

JRC REFERENCE MATERIALS REPORT

The certification of the mass concentrations of Cd, Cr, Hg, Ni, Pb and Tl in human blood. ERM[®]-DA634, ERM[®]-DA635 and ERM[®]-DA636

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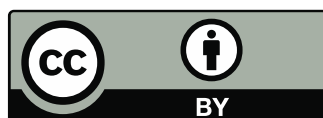
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Abstract

ERM-DA634, ERM-DA635 and ERM-DA636 are human blood reference materials certified for their mass concentrations of cadmium, chromium, mercury, nickel, lead and thallium. They were produced within the scope of ISO 17034 accreditation [1].

Human blood was lysed, spiked with the six elements, dispensed into vials and lyophilised. The CRMs are available in sets of three glass vials, each containing 1 mL freeze-dried blood.

Between-unit homogeneity was quantified and stability during transport and storage was assessed in accordance with ISO 33405:2024 [2]. The minimum sample size for one measurement is 150 mg for Hg and 250 mg for all other elements.

The material was characterised by an interlaboratory comparison of laboratories of demonstrated competence and adhering to ISO/IEC 17025:2017 [3] or ISO 15189 [4].

Uncertainties of the certified values were calculated in accordance with ISO 17034:2016 [1] and include uncertainties related to possible inhomogeneity, instability and characterisation.

The materials are intended for the assessment of method performance, calibration and quality control.

Before release of the CRMs, the certification project was subjected to peer-review involving both internal and external experts.

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1 Introduction

1.1. Background

Metals, pervasive in our environment, can have significant implications on human health.

Acute cadmium (Cd) poisoning is relatively rare, but chronic exposure can result in kidney disease and lung cancer [5]. Additionally, heightened Cd exposure is associated with an increased risk of breast cancer [6]. Cd enters the environment mainly through the atmosphere from processes like Cd production and processing; combustion of fossil fuels, garbage and sewage sludge. After deposition it is readily resorbed by plants. The main exposure routes for human is through food [7], but also smoking [8] is a major exposure route.

Chromium (Cr) exists in a trivalent and hexavalent form and its toxicity is largely dependent on its oxidation state. Cr(III) at low doses is an essential dietary nutrient whereas Cr(VI) is more toxic than Cr(III) and is cancerogenic [9]. Exposure to Cr is mainly through ingestion (food, water) but inhalation can form a significant route in occupational settings. Acute poisoning is rare, but the practice of adding lead chromate to turmeric to enhance its colour is a public health issue in Bangladesh [10].

The toxic effects of mercury (Hg) are well-documented, with the infamous Minamata disease being the most notable case. However, even low levels of Hg exposure can result in neurological, renal, and cardiovascular complications [11]. Exposure to Hg is primarily through consumption of seafood containing methylmercury and amalgam dental fillings [7]. Industrial exposure to inorganic Hg is typically confined to workers in the dental products, fluorescent lighting, and chloralkali electrolysis industries [11].

Exposure to nickel (Ni) can harm the lungs, stomach, and kidneys, and may also increase the risk of cancer [12]. In addition, Ni can cause skin eczema in sensitized persons [13]. Harmful exposure to Ni is largely confined to workers in Ni processing plants and the jewellery industry, but exposure through food can cause effects in sensitized humans [12, 13]

Lead (Pb) toxicity is also well-established and includes inhibition of the synthesis of the haem-complex of haemoglobin, obstruction of motoric nerves as well as fatigue, headache and tremor [7]. Pb is particularly detrimental to children and unborn babies, leading to low birth weight, miscarriage, and cognitive impairment [14]. Alarming, one-third of children worldwide have blood Pb levels above 50 µg/L [15], which can result in stunting and cognitive impairment. The major exposure route for the general population is through food [16] but exposure can also occur in occupational settings, and through inhalation. Residing near industrial processes can lead to increased blood Pb levels, as was observed in children living near an industrial site in Hoboken, Belgium [17].

To protect workers from these chemical risks, Council Directive 98/24/EC [18] has been introduced. This directive mandates the monitoring of workers' blood Pb levels and necessitates medical surveillance of workers with a blood Pb concentration exceeding 400 µg/L.

Chronic exposure to thallium (Tl) can affect kidney function, lead to preterm births and there are reports that Tl is associated with autism spectrum disorder [19]. While Tl was used as rat and ant poison in the past, its use for this purpose has been banned. Exposure today is mainly occupational, for example, cement production, coal-burning industries or smelting of sulphide ores. Exposure can also come from food grown in the vicinity of such sources [20].

Within the context of "One Health", monitoring of exposure routes (e.g. air, food, water etc.) contributes greatly to limit the exposure of the general population to harmful concentrations of metals. However, biological monitoring remains a very important tool to assess actual exposure to metals where monitoring of environmental contamination may not be a reliable proxy for exposure.

To make this monitoring meaningful and reliable, its results must be accurate and traceable. To support the quality of monitoring results, the European Commission has funded projects for the development of certified reference materials (CRMs) for Cd and Pb in blood: BCR-194, BCR-195 and BCR-196, three lysed bovine blood materials were released in 1985 [21]. A set of lysed human blood CRMs (BCR-634, BCR-635 and BCR-636) was released in 2004 [22]. These materials were exhausted. To ensure the constant quality of the biological monitoring for metals, the JRC decided to replace these materials.

1.2. Choice of the material

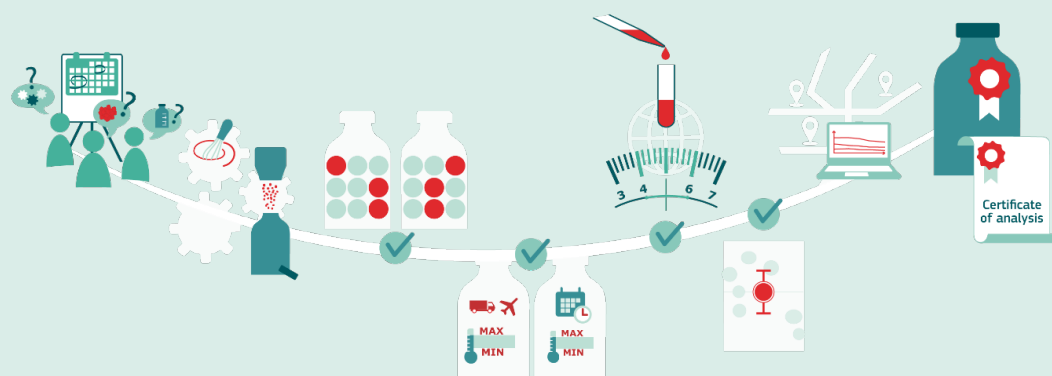
Given the popularity of the predecessor materials, it was decided that the new materials should resemble the previously produced ones as closely as possible. The material of choice was therefore also a lysed human blood. The Cd and Pb concentrations were chosen to closely match those of BCR-634, BCR-635 and BCR-636. The Cr, Hg, Ni and Tl concentrations were chosen after checking the ranges used in human blood proficiency testing and discussion with clinical laboratories. All element concentrations cover the relevant concentration range for biological monitoring.

Several clinical laboratories were contacted regarding the format (frozen or lyophilised) and the volume. These discussions confirmed that a lyophilised material was preferred, as it guarantees long-term stability.

Sourcing naturally incurred material with the desired metal levels was deemed impossible. It was therefore decided to spike the starting materials for all three concentrations.

1.3. Outline of the CRM project

Box 1. Reference material production



Reference material (RM) production is defined in ISO 17034 [1] as a project comprising planning and processing of the material, followed by homogeneity and stability testing, characterisation and assigning of one or more property values. Depending on the intended use of the RM a commutability study is carried out.

For certified reference materials (CRMs) a certificate is issued while for RMs a product information sheet is issued by the reference material producer (RMP).

CRMs and RMs are distributed globally and the stability of their assigned values is monitored throughout the life-time of the material.

Human blood from healthy donors without risk factors for elevated metal concentrations was acquired. The blood donations were lysed, spiked to achieve the intended element concentrations, filtered and lyophilised. Homogeneity was checked and the variation between units was quantified. The stability during transport was assessed using a 4-weeks stability study. Stability during storage for Pb and Cd was assessed using the data from the long-term stability monitoring of the previous certified reference materials. The findings of this assessment were confirmed by a 6-months stability study. Finally, the materials were characterised in an interlaboratory comparison among laboratories of demonstrated competence using different methods of sample preparation and quantification.

Uncertainties of certified values were assigned in compliance with ISO 17034 [1], which implements the basic principles of ISO/IEC Guide 98 (GUM) [23].

The CRM project, including the certification approach and the evaluation of the obtained measurement data, was subjected to peer-review involving both internal and external experts.

Certain commercial equipment, instruments, and materials are identified in this report to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

2 Participants

2.1 Project management and data evaluation

European Commission, Joint Research Centre, Directorate F – Health and Food, Geel, BE

(accredited to ISO 17034:2016 for production of certified reference materials, BELAC No. 268-RM)

2.2 Processing

European Commission, Joint Research Centre, Directorate F – Health and Food, Geel, BE

2.3 Homogeneity and stability measurements

ALS Scandinavia, Luleå, SE

(measurements performed under the scope of ISO/IEC 17025:2017 accreditation SWEDAC 2030)

2.4 Characterisation measurements

ALS Scandinavia, Luleå, SE

(measurements performed under the scope of ISO/IEC 17025:2017 accreditation SWEDAC 2030)

Institut za medicinska istraživanja i medicinu rada, Zagreb, HR

Institut Jožef Stefan, Ljubljana, SI

Laboratoire National de Métrologie et d'Essais, Trappes Cedex, FR

(measurements for Cd and Pb performed under the scope of ISO/IEC 17025:2017 accreditation COFRAC 2-54)

Medizinisches Labor Ostsachsen MVZ GbR, Görlitz, DE

(measurements performed under the scope of ISO 15189 accreditation DAkkS D-ML-13288-01-00)

Sciensano, Tervuren, BE

Työterveyslaitos, Helsinki, FI

(measurements for Cd, Cr, Hg and Pb performed under the scope of ISO/IEC 17025:2017 accreditation FINAS T013)

Umweltbundesamt GmbH, Wien, AT

(measurements under the scope of ISO/IEC 17025:2017 accreditation Akkreditierung Austria 0200)

All datasets are identified by a code (e.g. D01). The numbering is not related to the order of the laboratories presented above.

