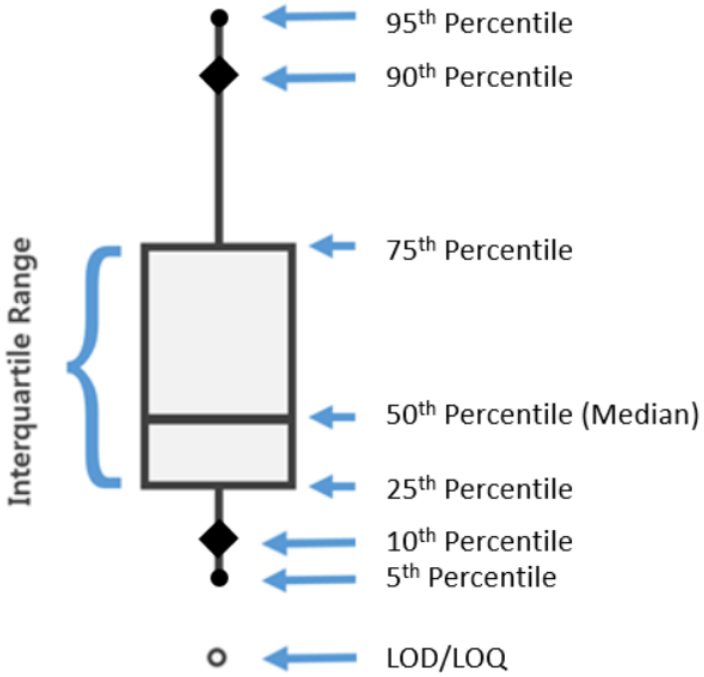
The normal ranges as used in clinical chemistry for blood serum and urine expressed in µmol/L (NVKC website) were converted into µg/L and shown below in Table 3. Normal range values encircle 95% of the analytical results obtained in a selected healthy population.

Table 3: Total copper normal ranges [1]

			µmol/L	µmol/L	µg/L	µg/L		
			Lower bound	Upper bound	**Lower bound**	**Upper bound**		
serum	Adults		11	23	**699**	**1462**	↔	**serum**
serum	Pregnancy	Raised by 2 or 3 times	22	69	**1398**	**4385**	↔	**serum**
serum	Use oral contraception	Raised by 2 times	22	46	**1398**	**2923**	↔	**serum**
serum	Newborns		8	11	**508**	**699**	↔	**serum**
serum	Prematures		3	8	**191**	**508**	↔	**serum**
urine	Undefined		0.15	0.95	**10**	**60**	↔	**urine**

[1] The values in columns 4 and 5 (in µmol/L) highlighed in green are from the original reference as well as the factors ('raised by 2 or 3 time') in column 3. The non-highlighted values in columns 4 and 5 (for pregnancy and use of oral contraception) are calculated using the factors 2 or 3 (in column 3) for multiplication of the 'lower and upper bound adult values'. All values in columns 4 and 5 (in µmol/L) were then converted and expressed in µg/L in columns 6 and 7.

The HBM data on Cu requested from the data collections are aggregated data both for the whole study population, and stratified for sex, Body Mass Index (BMI) and smoking status (stratification results were provided by part of the data owners). The plots shown below contain the limit of quantification (LOQ) or the limit of detection (LOD) (depending on the laboratory) as well as 7 percentile values, as illustrated here:



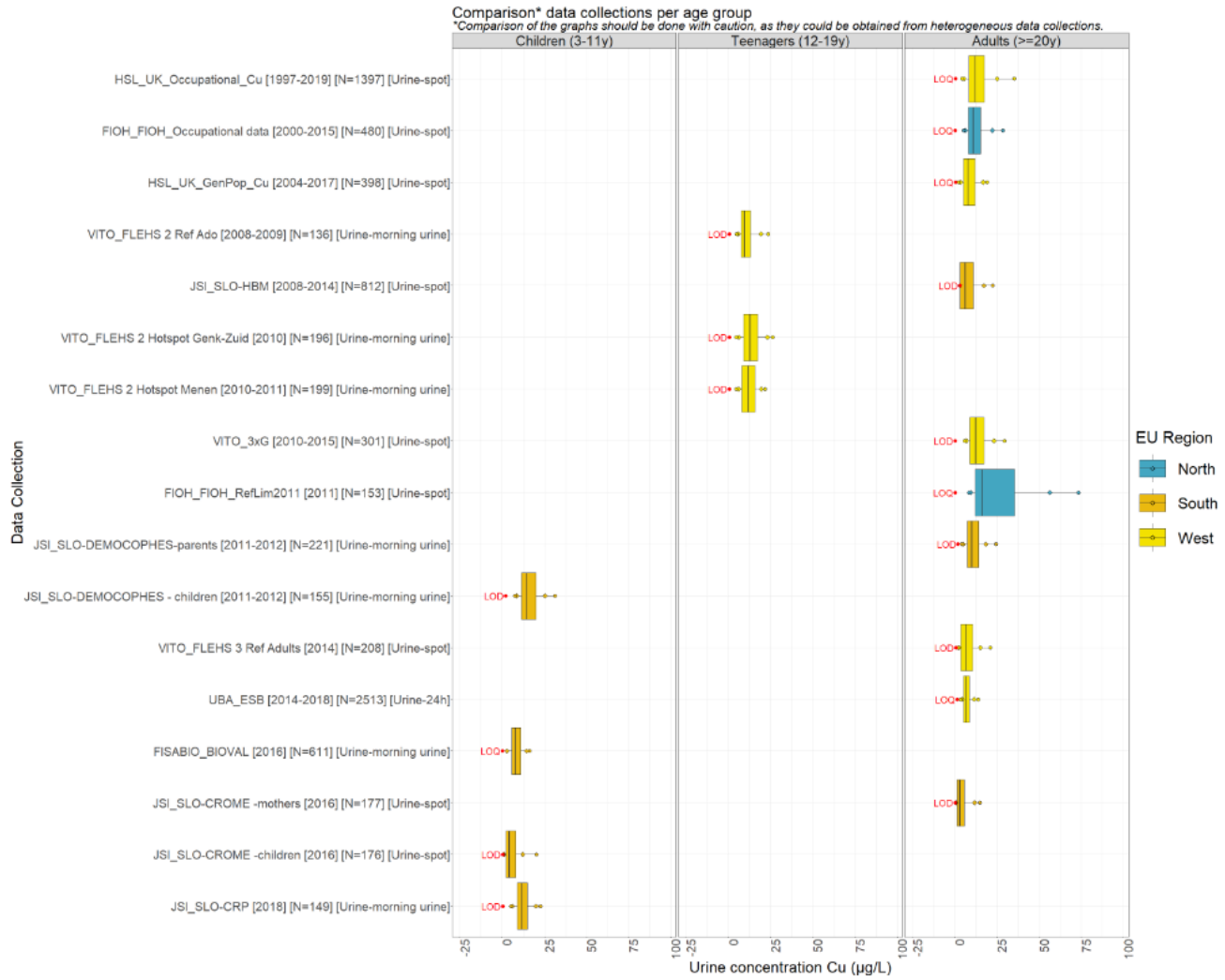
Most data collections obtained (35 in total) were from Western-Europe (16) and Southern-Europe (13) and only a few from Northern-Europe (5) and Eastern-Europe (1). Furthermore, a large part of the adult population data was retrieved from pregnant women and mothers that just have delivered. Consequently, females are being overrepresented compared to males. In addition, within female data, a subpopulation (see Table 3) may have higher copper values due to pregnancy and many women use oral contraceptives, which is known to raise blood Cu status (Berg et al., 1998).

The many differences between the data collections (blood matrix, urine sampling types, pregnancy, age) makes that there is a relatively small number of data collections per stratifier, implying that care should be taken not to overinterpret the data. See two figures below on blood and urine data for the different data collections obtained via HBM4EU partners by age group.

Figure 1: Copper in blood for the different study populations obtained *via* HBM4EU partners

It is noticed that in the adult data collections, the interindividual variation in blood Cu levels was higher than for newborns, children and teenagers. This might be because Cu levels are dependent of the use of oral contraceptives and may change during pregnancy. Both factors are not or are far less relevant for newborns, children and teenagers, respectively. A similar observation holds for the urinary data below.

