Copper isotope delta measurements in high purity materials: CCQM-P213 pilot study

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ABSTRACT

Accurate and precise isotope ratio measurements of heavy elements are playing an increasingly important role in modern analytical sciences and have numerous applications. Today, isotope ratio measurements are typically performed with two principal techniques: thermal ionization mass spectrometry (TIMS) and multiple collector-inductively coupled plasma mass spectrometry (MC-ICP-MS). To obtain accurate results by mass spectrometry, isotopic certified reference materials (iCRMs) are needed for mass bias correction and for the validation of the method used for analysis. Thus, it is of paramount importance to achieve measurement comparability of all data reported, and to assess measurement capability of each CRM producer/National Metrology Institute (NMI). Therefore, the international comparison (CCQM-P213) was performed to assess the analytical capabilities of NMIs for the accurate determination of copper isotope ratio delta values in high purity materials. The study was proposed by the coordinating laboratories National Research Council Canada (NRC), National Institute of Standards and Technology (NIST), Bundesanstalt für Materialforschung und –prüfung (BAM) and Physikalisch-Technische Bundesanstalt (PTB) as an activity of the Isotope Ratio Working Group (IRWG) of the *Consultative Committee for Amount of Substance – Metrology in Chemistry and Biology* (CCQM). Participants included six NMIs and one designated institute (DI) from the six countries. Although, no measurement method was prescribed by the coordinating laboratories, MC-ICP-MS with either standard-sample bracketing (SSB) or combined SSB with internal normalization (C-SSBIN) models for mass bias correction were recommended. Results obtained from the six NMIs and one DI were in good agreement.

1. INTRODUCTION

Accurate and precise isotope ratio measurements are playing an increasingly important role in modern analytical sciences. Significant and often unique applications include investigations involving geochronology, cosmochemistry, archaeology, provenance studies (chemical "finger-printing"), life/medical sciences, forensic sciences, environmental and atmospheric sciences as well as traditional analytical chemistry and physics[1,2]. As a native element metal, copper (Cu) exists widely in nature; it has two naturally occurring stable isotopes of ^{63}Cu and ^{65}Cu, with relative abundances of 69.17% and 30.83%, respectively.[3] Copper is one of the commonly studied elements for isotopic analysis because natural copper isotopic variations $n(^{65}\text{Cu})/n(^{63}\text{Cu})$ can provide insights into geological processes[4-8] and archaeological science.[9] Copper isotopes also provide a useful tool to trace back the source of Cu in the study of the evolution of metals in the environment.[10,11] Recently, copper isotopes have gained significant interest in medical science.[12,13] Copper is a micronutrient as well as a structural and catalytic cofactor of many significant enzymes involved in neoplastic tissue differentiation, which makes copper a relevant indicator in studies of monitoring the age,[14] sex,[15-18] diet,[16,17] disease pathologies[19-29] and other biological processes.[16,30-33]

Copper isotope ratio measurements are typically performed using two principal techniques: thermal ionization mass spectrometry (TIMS) and multiple collector-inductively coupled plasma mass spectrometry (MC-ICP-MS). Compared to TIMS, MC-ICP-MS has made high-precision analysis of Cu isotope ratio more efficient since MC-ICP-MS has simple sample introduction as well as high ionization efficiency and sensitivity[2]. However, MC-ICP-MS exhibits larger mass bias which needs to be properly corrected. Most published Cu isotopic data are reported in a delta notation (Eq. 1), allowing small isotopic differences to be expressed unambiguously without the need of the exact knowledge of the absolute isotope ratio of a common standard, usually an isotopic certified reference material (iCRM)[34].

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{std}}} - 1\right) \quad (1)$$

where R_{sample} is mass bias corrected ratio in the sample and R_{std} is mass bias corrected ratio/true ratio in the standard. Note Eq.1 can be simplified to use measured ratios, providing analyte and matrix in the sample and the standard are matched, and instrument is stable during a short measurement sequence of standard-sample-standard, etc.

An iCRM can be chosen as "delta-zero" material of an isotopic scale. It can play a crucial role for the correction of instrumental isotopic fractionation/mass bias of mass spectrometers including TIMS and MC-ICP-MS, and can be used for the validation of the methods used for isotopic analyses. Therefore, many National Metrology Institutes (NMIs) have devoted significant effort to addressing the growing need for new and replacement iCRMs over the past couple of decades. Currently, the National Institute of Standards & Technology (NIST) SRM 976 Cu is the internationally accepted "delta-zero" reference material for copper isotope ratios, however it is no longer available. Newer iCRMs, ERM®-AE633 and ERM®-AE647 were produced by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) and were certified for the Cu isotope amount ratio[35] using SRM 976 as base material (ERM®-AE633) and

as a calibrator (ERM®-AE647). While ERM®-AE647 is still commercially available, ERM®-AE633 has been discontinued. More recently, a new Cu iCRM called GBW04624 (certified for absolute Cu isotope ratio) was produced by the National Institute of Metrology China[36] and CRM 105-07-001 (certified for Cu delta value with use of SRM 976 as a calibrator) was produced by the Korea Research Institute of Standards and Science (KRISS) South Korea, as well as another Cu iCRM[37], HICU-1 (for absolute Cu isotope ratio), is in the production process at the National Research Council Canada. Also, an iCRM BAM-I020 (for Cu delta values), is in the production process at the Bundesanstalt für Materialforschung und-prüfung (BAM), Germany.

Several recent studies assessing the δ^{65}Cu value for SRM 976 relative to ERM®-AE647 have been reported. A δ^{65}Cu value of -0.21 ‰ ± 0.05 ‰ (uncertainty reported is the expanded uncertainty (*U*) with a coverage factor of two (*k* = 2)) was reported by Moeller *et al.*[38] while more recently, Sullivan *et al*.[37] confirmed this measurement (δ^{65}Cu = -0.21 ‰ ± 0.06 ‰, *U*, *k* = 2). In addition, the most recent study assessing the δ^{65}Cu value for SRM 976 relative to ERM®-AE647 by Song *et al.*[36] also confirmed the above findings. The calculated δ^{65}Cu value, based on the nominal certified values listed for SRM 976 ($R_{65/63}$ = 1/$R_{63/65}$ = 1/2.2440 = 0.44563 mol/mol ± 0.00042 mol/mol)[39] and ERM®-AE647 ($R_{65/63}$ = 0.44560 mol/mol ± 0.00072 mol/mol)[35], respectively, is0.067 ‰. Although these measured δ^{65}Cu values do agree with the calculated δ^{65}Cu values from of the 'absolute' values for these materials due to the larger uncertainties of the 'absolute' values, a bias does seem to potentially exist. These observations confirm the importance of measurement comparability of all data reported, and the need to assess the measurement capability of each CRM producer/NMI. Therefore, a pilot comparison, CCQM-P213, of δ^{65}Cu isotope ratio measurement in high purity copper was proposed by the coordinating laboratories National Research Council Canada (NRC), National Institute of Standards and Technology (NIST), Bundesanstalt für Materialforschung und –prüfung (BAM) and Physikalisch-Technische Bundesanstalt (PTB) as an activity of the Isotope Ratio Working Group (IRWG) of the *Consultative Committee for Amount of Substance – Metrology in Chemistry and Biology* (CCQM). No measurement method was prescribed by the coordinating laboratories, but MC-ICP-MS with either standard-sample bracketing (SSB) or combined SSB with internal normalization (C-SSBIN) models[2] for mass bias correction were recommended. Participants included six NMIs and one designated institute (DI) from six countries (see Table 1).