3 Material processing and processing control

Box 2. Reference material processing



RM processing covers the raw material conversion into a homogenous and stable material. It typically includes processing steps such as grinding or sieving and drying steps to enhance stability. When the processed material fulfils the specifications, the final material is filled into individual containers, referred to as RM units, such as bottles or ampoules and is labelled.

3.1 Origin of the starting material

A total of 28 frozen donations of 206 mL to 311 mL from healthy human donors (19 male, 9 female) of human blood were delivered by In.Vent Diagnostica GmbH (Hennigsdorf, DE). Each donation contained 2.21 mmol K_3EDTA as anticoagulant.

Each individual blood donation has been tested for HIV-1/2 antibodies, HCV antibodies and HBs antigen with methods cleared in compliance with the European Directive 98/79/EC, Annex II, List A and found negative or non-reactive.

The samples were collected according to the principles of the Declaration of Helsinki (DoH) and Taipei (DoT), processed and verified in a way that is ethically and legally compliant for the purposes of diagnostic research and development, production and quality assurance.

Furthermore, the samples were collected under ethic's approval. The ethic's approval was granted by the ethics commission review board of the Freiburger Ethik Kommission international under feki Code: 011/1763.

3.2 Processing

After thawing, the contents of each blood bag were transferred into a beaker and 2.21 mmol $CaCl_2$ (99.99 % on metal basis; Thermo Scientific, Kandel, Germany) was added to each donation to bind the EDTA. The beaker was placed on ice and the blood was lysed for 10 minutes using an ultrasonic probe (Q700, Qsonica, Newtown, US) with a total energy input of 50 - 65 J. The sonication was performed in two stages of 5 minutes to avoid overheating the blood. After lysing, the donations were placed in a 37 °C warm water bath for 2-3 h for clotting. Then lysates were pooled into 1-L bottles and were centrifuged at 4 °C at 12000 G (Sorvall RC6+, Thermo Fisher Scientific, Waltham, US) and filtered using a 0.45 μm polyethersulfone filter (Thermo Fisher Scientific, Waltham, US) followed by a second filtration through a 0.22 μm polyethersulfone filter (VWR, Haasrode, BE). At that stage, the lysate for each of the materials was pooled and spiked to obtain the desired metal levels. Afterward the pools were diluted by adding two times the volume of ultrapure water (resistivity 18.2 M Ω ·cm at 25 °C) and stirred overnight at 4 °C. The purpose of the dilution was to reduce the viscosity of the lysate and to increase the repeatability of the filling volume by filling a larger volume.

Five-mL amber glass vials were rinsed with 5 % nitric acid and rinsed with ultrapure water (resistivity 18.2 M Ω ·cm at 25 °C). Three mL of the spiked and diluted lysate were dispensed into each vial using a peristaltic pump. The lysate was stirred during the filling process. The lysate was then freeze-dried (Epsilon2-100D, Martin Christ, Osterode am Harz, DE). After freeze drying, the vacuum was broken with Ar and the vials were closed with silicone stoppers and capped. The dry matter content of each vial was about 200 mg. The number of vials retained of ERM-DA634, ERM-DA635 and ERM-DA636 was 2454, 1935, and 1314, respectively.

For the purpose of this report, the term 'unit' refers to one vial of ERM-DA634, ERM-DA635 and ERM-DA636. In this way it is ensured that the certified values with their uncertainty are valid for the individual unit. The materials were assembled into sets of three units, with the code xa, xb and xc with x being the set number. The labelling was done in a way that each set contains units from the complete filling sequence. If, for example, 2400 units were filled, labelling was done as follows: Unit 1 to 800 were labelled as "a", unit 801 to 1600 were labelled as "b" and units 1601 to 2400 were labelled as "c". The labelling within a, b and c was done in order of the filling sequence.

One set of CRM ERM-DA635 is shown in Figure 1 as an example for the three CRMs.

Figure 1. CRM ERM-DA635.



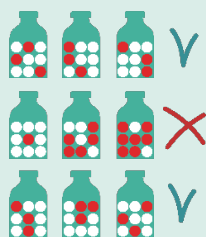
Source: JRC

3.3 Processing control

16 pre-weighed empty vials were entered at approximately every 100th position of the filling sequence. After filling, these units were removed and weighed. The relative standard deviations of the masses of ERM-DA634 and ERM-DA635 were 0.9 % and no trend was visible in the masses, showing that the filling was under control. A significant start-up effect was visible for the masses for ERM-DA636, where the first unit had a 5 % lower filling mass than later units. As the blood was subsequently lyophilised and reconstituted with the same volume of water regardless of the initial filling volume, this was expected to result in lower metal concentrations for these vials. This effect was confirmed by measurement of the metal concentration of six samples from unit number 3 to 126. Therefore, the first 50 sets were removed from the batch. The relative standard deviation of the filling masses of the remaining tested unit masses was 0.4 %, demonstrating sufficient control after the problems at the start.

4 Homogeneity

Box 3. Homogeneity assessment



A key requirement for any RM produced as a batch of units is equivalence between those units. It is important to know how much the variation between units contributes to the uncertainty of the certified value. Consequently, ISO 17034 [1] requires RMPs to quantify the between-unit variation in homogeneity studies.

The within-unit homogeneity is correlated to the minimum sample size, which is the minimum amount of sample that is, for a given measurand, representative of the whole unit and that should be used in an analysis. Using sample intakes equal to or above the minimum sample size guarantees the assigned value within its stated uncertainty.

4.1 Between-unit homogeneity

The between-unit homogeneity was evaluated to ensure that the certified values of the CRMs are valid for all units of the material, within the stated uncertainties.

The number of units selected corresponds to approximately the cube root of the total number of units produced for ERM-DA634, ERM-DA635 and ERM-DA636 (2454, 1935, and 1524, respectively). In total 15 units were selected using a random stratified sampling scheme covering the whole batch for the between-unit homogeneity test. This random stratified sampling involves dividing the total number of set numbers into five equal groups and randomly choosing one set from each group. As each set contains three units from different thirds of the batch, this setup ensures coverage of the whole batch in a random-stratified manner. The samples were reconstituted and three independent samples were taken from each selected unit and analysed by acid-digestion inductively coupled plasma sector field mass spectrometry (ICP-SFMS). The measurements were performed under repeatability conditions and in a randomised manner to separate a potential drift in the measurement results from a potential trend in the filling sequence.

Regression analyses were performed to evaluate potential trends in the measurement sequence. For several elements (see Table 1, Table 2 and Table 3) trends in the measurement sequence were visible, pointing at a changing parameter, e.g. a signal drift in the measurement system. Research has shown that the correction of even non-significant biases is beneficial. Such correction of non-significant biases has two advantages: on the one hand, the resulting uncertainties are smaller than for non-corrected biases. On the other hand, after correction the uncertainty interval has the highest probability of including the true value [24]. Correction of trends is therefore expected to improve the sensitivity of subsequent statistical analysis through a reduction in the variation of measurement results without masking potential between-unit heterogeneities. As the measurement sequence and the unit numbers were not correlated, trends significant on at least a 95 % confidence level were corrected as shown below:

$$x_{i_corr} = x_i - b \cdot i$$

Equation 1

b slope of the linear regression
 i position of the result in the measurement sequence

Regression analyses were performed on the (if necessary) trend-corrected data to evaluate potential trends in the filling sequence. For Pb in ERM-DA634 a trend in the filling sequence that was significant on a 95 % confidence level was detected. This is about what is expected by chance (5 % of 18 sequences = 0.9) and, as this trend is not visible for any other of the elements in the same material, might be a statistical artefact. Nevertheless, this trend was taken into consideration in the evaluation of u_{bb} of Pb.

The (if necessary) trend-corrected dataset was assessed for consistency using the single and double Grubbs outlier tests at a confidence level of 99 % on the individual results and on the unit means.

For ERM-DA634, two individual outliers were detected for Cr. These outliers only concerned one of the three individual replicates of one unit and were removed.

For ERM-DA635, two Individual outliers were detected for Cr. These outliers only concerned one of the three individual replicates of one unit and were removed. For none of the materials outlying unit means were detected.

For ERM-DA636, two outlying unit means were observed for Pb. These were flagged as outliers, although the results agreed within their confidence intervals with the other results. These two means were retained.

Quantification of between-unit inhomogeneity was undertaken by analysis of variance (ANOVA), which separates the between-unit variation (s_{bb}) from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability if the individual samples were representative for the whole unit.

Evaluation by ANOVA requires mean values per unit, which follow at least a unimodal distribution and results for each unit that follow unimodal distributions with approximately the same standard deviations. The distribution of the mean values per unit was visually tested using histograms and normal probability plots. Too few data are available for the unit means to make a clear statement of the distribution. Therefore, it was checked visually whether all individual data follow a unimodal distribution using histograms and normal probability plots. Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-unit standard deviations. The results of all statistical evaluations are given in Table 1, Table 2 and Table 3; the results of the homogeneity tests are shown in graphical form in Annex 1.

The unit means for Ni do not follow normal distributions. The distributions are broad with a hint of bimodality. Evaluation by ANOVA is therefore not applicable.

Table 1. Results of the statistical evaluation of the homogeneity study of ERM-DA634. Trends in the measurement sequence were corrected before further data treatment.

ERM-DA634	Trends ¹⁾		Outliers ²⁾		Distribution	
	Measure ment sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
Cd	yes	no	no	no	normal	normal
Cr	yes	no	2 (removed)	no	normal	normal
Hg	no	no	no	no	normal	normal
Ni	no	no	no	no	normal	not normal
Pb	yes	yes	no	no	unimodal	normal
Tl	yes	no	no	no	normal	normal

1) 95 % confidence level.

2) 99 % confidence level.

Source: JRC

Table 2. Results of the statistical evaluation of the homogeneity study of ERM-DA635: Trends in the measurement sequence were corrected before further data treatment.

ERM-DA635	Trends ¹⁾		Outliers ²⁾		Distribution	
	Measure ment sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
Cd	yes	no	no	no	unimodal	normal
Cr	yes	no	2 (removed)	no	normal	normal
Hg	yes	no	no	no	normal	normal
Ni	no	no	no	no	normal	not normal
Pb	no	no	no	no	normal	normal
Tl	yes	no	no	no	normal	normal

1) 95 % confidence level.