Figure 2: Copper in urine for the different study populations obtained *via* HBM4EU partners

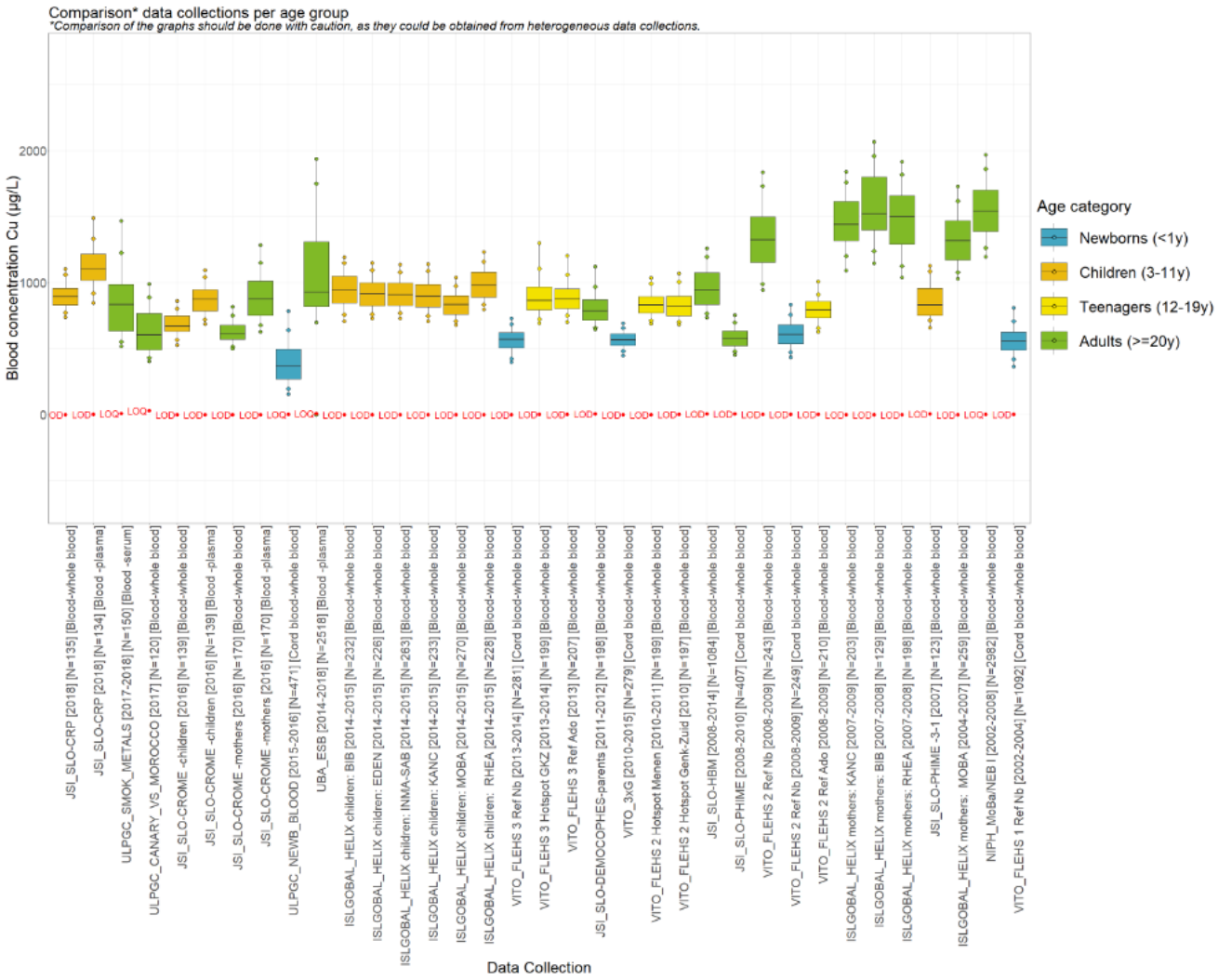


In addition, other findings that are more specific are described below.

3.2.1 Effect of age

There seems to be some correlation of blood Cu levels with age, where lower levels are observed for newborns (blue in plot below) and higher levels for adults (green in plot below). This contrasts somehow with literature where no correlation of serum Cu levels with age was reported in one Spanish study with 434 subjects ranging between 16 and 65 years (Schuhmacher et al., 1994). However, care should be taken as in the current report on the Rapid Response Mechanism of Cu, the vast majority of data collections contained whole blood data whereas the paper of Schuhmacher et al. presents data from Cu serum levels. Furthermore, Schuhmacher et al. did not include newborns in their study.

Figure 3: Copper in blood for the different study populations obtained *via* HBM4EU partners by age groups



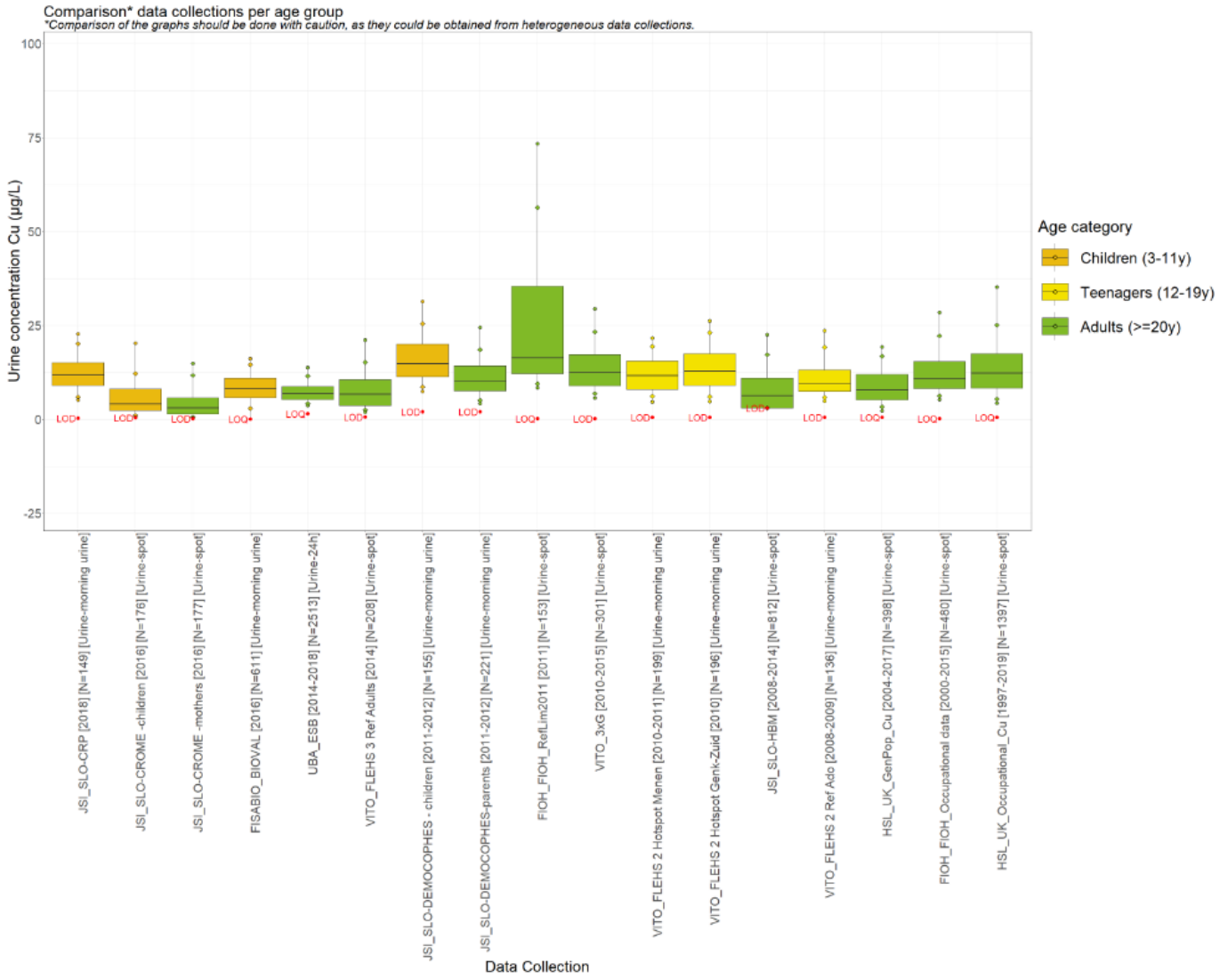
Even more relevant might be the fact that the data collections from exclusively pregnant women, seem to have higher median values than the other adult data collections in blood (Table 1). It is known that pregnancy increases blood Cu concentrations (see Table 3). Therefore, the apparent age-effect might not be an age-effect but a (recent) pregnancy effect.