Table 1. CCQM-P213: List of participating institutes

Lab Number	Institute	Country	Results reporting date
01	NRC	Canada	Dec. 17, 2020
02	PTB	Germany	Sept. 17, 2020
03	NIST	USA	Mar. 08, 2022
04	UNIIM	Russia	July. 27, 2021
05	BAM	Germany	Nov. 27, 2020
06	NIM	China	Apr. 12, 2021
07	KRISS	Republic of Korea	Apr. 23, 2021
08	UME	Turkey	withdrawn

2. Experimental section

2.1. Instrumentation

All laboratories used a Neptune Plus MC-ICP-MS except for NIST. They used a Neptune MC-ICP-MS, both from Thermo Fisher Scientific (Bremen, Germany) for Cu isotope ratio measurements in the low-resolution mode. The MC-ICP-MS instruments were equipped with nine Faraday cups, a quartz dual cyclonic spray chamber and a PFA self-aspirating nebulizer (Elemental Scientific, Omaha, NE, USA) at a flow rate of 50 - 400 µL min^{-1}. The instruments were tuned for high sensitivity while maintaining flat-top square peaks and stable signals. The gain calibration of the Faraday cups was performed to ensure the normalization of their efficiencies. Rotating amplifiers were chosen by most laboratories during the measurements to minimize amplifier calibration biases and to improve measurement precision. Cup configurations used by different laboratories were similar, and the measurement time was in a range of 1.5 to 3.1 min for each solution. Typical operating conditions are presented in Table 2.

Table 2. Typical MC-ICP-MS operating conditions

Instrument settings	
Radio-frequency power	1100 - 1250 W
Plasma gas flow rate	16.0 L min^{-1}
Auxiliary gas flow rate	0.70 - 1.00 L min^{-1}
Sample gas flow rate	0.9 - 1.2 L min^{-1}
Sampler cone orifice (Ni or Pt)	1.1 mm
Skimmer cone orifice (Ni or Pt)	0.8 mm
Lens settings	Optimized for high and stable analyte signal while maintaining a flat top peak
Data acquisition parameters	
Faraday cup configuration	NRC: L3 (^{58}Ni), L2 (^{60}Ni), L1 (^{61}Ni), C (^{62}Ni), H1 (^{63}Cu), H2 (^{64}Ni), H3 (^{65}Cu); PTB: L3 (^{60}Ni), L1 (^{62}Ni), C (^{63}Cu), H1 (^{65}Cu); NIST: L2 (^{60}Ni), C (^{62}Ni), H1 (^{63}Cu), H3 (^{65}Cu); UNIIM: L1 (^{60}Ni), C (^{62}Ni), H1 (^{63}Cu), H3 (^{65}Cu); BAM: L3 (^{63}Cu), C (^{65}Cu) and L3 (^{60}Ni), L2 (^{61}Ni), L1 (^{62}Ni), C (^{63}Cu), H3 (^{65}Cu); NIM: L3 (^{58}Ni), L1 (^{60}Ni), C (^{61}Ni), H1 (^{62}Ni), H2 (^{63}Cu), H4 (^{65}Cu); KRISS: L4 (^{58}Ni), L2 (^{60}Ni), L1 (^{61}Ni), C (^{62}Ni), H1 (^{63}Cu), H2 (^{64}Ni), H3 (^{65}Cu)
Mass resolution	Low
Signal integration time	0.524- 4.194 s
Numbers of integrations, cycles, and blocks	1, 3-60, 7-10

2.2. Reagents and solutions

High-purity nitric acid obtained by a sub-boiling distillation system (Milestone Inc., Shelton, CT, USA) of reagent grade feedstocks (Fisher Scientific, Ottawa, ON, Canada) and deionized water (DIW, 18.5 MΩ cm) obtained from a Milli-Q ion exchange system (Sigma Aldrich, Oakville, ON, Canada) were used to prepare the samples for the CCQM-P213 comparison. Polyethylene plastic bottles (Fisher Scientific, Ottawa, ON, Canada) were acid washed and dried prior to use.

Two natural copper materials were provided as comparison samples. Sample A (diluted SRM 3114) was prepared from the NIST SRM 3114 Copper Standard Solution in 2 % HNO_3 (volume fraction, *V/V*) (approximately 4 g at 500 mg kg^{-1}); and sample B (diluted BAM-I020) was prepared from BAM-I020 Cu standard solution (a candidate isotopic Certified Reference Material (iCRM)) in 2 % HNO_3 (*V/V*) (about 4 g at 500 mg kg^{-1}). Since both NIST SRM 3114 and BAM-I020 are in solution form, they are homogeneous in isotopic composition.

Two calibration reference materials (RMs) were also provided. RM A (diluted SRM 976): prepared from NIST SRM 976 Isotopic Standard for Copper in 2 % HNO_3 (*V/V*) (about 4 g at 500 mg kg^{-1}) as the bracketing standard/calibrator; and RM B (diluted SRM 986): prepared from NIST SRM 986 Isotopic Standard for Nickel in 2 % HNO_3 (*V/V*) (about 4 g at 500 mg kg^{-1}) as an internal standard for the C-SSBIN model.

2.3. Sample preparation and analysis

Samples, standard and internal standard were diluted in 1 to 2 % high purity HNO_3 (*V/V*) at each laboratory to achieve low blank level and adequate signals for measurements by MC-ICP-MS (with a common sequence of standard-sample-standard, etc.). Typical intensities in the blank and sample are shown in Table 3.

Table 3. Instrument employed and typical intensities obtained

Lab Number	Institute	Intensity, V Blank	Intensity, V Sample
01	NRC	^{58}Ni (0.033), ^{60}Ni (0.00031), ^{63}Cu (0.0032), ^{65}Cu (0.0015)	^{58}Ni (41), ^{60}Ni (17), ^{63}Cu (41), ^{65}Cu (20)
02	PTB	^{60}Ni (0.0005), ^{62}Ni (0.0003), ^{63}Cu (0.0008), ^{65}Cu (0.0005)	^{60}Ni (4.2)*, ^{62}Ni (0.62)*, ^{63}Cu (5.5), ^{65}Cu (2.6)
03	NIST	^{60}Ni (0.002), ^{62}Ni (0.0003), ^{63}Cu (0.0008), ^{65}Cu (0.0004)	^{60}Ni (1.8), ^{62}Ni (0.2), ^{63}Cu (4), ^{65}Cu (2)
04	UNIIM	^{60}Ni (0.0028), ^{62}Ni (0.0004), ^{63}Cu (0.0044), ^{65}Cu (0.0021)	^{60}Ni (7.0), ^{62}Ni (1.0), ^{63}Cu (17), ^{65}Cu (8)
05	BAM	^{60}Ni (<0.0009), ^{62}Ni (<0.0002), ^{63}Cu (<0.0007), ^{65}Cu (<0.0004)	^{60}Ni (7.2)*, ^{62}Ni (1.1)*, ^{63}Cu (4.5), ^{65}Cu (2.1)
06	NIM	^{60}Ni (0.0018), ^{62}Ni (0.0005), ^{63}Cu (0.001), ^{65}Cu (0.0004)	^{60}Ni (8)*, ^{62}Ni (1.15)*, ^{63}Cu (20), ^{65}Cu (10)
07	KRISS	^{60}Ni (0.003), ^{62}Ni (0.0004), ^{63}Cu (0.007), ^{65}Cu (0.003)	^{60}Ni (8), ^{62}Ni (1), ^{63}Cu (21), ^{65}Cu (10)

* Ni signals in sample solutions using C-SSBIN, and Ni signals at blank levels in sample solutions using SSB.