2) 99 % confidence level.

Source: JRC

Table 3. Results of the statistical evaluation of the homogeneity study of ERM-DA636. Trends in the measurement sequence were corrected before further data treatment.

ERM-DA636	Trends ¹⁾		Outliers ²⁾		Distribution	
	Measure ment sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
Cd	yes	no	no	no	normal	normal
Cr	no	no	no	no	normal	normal
Hg	no	no	no	no	normal	normal
Ni	no	no	no	no	normal	not normal
Pb	no	no	no	2 (retained)	normal	unimodal
Tl	yes	no	no	no	normal	normal

1) 95 % confidence level.

2) 99 % confidence level.

Source: JRC

It should be noted that $s_{bb, rel}$ and $s_{wb, rel}$ are estimates of the standard deviations and are therefore subject to random fluctuations. Therefore, the mean square between groups ($MS_{between}$) can be smaller than the mean squares within groups (MS_{within}), resulting in a negative number under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be less than zero. In this case, u_{bb}^* , the maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [25]. u_{bb}^* is comparable to the limit of detection (LOD) of a measurement method yielding the maximum degree of inhomogeneity that might be undetected by the given study setup.

Method repeatability ($s_{wb, rel}$) (equivalent to the within-unit standard deviation), between-unit standard deviation ($s_{bb, rel}$) and $u_{bb, rel}^*$ were calculated as:

$$s_{wb, rel} = \frac{\sqrt{MS_{within}}}{\bar{y}}$$

Equation 2

$$S_{bb, \text{rel}} = \frac{\sqrt{\frac{MS_{\text{between}} - MS_{\text{within}}}{n}}}{\bar{y}}$$

Equation 3

$$U_{bb, \text{rel}}^* = \frac{\sqrt{\frac{MS_{\text{within}}}{n}} \sqrt{\frac{2}{v_{MS_{\text{within}}}}}}{\bar{y}}$$

Equation 4

MS_{within} mean of squares within-unit from ANOVA
 MS_{between} mean of squares between-unit from ANOVA
 \bar{y} mean of all results of the homogeneity study
 n mean number of replicate analysis per unit
 $v_{MS_{\text{within}}}$ degrees of freedom of MS_{within}

For Pb in ERM-DA634, the trend in the filling sequence was significant on a 95 % confidence level. Therefore the uncertainty was assessed in a different way. Here, U_{rec} was estimated using a rectangular distribution between the highest and lowest unit mean [23] as given in Equation 5. The same approach was adopted for Ni (all materials), where the unit means do not follow normal distributions.

The Pb mass concentrations in ERM-DA636 are a special case: The unit means show two outliers with concentrations above the mean value (451 µg/L and 445 µg/L compared to the average of 432 µg/L). Removal of these two outliers reveals another outlier with a lower concentration (421 µg/L). Despite these outliers, the homogeneity of the Pb mass concentration is still sufficient for use. Throughout the other studies (stability, characterisation) no other such outliers were found supporting the assumption that the outliers in the homogeneity study indeed cover the highest and lowest concentrations that can be expected. Therefore, the uncertainty of homogeneity was also estimated using a rectangular distribution between the highest and lowest unit mean, hence taking the outliers into consideration (Equation 5).

$$U_{\text{rec}} = \frac{|\bar{x}_{\text{max}} - \bar{x}_{\text{min}}|}{2 \cdot \sqrt{3} \cdot \bar{y}}$$

Equation 5

\bar{y} mean of all results of the homogeneity study
 \bar{x}_{max} highest unit mean of the homogeneity study
 \bar{x}_{min} lowest unit mean of the homogeneity study

The results of the evaluation of the between-unit variation are summarised in Table 4, Table 5 and Table 6. In half of the cases, the uncertainty contribution for homogeneity was determined by the method repeatability.

Table 4. Results of the homogeneity study of ERM-DA634.

ERM-DA634	$S_{wb, \text{rel}}$ [%]	$S_{bb, \text{rel}}$ [%]	$U_{bb, \text{rel}}^*$ [%]	$U_{\text{rec, rel}}$ [%]	$U_{bb, \text{rel}}$ [%]
Cd	6.64	1.84	1.95	n.a. ¹⁾	1.95
Cr	11.86	n.c. ²⁾	3.62	n.a. ¹⁾	3.62
Hg	9.91	n.c. ²⁾	2.91	n.a. ¹⁾	2.91
Ni	13.53	n.a. ¹⁾	3.97	9.55	9.55
Pb	6.30	n.a. ¹⁾	1.85	3.67	3.67
Tl	6.05	3.48	1.78	n.a. ¹⁾	3.48

1) n.a.: not applicable as not evaluated by ANOVA.

2) n.c.: not calculated, as $MS_{\text{between}} < MS_{\text{within}}$

Source: JRC

Table 5. Results of the homogeneity study of ERM-DA635.

ERM-DA635	$S_{wb, rel}$ [%]	$S_{bb, rel}$ [%]	$U_{bb, rel}^*$ [%]	$U_{rec, rel}$ [%]	$U_{bb, rel}$ [%]
Cd	2.30	1.30	0.68	n.a. ¹⁾	1.30
Cr	3.15	1.18	0.96	n.a. ¹⁾	1.18
Hg	3.56	1.20	1.04	n.a. ¹⁾	1.20
Ni	3.73	n.a. ¹⁾	1.09	2.05	2.05
Pb	5.27	n.c. ²⁾	1.55	n.a. ¹⁾	1.55
Tl	3.92	n.c. ²⁾	1.15	n.a. ¹⁾	1.15

1) n.a.: not applicable. As not evaluated by ANOVA

2) n.c.: cannot be calculated as $MS_{between} < MS_{within}$.

Source: JRC

Table 6. Results of the homogeneity study of ERM-DA636.

ERM-DA636	$S_{wb, rel}$ [%]	$S_{bb, rel}$ [%]	$U_{bb, rel}^*$ [%]	$U_{rec, rel}$ [%]	$U_{bb, rel}$ [%]
Cd	3.16	1.17	0.94	n.a. ¹⁾	1.17
Cr	4.00	2.38	1.19	n.a. ¹⁾	2.38
Hg	5.07	n.c. ²⁾	1.51	n.a. ¹⁾	1.51
Ni	4.07	n.a. ¹⁾	1.21	2.65	2.65
Pb	4.94	n.a. ¹⁾	1.48	2.31	2.31
Tl	4.92	n.c. ²⁾	1.47	n.a. ¹⁾	1.47

1) n.a.: not applicable. As not evaluated by ANOVA

2) n.c.: cannot be calculated as $MS_{between} < MS_{within}$.

Source: JRC

The homogeneity studies showed outlying unit means for Pb in ERM-DA636, but the deviation was within the confidence intervals of the various means. Except for Pb in ERM-DA634, none of the trends in the filling sequence were significant on a 95 % confidence level. Therefore, the between-unit standard deviation s_{bb} or U_{bb}^* can be used as an estimate of u_{bb} . This holds for all elements except Pb in ERM-DA634 and Ni: these data do not follow normal distributions and hence a calculation of a standard deviation would be not valid. Therefore, u_{bb} was estimated from a rectangular distribution. As U_{bb}^* sets the limits of the study to detect inhomogeneity, the larger value of s_{bb} and U_{bb}^* is adopted as uncertainty contribution to account for potential inhomogeneity.

4.2 Within-unit homogeneity and minimum sample size

The within-unit inhomogeneity does not influence the uncertainty of the certified value when the minimum sample size is respected, but determines the minimum size of sample that is representative for the whole unit. Quantification of within-unit homogeneity is therefore necessary to determine the minimum sample size.

The minimum sample size was established based on the technically valid results of the characterisation and stability studies, using the method information supplied by the participants. The smallest sample intake that still yielded results with acceptable precision to be included in the respective studies was taken as minimum sample size. The following minimum sample sizes of reconstituted material are derived:

Hg: 150 mg which is for practical purposes equivalent to 150 μL .

Cd, Cr, Ni, Pb, Tl: 200 mg which is for practical purposes equivalent to 200 μL .

5 Stability

5.1 General

Box 4. Stability assessment



Stability testing is necessary to establish the conditions for storage as well as the transport conditions of the RMs to the customers. During transport, especially in summer, temperatures up to 60 °C can be reached, and stability under these conditions must be demonstrated if the RMs are to be transported without any additional cooling.

Time, temperature and light (including ultraviolet radiation) were regarded as the most relevant influences on the stability of the materials. The influence of ultraviolet and visible light is minimised by storing the material in amber glass units, which reduce light exposure. In addition, materials are stored in the dark and transported in boxes, thus removing any possibility of degradation by light. Therefore, only the influences of time and temperature needed to be investigated.

The stability studies were carried out using an isochronous design [26]. In this approach, units are stored for a particular length of time in different temperature conditions. Afterwards, the units are moved to conditions where further degradation can be assumed negligible (reference conditions). At the end of the isochronous storage, the samples are analysed simultaneously under repeatability/intermediate precision conditions. Analysis of the material (after various exposure times and temperatures) under repeatability/intermediate precision conditions greatly improves the sensitivity of the stability tests.

5.2 Transport stability

The conditions for the transport of the material to the customers were established in a short-term stability study. To this end, units were stored at 18 °C and 60 °C for 0, 1, 2 and 4 weeks (at each temperature). The reference temperature was set to -20 °C. Two units per storage time were selected using a random stratified sampling scheme. From each unit, three samples were measured by acid-digestion ICP-SFMS. The measurements were performed under repeatability conditions and a randomised sequence was used to differentiate any potential drift in the measurement results from a potential trend over storage time. The data were evaluated individually for each temperature. For ERM-DA636, only units with numbers > 70 were used because of the run-in effects found in the processing.

The results were screened for trends in the analytical sequence and trends were corrected if they were significant on a 95 % confidence level as described in section 4.1. The results were screened for outliers using the single and double Grubbs test on a confidence level of 99 %.

In addition, the data were evaluated against storage time, and regression lines of mass concentration versus time were calculated, to test for potential increases or decreases of the mass concentration due to shipping conditions. The slopes of the regression lines were tested for statistical significance.

The results of the measurements are shown in Annex 2. The results of the statistical evaluation of the short-term stability are summarised in Table 7, Table 8 and Table 9.

Table 7. Results of the short-term stability tests of ERM-DA634.