However, in urine (see figure below), there is not such a clear age-related increase in concentrations reported. Although the urine data sets contain a mix of unspecified spot urine samples, morning spot urine samples and 24 h urine samples, it seems there is no age-related increase in Cu concentrations, irrespective of the way urine was sampled.

Most levels found were in the normal range, as found at the NVKC website for Cu for newborns (500 – 700 µg/L blood serum), as well as for adults (700 – 1450 µg/L blood serum). Six data collections showed slightly higher ranges and they all appeared to represent pregnant mothers. This is as expected as the Cu levels are known to be raised in pregnant mothers.

For Cu in urine the observed concentrations (see plot below), with the exception of the P95 in one adult dataset, all are within the range of normal values that go from 10-60 µg/L (see Table above).

Figure 4: Copper in urine for the different study populations obtained *via* HBM4EU partners by age groups



3.2.2 Effect of Body Mass Index (BMI):

Higher BMI seems to be correlated with slightly higher total Cu concentrations in blood in some data collections (see plot below) but not in others. In urine, the P50 values are higher with higher BMI but the P25-P75 plots always overlap and there are only 4 data collections with this information (see plot below).
As the data sets are relatively small, possible confounders such as 'unhealthy food' could not be investigated.

Figure 5: Copper in blood for the different study populations obtained *via* HBM4EU partners stratified for BMI

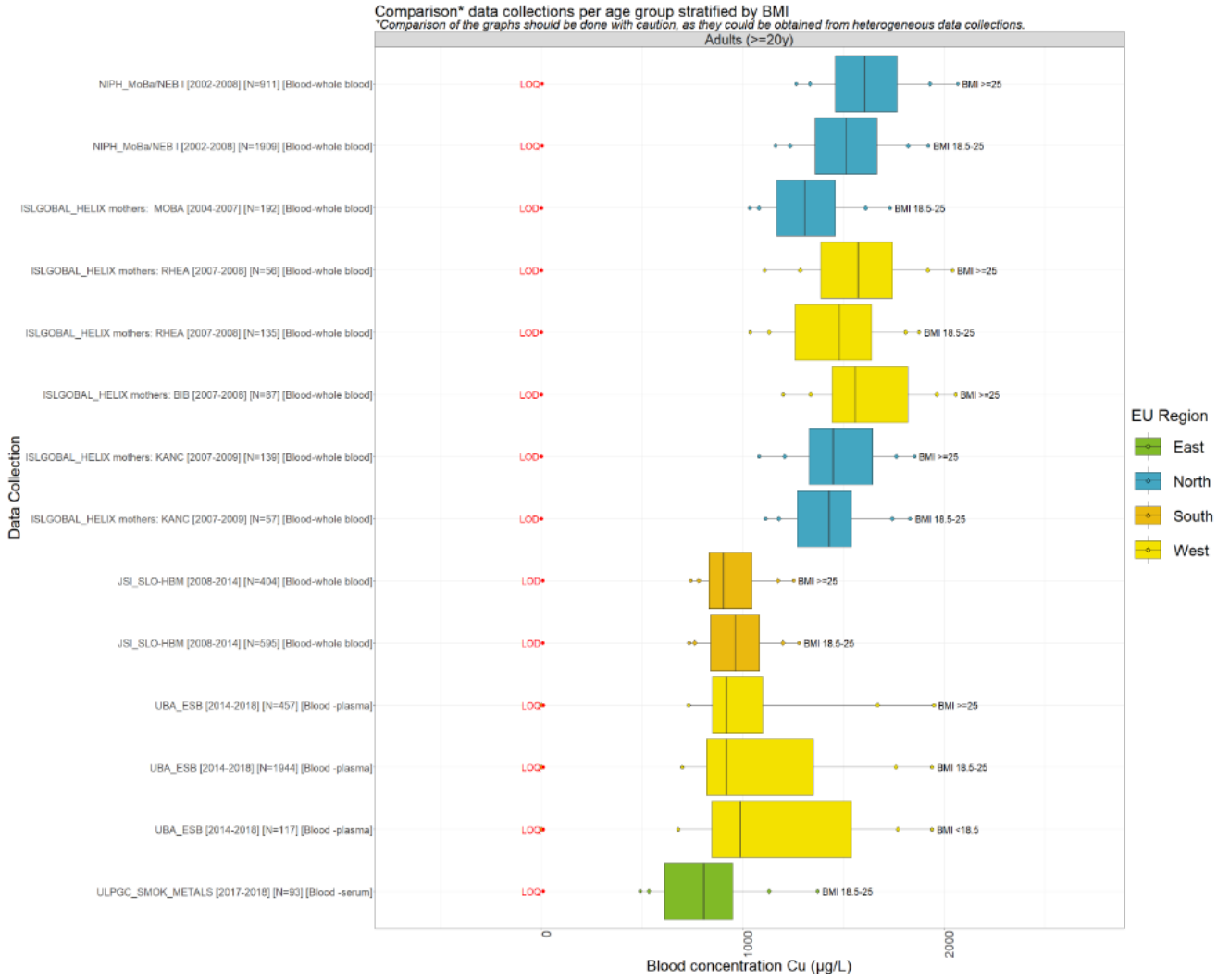
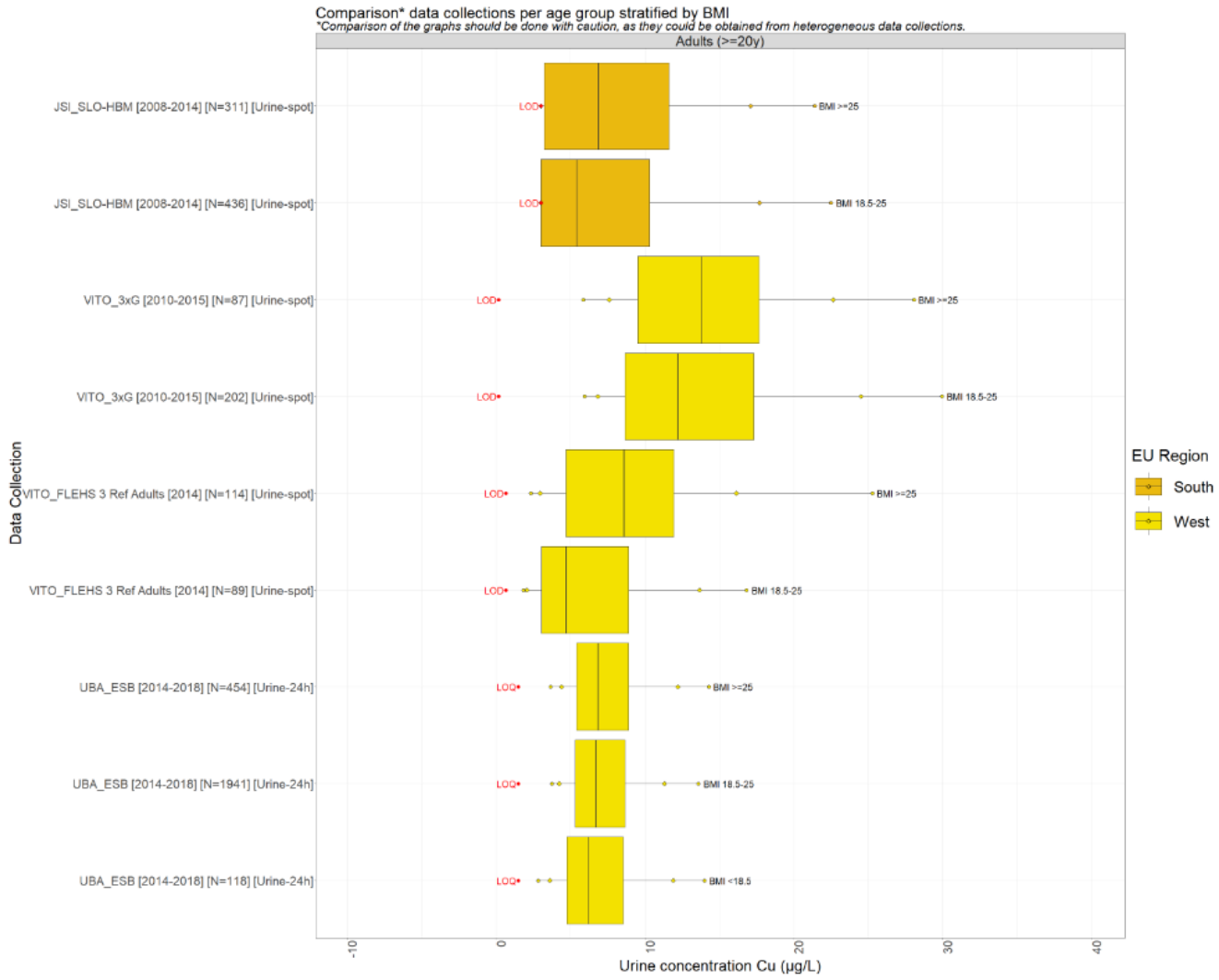