Note that PTB, BAM and NIM used separate single Cu sample solutions without internal standard Ni for SSB measurements.

2.4. Data reporting

The measurand for CCQM-P213 comparison was $\delta_{SRM\,976}(^{65}Cu/^{63}Cu)$ relative to NIST SRM 976 Isotopic Standard for Copper. Each participant was required to report a final value with combined uncertainty for the measurand in each sample solution, and the minimum replicate measurements was five. Each laboratory was required to provide an uncertainty assessment in accordance with JCGM 100:28 *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement*[40].

3. Results and Discussion

Results obtained using the standard-sample bracketing (SSB), and combined SSB with internal normalization (C-SSBIN) models for mass bias correction, are reported in Table 4.

Table 4. CCQM-P213: Reported results for $\delta_{SRM\,976}(^{65}Cu/^{63}Cu)$ relative to NIST SRM 976

	Lab ID	$\delta^{65}Cu_{SRM976}$, ‰	u, ‰ k = 1	U, ‰ k = 2	1SD, ‰	N
Sample A SSB	KRISS	-0.101	0.018	0.037	0.041	5
	NIST	-0.10	0.15	0.30	0.12	5
	PTB	-0.086	0.016	0.033	0.012	6
	NRC	-0.068	0.009	0.018	0.031	79
	BAM	-0.064	0.021	0.042	0.034	18
	NIM	-0.063	0.009	0.018	0.070	79
	UNIIM	0.020	0.045	0.090	0.10	5
Sample A C-SSBIN	NIM	-0.084	0.006	0.012	0.025	54
	KRISS	-0.0764	0.0023	0.0046	0.0051	5
	NRC	-0.075	0.003	0.006	0.010	79
	PTB	-0.074	0.0115	0.023	0.0145	6
	BAM	-0.073	0.008	0.016	0.014	18
	UNIIM	0.090	0.077	0.15	0.17	5
Sample B SSB	KRISS	1.453	0.015	0.030	0.034	5
	NIST	1.46	0.08	0.15	0.12	5
	NIM	1.470	0.010	0.020	0.050	38
	PTB	1.473	0.011	0.022	0.0064	6
	BAM	1.478	0.020	0.040	0.034	18
	NRC	1.487	0.0076	0.015	0.027	80
	UNIIM	1.490	0.046	0.092	0.10	5
Sample B C-SSBIN	UNIIM	1.360	0.022	0.044	0.05	5
	NIM	1.468	0.008	0.016	0.017	27
	KRISS	1.4734	0.0033	0.0067	0.0075	5
	NRC	1.477	0.003	0.006	0.011	80

PTB	1.480	0.007	0.014	0.0041	6
BAM	1.480	0.007	0.014	0.012	18

Note that results are listed in order of increasing delta value

As shown in Tables 3 and 4, it was noted that the instrument used by NIST was exhibiting an abnormal level of signal instability when the experiments were conducted, thus larger uncertainties (*u*) and/or standard deviations (SDs) were reported. UNIIM also reported similar larger uncertainties and/or SDs as compared to the other results. In general, no significant decreasing trends in uncertainties and/or SDs with increasing Cu isotope intensities were observed for the reported four sets of results, indicating that measurement uncertainties from the laboratories are not limited by counting statistics, rather reflecting their measurement uncertainties of the instrument conditions. Notably, the smallest uncertainties and/or SDs were reported by NRC, which may partially be due to the high signal intensities that further minimize counting statistics contribution as expected. As shown in Table 4, in general, smaller uncertainties and/or SDs were obtained using the C-SSBIN mass bias correction model as compared to the simple SSB model, this is because C-SSBIN can effectively correct the temporal drift of mass bias during the measurement sequence.

3.1. Pilot Comparison Reference Values (PCRVs)

Note that we have used the Guidance note[41] that applies to the calculation of key comparison reference values (KCRVs) here for the calculation of the PCRVs. Following the CCQM Guidance note in section 5.2. and Appendix 1 (page 18), a consistency check was applied for the four sets of data (Sample A SSB, Sample A C-SSBIN, Sample B SSB and Sample B C-SSBIN). Based on a chi-squared test, it was found that the data set for Sample B SSB was mutually consistent (as chi square value of χ^2_{obs} = 2.33 and *n*-1 chi square value of $\chi^2_{obs,n-1}$ = 0.388 obtained, *n* is the number of data points; for *n* = 7, 95% critical value of $\chi^2_{0.05\,,n-1}$ = 12.592), the data set for Sample B C-SSBIN (χ^2_{obs} = 29.77, $\chi^2_{obs,n-1}$ = 5.95, $\chi^2_{0.05\,,n-1}$ = 11.070 for *n* = 6,) was inconsistent, and data sets for Sample A SSB (χ^2_{obs} = 8.71, $\chi^2_{obs,n-1}$ = 1.45, $\chi^2_{0.05,n-1}$ = 12.592 for *n* = 7) and Sample A C-SSBIN (χ^2_{obs} = 6.71, $\chi^2_{obs,n-1}$ = 1.34, $\chi^2_{0.05,n-1}$ = 11.070 for *n* = 6) were both between the mutually consistent and inconsistent.

An outlier (1.360 ‰ ± 0.022 ‰, *u*, *k* = 1) in the data set of Sample B C-SSBIN was identified, based on median calculation and 99 % confidence level; the value is considered an outlier when outside $x_{\text{median}} \pm 3 \cdot u_{\text{median}}$ (1.475 ‰ ± 3·0.004 ‰, approximately 99 % confidence level), as suggested in section 6.3.2.6 of the CCQM Guidance note[2]. Note that no technical reason for the outlier could be identified, and the identified outlier was not used to calculate the pilot comparison reference values (PCRVs). Results are summarized in Table 6.

As suggested in section 6 of the CCQM Guidance note[41], although simple mean and median methods may be used for the calculation of PCRVs and associated combined uncertainties (for large and consistent data sets), they are, however, not suitable for this comparison since the data sets are rather small (*n* ≤ 7). Among the 4 data sets, only one data

set is mutually consistent. In addition, both mean and median methods ignore the individual uncertainty of each value. The proper uncertainty estimation of each individual value is essential in order to maintain measurement traceability for that NMI, and it should be considered in the final calculation of PCRVs and uncertainties. Similarly, the weighted mean (W-Mean) method can be used for consistent data sets. Of the four data sets, two data sets (Sample B SSB and Sample B C-SSBIN after rejecting outlier) were consistent and showed zero dark uncertainty present (based on the consistency check) and the other two data sets showed some dark uncertainty present.