ERM-DA634	Number of individual outlying results ¹⁾		Significance of the trend ²⁾	
	18 °C	60 °C	18 °C	60 °C
Cd	none	none	no	no
Cr	none	none	no	no
Hg	none	none	no	no
Ni	none	none	no	no
Pb	none	none	no	yes (95 %)
Tl	none	none	no	no

1) 99 % confidence level.

2) 95 % confidence level.

Source: JRC

Table 8. Results of the short-term stability tests of ERM-DA635.

ERM-DA635	Number of individual outlying results ¹⁾		Significance of the trend ²⁾	
	18 °C	60 °C	18 °C	60 °C
Cd	none	none	no	no
Cr	none	none	no	no
Hg	none	none	no	no
Ni	none	none	no	no
Pb	none	none	no	no
Tl	none	none	no	no

1) 99 % confidence level.

2) 95 % confidence level.

Source: JRC

Table 9. Results of the short-term stability tests of ERM-DA636.

ERM-DA636	Number of individual outlying results ¹⁾		Significance of the trend ²⁾	
	18 °C	60 °C	18 °C	60 °C
Cd	none	none	no	no
Cr	none	none	no	no
Hg	none	none	no	no
Ni	none	none	no	yes (95 %)
Pb	none	none	no	no
Hg	none	none	no	no

1) 99 % confidence level.

2) 95 % confidence level.

Source: JRC

The trends of mass concentration versus time at 60 °C were significant at a 95 % confidence level for Pb in ERM-DA634 and Ni in ERM-DA636. Two of 36 positive results is about the expected rate of false positives on a 95 % confidence level. The elements themselves are stable and any trend can only be caused by changes in the matrix, which should affect all elements. As none of the other elements showed a trend for these two materials, these trends are probably statistical artefacts. Nevertheless, they will be taken into consideration when assessing the uncertainties.

The material can be dispatched without further precautions under ambient conditions.

5.3 Storage stability

5.3.1 General considerations

Pb and Cd are stable elements that cannot degrade. However, degradation of the blood matrix is possible and therefore stability during storage must be assessed.

Storage conditions and shelf life guaranteeing the stability of the material and the certified values were established in a long-term stability study. Stability of the Pb and Cd mass concentrations was assessed using the stability data of BCR-634, BCR-635 and BCR-636. To verify that the data obtained from the stability monitoring of these CRMs produced and stored in the same way could be used to estimate the stability uncertainty contribution for ERM-DA634, ERM-DA635 and ERM-DA636, the data of the a 12-months stability study were compared to the stability monitoring data. The data of the 12-months stability study supports the assumption that the Cd and Pb mass concentrations in ERM-DA634, ERM-DA635 and ERM-DA636 are as stable as in BCR-634, BCR-635 and BCR-636 and therefore uncertainty estimations based on stability monitoring data can be used to estimate the uncertainty contribution relating to the storage of the CRM.

5.3.2 Stability data on BCR-634, -635 and -636

The CRMs BCR-194, BCR-195 and BCR-196 with certified values for Pb and Cd in bovine blood, have been for sale for 22 years (BCR-194) and 35 years (BCR-195, BCR-196), respectively. During this time, they were tested 15 times for stability with no indication of degradation.

BCR-634, BCR-635 and BCR-636 were certified in 2003. A 24-months stability study with time points of 0, 6, 12 and 24 months and a 48-months stability study with time points of 0, 12, 24 and 48 months were started after the release. In each of the studies, 6 independent measurements per time point were performed under repeatability conditions.

In addition, units were moved from the normal storage temperature at -20 °C to the reference temperature of -70 °C in October 2002. These reference samples were used to assess potential changes in the materials in the stability tests of 2009, 2011, 2013, 2015, 2017, 2019, 2021 and 2023 (82-222 months). For each of these tests, six independent measurements of samples stored at the storage temperature and six independent measurements of units stored at the reference temperature were performed.

A quantitative assessment for the possible degradation of the materials was made separately for Cd and Pb on the basis of the human materials (BCR-634, -635 and -636) as follows:

For each time point of the isochronous studies and the follow-up stability monitoring studies, the ratio of the mean result from normal stock to mean result from reference stock was calculated. These data are shown in a graph in Annex 3.

The data of BCR-634, -635 and -636 were pooled. This is justified as the three materials were processed in the same way and as the analytes under investigation are the same.

The data were checked for outliers and significance of the trends. The results of these investigations are shown in Table 10

Table 10. Evaluation of the pooled data of the stability monitoring of BCR-634, BCR-635 and BCR-636.

	Number of individual outlying results ¹⁾	Significance of the trend ²⁾
Cd	1 (removed)	yes
Pb	none	no

1) 99 % confidence level.

2) 95 % confidence level.

Source: JRC

The dataset for Cd contained one outlying ratio where the result at reference temperature was 16 % lower than at the storage temperature, indicating a more severe degradation at -70 °C than at -20 °C. The reason for this is that this result (time point 130 months) was close to the limit of quantification of the method. As this finding is unlikely and was also not confirmed by the results of BCR-635 and BCR-636 at this time point and neither from any other time, the outlier was removed.

The trend for Cd is statistically significant. This significance hinges at the results at 221 and 248 months. Removing one of these two time points means that the trend is no longer significant, which indicates a lack of robustness of the finding. In addition, the trend is small: at a rate of 0.11 % per year, it is technically not relevant. This means that despite the apparent trend, the Cd mass concentration is sufficiently stable even if this apparent trend is real. As a precautionary measure, the apparent degradation is included in the estimation of the uncertainty.

The data for Pb showed neither outliers nor a significant trend.

The uncertainty evaluation of the data is described in section 5.4.

5.3.3 12-months stability study

Units were stored at -20 °C for 0, 4, 8 and 12 months. The reference temperature was set to -70 °C. Two units per storage time were selected using a random stratified sampling scheme. From each unit, three samples were digested and measured by ICP-SFMS. The measurements were performed under repeatability conditions and in a random sequence to be able to separate any potential drift in the measurement results from a potential trend over storage time.

The results were screened for trends in the analytical sequence and trends were corrected if they were significant on a 95 % confidence level as described in section 4.1. Single and double Grubbs outliers tests at a confidence level of 99 % were performed.

Finally, the data were plotted against storage time and linear regression lines of mass concentration versus time were calculated. The slopes of the regression lines were tested for statistical significance (loss/increase due to storage).

The results of the statistical evaluation of the long-term stability study are summarised in Table 11 to Table 14.

Table 11. Results of the 12-months study of ERM-DA634.

Element	Number of individual outlying results ¹⁾	Significance of the trend in the analytical sequence ²⁾	Significance of the trend in time ²⁾
Cd	none	no	no
Cr	none	no	no
Hg	none	no	no
Ni	none	no	no
Pb	none	no	no
Tl	none	yes	no

1) 99 % confidence level. 2) 95 % confidence level.

Source: JRC

Table 12. Results of the 12-months study of ERM-DA635

Element	Number of individual outlying results ¹⁾	Significance of the trend in the analytical sequence ²⁾	Significance of the trend in time ²⁾
Cd	2 (removed)	yes	no
Cr	none	no	no
Hg	none	no	no
Ni	none	no	yes
Pb	1 (removed)	no	no
Tl	none	no	no

1) 99 % confidence level.

2) 95 % confidence level.

Source: JRC

Table 13. Results of the 12-months study of ERM-DA636.

Element	Number of individual outlying results ¹⁾	Significance of the trend in the analytical sequence ²⁾	Significance of the trend in time ²⁾
Cd	none	no	no
Cr	none	no	no
Hg	none	no	no
Ni	none	yes	yes
Pb	none	no	no
Tl	none	no	no

1) 99 % confidence level.

2) 95 % confidence level.

Source: JRC

5.4 Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can entirely rule out degradation of materials, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method precision, i.e. to estimate the uncertainty of stability. This means that, even under ideal conditions, the outcome of a stability study can only be that there is no detectable degradation within an uncertainty to be estimated.

The uncertainties of stability during transport and storage were estimated, as described in [27] for each element. In this approach, the uncertainty of the linear regression line with a slope of zero was calculated. The uncertainty contributions u_{sts} and u_{lts} were calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as:

$$u_{sts, rel} = \frac{s_{rel}}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{tt} \quad \text{Equation 6}$$

$$u_{lts, rel} = \frac{s_{rel}}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{sl} \quad \text{Equation 7}$$

s_{rel}	relative standard deviation of all results of the stability study
t_i	time elapsed at time point i
\bar{t}	mean of all t_i
t_{tt}	chosen transport time (1 week at 60 °C)
t_{sl}	chosen shelf life (18 months at 18 °C)

For Pb in ERM-DA634 (short-term), Ni in ERM-DA635 (1 year) and Ni in ERM-DA636 (1 year and short-term) where the slope b of the regression line was significant on a 95 % confidence level as well as for the assessment of the long-term stability for Cd, the potential effect of change was included in the uncertainty estimation. This effect was modelled as a rectangular distribution and added to the uncertainty of the regression line itself as shown below:

$$u_{sts, rel} = \sqrt{\frac{s_{yx}^2}{\sum (t_i - \bar{t})^2} + \frac{b_{rel}^2}{3}} \cdot t_{tt} \quad \text{Equation 8}$$

b	slope of the regression line
s_{yx}	relative standard error of the regression
t_i	time elapsed at time point i
\bar{t}	mean of all t_i
t_{tt}	chosen transport time (1 week at 60 °C)

The following uncertainties were estimated:

- $u_{sts, rel}$, the uncertainty of stability during transport. This was estimated from the 60 °C study. The uncertainty describes the possible change during a transport at 60 °C lasting for one week.
- $u_{lts, rel}$, the uncertainty of stability during storage. This uncertainty contribution was estimated from stability monitoring for Pb and Cd of the CRMs BCR-634, BCR-635 and BCR-636 and was estimated for 3 years of storage. For all other elements, the stability was estimated for 18 months of storage based on the results of the 1-year study.

The results of these evaluations are summarised in Table 15.

Table 14. Uncertainties of stability during transport and storage for ERM-DA634. $u_{\text{sts,rel}}$ was calculated for a temperature of 60 °C and 1 week; $u_{\text{lts,rel}}$ was calculated for a storage temperature of -20 °C and 18 months storage time.

