Perrine Hoet*, Chantal Jacquerye, Gladys Deumer, Dominique Lison and Vincent Haufroid

Reference values of trace elements in blood and/or plasma in adults living in Belgium

https://doi.org/10.1515/cclm-2020-1019
Received May 20, 2020; accepted October 9, 2020;
published online October 27, 2020

Abstract

Objectives: Trace elements (TEs) from natural and anthropogenic sources are ubiquitous. Essential or not, their relevance for human health and disease is constantly expanding. Biological monitoring is a widely integrated tool in risk assessment both in occupational and environmental settings. However, the determination of appropriate and accurate reference values in the (specific) population is a prerequisite for a correct interpretation of biomonitoring data. This study aimed at determining the reference distribution for TEs (Al, As, Sb, Be, Bi, Cd, Co, Cu, Mn, Hg, Mo, Ni, Pb, Se, Tl, Sn, V, Zn) in the blood and/or plasma of the adult population in Belgium.

Methods: Blood and plasma samples were analyzed for 178 males and 202 females, recruited according to an a priori selection procedure, by inductively coupled plasma mass spectrometry (ICP-MS).

Results: Reference values were established with high confidence for AsT, Cd, Cu, HgT, Mn, Mo, Pb, Sn, Se, Tl and Zn. Compared to previously published data in the Belgian population, a decreasing time trend is observed for Zn, Cd and Pb. Globally, the results also indicate that the current exposure levels to TEs in the Belgian population are similar to those from other recent national surveys.

Conclusions: These reference values and limits obtained through validated analytical and statistical methods will be useful for future occupational and/or environmental surveys. They will contribute to decision-making concerning both public health policies but also exposure assessments on an individual scale.

Keywords: Belgium; biological monitoring; blood; plasma; reference values; trace elements.

Introduction

Present in the Earth's crust, trace elements (TEs) are naturally and anthropogenically released into the environment. As TEs are non-biodegradable and highly persistent in the environment, human exposure can be a public health concern, in developed as well as in developing countries, in environmental as well as in occupational settings.

Some TEs are essentials to humans (Co, Cu, Mn, Mo, Se, Zn) [1] and deficiency may result in severe malfunctioning of the body. Others, often called "toxic TEs", have no known physiological role in humans. Overexposure to either essential or "toxic" TEs can be detrimental to human health, and documentation of exposure levels is a key component of the risk assessment.

Human biological monitoring (HBM) provides indications on the absorbed dose of chemicals, whatever their sources or routes of exposure. HBM is increasingly important in risk assessment and prevention at both individual and group levels and several tools have been developed to integrate biomonitoring data in risk assessment strategies [2, 3].

For the interpretation of HBM data and the identification of (groups of) individuals with extreme exposure, it is essential to document background exposure to the chemicals of interest in the appropriate reference population, and to derive reliable reference values (RVs).

The concept of RV, launched by a Scandinavian group in a session devoted to "normal values" during a Congress of Clinical Laboratory Medicine in 1969, has evolved over years, leading to the elaboration of worldwide-endorsed guidelines by the International Federation of Clinical

Current affiliation: Fédération des Mutualités socialistes du Brabant, Brussels, Belgium

***Corresponding author: Perrine Hoet**, Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Université catholique de Louvain, Institute of Experimental and Clinical Research (IREC), Brussels, Belgium, E-mail: perrine.hoet@uclouvain.be
Chantal Jacquerye, CESI, Occupational Health Service, Brussels, Belgium
Gladys Deumer, and Cliniques Universitaires Saint-Luc, Laboratory of Analytical Biochemistry, Brussels, Belgium
Dominique Lison, Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Université catholique de Louvain, Institute of Experimental and Clinical Research (IREC), Brussels, Belgium
Vincent Haufroid, Louvain Centre for Toxicology and Applied Pharmacology (LTAP), Université catholique de Louvain, Institute of Experimental and Clinical Research (IREC), Brussels, Belgium; and Cliniques Universitaires Saint-Luc, Laboratory of Analytical Biochemistry, Brussels, Belgium

Chemistry and Laboratory Medicine (IFCC) and the Clinical and Laboratory Standards Institute (CLSI) [4–7].

In the 80–90's, the EUROTERVIHT project aimed at producing RVs for TEs in human body fluids, and highlighted significant differences related to geological factors and diet among European countries, but also considerable uncertainty in TEs determination. The project identified a need for harmonization of the different steps of the procedure, from the control of pre-analytical factors to the statistical treatment [8, 9]. Under the scope of European Union, the HBM4EU project (2017–2021) was launched to coordinate HBM of chemicals, including TEs, in Europe, to provide evidence for chemical policymaking. Meanwhile, it was important to establish RVs for TEs in Belgium under strict quality control at all steps of the procedure.

We recently established the distribution and RVs for TEs in the urine of the adult population in Belgium [10]. The aim of the present study was to determine the distribution and RVs for blood and plasma levels of relevant TEs in the adult population in Belgium.

A comprehensive overview of the toxicity profile of these TEs, including tolerable levels, can be found elsewhere [11].

Materials and methods

Study population

Subjects were recruited on a voluntary basis by an occupational health service during annual medical check-ups and by the Louvain centre for Toxicology and Applied Pharmacology (LTAP). Recruitment took place between October 2016 and October 2017, in the 10 provinces of Belgium, covering urban, suburban, and rural areas. The *a priori* selection included the following criteria: 18–70 years old; healthy condition; living for a period of at least 1 year in Belgium; no history of known or suspected (extra-)occupational exposure to the TEs investigated. Pregnancy was an exclusion criterion. A one-page questionnaire collected information on age, gender, health status, medication use, smoking habits, area of residence, occupation and other factors likely to have an influence on TEs concentrations.

All participants signed an informed consent form in accordance with the Biomedical Ethical Commission of the Faculty of Health Sciences (Université catholique de Louvain, Brussels) that approved the study protocol (CEHF 2015/544-B403).

A total of 394 eligible subjects provided non-fasting blood samples. After disinfection of the skin with ethanol, two venous blood samples were drawn by well-trained professionals to ensure proper sample collection, handling and transport. Stainless steel needles and either Sarstedt Lithium Heparin – Trace Metal Analysis S-Monovette® or Becton Dickinson Vacutainer® Plus plastic tubes with K_2EDTA Royal Blue Stopper for TEs testing were used. For plasma processing, centrifugation at 2,000*g* for 10 min at RT was performed with in a maximum of 2 h after blood sampling. Specimens and questionnaires were anonymously coded at the point of collection, and samples were kept for a maximum of 2 days at +4 °C before being sent to the laboratory and stored frozen at −20 °C until analyzed.

Analyses

All samples were analyzed in the Laboratory of Analytical Biochemistry at the Cliniques Universitaires Saint-Luc (Brussels, Belgium) in a random sequence. Eighteen TEs were measured in whole blood and plasma by means of inductively coupled argon plasma mass spectrometry (ICP-MS) on an Agilent 7500cx instrument. Briefly, both specimens (500 µL) were diluted quantitatively (1+9) with a 1-butanol (2%w/v), EDTA (0.05%w/v), Triton X-100 (0.05%w/v), NH_4OH (1%w/v) solution containing Sc, Ge, Rh and Ir as internal standards. A micromist nebulizer was used for the plasma determination while a Babington nebulizer was preferred for the whole blood determination to avoid clotting problems. Limits of detection (LoD) and quantification (LoQ), given in Tables 1 and 2, were calculated as three and nine times, respectively, the standard deviation of the blank signal. Two sampling tubes from each lot were checked for the absence of contamination by leachable elements. BD tubes were excluded for the determination of Sb and Sn distribution because, as specified by the manufacturer, BD Vacutainer® PET-TEs should not be used for Sb determination, and no upper limit of contamination is provided by the manufacturer for Sn.

Traceability to international reference materials was guaranteed by the use of certified internal quality controls. For that purpose, Seronorm™ Trace Elements Whole Blood at three different levels (L1, ref. 210105; L2, ref. 210202; L3, ref. 210305) and Seronorm™ Trace Elements Serum (L1, ref. 201405) materials were used for whole blood and plasma analyses, respectively.

The laboratory has the highest level of certification (ISO15189, granted by BELAC) for the measurement of six (Cd, Co, Mn, Hg, Pb and Tl) and five (Al, Bi, Cu, Se and Zn) TEs in whole blood and plasma, respectively. Using validated ICP-MS methods, the laboratory has obtained successful results, for more than 10 years, in external quality assessment schemes organized by the Institute for Occupational, Environmental and Social Medicine of the University of Erlangen, Germany (G-EQUAS program) and act as a reference laboratory for these TEs, as well as by the Institut National de Santé Publique, Québec (PCI and QMEQAS programs).

Besides the ISO15189 certified TEs/matrix combinations, we also included in the study TEs/matrix combinations for which traceability could be guaranteed by the successful use of certified internal quality controls analyzed at the beginning and at the end of each analytical run (Al, As, Be, Bi, Cu, Mo, Ni, Sb, Se, Sn, V, and Zn in blood; Co, Ni, and Mn in plasma).