Figure 6: Copper in urine for the different study populations obtained *via* HBM4EU partners stratified for BMI



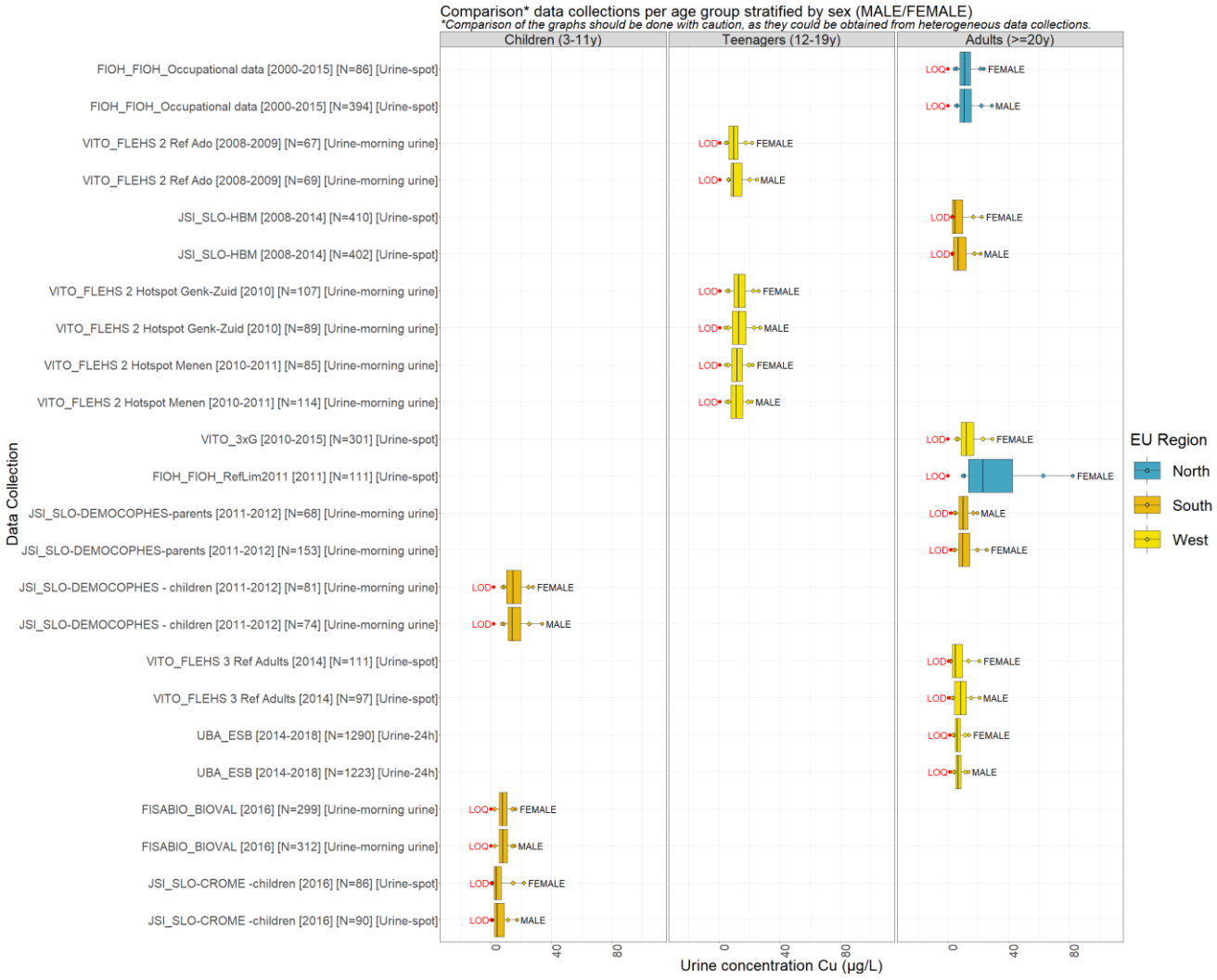
3.2.3 Sex

Higher total Cu concentrations are observed in blood in female *versus* male adults, as depicted in the plot below.

Figure 7: Copper in blood for the different study populations obtained *via* HBM4EU partners stratified for sex

From the urinary measurements where male and female data are present within one data collection (see plot below), overall, if there is a difference, females tend to show somewhat higher levels than females in teenagers and in the adult group for all percentiles. For newborns and children, no differences between total Cu levels in urine, for males *versus* females were observed.

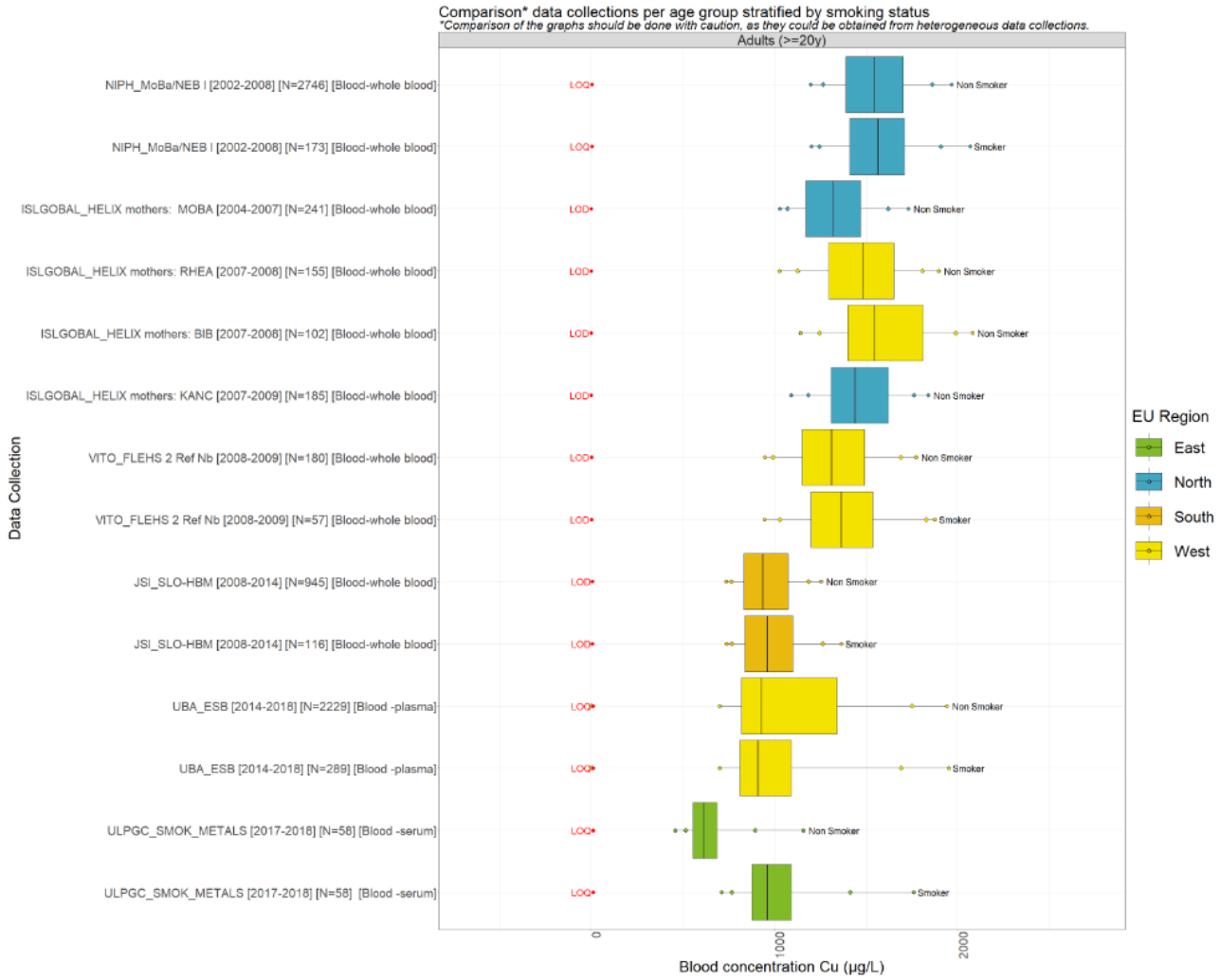
Figure 8: Copper in urine for the different study populations obtained *via* HBM4EU partners stratified for sex