ERM-DA634	$u_{\text{sts,rel}}$ [%]	$u_{\text{lts,rel}}$ [%]
Cd	0.48	0.12
Cr	1.39	8.00
Hg	0.60	2.73
Ni	1.72	4.65
Pb	0.67 (degradation included)	0.10
Tl	0.57	1.51

Source: JRC

Table 15. Uncertainties of stability during transport and storage for ERM-DA635. $u_{\text{sts,rel}}$ was calculated for a temperature of 60 °C and 1 week; $u_{\text{lts,rel}}$ was calculated for a storage temperature of -20 °C and 18 months storage time. .

ERM-DA635	$u_{\text{sts,rel}}$ [%]	$u_{\text{lts,rel}}$ [%]
Cd	0.32	0.12
Cr	0.54	4.55
Hg	0.38	2.49
Ni	0.70	3.53 (degradation included)
Pb	0.33	0.10
Tl	0.54	2.08

Source: JRC

Table 16. Uncertainties of stability during transport and storage for ERM-636. $u_{\text{sts,rel}}$ was calculated for a temperature of 60 °C and 1 week; $u_{\text{lts,rel}}$ was calculated for a storage temperature of -20 18°C and 18 months storage time.

ERM-DA636	$u_{\text{sts,rel}}$ [%]	$u_{\text{lts,rel}}$ [%]
Cd	0.54	0.12
Cr	0.43	2.90
Hg	0.42	2.02
Ni	1.1 (degradation included)	4.06 (degradation included)
Pb	0.33	0.10
Tl	0.51	1.70

Source: JRC

The calculation of the uncertainties shows that the statistically significant trends in the 60 °C study for Pb (ERM-DA634) and Ni (ERM-DA636) are technically insignificant. Therefore, the material can be shipped at ambient conditions without special precautions.

An increasing trend was found in the 1-year study for Ni in ERM-DA635. This would only be possible if the matrix degraded, which would be visible also for the other elements. The stability monitoring will show whether this trend is real. As a precautionary measure, the extent of the trend was included in the uncertainty estimation.

A decreasing trend was found in the 1-year study for Ni in ERM-DA636. This is very unlikely to be real as Ni itself is a stable element. A degradation could be due to changes in the matrix that result in incomplete reconstitution. However, this effect should be visible for the other elements as well, which is not the case. The stability monitoring will show whether this trend is real. As a precautionary measure, the extent of the trend was included in the uncertainty estimation.

The materials are included in the JRC's regular stability monitoring programme, to control their further stability.

Box 5. Stability monitoring



RMs are produced as batches that should last for ten years or longer. This long lifetime means that a storage stability study of limited duration cannot provide a definite “use by” date for the material. It therefore needs to be complemented by stability monitoring throughout the lifetime of the RM.

Therefore, the stability of RMs whose assigned values might change is regularly monitored. The monitoring frequency depends on the outcome of the storage stability assessment.

If the tests confirm the stability of the assigned values, the material remains on sale. If not, possible actions include the retraction of the value in question, retraction of the complete material or a change of the certified value. Customers are notified if the change is larger than the uncertainty of the assigned value.

5.5 Stability of reconstituted samples

One set of each material was used to test the stability of reconstituted samples. On day one of the study, the first unit of each set was reconstituted and stored at 4 °C. On day 3 of the study, the second unit was reconstituted and stored at 4 °C. On day 7, the third unit of the set was reconstituted. All reconstituted units were re-homogenised on day 8. Three separate digestions were performed on each of the vials and each digest was measured once.

The results were screened for individual outlying results using a Grubbs test on a 99 % confidence level. The results were evaluated using one-way ANOVA with the day of the reconstitution as the grouping variable. In addition, the trend of the results over the reconstitution day was tested for significance.

No outlier was detected for ERM-DA634. The variation between days was not significant on a 95 % confidence level for Cd, Cr, Hg, Pb and Tl. It was significant for Ni on a 95 %, but not on a 99 % confidence level. None of the trends was significant on a 95 % confidence level.

For ERM-DA635, one individual statistical outlier for Cd was removed. None of the variations between days was significant on a 95 % confidence level. None of the trends was significant on a 95 % confidence level.

No outlier was detected for ERM-DA636. None of the variations between days was significant on a 95 % confidence level. None of the trends was significant on a 95 % confidence level.

All the data are shown in graphical form in Annex 4.

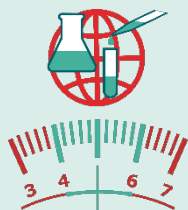
The results show that reconstituted samples can be stored for 1 week at 4 °C.

6 Characterisation

6.1 Characterisation principle

The material characterisation was based on an interlaboratory comparison, i.e. the element mass concentrations of the material were determined by combining independent datasets obtained in laboratories applying different measurement procedures to demonstrate the absence of a measurement bias. This approach converts the systematic bias of each dataset into a random variable, the combined effect of which is reduced by averaging over several datasets.

Box 6. Reference material characterisation



Material characterisation is the process of determining the property value(s) of an RM. While ISO 17034 [1] allows to characterise a RM in various ways.. Quality management procedures of the JRC are more stringent and allow characterisation only by either interlaboratory comparison or the use of a primary method confirmed by independent analysis.

6.2 Selection of participants

Eight laboratories were selected based on criteria that comprised both technical competence and quality management aspects. Each participating laboratory was required to operate a quality system and to deliver documented evidence of its laboratory proficiency in the determination of elements in biological matrices, preferably in blood by providing results for interlaboratory comparison exercises. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 [3] or ISO 15189 [4] was obligatory. All laboratories are accredited, but not all measurements were performed under the scope of accreditation. Where measurements were performed under the scope of accreditation, the accreditation number is stated in the list of participants (Section 2).

6.3 Study setup

Each laboratory provided one dataset. Not all laboratories measured all elements.

Laboratories received several units of ERM-DA634, ERM-DA635 and ERM-DA636 and were requested to provide for each material six independent results. The number of units per laboratory varied depending on the sample intake: laboratories with a sufficiently small sample intake received three units and performed two independent measurements per unit; laboratories with a sample intake above 1 mL pooled two or three units to obtain sufficient material for the analyses. The units for material characterisation were selected using a random stratified sampling scheme and covered the whole batch. The sample preparations and measurements had to be done on at least three days to ensure intermediate precision conditions. An independent calibration was performed for each day.

Each laboratory also received one unit each of the certified reference materials BCR-634 and BCR-636 as quality control for the Pb and Cd measurements. In addition, laboratories were instructed to perform one standard addition experiment for all elements on an independent preparation of ERM-DA635 as a quality control for all elements. The results of these measurements were used to support the evaluation of the characterisation results.

Laboratories were also requested to give estimations of the expanded uncertainties of the mean value of the six results. No approach for the estimation was prescribed, i.e. top-down and bottom-up [23] were regarded as equally valid procedures.

6.4 Measurement procedures used

A variety of digestion and dilution methods using pressurised digestion HNO_3 or $\text{HNO}_3\text{-H}_2\text{O}_2$, digestion at ambient pressure with HNO_3 , dilution with various media with different quantification steps (ICP-SFMS, ICP-QMS, ICP-Q3MS, ICP-IDCQMS, AAS) were used to characterise the material. The combination of results from methods all targeting the same measurand but based on completely different measurement principles mitigates undetected method bias.

All methods used during the characterisation study are summarised in Annex 5. The dataset code (e.g. D01-ICP-SFMS) is a random number and does not correspond to the order of laboratories in Section 2. The dataset code consists of a number assigned to each dataset (e.g. D01) and abbreviation of the measurement method used (e.g. ICP-SFMS).

6.5 Evaluation of results

The characterisation study resulted in 6-8 datasets per element. All individual datasets of the participating laboratories, grouped per element, are displayed in tabular and graphical form in Annex 5.

6.5.1 Technical evaluation

The obtained data were first checked for compliance with the requested instructions and for their validity based on technical reasons. The following criteria were considered during the evaluation:

- compliance with the instructions given: sample preparations and measurements performed on three days,
- absence of values given as below limit of detection (LOD) or below limit of quantification (LOQ)
- method performance, i.e. agreement of the measurement results with the certified values of the QC samples (BCR-634 and BCR-636) and the results of the standard addition experiments

Based on the above criteria, the following data or datasets were rejected as not technically valid (Table 18).

Cd: The result for Cd for the quality control sample BCR-634 of Dataset 06 (measured on day 1) were significantly above the certified value. The laboratory suspected a contamination of the blank and re-measured ERM-DA634, ERM-DA635 and ERM-DA636 (but not BCR-634) on day 2. The results of the re-measured samples are significantly lower than those obtained on day 1 and are in line with the results obtained on days 2 and 3. This confirms the suspicion of a contamination of day 1. The results of day 1 were therefore ignored and the data obtained from the re-measurement on day 2 were used.

The result of the standard addition of Dataset 03 disagreed with the added concentration on a 95 %, but not on a 99 % confidence level. This disagreement was therefore attributed to a statistical artefact and the results were retained.

Cr: One digest for ERM-DA635 of Dataset 03 showed markedly higher results than the other digests (36 $\mu\text{g/L}$ compared to 21 $\mu\text{g/L}$). This was the same digest that also showed higher results for Ni (see below). As contamination comes most likely through stainless steel, also this deviating result was attributed to a contamination of the digest and the results of this digest were not used for evaluation.

Dataset 08 also included results for Cr. The laboratory confirmed that the method was not yet validated for Cr. The results were obtained in the same measurement run as the other elements and the sample preparation was therefore the same; the results also agreed with the assigned value within the stated uncertainty. However, as the method was not validated, the results were not used for the calculation of the certified value.

Ni: The result of the standard addition of Dataset 03 disagreed with the added concentration on a 95 %, but not on a 99 % confidence level. This disagreement was therefore attributed to a statistical artefact and the results were retained. In addition, one digest for ERM-DA635 of Dataset 03 showed markedly higher results than the other digests (32 $\mu\text{g/L}$ compared to 18 $\mu\text{g/L}$). Re-measurement of the same digests gave the same results, as did a measurement of diluted digests. The laboratory attributed this deviating result to contamination and the result of this digest was therefore not used for the evaluation.

Pb: Dataset 02 showed low results for BCR-636. The laboratory reported that BCR-636 was not a homogeneous solution after reconstitution, which could explain the deviation. However, also the result for BCR-634 was low. Contrary to these low findings, the result of the standard addition experiment on ERM-DA635 was indeed the measured concentration of ERM-DA635 plus the added concentration of the spike.