Statistics

Statistical analyses were completed using Excel 2016 (Microsoft Office, Redmond, USA) and GraphPad Prism 8.4.2. (GraphPad Software, San Diego, USA). Statistics were performed as recommended by the IFCC-CLSI guidelines [7] and according to the procedure used previously for the determination of RVs of TEs in urine [10].

For each TE, we characterized the distribution and calculated the 2.5, 10, 25, 50, 75, 90, 95, 97.5th percentiles. Concentrations below the LoD were assigned a value of LoD/2 for statistical calculations. Distribution (log)normality was checked with the D'Agostino-Pearson test. The shape of most distributions was asymmetrical and positively skewed. The geometric mean (GM) was calculated if the proportion of values below the LoD was <40%. Outliers, identified by box-plots and

Table 1: Distribution of essential trace elements and derived LRL and URL (µg/L).

		LoD (%<LoD)	LoQ (%<LoQ)	P2.5 (90%CI)	P50	P97.5 (90%CI)	LRL	URL
Co	Plasma	0.47 (94.7)	1.38 (99.7)	<LoD	<LoD	0.60 (0.56–0.88)	–	0.8[a]
	Blood	0.10 (79.5)	0.16 (90)	<LoD	<LoD	0.44 (0.61–0.63)	–	0.6[a]
Cu	Plasma	1.60 (0)	5.25 (0)	598 (512–631)	948	1,998 (1,791–2,122)	520	2,100
	W			610 (502–663)	1,080	2,050 (1,950–2,233)	520	2,200
	M			581 (510–643)	814	1,095 (1,047–1,302)	520	1,300
	Blood	0.84 (0)	1.86 (0)	610 (571–622)	795	1,433 (1,343–1,595)	580	1,590
	W			649 (592–659)	899	1,462 (1,409–1,612)	600	1,600
	M			582 (543–611)	724	890 (847–965)	550	950
Mn	Plasma	0.61 (80.1)	1.60 (99.5)	<LoD	<LoD	0.90 (0.82–0.98)	–	0.9[a]
	Blood	1.09 (0)	2.95 (0)	5.43 (5.27–5.95)	9.24	17.6 (16.1–18.7)	5.30	18.0
	W			6.21 (5.38–6.51)	9.64	18.7 (16.8–19.1)	5.50	19.0
	M			5.39 (4.94–5.69)	8.81	14.1 (12.9–16.1)	5.00	15.0
Mo	Blood	0.43 (5)	0.95 (60)	<LoD	0.77	2.98 (2.45–4.20)	–	4.0
Se	Plasma	0.40 (0)	1.22 (0)	66.7 (60.0–70.3)	93.7	122 (116–132)	65	125
	W			61.6 (50.2–69.1)	91.7	122 (116–133)	55	130
	M			73.1 (63.0–76.5)	96.8	125 (119–142)	65	140
	Blood	0.11 (0)	0.2 (0)	83.2 (77.3–86.4)	110	148 (143–158)	80	155
Zn	Plasma	7.18 (0)	14.6 (0)	500 (462–556)	762	1,130 (1,066–1,163)	480	1,150
	W			473 (421–503)	716	1,105 (996–1,151)	430	1,150
	M			619 (562–654)	818	1,125 (1,066–1,162)	570	1,150
	Blood	1.98 (0)	6.29 (0)	4,005 (3,669–4,112)	5,477	7,029 (6,919–7,266)	3,700	7,250
	W			3,709 (3,350–4,010)	5,241	6,678 (6,412–6,866)	3,400	6,850
	M			4,388 (4,005–4,806)	5,801	7,258 (7,012–7,361)	4,000	7,350

In black: TEs traceable with internal and external QC and ISO15189 certification; in gray: TEs traceable with internal QC. LoD, limit of detection; LoQ, limit of quantification; LRL, lower reference limit; URL, upper reference limit; W, women; M, men; S, smokers; NS, non-smokers.
[a]RL below LoQ should be taken with great caution.

using the non-parametric algorithm based on the Tukey's box-plot method [12], were excluded only after a case-by-case analysis.

For essential TEs, a Lower Reference Limit (LRL) and an Upper Reference Limit (URL) were determined. The LRL was defined as the lower limit of the 90% confidence interval (90%CI) of P2.5, and the URL as the upper limit of the 90%CI of P97.5 using non-parametric ascending rank order statistics. For non-essential TEs, only URL were established. Values were rounded off within the CI.

Possible differences between genders or based on the smoking status were analyzed by the Mann–Whitney test. The level of statistical significance was set at 0.05. Since a minimum sample size of 120 acceptable observations is required to derive the nonparametric 90% CI for P2.5 and P97.5 [7], separate calculations for nonsmokers and smokers in each gender was not possible.

Results

Of the 394 individuals who provided blood, 14 were excluded because sampling or sample storage conditions were not optimal, leaving 380 individuals. The sample included 202 women and 178 men of similar age (mean (SD) [P50]: 35.4 (12.1) [31] years for women; 35.4 (12.7) [30] for men) and smoking status (71.7% non-smokers, 12.6% smokers and 15.7% ex-smokers for women; 72.0% non-smokers, 11.9% smokers and 16.1% ex-smokers for men).

Table 1 lists the P2.5 (90%CI), P50, P97.5 (90%CI) and derived LRL and URL for essential TEs. For non-essential TEs, P2.5, P50, P97.5 (90%CI) and derived URL are provided in Table 2. Distributions are displayed graphically in Figures 1 and 2. Values by gender and smoking status are reported when statistically significantly different. Women showed higher values than men for Cu in plasma and blood ($p<0.0001$), Mn in blood ($p=0.0004$), and Cd in blood ($p=0.0013$). Men had higher Zn concentrations in blood and plasma ($p<0.0001$), Se in plasma ($p=0.002$) and Pb concentrations in blood ($p=0.0001$) than women. No significant gender difference was observed for the other TEs. Smokers did not have different levels as compared with non-smokers for any TE, except for Cd in blood ($p<0.0001$). As highlighted in Tables 1 and 2, proposed URLs below LoQ should be taken with caution. URLs could not be set for TEs measured below the LoD.

Table 3 compares, for TEs detected in at least 60% of the subjects, the GM (95%CI) and P5–P95 (95%CI) intervals calculated for the Belgian adult population with the RVs reported in other national (or large scale) surveys published after 2000 [13–37].

Table 2: Distribution of non-essential trace elements and derived URL (µg/L).

		LoD (%<LoD)	LoQ (%<LoQ)	P2.5	P50	P97.5 (90%CI)	URL
Al	Plasma	3.10 (98.6)	9.04 (99.2)	<LoD	<LoD	<LoD	<3[b]
	Blood	15 (98.9)	29 (100)	<LoD	<LoD	<LoD	<15[b]
AsT	Blood	0.08 (0)	0.14 (0)	0.58	1.64	8.70 (6.82–9.21)	9
Be	Blood	0.03 (100)	0.08 (100)	<LoD	<LoD	<LoD	<0.03[b]
Bi	Plasma	0.19 (100)	0.63 (100)	<LoD	<LoD	<LoD	<0.2[b]
	Blood	0.07 (100)	0.11 (100)	<LoD	<LoD	<LoD	<0.07[b]
Cd	Blood	0.21 (0.3)	0.47 (21.4)	0.18	0.68	2.22 (2.04–2.85)	2.5
	NS	0.21 (2.3)	0.47 (20.0)	0.21	0.65	1.74 (1.39–2.18)	2.0
	S	0.21 (0.3)	0.47 (7.0)	0.22	1.32	3.19 (2.55–5.56)	5.5
	W	0.21 (0.5)	0.47 (14.8)	0.28	0.71	2.21 (2.12–2.54)	2.5
	M	0.21 (4.7)	0.47 (26)	0.17	0.65	2.19 (1.99–2.22)	2.2
HgT	Blood	0.41 (9.1)	1.31 (44)	<LoD	1.51	6.36 (4.75–7.15)	7.0
Ni	Plasma	0.54 (31.3)	1.66 (100)	<LoD	0.62	1.10 (1.00–1.25)	1.2[a]
	Blood	0.33 (98.9)	0.74 (100)	<LoD	<LoD	<LoD	<0.3[b]
Pb	Blood	0.14 (0)	0.35 (0)	4.28	11.1	31.6 (27.8–41.0)	40
	W			4.15	9.88	27.8 (26.2–35.5)	35
	M			4.70	10.9	35.4 (28.2–45.6)	45
Sb	Blood	0.08 (100)	0.13 (100)	<LoD	<LoD	<LoD	<0.08[b]
Sn	Blood	0.07 (72.2)	0.17 (92.8)	<LoD	<LoD	0.20 (0.18–0.45)	0.4
Tl	Blood	0.01 (56.2)	0.02 (84.8)	<LoD	<LoD	0.05 (0.04–0.06)	0.06
V	Blood	0.14 (98.9)	0.25 (99.7)	<LoD	<LoD	<LoD	<0.14[b]

In black: TEs traceable with internal and external QC and ISO15189 certification; in gray: TEs traceable with internal QC. LoD, limit of detection; LoQ, limit of quantification; LRL, lower reference limit; URL, upper reference limit; W, women; M, men; S, smokers, NS, non-smokers.
[a]RL below LoQ should be taken with great caution;
[b]RL below LoD.