The DerSimonian-Laird (DSL) estimator[41, 42] is a simple direct calculation, in which individual uncertainty is considered, and the DSL has been suggested as a preferred calculation where calculation simplicity is desired (page 28 in the CCQM Guidance note[41]). Thus, DSL was selected for the final calculation of PCRVs and associated uncertainties for all 4 data sets. The DSL-mean (x_{DSL}) and its uncertainty (u_{DSL}) are calculated using Eqs. 2 through 7 from the individual result (x_i) and its uncertainty (u_i). Note that p is the number of data sets.

$$x_{DSL} = \frac{\sum_i w_i^* \cdot x_i}{\sum_i w_i^*} \tag{2}$$

where

$$w_i^* = \frac{1}{u_i^2 + \lambda} \tag{3}$$

$$\lambda = \max\left[0, \frac{\sum_{i=1}^{p} (w_i (x_i - \bar{x})^2 - p + 1)}{w_1 - w_2 / w_1}\right] \tag{4}$$

$$\bar{x} = \frac{1}{w_1} \sum_{i=1}^{p} w_i x_i \text{ and} \tag{5}$$

$$w_i = \frac{1}{u_i^2} \ (i=1, \ldots p),\ w_1 = \sum_{i=1}^{p} w_i;\ w_2 = \sum_{i=1}^{p} w_i^2 \tag{6}$$

$$u_{DSL}^2 = \frac{1}{\sum_i w_i^*} \tag{7}$$

Table 5. CCQM-P213 PCRVs, *u*(PCRV), *U*(PCRV) and U_r(PCRV)

		PCRV ‰	*u*(PCRV) ‰, *k* = 1	*U*(PCRV) ‰, *k* = 2	U_r(PCRV) %
Sample A SSB	Mean	-0.066	0.016	0.031	47
	Median	-0.068	0.013	0.025	37
	W-Mean	-0.0697	0.0065	0.013	19
	DSL-Mean	**-0.0709**	**0.0075**	**0.015**	**21**
Sample A C-SSBIN	Mean	-0.049	0.028	0.056	114
	Median	-0.0745	0.0013	0.0026	3.5
	W-Mean	-0.0763	0.0020	0.0039	5.1
	DSL-Mean	**-0.0764**	**0.0024**	**0.0048**	**6.2**
Sample B SSB	Mean	1.476	0.0045	0.009	0.6
	Median	1.476	0.0081	0.016	1.1
	W-Mean	1.479	0.0032	0.006	0.4
	DSL-Mean	**1.479**	**0.0051**	**0.010**	**0.7**
Sample B C-SSBIN	Mean	1.476	0.0023	0.005	0.3
	Median	1.477	0.0025	0.005	0.3
	W-Mean	1.476	0.0015	0.003	0.2
	DSL-Mean	**1.476**	**0.0020**	**0.004**	**0.3**

Note that *u*(PCRV) is the combined uncertainty, *U*(PCRV) is the expanded uncertainty at *K* = 2 and U_r(PCRV) is the relative expanded uncertainty. The outlier identified in Sample B C-SSBIN was not used for the calculation of PCRV.

As shown in Table 5, it is evident that the DSL method provides very similar results to other approaches, especially for the consistent data sets of Sample B SSB or C-SSBIN (after rejecting the outlier).

3.2. Measurand graphic results

The reported results for Sample A SSB, Sample A C-SSBIN, Sample B SSB and Sample B C-SSBIN are presented in Figures 1-4. PCRVs for $\delta_{SRM\,976}(^{65}Cu/^{63}Cu)$, in short $\delta^{65}Cu$, and their uncertainties (green dashed lines) are based on the DSL method described earlier in section 3.1.

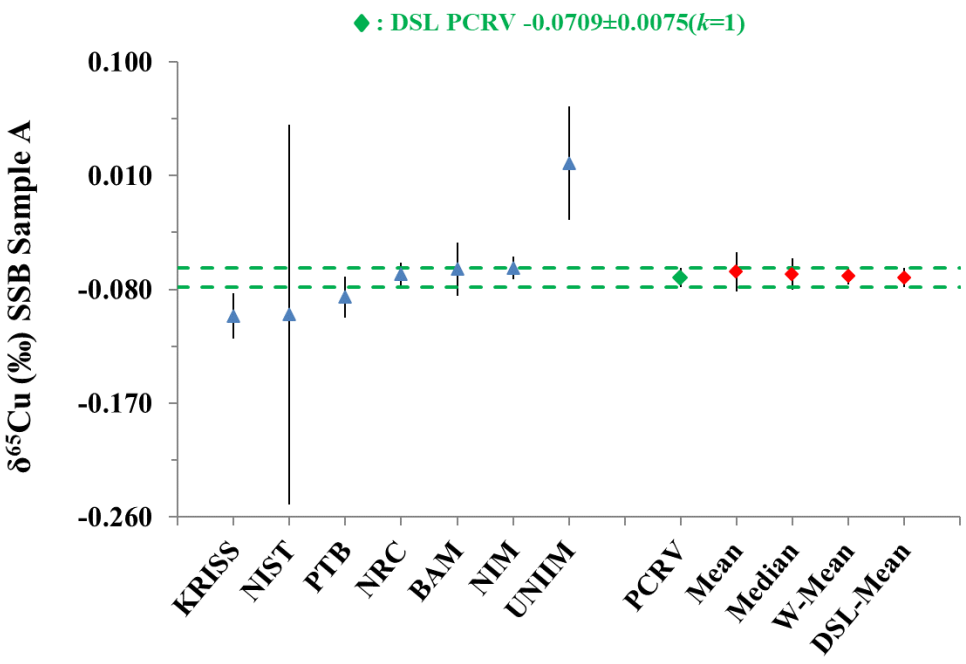

Figure 1. $\delta_{SRM\ 976}(^{65}Cu/^{63}Cu)$ in CCQM-P213 Sample A using SSB model (*u*, *k* = 1)

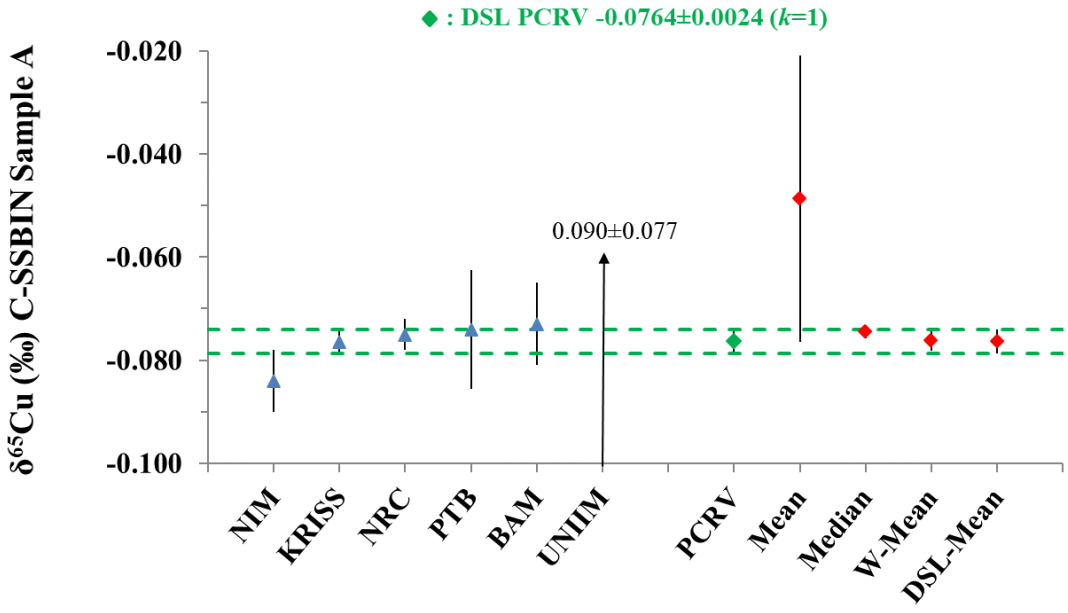

Figure 2. $\delta_{SRM\ 976}(^{65}Cu/^{63}Cu)$ in CCQM-P213 Sample A using C-SSBIN model (*u*, *k* = 1)