3.2.4 Smoking

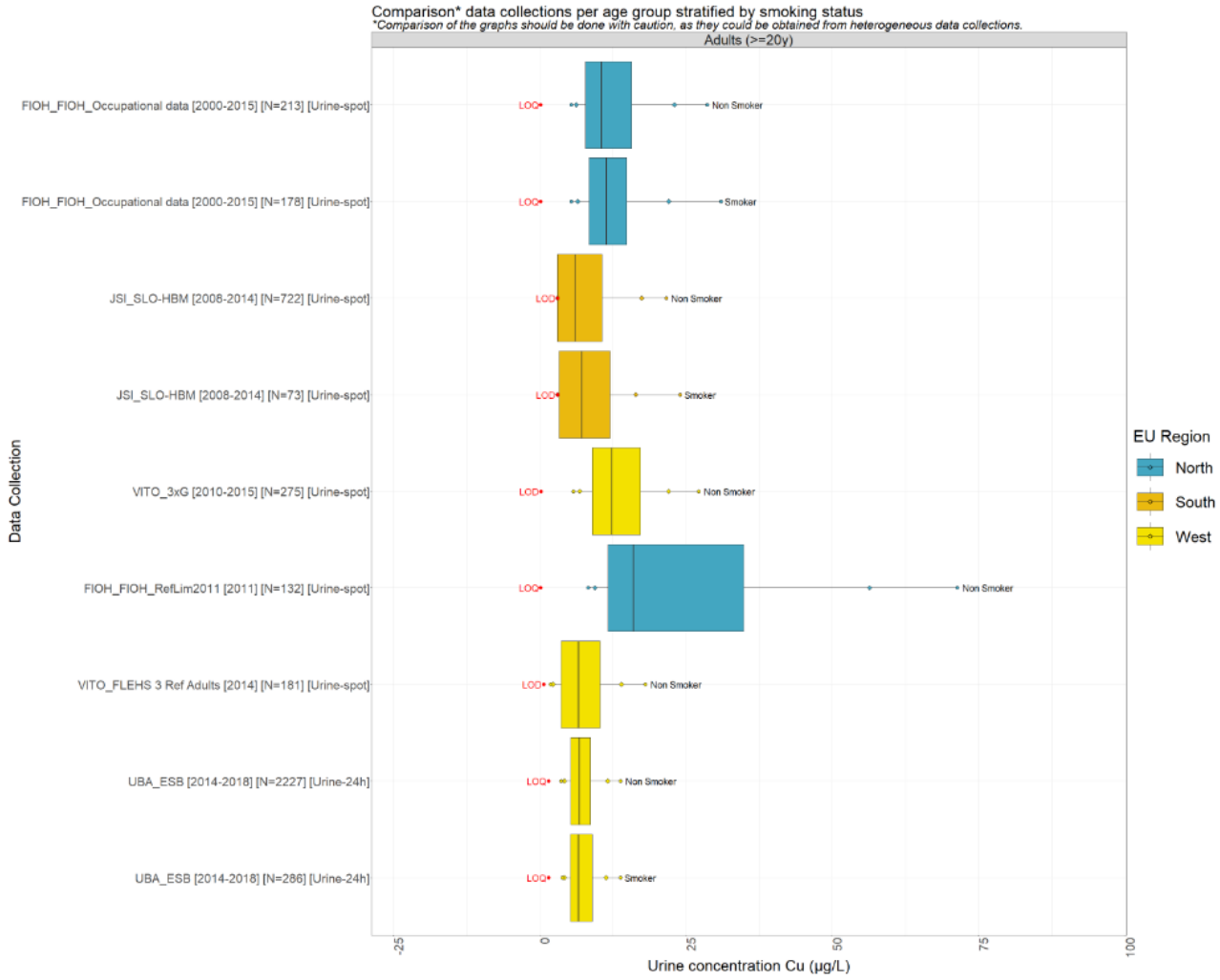
Five data collections on blood Cu can be stratified based on smoking status. Significant differences between Cu levels in smokers and non-smokers are absent in the upper four of those five in the plot. The first three are based on **whole blood**; the fourth one used **blood plasma**. Only in the lower plot (data from ULPGC) there seems to be a significant difference in blood (**blood serum**) levels with smokers showing significantly higher total Cu values compared to non-smokers (P25-P75 range smokers completely separated from P25-P75 range in non-smokers).

Figure 9: Copper in blood for the different study populations obtained *via* HBM4EU partners stratified for smoking



Where stratification for smoking was possible within 3 data collections in urine, no differences were observed between smokers and non-smokers (see plot below).

Figure 10: Copper in urine for the different study populations obtained *via* HBM4EU partners stratified for smoking



3.2.5 Occupational exposure

From the two data collections that were from the UK (Health and Safety Laboratories, UK; see last two lines in the table of section 3.1.1. containing the arithmetic means and the 95 CI), it appears that workers exhibited higher urinary copper values than the general population. Those workers had occupationnal activities related to additive manufacturing, aerospace, foundry, metals, timber treatment, waste treatment and recycling, or exposed to diesel engine exhaust emissions. Unfortunately, no further information or detailed contextual data on specific copper exposure are available. Moreover, this was under the assumption that the data presented for general population were from “white collar” colleagues in the same company, that were sufficiently comparable with respect to age, sex and smoking habits. The arithmetic mean value was 15.3 µg/L (14.5 – 16.1 µg/L lower and upper 95%CI) and 9.1 µg/L (8.5 – 9.6 µg/L lower and upper 95%CI) for workers and general population respectively. Although the worker values are clearly higher, they are still within or close to the normal range (see table at the beginning of this chapter).
It is noted, however, that many arithmetic mean values as presented in the table 1 are lower than 10 µg/L. This may be due to different normalisation procedures (per mg creatinine excreted, or to osmolality). There is no clarity about the procedure for normalisation used to provide the normal range.

3.2.6 General findings

From the blood and urinary HBM data available, no strong conclusions can be drawn. The only relation clearly observed, for blood copper, is the one with very young age (children have higher levels than newborns). Another link is found with occupational exposure. However, no information is available on what kind of occupation the volunteers had. It can be carefully concluded that the higher values in adults are mostly based on female data. It is known that blood copper values are significantly increased by pregnancy as well as by copper-containing intrauterine contraceptives as well as post-menopausal hormone therapy.

The observed blood and urine levels were within the normal ranges as indicated by Table 3. No conclusions can be drawn with respect to possible health effects as neuropsychological development in 1-year old children might be affected at maternal serum copper levels otherwise regarded as normal values (Amorós et al., 2019). The upper range levels in blood were all found in mothers and did not clearly exceed 2000 µg/L whereas the normal range with use of oral contraceptives goes up to almost 3000 µg/L and pregnancy can raise levels to more than 4000 µg/L. In urine, only the P95 in the study named FI_FIOH_RefLim2011 exceeded the upper bound value of the normal range of concentration of 60 µg/L.