Discussion

This study provides RVs for TEs in blood and plasma in the adult population in Belgium. Lower and upper limit levels are established for essential TEs, and upper limit levels for non-essential TEs. Percentiles provide additional information on the shape of the distribution in the population.

Blood is the primary compartment through which chemicals travel within the body before reaching tissues. Although more invasive than urine collection, blood sampling can be accomplished with lower risk of contamination. Blood may also offer the advantage of a relatively constant gross composition, eliminating the need to adjust the measured biomarker concentration for parameters such as diuresis for urinary samples [38]. Yet, TEs concentrations in blood do not necessarily reflect concentrations in the whole body or in target organs. Moreover, toxicokinetic parameters, such as excretion route and elimination half-life, determine the preference of measuring TEs in urine or blood.

Whole blood is generally the specimen of choice for TEs such as Cd and Pb, which are concentrated in the cellular compartment, in particular in red blood cells (RBC). In contrast, plasma/serum (P/S) samples are preferred for elements that bind to serum proteins, such as Cu bound to ceruloplasmin [39]. This rule is, however, not systematic. Zn, for example, is commonly measured in plasma or serum, although 75% of the blood content is in RBC.

Zinc, copper, and selenium deficiencies are more likely to occur than overload. A homeostatic control of essential TEs concentrations restricts major variations in circulating concentrations [39]. The assessment of these TEs, which is widely performed on P/S samples, has some interest to document a deficiency status at the population level but reliable indicators of their status at the individual level are currently lacking [40, 41]. RVs for Zn-P/S and Cu-P/S are few, but as seen in Table 3, the population in Belgium tends to have lower mean levels than in USA and Korea. The current Zn-P level is also lower than that recorded in a large-scale study conducted in Belgium in the late 80s. Over the past three decades, Zn-S (GM) fell from 857 to 827 µg/L in men and from 824 to 724 µg/L in women [42]. Zn-B is also lower in our population than in the French and Canadian populations. Cu-B is lower than in the Canadian and Brazilian populations, but higher than in China (Table 3). We measured Zn and Cu in plasma, whereas in the other surveys serum concentrations were determined. Serum and plasma concentrations are regarded as equivalent for Zn and Cu [40, 41].

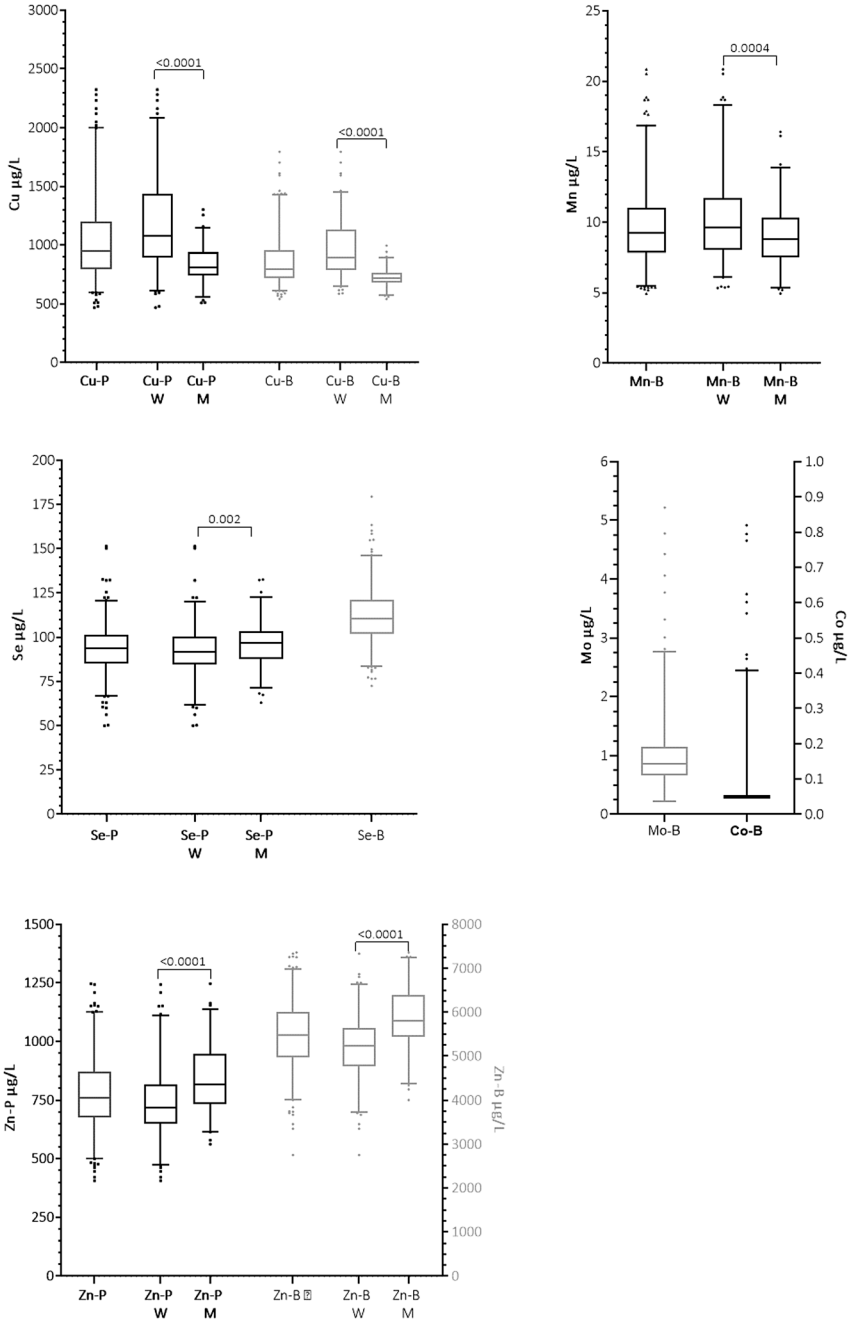

Figure 1: Distribution of essential TEs in blood and in plasma.
In black: TEs traceable with internal and external QC and ISO15189 certification; in gray: TEs traceable with internal QC. Boxes represent median and interquartile range and whiskers depict P2.5 and P97.5. Extreme data points are identified by dots. Outliers, excluded after a case-by-case analysis, are not represented. Distribution by gender (W, women; M, men) and smoking status (S, smokers; NS, non-smokers) are shown when statistically significantly different. Cu-B, Cu-P and Mn-B were significantly higher in women than in men; Zn-B, Zn-P and Se-P were significantly higher in men than in women.

However, Zn-S would be 5–15% higher than Zn-P due to the release of Zn from blood cells during clotting and centrifugation, and an osmotic fluid shift from cells when anticoagulants are used. Therefore, Zn-P should probably be preferred [43]. In agreement with other surveys, women showed lower Zn but higher Cu blood and plasma levels than men in our population.

Compared to data collected in Belgian adults in previous years [44], Se-P levels in the present study are roughly similar or very slightly higher. The present values are, however, substantially lower than in the US and the Canadian populations (Table 3), reflecting the suboptimal Se status throughout Europe and the Middle East [45]. As typically reported [40, 46–48], we found a gender difference, women showing lower levels than men. Se-P/S concentrations required to achieve full glutathione peroxidase activity (GPx) and selenoprotein P (SEPP1) expression are believed to be around 70–120 µg/L [45–50]. Assuming that Se-P below 100 µg/L indicates a suboptimal activity of these selenoproteins, about 75% of adults in Belgium would have a suboptimal Se status according to the present survey.