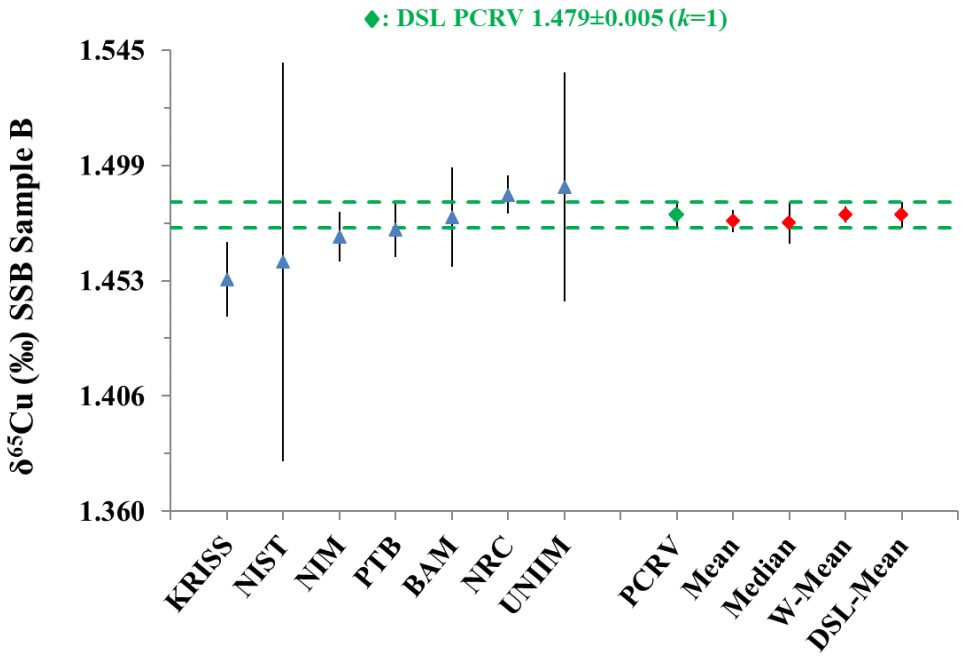

Figure 3. $\delta_{SRM\ 976}(^{65}Cu/^{63}Cu)$ in CCQM-P213 Sample B using SSB model (*u*, *k* = 1)

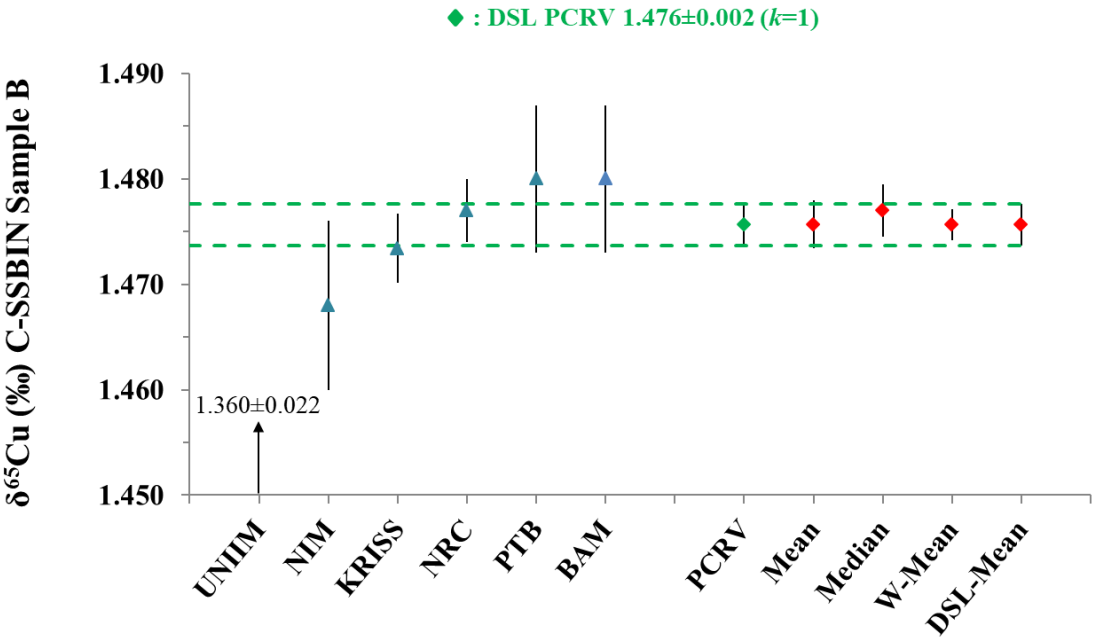

Figure 4. $\delta_{SRM\ 976}(^{65}Cu/^{63}Cu)$ in CCQM-P213 Sample B using C-SSBIN model (*u*, *k* = 1)

3.3. Equivalence statements

The degree of equivalence (DoE, d_i) and its uncertainty ($u(d_i)$) of a measurement result (x_i) reported by a participant relative to the PCRV based on DSL calculation were calculated using Eqs. 8-10, as outlined on page 28 of the CCQM Guidance note[41].

$$d_i = x_i - x_{DSL} \quad (8)$$

$$u^2(d_i) = u_i^2 + \lambda - u_{DSL}^2 \quad (9)$$

where the value x_i is included in the calculation of PCRVs.

$$u^2(d_i) = u_i^2 + \lambda + u_{DSL}^2 \quad (10)$$

where the value x_i is not included in the calculation of PCRVS.

Note that λ (calculated from Eq. 4) is the excess variance due to differences between the submitted results from participating labs and its contribution was included in the uncertainty of the DoE. Eq 9 was used to calculate each uncertainty ($u(d_i)$) of DoE for all data with exception of the identified outlier (1.360 ‰ ± 0.022 ‰, u, k = 1) in the data set of Sample B C-SSBIN wherein Eq 10 was used. Results of DoE are shown in Table 6 and Figures 5-8. Clearly, the majority of the $|d_i/U(d_i)|$ values from the different participants are less than 1, confirming the equivalence of the results obtained.