In summary, all values were within the normal range as published for children, adults, pregnant women and intrauterine contraceptives using women.

4. PBTK modelling

As mentioned earlier, copper in blood is tightly regulated by homeostatic mechanisms and plasma Cu does not reflect medium and low levels long-term exposure. Plasma biomonitoring is becoming even more complex by the fact that most Cu in serum is in the protein ceruloplasmin, and its regulation may reflect regulation of the protein, rather than of Cu levels. Similarly, urinary concentrations reflect blood concentrations of unbound copper in plasma; however, copper levels in urine are subject to the uncertainties related to urinary dilution and excretion rate, introducing additional uncertainties in the reflection of urinary HBM levels and potential exposure. In addition, overall elimination via urine reflects a small portion of the daily intake, in the range of 1-2% (Bost et al. 2016). Hair seems to reflect long-term plasma levels; yet, hair biomonitoring is subject to uncertainties related to the rate of hair growth, pigment, as well as to the potential considerable uptake of Cu from exogenous sources such as shampoos. Given the above, it is highly unlikely that limited differences (up to 3-4 times) in the general population exposure could be captured through biomonitoring.

The kinetics of copper transfer among the various body compartments, and especially the ones that regulate blood homeostasis; have characteristic times in the range of many hours. As a result, increased exposure levels through occupational activities that result in higher copper exposure (e.g. workers in metals industry, pesticide applicants and bystanders) will be reflected in short term exposure monitoring of serum (and eventually urine) as well as in the longer term in hair.

Initially, our modelling efforts relied on the toxicokinetic data given by Harvey et al (2005). By working more in depth on this model, we concluded that the model lacks a physiological basis. Thus, it is able only to partially describe toxicokinetics of short-term exposure regimes, i.e. it is able to describe changes that occur after a single dose for a few days, but it is not able to describe the time course of chronic intake. For this purpose, we focused on the parameterisation of the generic PBTK model of the INTEGRA platform (Sarigiannis et al. 2014; 2016) for integrated exposure assessment.
The major effort was spent on the adjustment of hepatobiliary recirculation, so that it may reflect internal excretion in order to match better the intake-dependent intestinal absorption and internal excretion. For this purpose, we used the data of the diet-dependent excretion from Turnlund et al. (1998). For the usual intake levels, the absorption ranges between 44 to 54%; it is reduced in the case of high copper intake, while it increases in the light of long-er term copper deficiency, aiming in all cases to maintain the homeostatic blood levels. In addition, tissue:blood partition coeficients for major copper storage tissues (liver, bone and muscles) were estimated based on the data provided by Bost et al. (2016), aiming at the amount of copper found in these tissues in steady state. Excretion through bile and excretion through urine, were adjusted based on the pooled concentrations of the whole blood and urinary levels found in Table 1, assuming age-dependent daily intakes provided by Bost et al. (2016).
At the same time, we have been trying to use HBM data from occupational studies, where statistically significant higher levels of copper in blood (Li et al, 2004; Bizon et al, 2016; Ivanenko et al, 2013, Rainska et al, 2007) and urine (UK_HSL_GenPop vs UK_HSL_Occupational_Urine_Cu; Ivanenko et al, 2013, Rainska et al, 2007) have been

identified. Additional work has been done here in order to combine the input of human respiratory tract deposition modelling of particles, since copper is adsorbed on particles and its bioavailability depends on the amount of particles retained in the lower respiratory tract and is actually translocated into the systemic circulation. Inhalation absorption depends on the size distribution of the particles, as well as the inhalation rate, which depends further on age, gender and activity. The respective absorption fraction for a given exposure regime is around 35 to 60% (higher absorption for lower PM size distribution). Integration of inhalation exposure is particularly important; Cu administered through inhalation is indeed not subject to first-pass metabolism; on the other hand, it is not immediately subject to hepatobiliary recirculation, which is the mechanism that controls internal excretion. This is one more reason for which exposure to inhaled Cu in occupational settings allows the attainment of higher levels of internal exposure. These are also reflected in blood and urine HBM data observed in the occupational studies mentioned above. Considering that exposure to pesticide applicants occurs through inhalation, falls within the interest of DG SANTE and this is something that has to be further explored. Overall, due to lack of specific external exposure data, the limited HBM data from occupational studies were not particularly helpful for the model parameterisation. However, interpretation of external exposure data in occupational studies, where airborne Cu has been measured (Dasch and D'Arcy, 2008; Julander et al, 2014; Lau et al, 2014) has been carried out, including the translation of occupational Cu exposure (in addition to the typical dietary intake) into expected blood and urinary levels. However, the effort for mining occupational studies that eventually combine good exposure and biomonitoring data is ongoing.

From the moment the different Cu forms are absorbed, these are treated as Cu, since the various forms of Cu present similar elimination, distribution and excretion behaviour (EU RAR, 2008). From that point onwards, independently of the maximum intestinal absorption, overall actual absorption will be regulated by the internal excretion as already mentioned. Thus, a dose additivity approach of the various Cu forms seems to be sufficient for toxicokinetics.

The generic PBTK model adaptation to the Cu homeostatic model has been performed. The model is able to describe the lifelong exposure toxicokinetics for the general population. In the following figure, the lifecourse of the Cu concentration in whole blood corresponds excellently with the data obtained from the available HBM studies. What is of particular interest is that the significantly lower concentration related to neonates (which is not attributed to the lower bodyweight normalized exposure for the different developmental stages) is captured very well. In addition, when the Cu concentration in whole blood is in steady state, the amount of Cu stored in the body (~110 mg) is distributed in the tissues (Bost et al. 2016). The partitioning equilibria in particular tissues (28 mg in muscle, 46 mg in bones and 10 mg in liver) reported in the literature are explicitly captured by the model. The HBM data collected from WP10 (presented in Chapter 3) were usefull to allow us to identify the age-dependent differences of Cu levels in whole blood. A summary of the modelling work is presented in Figure 11, where the lifetime course of Cu in whole blood is described (continuous line) together with the measured data (box plots reflecting 5 %, 25 %, median, 75 % and 95 %). All collected data regarding the studies where whole blood Cu levels are included in Table 1, were pooled into one data set for each age group. The selected age groups (neonates, children and adults) are displayed, because dinstinct differences in the Cu whole blood levels have been identified.

In addition, the model was able to reproduce the experimental data delivered by Turnlund et al. (1998), where eleven young men were confined to a metabolic research unit for 90 d. The study was divided into three periods, where dietary copper intake was 0.66 mg/d for 24 d, 0.38

mg/d for 42 d, and 2.49 mg/d for 24 d. Overall, the study identified that the amount of Cu excreted was lower when dietary Cu was low and increased with higher intakes of dietary Cu, clearly affecting the overall amount of Cu actually absorbed.

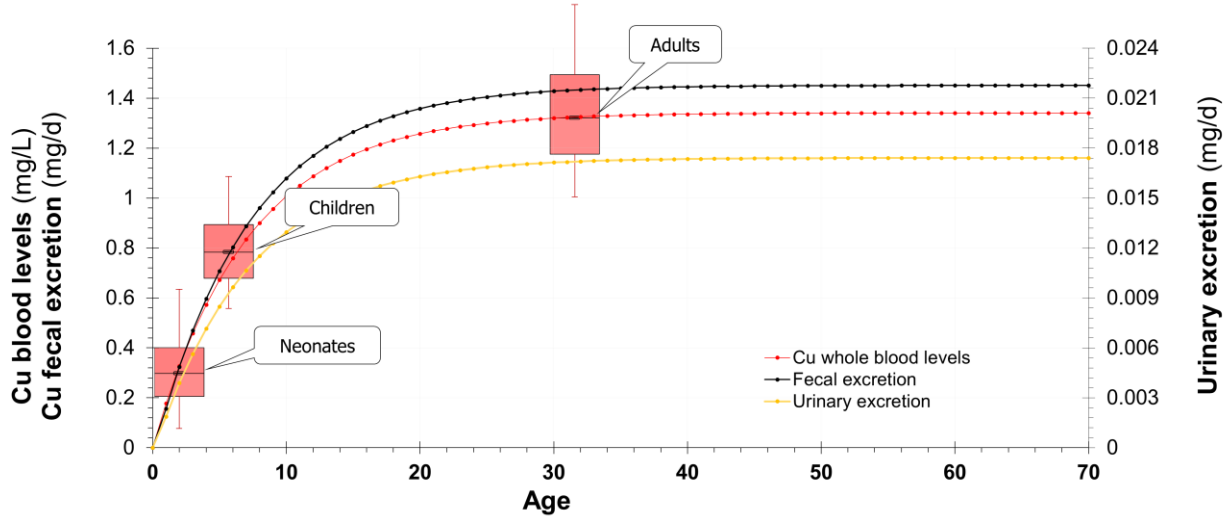


Figure 11. Lifetime course of copper in whole blood. The continuous line represents the modelled levels, while the box plots reflect the collected biomonitored levels from Table 1 (Chapter 3.1).