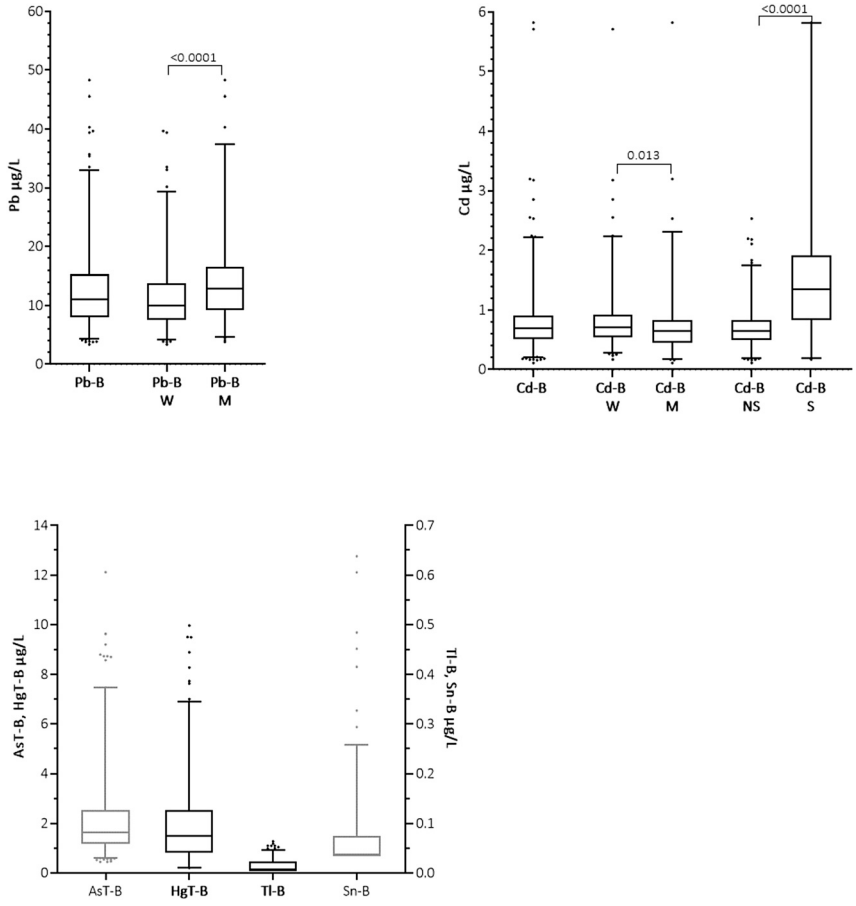

Figure 2: Distribution of non-essential TEs in blood.
In black: TEs traceable with internal and external QC and ISO15189 certification; in gray: TEs traceable with internal QC. Boxes represent median and interquartile range and whiskers depict P2.5 and P97.5. Extreme data points are identified by dots. Outliers, excluded after a case-by-case analysis, are not represented. Distribution by gender (W, women; M, men) and smoking status (S, smokers; NS, non-smokers) are shown when statistically significantly different. Pb-B was significantly higher in men than in women; Cd-B was significantly higher in women than in men, and in smokers then in non-smokers.

Most of the manganese blood content is in cellular components, plasma containing less than 5% [43]. Mn-B has a short elimination half-life (hours, days) and poorly reflects the Mn concentration in the brain, the main target organ, where its half-life is longer [46, 51]. Mn-B may have some utility for distinguishing occupationally exposed from unexposed subjects at the group level, but not at the individual level [51]. Mn-B in adults in Belgium lies within values reported in other surveys, similar to the levels reported in USA and Canada, higher than in France and Italy, lower than in Brazil (Table 3). In agreement with other surveys, women from the present study showed higher values than men. This difference is generally explained by an increased gastrointestinal absorption rate related to a lower iron status in women [14, 15, 18–20, 31, 33, 37].

Data on molybdenum in blood are few. Compared with the Chinese [33] and the Canadian [20] populations, the GM of Mo-B in our population is respectively 71 and 26% higher but it is 28% lower than the one reported for the Italian population [15]. Rentschler et al. [52] measured a median Mo-B of 2.0 µg/L in adult women from six European and three non-European countries. A significant ($p<0.001$) variation (2.9 times) was observed between the nine countries. In the present study, the median concentration is 0.77 µg/L without gender difference.

Blood cobalt is lower in our population (P50–P95<0.10–0.26 µg/L) than in Canada (0.22–0.40) [20], USA (0.13–0.40 µg/L) [18], Italy (0.14–0.44 µg/L) [15] and France (0.29–0.54 µg/L) [14] despite the higher LoD in our study. Being below the LoQ, our upper reference limit for cobalt should be taken with caution.

Health-based guidance values to define deficiency or toxicity of Zn, Se, Cu, Co, Mn and Mo are not available.

Among the numerous TEs without any (known) physiological function, inorganic arsenic, cadmium, mercury and lead are of high toxicological concern.

The most common biomarker of exposure for inorganic arsenic (iAs) is As in urine (As-U). The identification of the determination of As-B is generally limited to suspicion of recent high exposure. Data obtained from a cohort of subjects environmentally exposed to iAs, suggest that As-B might reflect an individual's total As body burden [53]. The mean total As-B (AsT-B) in the present study is similar that recorded in France, respectively 37 and 51% higher than in Italy and in Canada, and about two times lower than in Brazil (Table 3). Organic forms of arsenic, including

Table 3: Comparison of TEs RVs in the Belgian adult population with data of other main surveys worldwide (µg/L).

	Europe				**North-America**		**South-America**	**Asia**
	This survey	France[a,b]	Italy[c]	Germany – DE[d] Spain – Sp[e] Czech Rep – CZ[f]	USA[g]	Canada[h] Québec[i]	Brazil[j]	Korea – KR[k] China – CN[l]
Cu								
Serum/plasma								
% <LoD	*0% <1.60*				n.i. <25[ga]			
GM [95%CI]	*1,001 [970–1,030]*				1,160 [1,130–1,190][ga]			
P5 [95%CI]	*666 [623–679]*							
P95 [95%CI]	*1,771 [1,689–1,907]*				1,750 [1,650–1,830][ga]			
Blood								
% <LoD (LoQ)	0%<0.84					0%<20[hb]	n.i. <0.5[ja]	CN: 0%<1[lb]
GM [95%CI]	847 [828–867]					900 [900–910][hb]		CN: 795 [791–799][lb]
P5 [95%CI]	639 [612–651]						712[ja]	CN: 634[lb]
P95 [95%CI]	1,303 [1,260–1,364]					1,200 [1,200–1,300][hb]	1,732[ja]	CN: 999[lb]
Mn-B								
% <LoD (LoQ)	0%<1.09	0%<0.057[b]	0%<0.78		n.i.<0.99[ga]	0%<0.5[hb] n.i. <0.11[i]	n.i. <0.09[ja] n.i. <0.009[jb]	KR: n.i.<0.8[kb] CN: 0% <(0.85)[lb]
GM [95%CI]	9.40 [9.15–9.66]	7.71 [7.60–7.81] [b]	8.19 [8.04–8.35]		9.34 [9.11–9.58] [ga] P2.5: 4.9 [4.7–5.11][gb]	9.8 [9.5–10] [hb] 9.34[i]	12.8[jb]	KR: 10.8 [10.4–11.1][kb] CN: 8.98[la] ; 12.4[lb]
P5 [95%CI]	6.36 [5.69–6.59]		4.41		5.5 [5.3–5.59][ga]	P2.5: 4.84[i]	6.90[ja]; 6.00[jb]	CN: 8.09[lb]
P95 [95%CI]	14.8 [14.1–16.6]	12.9 [12.6–13.3][b]	14.5		16.1 [15.6–16.9][ga]	15.6 [14.0–16.3][hb] P97.5: 16.7[i]	18.4[ja] 28.6 [27.8–29.3][jb]	KR: 19.8 [18.7–21.7][kb] CN: 18.5[lb]
Mo-B								
% <LoD	5%<0.43		0%<0.31			0.18%<0.1[hb] n.i. <0.38[i]		
GM [95%CI]	0.87 [0.81–0.89]		1.21 [1.19–1.23]			0.65 [0.63–0.67][hb] 1.14[i]		CN: 0.25[la]
P5 [95%CI]	<LoD		0.69			P2.5 : 0.45 [i]		
P95 [95%CI]	2.21 [1.97–2.75]		2.05			1.6 [1.4–1.7][hb α] P97.5: 2.33[i]		
Se								
Serum/plasma								
% <LoD	*0%<0.40*				n.i. <4.5[ga]	n.i. <7.9[i]		
GM [95%CI]	*92.7 [91.4–94.1]*				129 [127–131][ga]	134[i]		
P5 [95%CI]	*73.6 [72.1–75.3]*					P2.5: 106[i]		
P95 [95%CI]	*114 [111–116]*				161 [157–162][ga]	P97.5: 178[i]		

Table 3: (continued)