Table 6. CCQM-P213 Equivalence statement for $\delta_{SRM\ 976}(^{65}Cu/^{63}Cu)$

	Institute	d_i, ‰	$U(d_i)$, ‰, $k = 2$	$\lvert d_i/U(d_i)\rvert$
Sample A SSB	KRISS	-0.030	0.039	0.77
	NIST	-0.029	0.300	0.10
	PTB	-0.015	0.036	0.43
	NRC	0.0029	0.023	0.12
	BAM	0.0069	0.044	0.15
	NIM	0.0079	0.023	0.34
	UNIIM	0.091	0.091	1.00
Sample A C-SSBIN	NIM	-0.0076	0.012	0.61
	KRISS	0.0000	0.006	0.002
	NRC	0.0014	0.007	0.21
	PTB	0.0024	0.023	0.10
	BAM	0.0034	0.016	0.21
	UNIIM	0.17	0.15	1.08
Sample B SSB	KRISS	-0.026	0.028	0.92
	NIST	-0.019	0.160	0.12
	NIM	-0.0089	0.017	0.52
	PTB	-0.0059	0.020	0.30
	BAM	-0.0009	0.039	0.02
	NRC	0.0081	0.011	0.72
	UNIIM	0.011	0.091	0.12
Sample B C-SSBIN	UNIIM	-0.116	0.044	2.62
	NIM	-0.0077	0.016	0.49
	KRISS	-0.0023	0.005	0.43
	NRC	0.0013	0.005	0.30
	PTB	0.0043	0.013	0.32
	BAM	0.0043	0.013	0.32

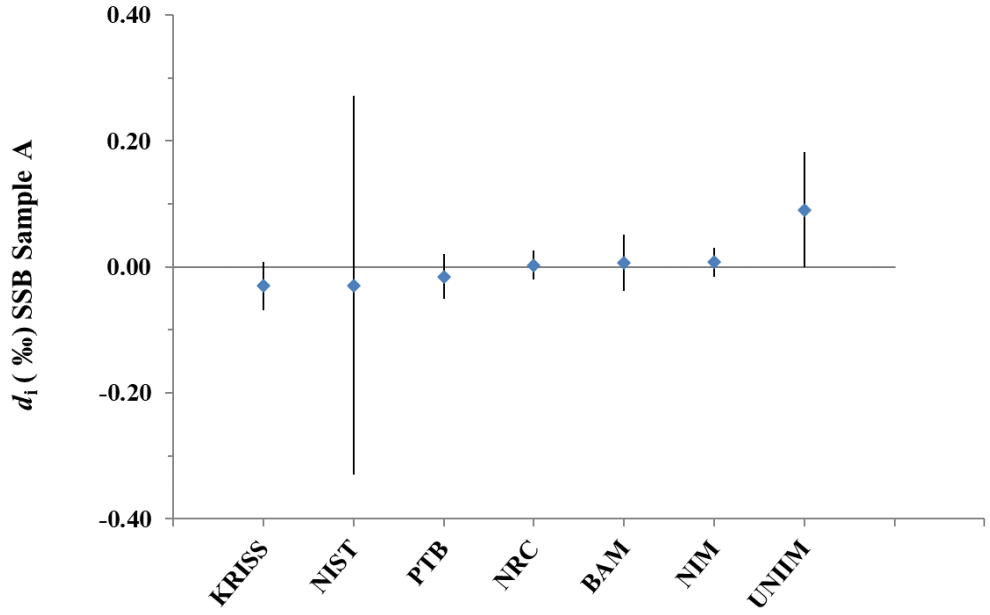

Figure 5. Equivalence statement for $\delta_{SRM\ 976}(^{65}Cu/^{63}Cu)$ in CCQM-P213 Sample A using SSB model (k = 2)

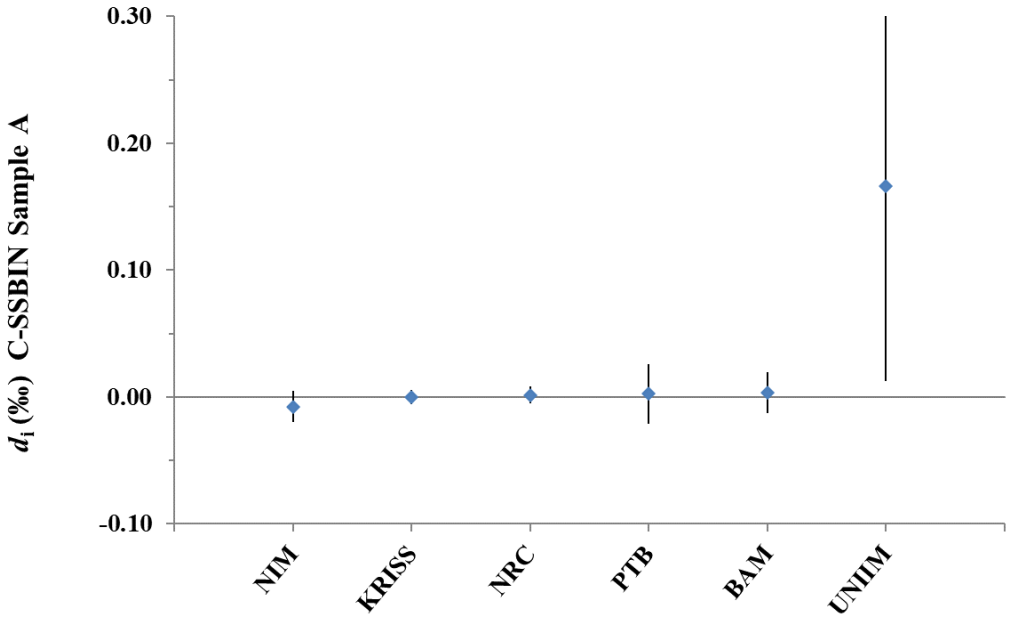

Figure 6. Equivalence statement for $\delta_{SRM\ 976}(^{65}Cu/^{63}Cu)$ in CCQM-P213 Sample A using C-SSBIN model (k = 2)

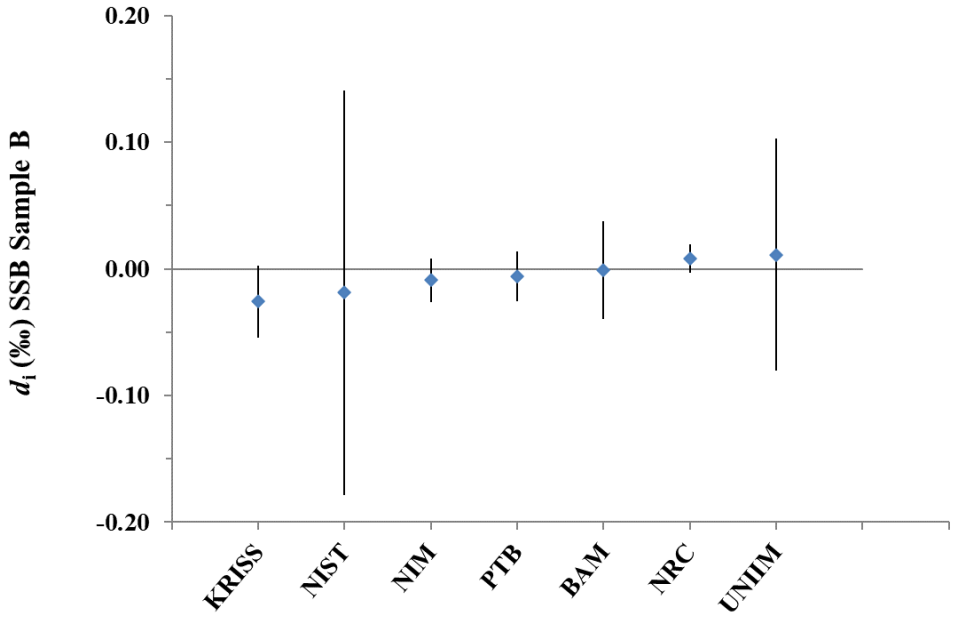

Figure 7. Equivalence statement for $\delta_{SRM\ 976}(^{65}Cu/^{63}Cu)$ in CCQM-P213 Sample B using SSB model (k = 2)