Nevertheless, the low findings on the two BCR-materials cast doubt on the result and the complete dataset for Pb was not used for evaluation.

For ERM-DA634, Dataset 07 showed a markedly lower results for day 1 than on day 2 and day 3. The laboratory confirmed that the concentration level is close to the LOQ and quite far below the lowest calibration point of 85 µg/L. In addition, day 2 and 3 had been measured with a fresh graphite furnace, which tends to have better sensitivity for Pb. For this reason, the values for Pb for ERM-DA634 were not included in the calculation of the certified value, although the average is in agreement with all other results.

Table 17. Datasets that showed non-compliance with the instructions given and technical specifications, and action taken.

	Dataset code	Description of problem	Action taken
Cd, all CRMs	D06	Contamination of the blank on measurement day 1	Data of the re-measured samples on day 2 were used.
Cd, standard addition	D03	Results for the standard addition deviated from the added value on a 95 %, but not on a 99 % confidence level.	Results retained
Cr, ERM-DA635	D03	One digest gave markedly higher results.	Results of this digest not used for evaluation
Cr, all CRMs	D08	Method not validated for Cr	Not used for evaluation
Ni, standard addition Ni, ERM-DA635	D03	One digest gave markedly higher results. Results for the standard addition deviated from the added value on a 95 %, but not on a 99 % confidence level.	Results of this digest not used for evaluation Results retained
Pb, all materials	D02	Results for BCR-634 and BCR-636 do not agree with the certified values.	Data not used for evaluation
Pb, ERM-DA634	D07	Result close to LOQ and far below lowest calibration point	Data not used for evaluation

Source: JRC

6.5.2 Statistical evaluation

The datasets accepted based on technical reasons were tested for normality of dataset means using normal probability plots and were tested for outlying means using the Grubbs test and using the Cochran test for outlying standard deviations (both at a 99 % confidence level). Standard deviations within (s_{within}) and between ($s_{between}$) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 19 to Table 21. The individual data are shown as table and as charts in Annex 6.

Table 18. Statistical evaluation of the technically accepted datasets for ERM-DA634. p : number of technically valid datasets. n.a. not applicable, as the data do not follow a normal distribution

Element	p	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean [μg/L]	s [μg/L]	$s_{between}$ [μg/L]	s_{within} [μg/L]
Cd	8	no	no	yes	1.289	0.088	0.079	0.095
Cr	6	no	D02, D07	yes	1.462	0.306	0.285	0.267
Hg	7	no	D06	yes	1.614	0.274	0.252	0.259
Ni	6	no	D02, D03, D08	no	3.069	0.831	n.a.	0.617
Pb	6	no	no	yes	18.62	1.15	1.13	0.54
Tl	6	no	D06, D08, D02	yes	0.809	0.062	0.060	0.037

Source: JRC

Table 19. Statistical evaluation of the technically accepted datasets for ERM-DA635. p : number of technically valid datasets.

Element	p	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean [μg/L]	s [μg/L]	$s_{between}$ [μg/L]	s_{within} [μg/L]
Cd	8	no	D05	yes	5.655	0.377	0.369	0.183
Cr	6	no	no	yes	22.03	1.33	1.27	0.93
Hg	7	no	D06	yes	25.52	3.31	3.27	1.31
Ni	6	no	D8	yes	18.27	0.50	0.30	0.96
Pb	7	no	no	yes	182.0	11.2	11.0	4.5
Tl	6	no	no	yes	8.311	0.601	0.593	0.247

Source: JRC

Table 20. Statistical evaluation of the technically accepted datasets for ERM-DA636. p : number of technically valid datasets.

Element	p	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean [μg/L]	s [μg/L]	$s_{between}$ [μg/L]	s_{within} [μg/L]
Cd	8	no	D05	yes	10.85	0.65	0.63	0.35
Cr	6	no	D07	yes	41.93	2.99	2.95	1.86
Hg	7	no	no	yes	56.36	6.00	5.85	2.39
Ni	6	no	D08	yes	35.96	2.87	2.63	2.78
Pb	7	no	D07	yes	438.8	34.7	33.9	9.0
Tl	6	no	no	yes	16.57	1.44	1.42	0.46

Source: JRC

The results for Ni in ERM-DA634 show three groups of results: one at 2.0 µg/L, three at (2.7-2.9) µg/L and two at 4.0 - 4.2 µg/L. The lowest dataset (2.0 µg/L) was the only one obtained by a sector field instrument at a higher mass resolution (4000) than the other data; these results were also obtained using a different isotope (^{62}Ni rather than ^{60}Ni). The Ni-level of this material is also rather close to the LOQ for other methods and how the intercept of the calibration curve is drawn becomes increasingly important. In addition, Ni is prone to contamination. There is therefore significant doubt on what the correct value for Ni for ERM-DA634 is and consequently no certified value is assigned. The range is given as information value.

All other dataset means follow normal distributions. None of the data contains outlying means. The statistical evaluation flagged several datasets as outlying variances. This merely reflects the fact that different methods have different intrinsic variability. As all measurement methods were found technically sound, all results were retained.

The datasets are therefore consistent and the mean of means is a good estimate of the true value. Most of the standard deviations between dataset means are considerably larger than the standard deviation within datasets, showing that confidence intervals of replicate measurement results are unsuitable as estimates of measurement uncertainty.

The uncertainty related to the characterisation is estimated as the standard error of the mean of means (s/\sqrt{p}) (Table 22 to Table 24).

Table 21. Uncertainty of characterisation for ERM-DA634.

Element	p	Mean [µg/L]	s [µg/L]	u_{char} [%]
Cd	8	1.289	0.088	2.41
Cr	6	1.462	0.306	8.53
Hg	7	1.614	0.274	6.42
Pb	6	18.62	1.15	2.53
Tl	6	0.809	0.062	3.13

Source: JRC

Table 22. Uncertainty of characterisation for ERM-DA635.

Element	p	Mean [µg/L]	s [µg/L]	u_{char} [%]
Cd	8	5.655	0.377	2.36
Cr	6	22.03	1.33	2.46
Hg	7	25.52	3.31	4.90
Ni	6	18.27	0.50	1.12
Pb	7	182.0	11.2	2.32
Tl	6	8.311	0.60	2.95

Source: JRC

Table 23. Uncertainty of characterisation for ERM-DA636.

Element	p	Mean [µg/L]	s [µg/L]	u_{char} [%]
Cd	8	10.85	0.65	2.11
Cr	6	41.93	2.99	2.91
Hg	7	56.36	6.00	4.02
Ni	6	35.96	2.87	3.26
Pb	7	438.8	34.7	2.99
Tl	6	16.57	1.44	3.54

Source: JRC

7 Value Assignment

Box 7. Assignment of values to a reference material



Based on the outcome of characterisation measurements three types of values can be assigned, namely certified, indicative or additional material information values.

Certified values are values that fulfil the highest standards of accuracy. Procedures at JRC Directorate F require a sufficient number of datasets to assign certified values. Full uncertainty budgets in accordance with ISO 17034 [1] and ISO Guide 35 [2] are required. Certified values of a CRM can be used for calibration and trueness controls.

Indicative values are values where either the uncertainty is deemed too large or too few independent datasets are available to allow certification. Indicative values of an RM can be used for statistical quality control (homogeneity and stability have been assessed) but not for calibration, demonstration of method or laboratory proficiency or method trueness.

Additional material information values are values for which homogeneity and stability have usually not been assessed and insufficient data for characterisation is available. Consequently, an estimate of the reliability of the values is not possible and no uncertainty is given. Additional material information values cannot be used for calibration, demonstration of method or laboratory proficiency or method trueness. They can be used to e.g. anticipate possible interferences in measurement processes.

7.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table 19 to Table 21 was assigned as certified value for each parameter.

The assigned uncertainty consists of uncertainties relating to characterisation (u_{char}), potential between-unit inhomogeneity (u_{bb}), and potential degradation during transport (u_{sts}), and storage (u_{its}). These different contributions were combined to estimate the relative expanded uncertainty of the certified value ($U_{\text{CRM, rel}}$) with a coverage factor k given as:

$$U_{\text{CRM, rel}} = k \cdot \sqrt{u_{\text{bb, rel}}^2 + u_{\text{sts, rel}}^2 + u_{\text{its, rel}}^2 + u_{\text{char, rel}}^2} \quad \text{Equation 9}$$

- u_{char} was estimated as described in Section 6.5
- u_{bb} was estimated as described in Section 4.1
- u_{sts} and u_{its} were estimated as described in Section 5

The mass concentration is in principle dependent on the temperature because of the thermal expansion of water. However, in the range of from 4 °C to 25 °C, the change of volume compared to 20 °C caused by thermal expansion amounts to 0.17 % and 0.12 %, respectively, which is negligible compared to the other uncertainty contributions.

The number of effective degrees of freedom were calculated using the Welch-Satterthwaite equation [23] and are shown in Table 25 to Table 27. The number of degrees of freedom are sufficient to justify a coverage factor (k) of $k=2$ for all elements.

Table 24. Certified values and their uncertainties for ERM-DA634.

CRM	Element	Certified value [µg/L]	$U_{\text{char, rel}}$ [%]	$U_{\text{bb, rel}}$ [%]	$U_{\text{sts, rel}}$ [%]	$U_{\text{its, rel}}$ [%]	ν_{UCRM}	$U_{\text{CRM, rel}}$ [%]	$U_{\text{CRM}}^{1)}$ [µg/L]
ERM-DA634	Cd	1.29	2.41	1.95	0.48	0.12	18	6.3	0.09
	Cr	1.5	8.53	3.62	1.39	8.00	18	24.6	0.4
	Hg	1.6	6.42	2.91	0.60	2.73	11	15.2	0.3
	Pb	18.6	2.53	3.67	0.67	0.10	34	9.0	1.7
	Tl	0.81	3.13	3.48	0.57	1.51	20	9.9	0.08

1) Expanded ($k = 2$) and rounded uncertainty; uncertainties are always rounded up [28] and in a way that the rounding error corresponds to 3 % to 30 % of the uncertainty.

Source: JRC

Table 25. Certified values and their uncertainties for ERM-DA635.