	Europe				North-America		South-America	Asia
Blood								
% <LoD	0% <0.11				n.i. <24.5[ga]	0% <32[hd] n.i. <7.9[i]	n.i. <0.1[ja]	
GM [95%CI]	111 [109–113]				194 [190–198][ga]	170 [170–170][hd] 221[i]		
P5 [95%CI]	88.8 [86.5–89.9]				153 [150–157][ga] P2.5: 152 [149–155][gb]	P2.5: 166[i]	68[ja]	
P95 [95%CI]	140 [136–145]				237 [230–243][ga] P97.5: 251 [244–258][gb]	210 [200–210][hd]P97.5: 284[i]	245[ja]	
Zn								
Serum/plasma								
% <LoD (µg/L)	*0%<7.18*				n.i. <2.9[ga]			
GM [95%CI]	*764 [753–778]*				801 [779–825][ga]			
P5 [95%CI]	*563*							
P95 [95%CI]	*1,037 [1,010–1,120]*				1,090 [1,030–1,140][ga]			
Blood								
% <LoD (LoQ)	0%<1.98	0%<1.03[b]				0%<1[hb]		CN: 0%<1[lb]
GM [95%CI]	5,432 [5,352–5,509]	5,810 [5,760–5,850][b]				6,000 [5,900–6,100][hb]		CN: 5,850[lb] 3,996 [3,976–4,015][la]
P5 [95%CI]	4,270 [4,253–4,298]							CN: 4,320[lb]
P95 [95%CI]	6,770 [6,683–6,967]	7,270 [7,200–7,390][b]				7,300 [7,100–7,600][hb]		CN: 7,750[lb] P97.5: 9,492[lb]
AsT-B								
% <LoD (LoQ)	0%<0.08	2.2%<0.022[b]	4.4%<0.27			7.2%<0.2[ha] n.i. <0.67[i]	n.i. <0.08[ja] n.i. <0.014[jb]	CN: 0% <(0.23)[lb]
GM [95%CI]	1.8 [1.69–1.91]	1.67 [1.58–1.75][b]	1.14 [1.09–1.20]			0.89 [0.74–1.06][ha] 0.24[i]	4.19 [jb]	CN: 1.19[la]; 2.25[lb]
P95 [95%CI]	5.61 [4.45–7.16]	6.72 [6.26–7.24][b]	5.32			4.08 [2.94–5.23][ha] 2.00 [1.80–2.20][ha α,β] P97.5: 6.02[i]	3.2[ja] 9.62 [9.37–9.87][jb]	CN: 5.59[la]; 8.14[lb]
Cd-B								
% <LoD (LoQ)	0.3%<0.21	0.45%<0.0007[b]	1.1%<0.10	DE: 18%<0.12 CZ: n.i. <0.20		12.1%<0.097[hd] n.i. <0.11[i]	n.i. <0.04[ja] n.i. <0.003[jb] 65% <(0.10)[jc]	KR: n.i. <0.01[ka] CN: 0% <(0.06)[lb]
GM [95%CI]	0.68 [0.64–0.72]	0.39 [0.38–0.41][b]	0.53 [0.51–0.55]	DE: 0.44 [0.42–0.45] CZ: 0.6	0.30 [0.28–0.31][ga]	0.28 [0.25–0.30][hd]	0.09[jb] 0.08 [0.08–0.09][gc]	KR: 1.02 [1.00–1.05][ka] CN: 0.49[lb]; 0.70[lb]
P95 [95%CI]	1.79 [1.56–2.19]	1.67 [1.56–1.83][b]	1.42	DE: 2.34 (P98: 3.18) CZ: 3.0 [2.7–3.3]	1.35 [1.22–1.48][ga]	2.90 [2.40–3.30][hd] 0.83 [0.72–0.95][hc α]	1.1[ja] 0.82 [0.77–0.87] [jb] 0.50 [jc]	KR: 2.62[ka] CN: 6.44[lb]
P97.5 [95%CI]	NS : 1.74 [1.39–2.18]; S : 3.19 [2.55–5.56]					NS: 1.58; S: 6.88[i]		

Table 3: (continued)

	Europe				North-America		South-America	Asia
HgT-B								
% <LoD (LoQ)	9.1% <0.41	3.2% <0.036[b]	2.6% < 0.29	DE: 12.6% <(0.2) Sp: 0.26% <(0.1) CZ: n.i. <0.20	n.i. <0.28[ga]	17.1% <0.20[hd] n.i. <0.2[i]	1.5% <(0.2)[jc]	KR: 0.02% <n.i.[kc] CN: 6.54% <(1.15)[lb]
GM [95%CI]	1.38 [1.26–1.50]	1.38 [1.32–1.45][b]	1.19 [1.15–1.25]	DE: 0.58 [0.57–0.60] Sp: 6.35 [6.00–6.72] CZ: 0.82	0.81 [0.74–0.89][ga]	0.64 [0.54–0.75][hd] 0.74[i]	0.99 [0.90–1.05][jc]	KR: 3.11 [3.02–3.21][kc] CN: 1.90[lb]
P95 [95%CI]	4.64 [4.36–5.63]	5.06 [4.80–5.51][b]	5.16	DE: 2.3 ; P98: 3.3 Sp: 19.3 CZ: 3.45 [3.06–3.69]	4.66 [3.91–5.96][ga]	3.8 [2.9–4.8][hd] 2.3 [1.1–3.5][hc α,β] P97.5: 3.21[i]	4.10 [3.50–4.40][jc]	KR: 9.05[kc] CN: 5.97[lb]
Pb-B								
% <LoD (LoQ)	0% <0.14	n.i. <2[a] 0%<0.013[b]	0.07%<1.03	DE: 0.2% <(4) Sp: 0% <(0.10) CZ: n.i. <7	n.i. <0.07[ga]	0.3%<0.17[hd] n.i. <0.21[i]	n.i. <0.04[ja] 0%<2.5[jc]	KR: 0.02% <n.i.[kc] CN: 0% <(0.95)[lb]
GM [95%CI]	11.4 [10.8–12.0]	25.7 [24.9–26.5][a] 18.8 [18.3–19.3][b]	19.9 [19.2–20.5]	DE: 30.7 [30.2–31.2] Sp: 24.0 [23.0–25.1] CZ: 33	9.20 [8.62–9.82][ga]	9.3 [8.5–10][hd] 20.7[i]	23.7 [22.4–24.7][jc]	KR: 19.4 [18.9–19.9][kc] CN: 34.9[la] ; 17.8[lb]
P95 [95%CI]	26.8 [25.2–30.1]	73 [68–77][a] 49.3 [45.8–54.0][b]	51.7	DE: 71 (P98: 94) Sp: 56.8 CZ: 72 [69–80]	28.9 [26.5–30.7][ga]	25 [21–29][hd] 33 [31–36][hc α]P97.5: 66.2[i]	163[ja]56.2 [48.9–66.1][jc]	KR: 40.9[kc] CN: 48.1[lb]
Tl-B								
% <LoD (LoQ)	56.2%<0.01	9.33%<0.0016[b]	1.5%<0.015					CN: 63.1% <(0.05)[lb]
GM [95%CI]		0.02 [0.02–0.03][b]	0.04 [0.07–0.04]					CN: <0.05[lb]
P95 [95%CI]	0.04 [0.04–0.04]	0.14 [0.13–0.16][b]	0.10					CN: 0.09[lb]

LoD, limit of detection; LoQ, limit of quantification; GM, geometric mean; P2.5–97.5: 2.5–97.5th Percentile; [95%CI], confidence interval 95%; n.i., no information; S, smokers; NS, non-smokers France. [a] ENNS (Etude Nationale Nutrition Santé) (2006–2007; 1245F, 704M; 18–74 yrs) [12]. [b]IMEPOGE (Northern France; 2008–2010; 1016F, 976M; 20–59 years) [13].Italy. [c]PROBE (Programme for biomonitoring the Italian population exposure) – (2008–2010; 470F, 953M; 18–65 years) [14].Germany – GE. [d]GerES (German Environmental Surveys) – (1998; 2303F, 2342M; 18–69 yrs) [15]. Spain – Sp. [e] BIOAMBIENT.ES – (918F, 962M; 18–65 years) [22, 23].Czech Republic – CZ. [f]CZ-HBM - (2001–2003; 325F, 863M; 18–58 yrs) [21].USA. [ga]CDC-NHANES (National Health and Nutrition Survey) (2015–2016; 2610F+M; ≥20 years) [17]. [gb]CDC-NHANES (2011–2012; ≥20 years) [19] Canada. [h]Health Canada-CHMS (Canadian Health Measures Survey Cycle) [18]. [ha]Cycle 1 (2007–2011; 2743F, 2576M; 6–79 years), August 2010. [hb]Cycle 2 (2009–2011; 3130F, 2940M; 6–79 years), April 2013. [hc]Cycle 3 (2012–2013; 2769F, 27690M; 6–79 years), July 2015. [hd]Cycle 5 (2016–2017; 2260F, 2257M; 3–79 yrs). [α]When available, only adults' values are provided [20]. [β]seafood consumption >3 times/month excluded. [i]Québec [19] (2000–2003 ; 472 F+M; 18–65 yrs). [j]Brazil. [ja](five States; 2007–2008; 619F, 506M; 18–60 yrs) [24]. [jb](North Brazil, Acre State, 2010–2011; 293F, 890M; 18–65 yrs) [25]. [jc](Metropolitan Area of São Paulo; 2006; 289F, 364M; nonsmoking; 18–65 yrs) [26]. [k]South Korea-NHANES. (Korean National Environmental Health Survey (KoNEHS) part of the National Health and Nutrition Survey). [ka](2008; 1546F, 800M; ≥20 yrs) [27]. [kb](2008; 3531F, 1556M; ≥20 yrs) [29]. [kc](2012–2014; 3689F, 2766M; ≥20 yrs) [28]. (only more recent RVs are shown)[l]CHINA. [la]National study, eight provinces (2009–2010; 18120 F+M; 6–60 yrs) [30–34 (only abstracts available in English); [lb](Beijing suburb; 2009–2010; 330F, 318M; 12–60 yrs) [35].

arsenobetaine and arsenocholine, make up the majority of arsenic in seafood, while in other foods, inorganic arsenic may represent the predominant form. A higher AsT-B level in seafood consumers than in non-consumers was reported in the Chinese population (2.59 vs 1.47 µg/L, $p<0.05$) [36]. Using CHMS data to establish the RV95 (2.0 µg/L), Saravanabhavan et al. [22] excluded from the reference population individuals who ate seafood more than three times per month because of a significant influence on AsT-B ($p<0.0001$). In agreement with this, we observed that subjects who ate seafood within 4 days before blood collection showed a higher AsT-B level than those who did not (GM 2.00 vs. 1.50 µg/L, $p=0.055$).