The results of the measured (green points) *versus* the modeled (blue points) fecal excretion rate during the experimental period are presented in Figure 12. This was the result of adjusting the hepatobiliary recirculation as a function of the homeostatic levels of Cu in human body. In addition, the whole blood concentration (red line) and the urinary excretion (yellow line) are illustrated as well. The agreement of the model with the experimental data of this study is very important, because it reflects the capacity of the model to capture the Cu homeostasis under real life intake patterns. More details on the modelling work of Cu within the frame of HBM4EU can be found in the additionnal deliverable AD12.11.

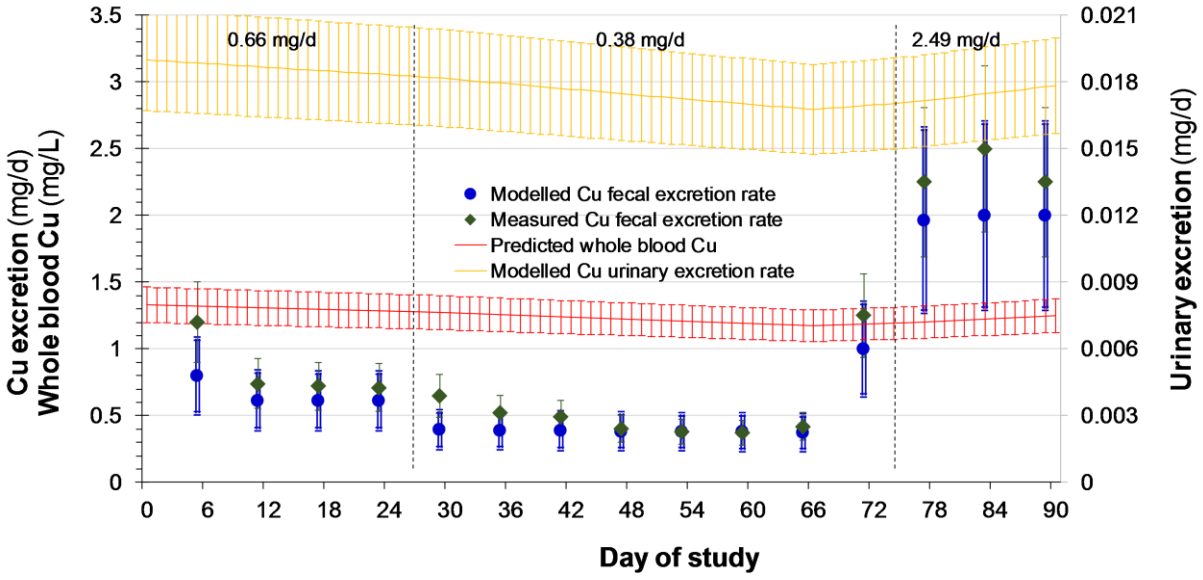


Figure 12. Measured (Turnlund et al. 1998) versus modelled excretion rate of copper during different intake periods, whole blood concentration and urinary excretion rate.

5. Conclusion

In conclusion, Cu is tightly regulated by homeostatic mechanisms and the current dataset does not allow to relate changes in dietary intake to differences in biomarkers in blood and urine. A good biomarker of exposure for copper is needed to monitor internal Cu exposure levels that could help avoiding chronic health effects in large populations due to high Cu exposures, and to give an 'early warning' in sensitive populations (infants, pregnant or lactating women, individuals with idiopathic or genetic changes in metabolism, elderly, diseased people), before any tissue damage occurs.
Rather than testing each of the cuproenzymes, Cu-binding proteins or Cu chaperones for potential use as a Cu biomarker, the advent of high-throughput technologies has made it possible to screen for potential biomarkers in the whole proteome of a cell. Since Cu is involved in so many biological processes, a good biomarker may be a downstream product, with no immediate role in Cu metabolism (Danzeisen et al., 2007).

Furthermore, according to the HBM data provided *via* the National hubs and the data owners, no strong conclusions can be drawn from blood and urinary data, except for the very young children that have higher blood Cu levels than newborns. Higher values in adults are mostly based on female data. This can be explained by the fact that blood Cu values are significantly increased by pregnancy, by Cu-containing intrauterine contraceptives as well as post-menopausal hormone therapy. Higher urinary Cu values were observed if occupationally exposed workers are compared with values from the general population. However little information is available regarding the type of occupation the volunteers had. Almost all values obtained were within the normal ranges as published for children, adults, pregnant women, and women that use intrauterine contraceptives.

Regarding the PBTK modelling, additional toxicokinetic data than the ones from the study of Harvey et al. (2005) are needed to cover higher exposure patterns related to occupational exposure, as well as to capture the short exposure regime dynamics and how they may affect Cu homeostasis. Ideally, the occupational HBM data should be accompanied with measurements / estimates about external exposure (e.g. copper levels in ambient air).
Moreover, from our lifelong exposure/PBTK model, it seems that long-term Cu intake levels are reflected to some extent in the overall body burden. To explain the observed differences in the blood Cu levels detailed information about the dietary intake or exposure through other exposure routes is needed. By reconstructing exposure based on the levels of HBM in blood from the various studies, daily intake has been estimated for the various age groups. The respective daily intake estimates (0.8 to 2 mg/d) are within the range of reported intake estimates based on food consumption data and Cu levels in food items.
Therefore, in order to concretely answer the questions asked by DG SANTE, the following two alternative strategies are proposed to be explored in the immediate future:

I. Ideally, at least two studies need to be identified comprising a sufficient number of individuals (n~200) at various age groups, in which:
    a. detailed food diaries will be available, that will allow us to estimate Cu intake at the individual level and
    b. whole blood levels of Cu are measured.

Studies designed as such would allow us to identify the extent to which differences in Cu intake explain the variance of the Cu levels in the whole blood. In this context, data of Cu levels in consumed food items, based on the FoodEx L2 classification, for the countries of the recruitment are needed in addition.

II. If the studies described above cannot be made available for the concrete interpretation of the already collated aggregate HBM data, that is to identify whether the differences in the HBM data are resulting from differences in Cu intake, we will need to calculate the intake levels. Towards this aim, and to deliver country-specific daily Cu intakes, we need to have data of the Cu levels in the consumed food items per EU Member State, based on the FoodEx L2 classification. This information could be provided by EFSA on top of in-house available knowledge.

We propose to proceed according to strategy II above at first, until the two complete population studies described above as *sine qua non* input to the implementation of strategy I are identified. Thus, if EFSA has food contamination data regarding Cu for the EU Member States for which we have HBM data available and is able to disclose it, HBM4EU would be able to identify whether the differences in HBM data result from differences in intake.

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7. Annexes

Annex 1 - Two studies measuring true absorption of Cu in humans

Harvey, L.J. et al. (2003). Adaptive responses in men fed low- and high-copper diets. British Journal of Nutrition 90, 161–168

The aim of the present study was to employ stable-isotope techniques to measure Cu absorption and endogenous losses in adult men adapted to low, moderate and high Cu-supplemented diets. Twelve healthy men, aged 20–59 years, were given diets containing 0.7, 1.6 and 6.0 mg Cu/d for 8 weeks, with at least 4 weeks intervening washout periods. After 6 weeks adaptation, apparent and true absorption of Cu were determined by measuring luminal loss and endogenous excretion of Cu following oral administration of 3 mg highly enriched ^{65}Cu stable-isotope label. Apparent and true absorption (41 and 48 % respectively) on the low-Cu diet were not significantly different from the high-Cu diet (45 and 48 %, respectively). Endogenous losses were significantly reduced on the low- (0.45 mg/d; $P<0.001$) and medium- (0.81 mg/d; $P<0.001$) compared with the high-Cu diet (2.46 mg/d). No biochemical changes resulting from the dietary intervention were observed. Cu homeostasis was maintained over a wide range of intake and more rapidly at the lower intake, mainly through changes in endogenous excretion.