CRM	Element	Certified value [µg/L]	$U_{\text{char, rel}}$ [%]	$U_{\text{bb, rel}}$ [%]	$U_{\text{sts, rel}}$ [%]	$U_{\text{its, rel}}$ [%]	ν_{UCRM}	$U_{\text{CRM, rel}}$ [%]	$U_{\text{CRM}}^{1)}$ [µg/L]
ERM-DA635	Cd	5.7	2.36	1.30	0.32	0.12	11	5.5	0.4
	Cr	22.0	2.46	1.18	0.54	4.55	30	10.7	2.4
	Hg	25.5	4.90	1.20	0.38	2.49	10	11.3	2.9
	Ni	18.3	1.12	2.05	0.70	3.53	44	8.6	1.6
	Pb	182	2.32	1.54	0.33	0.10	12	5.6	11
	Tl	8.3	2.95	1.15	0.54	2.08	13	7.7	0.7

1) Expanded ($k = 2$) and rounded uncertainty; uncertainties are always rounded up [28] and in a way that the rounding error corresponds to 3 % to 30 % of the uncertainty.

Source: JRC

Table 26. Certified values and their uncertainties for ERM-DA636.

CRM	Element	Certified value [µg/L]	$U_{\text{char, rel}}$ [%]	$U_{\text{bb, rel}}$ [%]	$U_{\text{sts, rel}}$ [%]	$U_{\text{its, rel}}$ [%]	ν_{UCRM}	$U_{\text{CRM, rel}}$ [%]	$U_{\text{CRM}}^{1)}$ [µg/L]
ERM-DA636	Cd	10.9	2.11	1.17	0.54	0.12	12	5.0	0.6
	Cr	42	2.91	2.38	0.43	2.90	26	9.5	4
	Hg	56	4.02	1.51	0.42	2.02	11	9.5	6
	Ni	36	3.26	2.65	1.1	4.06	30	11.9	5
	Pb	$0.44 \cdot 10^3$	2.99	2.31	0.33	0.10	15	7.6	$0.04 \cdot 10^3$
	Tl	16.6	3.54	1.47	0.51	1.70	9	8.4	1.4

1) Expanded ($k = 2$) and rounded uncertainty; uncertainties are always rounded up [28] and in a way that the rounding error corresponds to 3 % to 30 % of the uncertainty.

Source: JRC

7.2 Additional material information

The data provided in this section should be regarded as informative only on the general composition of the material and cannot, in any case, be used as certified or indicative value.

The range of the Ni mass concentrations in ERM-DA634 is given as information value only because of the disagreement of results in the characterisation study.

Table 27. Additional material information for ERM-DA634.

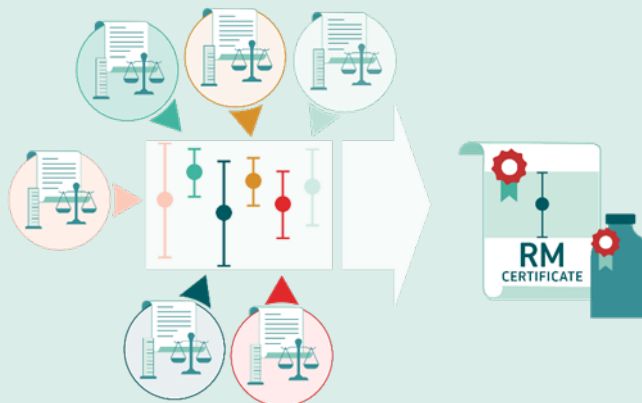
CRM	Element	Additional material information [µg/L]
ERM-DA634	Ni	2.0 - 4.2

Source: JRC

8 Metrological traceability and commutability

8.1 Metrological traceability

Box 8. Metrological traceability



Metrological traceability of measurement results is a key requirement for ensuring the comparability of data. As CRMs are used to make measurement results traceable, metrological traceability of its certified values to a stated reference is essential.

The certified value of a CRM is metrologically traceable if the measurements used for establishing it can be related to a reference through an unbroken chain of calibrations.

This requires that these measurements

- refer to the same property (e.g. Pb) and the same (kind of) quantity (e.g. Pb content),
- result in a number and its uncertainty (e.g. 6 ± 2) expressed in the same measurement unit (e.g. $\mu\text{g/kg}$).

The concept of traceability rests on several anchor points, namely identity, quantity value and measurement unit. The identity of a measurand can be defined by its structure alone or can be operationally defined, the quantity value of the measurand can refer to the SI or to other appropriate references.

8.1.1 Identity

Cd, Cr, Hg and Pb are chemically clearly defined analytes. The mass concentration data were obtained by applying different measurement principles for the sample preparation as well as for the final determination, demonstrating absence of measurement bias. The measurands are therefore structurally defined and independent of the measurement methods.

Also Ni and Tl are clearly defined analytes. All determinations are based on ICP-MS techniques. The MS techniques vary widely, from sector field-, single- and triple quadrupole- MS, which enhances the confidence in the absence of method bias. However, as all mass concentration data were obtained by ICP-MS, the identity for Ni and Tl is therefore operationally defined by ICP-MS.

8.1.2 Quantity value

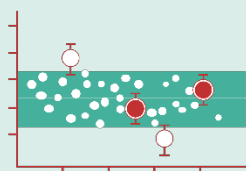
Only validated methods were used during the characterisation study. Different calibrants of known concentration and specified traceability of their assigned values were used. Investigation of the method and measurement details of the individual results show that all relevant input parameters of each technically accepted dataset have been properly calibrated. All technically accepted datasets are therefore traceable to the same reference, namely the SI. This traceability to the same reference is also confirmed by the agreement of results within their respective uncertainties. As the assigned values are combinations of agreeing results individually traceable to the International System of Units (SI), the assigned quantity values themselves are traceable to the SI as well.

8.2 Commutability

Box 9. Commutability

Commutability is a prerequisite for RMs intended to be used for calibration or quality control of different measurement procedures targeting the same measurand. The concept of commutability of an RM is defined by the VIM [29] as:

“property of a reference material, demonstrated by the closeness of agreement between the relation among the measurement results for a stated quantity in this material, obtained according to two given measurement procedures, and the relation obtained among the measurement results for other specified materials”



Commutability is a property of an RM indicating how well an RM mimics the characteristics of a typical routine sample in various measurement procedures for a stated measurand.

The same RM may be commutable for some measurement procedures but non-commutable for others. A commutability statement is therefore only valid for the mentioned measurement procedure(s).

Sample preparation in routine laboratories consists often only of a dilution step, compared to the complete digestion applied in the characterisation study of these materials. In addition, routine samples consist of whole blood whereas these CRMs are lysed, which can also lead to a lack of commutability.

The commutability study was carried out according to the guidelines of the IFCC working group on Commutability [30] using the ‘difference in bias analysis’. In this commutability study the CRMs were compared to 20 fresh, single donation patient samples of persons with a higher risk profile for elevated metal concentrations in blood. In addition, 22 spiked, fresh blood samples were included in the study to ensure that the whole concentration range was covered. The following methods were tested: ICP-MS after dilution (all elements), ET-AAS after dilution (Cd and Pb only) and ICP-MS after digestion (all elements). Commutability of the CRM was assessed for each combination of digestion-ICP-MS with one routine method (dilution ICP-MS or ET-AAS). The commutability criteria were set on the basis of the performance criteria of external quality assessment schemes and ranged from 3.7 % to 17.9 %. The results are shown in Annex 7.

Due to the narrow criteria, the results were mostly inconclusive, as shown in Table 28.

Table 28. Result of the commutability study.

Material	Method	Cd	Cr	Hg	Ni	Pb	Tl
ERM-DA634	Dilution-ICP-MS	I	NC	C	NC	C	I
	Dilution-ET-AAS	I	n.a.	n.a.	n.a.	I	n.a.
ERM-DA635	Dilution-ICP-MS	I	I	I	I	I	I
	Dilution-ET-AAS	I	n.a.	n.a.	n.a.	NC	n.a.
ERM-DA636	Dilution-ICP-MS	I	I	I	I	I	I
	Dilution-ET-AAS	I	n.a.	n.a.	n.a.	I	n.a.

C: Commutable. I: Inconclusive; NC: Not commutable; n.a. not applicable, as it was not measured)

Source: JRC

Although the results are mainly inconclusive, the graphs in Annex 5 show that normalising the results to the result of the CRMs would in all cases have reduced the bias for routine sample between the methods. The commutability study therefore shows that these three CRMs are an effective way to reduce the bias between laboratories. A detailed description of the evaluation is given in [31].

9 Instructions for use

9.1 Safety information

Each individual blood donation has been tested for HIV-1/2 antibodies, HCV antibodies and HBs antigen with methods cleared in compliance with the European Directive 98/79/EC, Annex II, List A and found negative or non-reactive. Nevertheless, biological specimens should be treated as potentially infectious. Therefore, standard precautions for a safe handling and disposal are advised.

9.2 Storage conditions

The materials should be stored at $(-20 \pm 5) ^\circ\text{C}$ in the dark.

Reconstituted samples stored at $4 ^\circ\text{C}$ were found to be stable for 7 days, i.e. certified values are valid over this period.

For more information regarding the shelf life of reference materials please consult ERM Application Note 7 [32].

The European Commission cannot be held responsible for changes that happen to samples after reconstitution when the material is stored differently from the stated storage conditions at the customer's premises.

9.3 Use and reconstitution of the material

To make it ready for use, the material has to be reconstituted according to the following procedure:

- Allow the unit to reach ambient temperature before opening.
- Tap the bottom of the unit to loosen any blood material adhering to the stopper and carefully remove the rubber stopper.
- Gently add 1.00 mL water ($20 \pm 4) ^\circ\text{C}$ and swirl by hand or vortex for 2 minutes

Samples need to be reconstituted on the day before and should be re-homogenised on the day of the measurement. Subsampling of dry material of one vial is not acceptable.

Dispose in accordance with good laboratory practice.

For general information on handling of reference materials, please consult ERM Application Note 6 [33].

9.4 Minimum sample size

The minimum sample size representative for Hg is 150 μL and 200 μL of the reconstituted material for all other elements.

9.5 Use of the certified values

The intended use of these materials is to assess method performance, i.e. for checking accuracy of measurement results/calibration. It can also be used for the calibration of methods, thereby making measurements results metrologically traceable.

The material is intended for in vitro analysis only.