Cadmium is a highly toxic and cumulative element, with an elimination half-life of up to 20 years. Cd-B is mainly influenced by relatively recent exposure but may also include a contribution from the body burden. Belgium has had a long-standing production of Cd from the refining of zinc ores, and some areas of the country have been polluted by past emissions [54]. Compared to the 80s, when mean Cd-B (GM) in the general population was 1.12 µg/L (range 0.1–15.5) [42], a marked decrease to 0.68 µg/L (0.1–5.7) was recorded in the present study. This GM is still higher than in most occidental countries, but the P95 lies in the same range. The mean Cd-B in the present study is also somewhat higher than in adults aged 50–65 years living in the north of Belgium [55] (GM 0.42 µg/L vs. 0.61 µg/L for the corresponding age group in the present study). A substantial reduction in Cd-B has also been noted over time in Japan, where Cd has been of major public health concern; the GM in women in Japan dropped from 3.58 µg/L in the end 70s to 1.98 µg/L in the 90s [56]. The current level in Japan is, however, still high. A national study of pregnant women (2011–2014) reported a GM of 0.71 µg/L (P95 1.55) [57]. A 30% decrease has also been observed between 2005 and 2008 (GM from 1.5 to 1.02 µg/L) in South Korea [29]. Smokers have typically two to five times higher Cd-B than nonsmokers [46], and our data confirm a similar difference between smokers and non-smokers. Besides differences in the environmental exposure level, varying proportions of smokers in the reference populations may greatly influence the values. The higher Cd-B observed in women, is also documented in other surveys but to a lesser degree and not consistently. It is generally attributed to an increased gastro-intestinal absorption of Cd when iron stores are low, a status that is more prevalent in women [14–23, 37]. Our data shows that 4–0.5% of the adult population in Belgium would reach the lowest Cd-B level related to a risk of adverse effect on kidney and bone , estimated to be around 2–5 µg/L (corresponding to Cd in urine around 2–5 µg/g creatinine) [58–60].

Lead in blood is the gold standard for the assessment of lead exposure, both for screening and diagnostic purposes. Pb-B mainly reflects "recent" exposure and concentration in soft tissue. It is also affected by past exposure as a result of Pb mobilization from the skeleton that constitutes about 90% of the lead body burden in adults. Pb-B does, however, not necessarily reflect the total body burden [46, 61]. The progressive lowering and banning of Pb in petrol and control of industrial emissions over the last several decades have resulted in a general decrease in Pb-B [13, 18, 19]. In Belgium, the median Pb-B dropped from 170 to 78 µg/L between 1978 and 1989 [62] to reach 11.1 µg/L in the present survey. The present Pb-B level is similar to that in USA and Canada, much lower than in many other countries (see Table 3), and lower than in Flanders in the 2002–2006 survey (GM 11.4 µg/L vs. 39.6 µg/L) [55]. The higher Pb-B concentration that we observed in men than in women is reported in most studies [13, 15, 18–20, 30]. Although often assigned to a higher blood hematocrit level in men, even corrected for hematocrit, a higher Pb-B has been reported in men in some surveys [29, 31, 61]. Smoking has been associated to increased Pb-B [13, 14, 37], but we did not confirm this observation in the present database. The epidemiological studies continue to provide evidence of health effects at lower and lower blood lead levels. The current evidence provides support for adverse health effects in adults at Pb-B below 100 µg/L, and even below 50 µg/L [63–65]. The Pb-B reference level (= case definition for an elevated Pb-B) set by the CDC at 50 µg/L [65] was never exceeded in our population. However, almost 27% of our population exceeded the EFSA [63] critical blood lead level of 15 µg/L.

Mercury, a pollutant of global concern, occurs in various chemical forms with different toxicokinetics properties. Total mercury in blood (HgT-B) reflects recent exposure to methylmercury (MeHg) and inorganic/elemental (Hg°) Hg, but not the level of Hg in brain or the body burden. More than 90% of MeHg is found in RBC, whereas, in case of exposure to Hg°, the Hg ratio RBC:plasma is 1:1 [46, 66, 67]. In the general population, HgT-B is mostly associated with dietary intake of MeHg-contaminated fish [18], and whole blood is, with scalp hair, the best matrix for assessing MeHg exposure. HgT-B is also useful when measured soon after a short-term, high-level exposure to Hg°, but the blood level decreases within days of exposure [68]. The background exposure level in our reference population is similar to that reported in occidental populations (Table 3). It is also in agreement with a systematic search of the scientific literature mainly based on national biomonitoring studies, which concluded that the general population worldwide has a

median HgT-B level generally below 5 µg/L [69]. Considering populations of concern because of the consumption of fish, the pooled median was 8.6 µg/L (IQR: 2.9–21.2 µg/L) with 38.6 µg/L as upper bound median value [69]. Fish consumption is the primary contributor to HgT-B levels [28, 29]. Seafood intake during the last 3 days before blood sampling affected HgT-B in the Asian studies [29], but not in our study, probably because of a lower seafood intake and perhaps consumption of less contaminated fish. An overview of the worldwide trends for HgT-B during the last half-century suggests the highest levels in South America, followed by Africa or Asia, the populations from Europe or North America displaying the lowest levels. HgT-B in Europe showed a steeper decline with time [69]. The HBM commission of the German Federal Environment Agency [70] established the HgT-B level below which there is no risk for adverse health effects (HBM-I) at 5 µg/L; this level was exceeded by 4.4% of our population. The concentration above which this Commission considers there is an increased risk for adverse health effects (HBM-II), i.e., 15 µg/L, was never reached in our population.

Sn-B and Tl-B appear to be much lower than in other countries. For Tl-B, P95 (0.04 µg/L) is 2.3 and 3.3 times less than in Italy (0.098 µg/L) [15] and France (0.14 µg/L) [14], respectively. The difference is even greater for Sn-B when compared to Italy (0.17 vs. 2.25 µg/L) [15]. The levels of Al, Be, Bi, Ni, Sb and V were below the LoD and the URL could not be determined for these TEs. A limitation of the study relates to the sensitivity of the analytical method used. Indeed, the use of a Babington type nebulizer for the quantification of TEs in the whole blood matrix, while increasing the robustness of the analytical method, clearly also decreases its analytical sensitivity. Therefore, LOQ values obtained do not make it possible to propose reference values for all the TEs measured.

RVs vary between studies, but comparisons are not immediately straightforward. Indeed, establishing RVs is challenging. Several methodological considerations may affect the determination of RVs. Some discrepancies in RVs are probably, to some extent, attributable to factors independent of the actual levels of exposure, as for example criteria used to define the reference population, time of sampling, collection material (needles, tubes, anticoagulant), sampling and handling, analytical method and LoD/LoQ, statistical issues such as treatment of left-censored values and outliers.

RVs reflect the distribution in a reference population, a group consisting of reference individuals selected for testing on well-defined criteria. In the present study, recruitment was done by occupational physicians specially trained in toxicology, using an *a priori* selection based on inclusion and exclusion criteria. This procedure is likely to confer several advantages, including the exclusion of subjects exposed to the measured TEs through occupation, hobbies, drugs, etc., assessment of health status, reduction of the number of individuals needed. Results obtained on a larger sample may be viewed as more robust, but recruiting a high number of reference individuals is not easy, especially for blood sampling, and controlling pre-analytical variables is more challenging.

Though sophisticated statistical methods exist for handling left-censored data, we used a most common approach in epidemiological investigations, which is to assign a value of LoD/2 to non-detected values [15, 20, 23, 27, 71]. US-NHANES used a value of LoD divided by the square root of two [18]. Others assigned a value of LoQ/2 to concentrations below the LoQ [17, 28]. More unusual methods are to assume results < LoD to be zero [72] or to replace concentrations < LoD with the value LoD/2 and those between LoD and LoQ by the value (LoD + LoQ)/2 [14]. These different approaches might have some impact on the levels of TEs having a high percentage of values < LoD.

An implicit assumption when estimating RLs is that the set of measured RVs represents a "homogeneous" collection of observations [7]. Handling of outliers is tricky and often clouded, especially when data are not normally distributed. Several statistical methodologies exist to identify them, but, once detected, a most difficult decision is how to deal with it. Outliers can be a result of pre-, post-, or analytical error, or it can be an indication of variance in data. Simply discarding these extreme values is generally not recommended. A careful checking, case by case, as we did, is possible when there are not too many of these data points.

Presentation of RVs, data provided and their interpretation also vary across the surveys. Differences might be partly explained by the objective of the studies, which complicates the comparisons, and caution is advised. Some studies only provide a distribution with selected percentiles; others specify reference intervals, or determine RLs, which can be set at different percentiles, taking or not the CI into account, and rounding values or not. We chose to follow the IFCC-CLSI guidelines that define reference intervals for a biomarker based on P2.5 and P97.5 estimates, using the 90%CI for the endpoints. Actually, P95 appears more often used than P97.5. An argument is that in terms of human exposure to environmental chemicals, an exposure as low as possible would probably be the best option for "toxic" TEs [22]. The German Human Biomonitoring Commission defined RV95 as "the P95 of the

measured pollutant concentration levels in the relevant matrix of the reference population. To derive it, it is rounded off within the 95%CI" [70]. This "limit" has often been adopted to identify subjects with "elevated exposure". However, the terminology used is sometimes confusing; "RVs" regularly appear to be considered as URLs and used without specifying the corresponding percentile [13, 37, 71]. To derive "RVs" in Slovenia, P95 (95%CI) was used for "toxic elements", P2.5 and P97.5 for essential TEs [71]. For people of Bejing, the "RV" for Pb and Cd were set at P95, whereas for "nontoxic elements" (Mn, Cu, Zn), P2.5 and P97.5 were used [37].