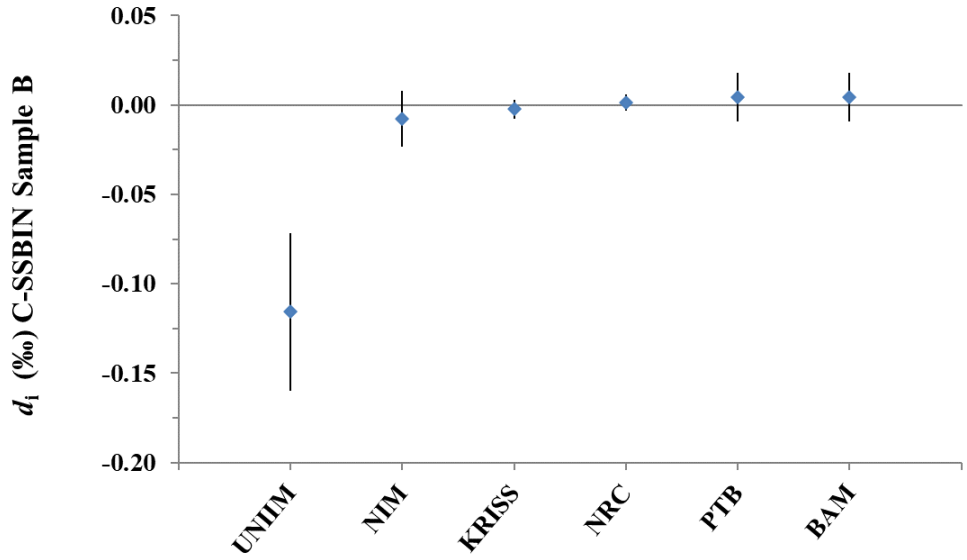

Figure 8. Equivalence statement for $\delta_{SRM\ 976}(^{65}Cu/^{63}Cu)$ in CCQM-P213 Sample B using C-SSBIN model (k = 2)

4. Conclusion

The pilot study CCQM-P213 was a successful comparison, as indicated by the agreement of the results of the majority of the NMIs/DIs with the PCRVs within their expanded uncertainties. Smaller uncertainties were obtained using C-SSBIN mass bias correction as compared to the simple SSB model; this is because C-SSBIN can effectively correct the temporal drift of mass bias during the measurement sequence. However, a future key comparison should be planned to demonstrate equivalence of results for this type of measurement among participants so that calibration and measurement capabilities (CMC) claims can be supported.

Disclaimer: The full description of the procedures used in this paper requires the identification of certain commercial products and their suppliers. The inclusion of such information should in no way be construed as indicating that such products or suppliers are endorsed by NIST or are recommended by NIST or that they are necessarily the best materials, instruments, software, or suppliers for the purpose described.

REFERENCES

(1) Yang, L. Accurate and precise determination of isotopic ratios by mc-icp-ms: A review. *Mass Spectrom. Rev.* **2009,** *28* (6), 990.

(2) Yang, L.; Tong, S.; Zhou, L.; Hu, Z.; Mester, Z.; Meija, J. A critical review on isotopic fractionation correction methods for accurate isotope amount ratio measurements by MC-ICP-MS. *Journal of Analytical Atomic Spectrometry* **2018,** *33* (11), 1849.

(3) Meija, J.; Coplen, T. B.; Berglund, M.; Brand, W. A.; Bièvre, P. D.; Gröning, M.; Holden, N. E.; Irrgeher, J.; Loss, R. D.; Walczyk, T.et al. Isotopic compositions of the elements 2013 (IUPAC Technical Report). *Pure and Applied Chemistry* **2016,** *88* (3), 293.

(4) Zhu, X.; O'nions, R.; Guo, Y.; Belshaw, N.; Rickard, D. Determination of natural Cu-isotope variation by plasma-source mass spectrometry: implications for use as geochemical tracers. *Chemical Geology* **2000,** *163* (1-4), 139.

(5) Vance, D.; Archer, C.; Bermin, J.; Perkins, J.; Statham, P. J.; Lohan, M. C.; Ellwood, M. J.; Mills, R. A. The copper isotope geochemistry of rivers and the oceans. *Earth and Planetary Science Letters* **2008,** *274* (1-2), 204.

(6) Moynier, F.; Vance, D.; Fujii, T.; Savage, P. The Isotope Geochemistry of Zinc and Copper. *Reviews in Mineralogy and Geochemistry* **2017** *82*, 543.

(7) Kempton, P. D.; Mathur, R.; Harmon, R. S.; Bell, A.; Hoefs, J.; Shaulis, B. Cu-Isotope Evidence for Subduction Modification of Lithospheric Mantle. *Geochemistry, Geophysics, Geosystems* **2022,** *23* (8), e2022GC010436.

(8) Markl, G.; Lahaye, Y.; Schwinn, G. Copper isotopes as monitors of redox processes in hydrothermal mineralization. *Geochimica et Cosmochimica Acta* **2006,** *70* (16), 4215.

(9) Gale, N. H.; Woodhead, A. P.; Stos-Gale, Z. A.; Walder, A.; Bowen, I. Natural variations detected in the isotopic composition of copper: possible applications to archaeology and geochemistry. *International Journal of Mass Spectrometry* **1999,** *184* (1), 1.

(10) Bigalke, M.; Weyer, S.; Kobza, J.; Wilcke, W. Stable Cu and Zn isotope ratios as tracers of sources and transport of Cu and Zn in contaminated soil. *Geochimica Et Cosmochimica Acta* **2010,** *74* (23), 6801.

(11) Ren, M.; Zheng, L.; Wang, D.; Chen, X.; Dong, X.; Wei, X.; Cheng, H. Copper isotope ratios allowed for quantifying the contribution of coal mining and combustion to total soil copper concentrations in China. *Environmental Pollution* **2022,** *308*, 119613.

(12) Télouk, P.; Puisieux, A.; Fujii, T.; Balter, V.; Bondanese, V. P.; Morel, A.-P.; Clapisson, G.; Lamboux, A.; Albarede, F. Copper isotope effect in serum of cancer patients. A pilot study. *Metallomics* **2015,** *7* (2), 299.

(13) Balter, V.; Nogueira da Costa, A.; Bondanese, V. P.; Jaouen, K.; Lamboux, A.; Sangrajrang, S.; Vincent, N.; Fourel, F.; Télouk, P.; Gigou, M.et al. Natural variations of copper and sulfur stable isotopes in blood of hepatocellular carcinoma patients. *Proceedings of the National Academy of Sciences* **2015,** *112* (4), 982.

(14) Jaouen, K.; Gibert, M.; Lamboux, A.; Telouk, P.; Fourel, F.; Albarède, F. Is aging recorded in blood Cu and Zn isotope compositions? . *Metallomics* **2013,** *5*, 1016.

(15) Jaouen, K.; Balter, V.; Herrscher, E.; Lamboux, A.; Telouk, P.; Albarède, F. Fe and Cu stable isotopes in archeological human bones and their relationship to sex. *American Journal of Physical Anthropology* **2012,** *148*, 334.

(16) Heghe, L. V.; Deltombe, O.; Delanghe, J.; Depypere, H.; Vanhaecke, F. The influence of menstrual blood loss and age on the isotopic composition of Cu, Fe and Zn in human whole blood. *Journal of Analytical Atomic Spectrometry* **2014,** *29*, 478.