From the results presented it appears that Cu homeostasis is maintained through control of only endogenous excretion and not absorption. Although the present study does not include data on urinary output, it has been reported that this form of excretion is minimal and is not dependent on dietary Cu intake [See below, Turnlund et al. (1989)].

Turnlund, J.R. et al. (1989). Copper absorption and retention in young men at three levels of dietary copper by use of the stable isotope 65Cu. Am J Clin Nutr 49, 870-878

This study was performed to establish the effect of the level of 3 different dietary levels of Cu on Cu absorption, retention and loss. Eleven volunteers were confined to a metabolic research unit for the 90-day duration of the study. They were under constant medical supervision. They were given a low-Cu diet, a liquid formula calorie supplement with added minerals, and a multivitamin tablet every day for the 90 days of the study. The calorific intake of each subject was calculated to maintain static bodyweight. The study was divided into three metabolic periods (MPs); an initial equilibration period with adequate Cu (MP1), a low-Cu period (MP2) and a high-Cu period (MP3). A solution containing Cu SO4 was added to the liquid formula calorie supplement to create the levels of Cu required. On one occasion (MP 1 and MP3) or four occasions (MP2), the dietary Cu was supplied as 65CuO in diluted HCl, for absorption determinations. Capsules containing a total of 6.0 mg polyethylene glycol (PEG), a faecal marker not absorbed by the body, were fed to each subject on each day the 65Cu was fed. Complete duplicate diet samples were taken, stored frozen and later analysed for Cu content. Complete faecal and urine collections were made throughout the study. Blood samples were taken weekly for plasma Cu, ceruloplasmin, and superoxide dismutase (SOD) levels. Samples of sweat and saliva were also taken once or twice during each MP. Cu content of plasma, urine, sweat, saliva, diet and faecal samples was determined by atomic absorption spectrophotometry. PEG content of faeces was determined by a turbidimetric method. 65Cu:63Cu ratio was determined by mass spectrometer. Ceruloplasmin was determined by o-dianisidine oxidation and SOD by autooxidation of pyrogallol.

The bodyweights of the volunteers at the end of the study was within 3 kg of initial weight. All were in good health. Cu absorption averaged 36 % in MP1 (adequate Cu), 55.0 % early in MP2 and 56.2 % later in MP2 (low Cu), and 12.4 % in MP3 (high Cu). The effect of varying the amount of Cu in the diet on Cu absorption is shown in the Table below.

Effect of dietary Cu level on Cu absorption

	Metabolic Period (MP)		
	MP1	MP2	MP3
Duration (days)	24	42	24
65Cu administered on day of MP	13	7,8,31,32	13
Dietary Cu (mg/day)			
Calculated	2.0	0.8	8.0
Measured	1.68	0.785	7.53
On 65Cu-days	1.99	0.900	7.78
Mean percent 65Cu absorbed (n=11)	36.3	55.6	12.4
Actual 65Cu absorbed (mg)	0.61	0.44	0.93
Endogenous faecal losses (mg)	0.61	0.35	0.97

Absorption of Cu varied significantly between MPs, but did not differ significantly between subjects or between the two feedings in MP2. The data demonstrated the Cu absorption varied with intake; that subjects on a low Cu diet absorbed proportionately more Cu, and subjects receiving an excess of Cu absorbed less. This result agrees with the concept of homeostatic control of Cu levels in the body. The subjects on the high Cu diet received 10 times the amount in the low Cu diet, yet absorption was only increased two-fold.

Excretion of endogenous Cu in the high Cu MP matched the increased absorption. From the data for MP3, it appears that absorption is the first point of regulation of Cu metabolism, and that this is followed by additional regulation through endogenous loss. Data from MP2 also suggest that adaptation to low Cu diet is more rapid than adaptation to high Cu diet. The more rapid response of the body would indicate that Cu deficiency (i.e. change to a low Cu diet) needs a more rapid reaction because deficiency is potentially a more deleterious state than mild excess. A diet of 8 mg Cu/person /day for at least 24 days was without ill-effect. Urinary, sweat and salivary Cu were not affected by the amount of Cu in the diet. Urinary losses averaged about 0.3 µmol/day, so low that they do not affect Cu balance. Cu content of sweat was very low. Cu content of saliva (from the parotid salivary gland) was low, about 1 µmol/day, and was not affected by dietary Cu. Most saliva is swallowed. Cu status, based on plasma Cu, ceruloplasmin Cu content, and SOD were unaffected by diet, and remained roughly static for each individual.

Absorption of Cu from the diet varied with dietary content. A higher proportion of Cu was absorbed from a low Cu diet, and a lower proportion of Cu was absorbed from a high Cu diet, indicating that the amount absorbed will vary with the nutritional content of the diet to maintain Cu balance in the body. Levels of Cu in the plasma, in ceruloplasmin and levels of SOD were unaffected by dietary Cu even at the high level of 8 mg/person/day. Dietary levels up to 8 mg/person/day were tolerated with no ill-effects

Conclusion
In 2 studies in humans, faecal excretion of absorbed Cu was taken into account to estimate true Cu absorption from dietary Cu intakes ranging from 0.7 to 6 mg/day, and true Cu absorption ranged from 45 to 49 %. Based on these studies, EFSA has considered that Cu absorption in humans from the diet is around 50% for all age and life-stage groups in the recent Scientific opinion on Dietary Reference Values for Cu document (EFSA Journal 2015).
This value includes the amount of endogenous losses in the calculation of oral absorption.

Annex 2 - Simple compartmental model for copper

Harvey, J.L. et al. (2005). Use of mathematical modeling to study copper metabolism in humans. American Journal of Clinical Nutrition, 81, 807-813
The objectives of the present study were to validate a method for estimating endogenous losses of Cu, test whether a simple model can predict true absorption from the plasma appearance of labeled Cu, and develop a compartmental model for Cu metabolism by using stable isotopes. The design was as following: a stable isotope of Cu was intravenously administered to 6 men, and fecal samples were collected for 14 days. Four weeks later the study was repeated, but with an oral dose, and blood samples were collected for 7 days and fecal samples for 14 days.
The oral and intravenous (IV) isotope excretion and oral absorption estimates are summarized in the table below.

Oral (3 mg) and intravenous (IV; 0.5 mg) isotope excretion in feces and estimated absorption of the oral dose in feces and plasma

Subjects (n=6)	Amount of IV dose excreted	Amount of oral dose excreted	Apparent absorption	True absorption based on excretion of IV dose	True absorption based on excretion of oral dose
Mean	32%	35%	33%	48%	49%
SD	5	2	3	5	4

The amount of IV Cu isotope excreted (32 ± 5%) was not significantly different from the estimate of excretion of the absorbed oral Cu label (35 ± 2%). The mean apparent absorption from the oral dose was 33 ± 3%. When a correction was made to the apparent absorption for the amount of oral dose absorbed and then excreted, the mean true absorption was estimated to be 49 ± 4%. This was not significantly different (P = 0.48) from the true absorption (48 ± 5%) when the correction was made by using the estimation of the quantity of the IV dose excreted. A simple mathematical model fitted to plasma isotope appearance data estimated true absorption to be 8 +/- 2% compared with 48-49% measured by fecal monitoring. A more complicated compartmental model predicted that, when newly absorbed Cu first enters the blood, 74% is removed by the liver and 99% is bound to ceruloplasmin in the plasma. The exchangeable pool of Cu was estimated to be 43 +/- 30 mg. Daily endogenous losses were predicted to be 2.4 mg.
In conclusion, the results showed that fecal monitoring is the only method that can reliably measure labeled Cu absorption, and it is not necessary to administer an IV dose of Cu to estimate endogenous losses. The compartmental model provides new insights into human Cu metabolism.