In summary, our study reports updated blood and plasma reference levels for selected TEs in the adult population in Belgium. These new data obtained through validated analytical and statistical methods will contribute to decision-making concerning both public health policies but also exposure assessments on an individual scale. The proposed reference values have no intrinsic physiological or toxicological meaning and do not constitute a red flag indicating a health risk.

Acknowledgments: The authors wish to thank Francis Desmedt and Marleen Kestens (LTAP) for skillful (pre) analytical handling of the samples.
Research funding: Part of this study was performed with the financial support of CESI (Occupational Health Service, Brussels, Belgium).
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflict of interest.
Informed consent: Informed consent was obtained from all individuals included in this study.
Ethical approval: The Biomedical Ethical Commission of the Faculty of Health Sciences (Université catholique de Louvain, Brussels) approved the study protocol (CEHF 2015/544-B403).

References

1. Maret W. The metals in the biological periodic system of the elements: concepts and conjectures. Int J Mol Sci 2016;17:66.
2. Ganzleben C, Antignac JP, Barouki R, Castaño A, Fiddicke U, Klánová J, et al. Human biomonitoring as a tool to support chemicals regulation in the European Union. Int J Hyg Environ Health 2017;220:94–7.
3. National Research Council (NRC), Board on Environmental Studies and Toxicology. Using 21st Century Science to Improve Risk-Related Evaluations. Washington, DC: National Academies Press (US); 2017. Available from https://doi.org/10.17226/24635.
4. Solberg HE, International Federation of Clinical Chemistry (IFCC), Scientific Committee, Clinical Section, Expert Panel on Theory of Reference Values, International Committee. Approved Recommendation (1986) on the theory of reference values. Part 1. The concept of reference values. J Clin Chem Clin Biochem 1987; 25:337–42.
5. Gräsbeck R. The evolution of the reference value concept. Clin Chem Lab Med 2004;42:692–7.
6. Henny J, Vassault A, Boursier G, Vukasovic I, Mesko Brguljan P, Lohmander M, et al. Recommendation for the review of biological reference intervals in medical laboratories. Clin Chem Lab Med 2016;54:1893–900.
7. Clinical and Laboratory Standards Institute (CLSI). Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline, 3rd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2008. CLSI Document C28-A3c.
8. Cornelis R, Sabbioni E, Van der Venne MT. Trace element reference values in tissues from inhabitants of the European Community. VII. Review of trace elements in blood, serum and urine of the Belgian population and critical evaluation of their possible use as reference values. Sci Total Environ 1994;158:191–226.
9. Minoia C, Pietra R, Sabbioni E, Ronchi A, Gatti A, Cavalleri A, et al. Trace element reference values in tissues from inhabitants of the European Community. III. The control of preanalytical factors in the biomonitoring of trace elements in biological fluids. Sci Total Environ 1992;120:63–79.
10. Hoet P, Jacquerye C, Deumer G, Lison D, Haufroid V. Reference values and upper reference limits for 26 trace elements in the urine of adults living in Belgium. Clin Chem Lab Med 2013;51: 839–49.
11. Nordberg GF, Fowler BA, Nordberg M. Handbook on the toxicology of metals. 4th ed. Cambridge: Academic Press, Elsevier; 2015.
12. Patterson N, Robust A. Non-parametric method to identify outliers and improve final yield and quality. In: CS MANTECH Conference; 2012 April 23rd–26th, 2012. Boston, Massachusetts, USA.
13. Fréry N, Saoudi A, Garnier R, Zeghnoun A, Falq G. Exposition de la population française aux substances chimiques de l'environnement. Tome 1 – Présentation générale de l'étude – Métaux et métalloïdes. Saint-Maurice: Institut de Veille Sanitaire; 2011.
14. Nisse C, Tagne-Fotso R, Howsam M. Members of Health Examination Centres of the Nord – Pas-de-Calais region, Richeval C, Labat L, et al. Blood and urinary levels of metals and metalloids in the general adult population of Northern France: the IMEPOGE study, 2008-2010. Int J Hyg Environ Health 2017;220:341–63.
15. Alimonti A, Bocca B, Mattei D, Pino A. Programme for biomonitoring the Italian population exposure (PROBE): internal dose of metals. Rapporti Istisan 11/9. Istituto Superiore di Sanità; 2011.
16. Umweltbundesamt (UBA), German Environment Agency. German Environmental Surveys (GerES). Available from: https://www.umweltbundesamt.de/en/topics/health/assessing-environmentally-related-health-risks/german-environmental-survey-geres.
17. Becker K, Kaus S, Krause C, Lepom P, Schulz C, Seiwert M, et al. German Environmental Survey 1998 (GerES III): environmental

pollutants in blood of the German population. Int J Hyg Environ Health 2002;205:297–308.
18. Centers for Disease Control and Prevention (CDC), National Report on Human Exposure to Environmental Chemicals. National Health and Nutrition Examination Survey (NHANES). Available from: https://www.cdc.gov/exposurereport/.
19. Jain RB, Choi YS. Normal reference ranges for and variability in the levels of blood manganese and selenium by gender, age, and race/ethnicity for general U.S. population. J Trace Elem Med Biol 2015;30:142–52.
20. Health Canada, Canadian Health Measures Survey (CHMS). Reports on Human Biomonitoring of Environmental Chemicals in Canada. [updated 2019-11-13]. Available from: https://www.canada.ca/en/health-canada/services/environmental-workplace-health/environmental-contaminants/human-biomonitoring-environmental-chemicals/canadian-health-measures-survey.html.
21. LeBlanc A, Lapointe S, Beaudet A, Côté I, Dumas P, Labrecque F, et al. Étude sur l'établissement de valeurs de référence d'éléments traces et de métaux dans le sang, le sérum et l'urine de la population de la grande région de Québec. Institut National de Santé Publique du Québec; 2003.
22. Saravanabhavan G, Werry K, Walker M, Haines D, Malowany M, Khoury C. Human biomonitoring reference values for metals and trace elements in blood and urine derived from the Canadian Health Measures Survey 2007-2013. Int J Hyg Environ Health 2017;220:189–200.
23. Batariova A, Spevackova V, Benes B, Cejchanova M, Smid J, Cerna M. Blood and urine levels of Pb, Cd and Hg in the general population of the Czech Republic and proposed reference values. Int J Hyg Environ Health 2006;209:359–66.
24. Canas AI, Cervantes-Amat M, Esteban M, Ruiz-Moraga M, Perez-Gomez B, Mayor J, et al. Blood lead levels in a representative sample of the Spanish adult population: the BIOAMBIENT.ES project. Int J Hyg Environ Health 2014;217:452–9.
25. Castano A, Pedraza-Diaz S, Canas AI, Perez-Gomez B, Ramos JJ, Bartolome M, et al. Mercury levels in blood, urine and hair in a nation-wide sample of Spanish adults. Sci Tot Environ 2019;670:262–70.
26. Nunes JA, Batista BL, Rodrigues JL, Caldas NM, Neto JA, Barbosa F Jr. A simple method based on ICP-MS for estimation of background levels of arsenic, cadmium, copper, manganese, nickel, lead, and selenium in blood of the Brazilian population. J Toxicol Environ Health A 2010;73:878–87.
27. Freire C, Koifman RJ, Fujimoto D, de Oliveira Souza VC, Barbosa F Jr., Koifman S. Reference values of cadmium, arsenic and manganese in blood and factors associated with exposure levels among adult population of Rio Branco, Acre, Brazil. Chemosphere 2015;128:70–8.
28. Kuno R, Roquetti MH, Becker K, Seiwert M, Gouveia N. Reference values for lead, cadmium and mercury in the blood of adults from the metropolitan area of Sao Paulo, Brazil. Int J Hyg Environ Health 2013;216:243–9.
29. Son JY, Lee J, Paek D, Lee JT. Blood levels of lead, cadmium, and mercury in the Korean population: results from the second Korean National Human Exposure and Bio-monitoring Examination. Environ Res 2009;109:738–44.
30. Choi W, Kim S, Baek YW, Choi K, Lee K, Kim S, et al. Exposure to environmental chemicals among Korean adults-updates from the second Korean National Environmental Health Survey (2012-2014). Int J Hyg Environ Health 2017;220:29–35.
31. Lee JW, Lee CK, Moon CS, Choi IJ, Lee KJ, Yi SM, et al. Korea National Survey for environmental pollutants in the human body 2008: heavy metals in the blood or urine of the Korean population. Int J Hyg Environ Health 2012;215:449–57.
32. Pan X, Ding C, Pan Y, Zhang A, Wu B, Huang H, et al. [Distribution of copper and zinc in blood among general population from 8 provinces in China]. Zhonghua Yu Fang Yi Xue Za Zhi 2014;48:109–13.
33. Pan Y, Ding C, Zhang A, Wu B, Huang H, Zhu C, et al. [Distribution of manganese, cobalt and molybdenum in blood and urine among general population in 8 provinces of China]. Zhonghua Yu Fang Yi Xue Za Zhi 2014;48:784–90.
34. Ding CG, Pan YJ, Zhang AH, Wu BH, Huang HL, Zhu C, et al. [Distribution of chromium in whole blood and urine among general population in China between year 2009 and 2010]. Zhonghua Yu Fang Yi Xue Za Zhi 2012;46:679–82.
35. Ding C, Pan Y, Zhang A, Wu B, Huang H, Zhu C, et al. [Study of distribution and influencing factors of lead and cadmium in whole blood and urine among population in 8 provinces in China]. Zhonghua Yu Fang Yi Xue Za Zhi 2014;48:91–6.
36. Ding C, Pan Y, Zhang A, Wu B, Huang H, Zhu C, et al. [Study of distribution and influencing factors of arsenic in whole blood and urine among population in 8 provinces in China]. Zhonghua Yu Fang Yi Xue Za Zhi 2014;48:97–101.
37. Zhang LL, Lu L, Pan YJ, Ding CG, Xu DY, Huang CF, et al. Baseline blood levels of manganese, lead, cadmium, copper, and zinc in residents of Beijing suburb. Environ Res 2015;140:10–7.
38. Hoet P, Deumer G, Bernard A, Lison D, Haufroid V. Urinary trace element concentrations in environmental settings: is there a value for systematic creatinine adjustment or do we introduce a bias? J Expo Sci Environ Epidemiol 2016;26:296–302.
39. Cornelis R, Heinzow B, Herber R, Christensen JM, Poulsen O, Sabbioni E, et al. Sample collection guidelines for trace elements in blood and urine. J Trace Elem Med Biol 1995;10:103–27.
40. EFSA (European Food Safety Authority). Dietary Reference Values for nutrients. Summary report. EFSA Supporting publication 2017:e15121.
41. de Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C. Conclusions of the Joint WHO/UNICEF/IAEA/IZiNCG interagency Meeting on zinc status indicators. Food Nutr Bull 2007;28:S480–4.
42. Thijs L, Staessen J, Amery A, Bruaux P, Buchet JP, Claeys F, et al. Determinants of serum zinc in a random population sample of four Belgian towns with different degrees of environmental exposure to cadmium. Environ Health Perspect 1992;98:251–8.
43. Rifai N, Horvath AR, Wittwer C. Tietz textbook of clinical chemistry and molecular diagnostics. St Louis, Missouri: Elsevier; 2018.
44. Van Cauwenbergh R, Robberecht H, Van Vlaslaer V, De Smet A, Emonds MP, Hermans N. Plasma selenium levels in healthy blood bank donors in the central-eastern part of Belgium. J Trace Elem Med Biol 2007;21:225–33.
45. Stoffaneller R, Morse NL. A review of dietary selenium intake and selenium status in Europe and the Middle East. Nutrients 2015;7:1494–537.
46. Lauwerys R, Hoet P. Industrial chemical exposure. Guidelines for biological monitoring, 3rd ed. Boca Raton, Florida: Lewis Publisher. CRC Press, Inc.; 2001.