(17) Jaouen, K.; Herrscher, E.; Balter, V. Copper and zinc isotope ratios in human bone and enamel. *American Journal of Physical Anthropology* **2017,** *162*, 491.
(18) Boucher, R. D.; Alavi, S. E.; de Jong, H. N.; Godfrey, L. V.; Vogel, E. R. Stable isotope evidence (Fe, Cu) suggests that sex, but not aging is recorded in rhesus macaque (Macaca mulatta) bone. *American Journal of Physical Anthropology* **2021,** *176* (1), 80.
(19) Aramendía, M.; Rello, L.; Resano, M.; Vanhaecke, F. Isotopic analysis of Cu in serum samples for diagnosis of Wilson's disease: a pilot study. *Journal of Analytical Atomic Spectrometry* **2013,** *28*, 675.
(20) Balter, V.; Costa, A. N.; V. P. da, B.; Jaouen, K.; Lamboux, A.; Sangrajrang, S.; Vincent, N.; Fourel, F.; Télouk, P.; Gigou, M.et al. Natural variations of copper and sulfur stable isotopes in blood of hepatocellular carcinoma patients. *Proceedings of the National Academy of Sciences of the United States of America* **2015,** *112* 982.
(21) Costas-Rodríguez, M.; Anoshkina, Y.; Lauwens, S.; Vlierberghe, H. V.; Delanghe, J.; Vanhaecke, F. Isotopic analysis of Cu in blood serum by multi-collector ICP-mass spectrometry: a new approach for the diagnosis and prognosis of liver cirrhosis? *Metallomics* **2015,** *7* 491.
(22) Télouk, P.; Puisieux, A.; Fujii, T.; Balter, V.; Bondanese, V. P.; Morel, A. P.; Clapisson, G.; Lamboux, A.; Albarede, F. Copper isotope effect in serum of cancer patients. A pilot study. . *Metallomics* **2015,** *7*, 299.
(23) Lauwens, S.; Costas-Rodríguez, M.; Vlierberghe, H. V.; Vanhaecke, F. Cu isotopic signature in blood serum of liver transplant patients: a follow-up study. . *Scientific Reports* **2016,** *6*, 30683.
(24) Sauzéat, L.; Bernard, E.; Perret-Liaudet, A.; Quadrio, I.; Vighetto, A.; Krolak-Salmon, P.; Broussolle, E.; Leblanc, P.; Balter, V.; . Isotopic evidence for disrupted copper metabolism in amyotrophic lateral sclerosis. . *Science* **2018** *6*, 264.
(25) Miller, K. A.; Vicentini, F. A.; Hirota, S. A.; Sharkey, K. A.; Wieser, M. E. Antibiotic treatment affects the expression levels of copper transporters and the isotopic composition of copper in the colon of mice. *Proceedings of the National Academy of Sciences of the United States of America* **2019,** *116* (13), 5955.
(26) Moynier, F.; Creech, J.; Dallas, J.; Le Borgne, M. Serum and brain natural copper stable isotopes in a mouse model of Alzheimer's disease. *Scientific Reports* **2019,** *9* (1).
(27) Kazi Tani, L. S.; Gourlan, A. T.; Dennouni-Medjati, N.; Telouk, P.; Dali-Sahi, M.; Harek, Y.; Sun, Q.; Hackler, J.; Belhadj, M.; Schomburg, L.et al. Copper Isotopes and Copper to Zinc Ratio as Possible Biomarkers for Thyroid Cancer. *Frontiers in Medicine* **2021,** *8*.
(28) Wang, W.; Li, Z.; Lu, Q.; Zhang, L.; Lu, D.; Yang, H.; Yang, X.; Zhang, L.; Zhang, Y.; Liu, Q.et al. Natural copper isotopic abnormity in maternal serum at early pregnancy associated to risk of spontaneous preterm birth. *Science of The Total Environment* **2022,** *849*, 157872.
(29) Solovyev, N.; El-Khatib, A. H.; Costas-Rodríguez, M.; Schwab, K.; Griffin, E.; Raab, A.; Platt, B.; Theuring, F.; Vogl, J.; Vanhaecke, F. Cu, Fe, and Zn isotope ratios in murine Alzheimer's disease models suggest specific signatures of amyloidogenesis and tauopathy. *Journal of Biological Chemistry* **2021,** *296*, 100292.
(30) Albarède, F.; Telouk, P.; Lamboux, A.; Jaouen, K.; Balter, V. Isotopic evidence of unaccounted for Fe and Cu erythropoietic pathways. . *Metallomics* **2011,** *3* 926.
(31) Jaouen, K.; Balter, V. Menopause effect on blood Fe and Cu isotope compositions: Menopause Effect on Blood Fe and Cu Isotopes. . *American Journal of Physical Anthropology,* **2014** *153*, 280.
(32) Cadiou, J. L.; Pichat, S.; Bondanese, V. P.; Soulard, A.; Fujii, T.; Albarède, F.; Oger, P. Copper transporters are responsible for copper isotopic fractionation in eukaryotic cells. *Scientific Reports* **2017,** *7*.

(33) Flórez, M. R.; Costas-Rodríguez, M.; Grootaert, C.; Van Camp, J.; Vanhaecke, F. Cu isotope fractionation response to oxidative stress in a hepatic cell line studied using multi-collector ICP-mass spectrometry. *Analytical and Bioanalytical Chemistry* **2018,** *410* (9), 2385.
(34) Brand, W. A.; Coplen, T. B.; Vogl, J.; Rosner, M.; Prohaska, T. Assessment of international reference materials for isotope-ratio analysis (IUPAC Technical Report). *Pure Appl. Chem.* **2014,** *86*, 425.
(35) IRMM. Certificate of analysis ERM®-AE647. *https://crm.jrc.ec.europa.eu/p/40454/40476/By-application-field/Stable-isotopes/ERM-AE647-COPPER-NATURAL-SPIKE-NITRATE-SOLUTION/ERM-AE647, Certificate of analysis ERM®-AE647, Geel, Belgium* **2013**.
(36) Song, P.; Zhou, T.; Wang, J.; Wang, S.; Ren, T.; Lu, H. An improved calibrated mass spectrometry for absolute copper isotope-ratio measurement using two strategies for mass bias correction factor. *Analytica Chimica Acta* **2022,** *1211*, 339905.
(37) Sullivan, K.; Layton-Matthews, D.; Leybourne, M.; Kidder, J.; Mester, Z.; Yang, L. Copper isotopic analysis in geological and biological reference materials by MC-ICP-MS. *Geostandards and Geoanalytical Research* **2019,** *xx*, xx.
(38) Moeller, K.; Schoenberg, R.; Pedersen, R. B.; Weiss, D.; Dong, S. Calibration of the New Certified Reference Materials ERM-AE633 and ERM-AE647 for Copper and IRMM-3702 for Zinc Isotope Amount Ratio Determinations. *Geostandards and Geoanalytical Research* **2012,** *36* (2), 177.
(39) NIST. Isotopic standard for copper,. *https://www-s.nist.gov/srmors/view_detail.cfm?srm=976, Certificate of analysis, Standard Reference Material 976, Gaithersburg, USA* **1994**.
(40) ISO Guide 35, Reference materials — Guidance for characterization and assessment of homogeneity and stability. 2017, Switzerland.
(41) CCQM Guidance note: Estimation of a consensus KCRV and Associated Degrees of Equivalence, Version 10, 2013-04-12. **2013**.
(42) DerSimonian, R.; Laird, N. Meta-analysis in clinical trials. *Controlled Clinical Trials* **1986,** *7*, 177.