47. Neve J. New approaches to assess selenium status and requirement. Nutr Rev 2000;58:363–9.
48. Combs GF, Jr. Biomarkers of selenium status. Nutrients 2015;7: 2209–36.
49. Institute of Medicine (US) PoDAaRC. Dietary reference intakes: vitamin C, vitamin E, selenium, and carotenoids. Washington, DC: National Academies Press (US); 2000.
50. Thomson CD. Assessment of requirements for selenium and adequacy of selenium status: a review. Eur J Clin Nutr 2004;58: 391–402.
51. Hoet P, Roels H. Significance and usefulness of biomarkers of exposure to Manganese. In: Costa LG, Aschner M, editors. Manganese in health and disease. Issues in toxicology 22. Cambridge, UK: Royal Society of Chemistry; 2015.
52. Rentschler G, Rodushkin I, Cerna M, Chen C, Harari F, Harari R, et al. Platinum, palladium, rhodium, molybdenum and strontium in blood of urban women in nine countries. Int J Hyg Environ Health 2018;221:223–30.
53. Hall M, Chen Y, Ahsan H, Slavkovich V, van Geen A, Parvez F, et al. Blood arsenic as a biomarker of arsenic exposure: results from a prospective study. Toxicology 2006;225:225–33.
54. Lauwerys R, Bernard A, Buchet JP, Roels H, Bruaux P, Claeys F, et al. Does environmental exposure to cadmium represent a health risk? Conclusions from the Cadmibel study. Acta Clin Belg 1991;46:219–25.
55. Schoeters G, Den Hond E, Colles A, Loots I, Morrens B, Keune H, et al. Concept of the Flemish human biomonitoring programme. Int J Hyg Environ Health 2012;215:102–8.
56. Watanabe T, Nakatsuka H, Shimbo S, Iwami O, Imai Y, Moon CS, et al. Reduced cadmium and lead burden in Japan in the past 10 years. Int Arch Occup Environ Health 1996;68:305–14.
57. Nakayama SF, Iwai-Shimada M, Oguri T, Isobe T, Takeuchi A, Kobayashi Y, et al. Blood mercury, lead, cadmium, manganese and selenium levels in pregnant women and their determinants: the Japan Environment and Children's Study (JECS). J Expo Sci Environ Epidemiol 2019;29:633–47.
58. Hartwig A, Bolt HM, Levy L, Manno M, Papameletiou D, Klein CL. SCOEL/OPIN/336 – cadmium and its inorganic compounds. Opinion from the Scientific Committee on Occupational Exposure Limits; 2017. Available from https://op.europa.eu/en/publication-detail/-/publication/3325374b-0a14-11e7-8a35-01aa75ed71a1.
59. Nordberg GF, Bernard A, Diamond GL, Duffus JH, Illing P, Nordberg M, et al. Risk assessment of effects of cadmium on human health (IUPAC Technical Report). Pure Appl Chem 2018; 90:755–808.
60. Agence Nationale Sécurité Sanitaire de l'alimentation, de l'environnement et du Travail (ANSES). Valeurs limites d'exposition en milieu professionnel. Evaluation des indicateurs biologiques d'exposition et recommandation de valeurs limites biologiques et de valeurs biologiques de référence pour le cadmium et ses composés. Rapport d'expertise collective; 2018. Available from https://www.anses.fr/fr/system/files/VLEP2007SA0425Ra.pdf.
61. Barbosa F Jr., Tanus-Santos JE, Gerlach RF, Parsons PJ. A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ Health Persp 2005;113:1669–74.
62. Ducoffre G, Claeys F, Bruaux P. Lowering time trend of blood lead levels in Belgium since 1978. Environ Res 1990; 51:25–34.
63. European Food Safety Authority (EFSA), Panel on Contaminants in the Food Chain (CONTAM), Scientific opinion on lead in food [151 pp.]. revised in 2013. EFSA J 2010;8:1570. Available from www.efsa.europa.eu.
64. National Toxicology Program (NTP). NTP Monograph. Health effects of low-level lead. U.S. Department of Health and Human Services; 2012. Available from https://ntp.niehs.nih.gov/publications/monographs/index.html.
65. Centers for Disease Control and Prevention (CDC), The National Institute for Occupational Safety and Health (NIOSH). Adult blood lead epidemiology & surveillance (ABLES). Atlanta, GA: US Department of Health and Human Services. [last reviewed May 2018]. Available from https://www.cdc.gov/niosh/topics/ables/description.html.
66. Boerleider RZ, Roeleveld N, Scheepers PTJ. Human biological monitoring of mercury for exposure assessment. AIMS Environ Sci 2017;4:251–76.
67. Basu N, Horvat M, Evers DC, Zastenskaya I, Weihe P, Tempowski J. A state-of-the-science review of mercury biomarkers in human populations worldwide between 2000 and 2018. Environ Health Persp 2018;126:106001.
68. Sheehan MC, Burke TA, Navas-Acien A, Breysse PN, McGready J, Fox MA. Global methylmercury exposure from seafood consumption and risk of developmental neurotoxicity: a systematic review. Bull World Health Organ 2014;92:254–69F.
69. Sharma BM, Sanka O, Kalina J, Scheringer M. An overview of worldwide and regional time trends in total mercury levels in human blood and breast milk from 1966 to 2015 and their associations with health effects. Environ Int 2019;125:300–19.
70. Umweltbundesamt (UBA). German Environment Agency. Human Biomonitoring Commission. Available from: https://www.umweltbundesamt.de/en/topics/health/commissions-working-groups/human-biomonitoring-commission-hbm-commission.
71. Snoj Tratnik J, Falnoga I, Mazej D, Kocman D, Fajon V, Jagodic M, et al. Results of the first national human biomonitoring in Slovenia: trace elements in men and lactating women, predictors of exposure and reference values. Int J Hyg Environ Health 2019; 222:563–82.
72. Grandjean P, Nielsen GD, Jørgensen PJ, Hørder M. Reference intervals for trace elements in blood: significance of risk factors. Scand J Clin Lab Invest 1992;52:321–37.