Use as a calibrant

If the materials are used as calibrants, the uncertainty of the certified value shall be taken into account in the estimation of the measurement uncertainty.

Comparing a measurement result with the certified value

A result is unbiased if the combined standard uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1 [34]).

When assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is summarised here:

- Calculate the absolute difference between mean measured value and the certified value (Δ_{meas}).
- Combine the measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}): $u_{\Delta} = \sqrt{u_{\text{meas}}^2 + u_{\text{CRM}}^2}$
- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty (u_{Δ}) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %.
- If $\Delta_{\text{meas}} \leq U_{\Delta}$ then no significant difference exists between the measurement result and the certified value, at a confidence level of approximately 95 %.

Use in quality control charts

The materials can be used for quality control charts. Using CRMs for quality control charts has the added value that a trueness assessment is built into the chart.

10 Conclusions

ERM-DA634, ERM-DA635 and ERM-DA636 are human blood reference materials certified for their mass concentrations of Cd, Cr, Hg, Ni, Pb and Tl. These materials were produced and certified in accordance with ISO 17034:2016 [1] and ISO 33405:2024 [2]. They were produced within the scope of ISO 17034 accreditation.

The material helps implementing of biomonitoring schemes for the exposure of humans to metals and ensures that data collected as mandated by Council Directive 98/24/EC are reliable.

The following values were assigned:

Table 29. Certified values assigned to ERM-DA634, ERM-DA635 and ERM-DA636.

Mass concentration in the reconstituted material				
Material	Element	Certified value ³⁾	Uncertainty ⁴⁾	Unit
ERM-DA634	Cd ¹⁾	1.29	0.09	µg/L
	Cr ¹⁾	1.5	0.4	µg/L
	Hg ¹⁾	1.6	0.3	µg/L
	Pb ¹⁾	18.6	1.7	µg/L
	Tl ²⁾	0.81	0.08	µg/L
ERM-DA635	Cd ¹⁾	5.7	0.4	µg/L
	Cr ¹⁾	22.0	2.4	µg/L
	Hg ¹⁾	25.5	2.9	µg/L
	Ni ²⁾	18.3	1.6	µg/L
	Pb ¹⁾	182	11	µg/L
	Tl ²⁾	8.3	0.7	µg/L
ERM-DA636	Cd ¹⁾	10.9	0.6	µg/L
	Cr ¹⁾	42	4	µg/L
	Hg ¹⁾	56	6	µg/L
	Ni ²⁾	36	5	µg/L
	Pb ¹⁾	0.44	0.04	mg/L
	Tl ²⁾	16.6	1.4	µg/L
<p>1) Independent of the measurement method</p> <p>2) As obtained by ICP-MS</p> <p>3) Certified values are values that fulfil the highest standards of accuracy. The given values represent the unweighted mean values of the means of accepted sets of data, each set being obtained in a different laboratory and/or with a different method of determination. The certified value and its uncertainty are traceable to the International System of Units (SI). The values are valid in the temperature range from 4 ° to 25 °C.</p> <p>4) The uncertainty of the certified value is the expanded uncertainty with a coverage factor $k = 2$ corresponding to a level of confidence of 95 %, estimated in accordance with ISO 17034:2016 and ISO 33405:2024</p>				

Source: JRC

The materials are intended for the assessment of method performance calibration of methods and quality control.

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List of abbreviations

AAS (DMA)	Atomic absorption spectrometry (direct mercury analyser)
ANOVA	Analysis of variance
b	Slope in the equation of linear regression $y = a + bx$
BCR [®]	Trademark owned by the European Commission; used by the JRC for reference materials
c	Mass concentration $c = m/V$ (mass/volume)
CI	Confidence interval
CRM	Certified reference material
CV-AAS	Cold vapour- atomic absorption spectrometry
EC	European Commission
EDTA	Ethylenediaminetetraacetic acid
ERM [®]	Trademark owned by the European Commission; used by the JRC for reference materials
EU	European Union
ET-AAS	Electrothermal atomic absorption spectrometry
GUM	Guide to the Expression of Uncertainty in Measurement
ICP	Inductively coupled plasma
ICP-IDCQMS	ICP isotope dilution collision cell quadrupole mass spectrometry
ICP-QMS	ICP-quadrupole mass spectrometry
ICP-Q3MS	ICP-triple quadrupole mass spectrometry
ICP-SFMS	ICP-sector field mass spectrometry
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
ISO	International Organization for Standardization
JRC	Joint Research Centre of the European Commission
k	Coverage factor
LOD	Limit of detection
LOQ	Limit of quantification
MS_{between}	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicate analysis per unit
N	Number of units analysed
n.a.	Not applicable
n.c.	Not calculated
p	Number of technically valid datasets
QC	Quality control
rel	Index denoting relative figures (uncertainties etc.)
RM	Reference material
RMP	Reference material producer

s	Standard deviation
s_{bb}	Between-unit standard deviation; an additional index "rel" is added when appropriate; this parameter is linked to the homogeneity of the material
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an additional index "rel" is added as appropriate
SI	International System of Units
s_{meas}	Standard deviation of measurement data; an additional index "rel" is added as appropriate
s_{wb}	Within-unit standard deviation; this parameter is linked to the homogeneity of the material
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
T	Temperature
t	Time
t_i	Time point for each replicate
$t_{\alpha, df}$	Critical t -value for a t -test, with a level of confidence of $1-\alpha$ and df degrees of freedom
t_{sl}	Proposed shelf life
t_{tt}	Proposed transport time
u	Standard uncertainty
U	Expanded uncertainty
u_{bb}^*	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability/intermediate precision; an additional index "rel" is added as appropriate
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; an additional index "rel" is added as appropriate
u_c	Combined standard uncertainty; an additional index "rel" is added as appropriate
u_{char}	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate
U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate
u_{Δ}	Combined standard uncertainty of measurement result and certified value
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
u_{meas}	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_{rec}	Standard uncertainty related to possible between-unit inhomogeneity modelled as rectangular distribution; an additional index "rel" is added as appropriate
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel" is added as appropriate
VIM	International Vocabulary of Metrology – Basic and General Concepts and Associated Terms

\bar{x}	Arithmetic mean
\bar{x}_{\max}	Highest unit mean of the homogeneity study
\bar{x}_{\min}	Lowest unit mean of the homogeneity study
Δ_{meas}	Absolute difference between mean measured value and the certified value
$\nu_{MS_{\text{within}}}$	Degrees of freedom of MS_{within}
ν_{UCRM}	Degrees of freedom of U_{CRM}
\bar{y}	Mean of all results of the homogeneity study

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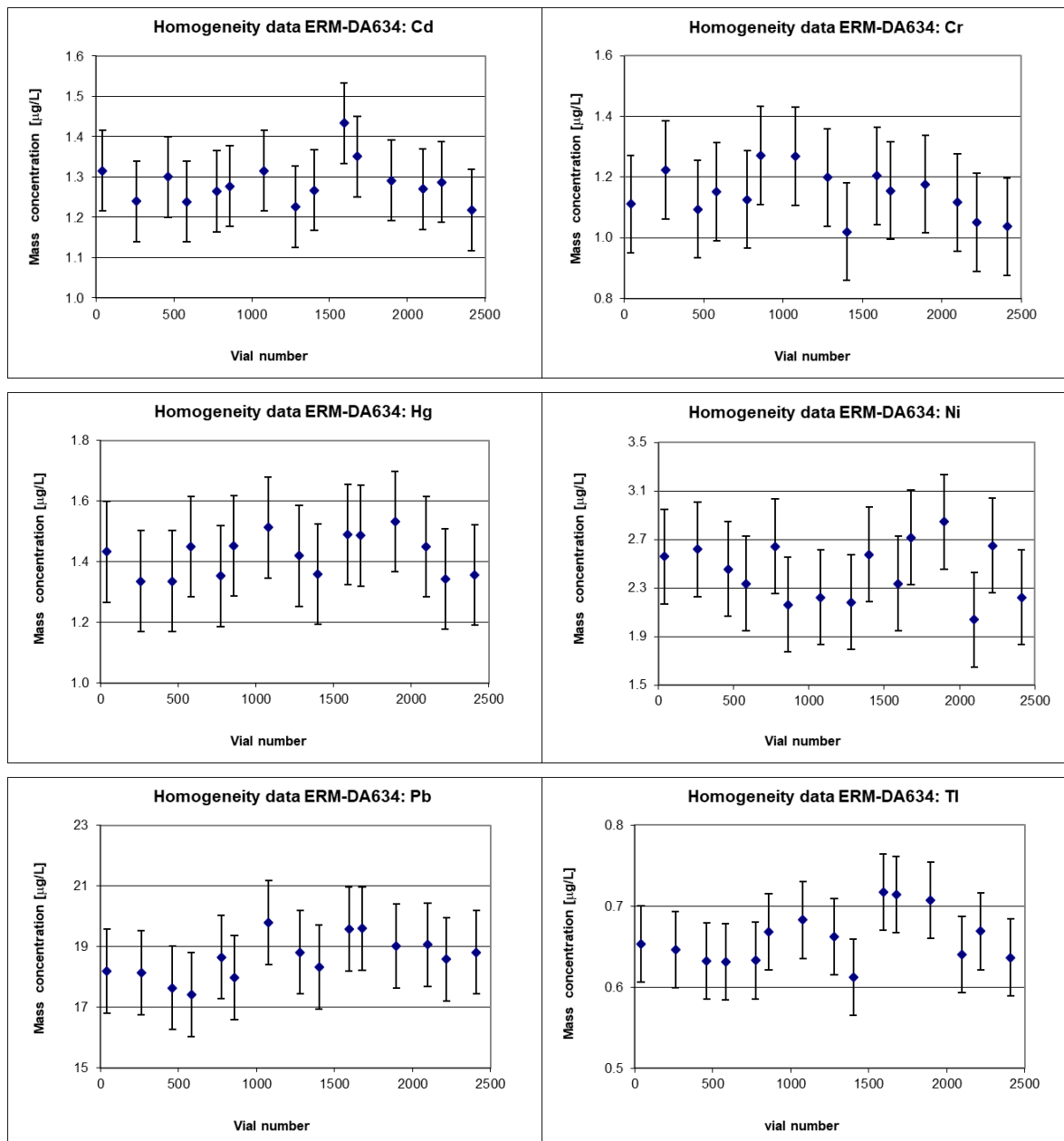
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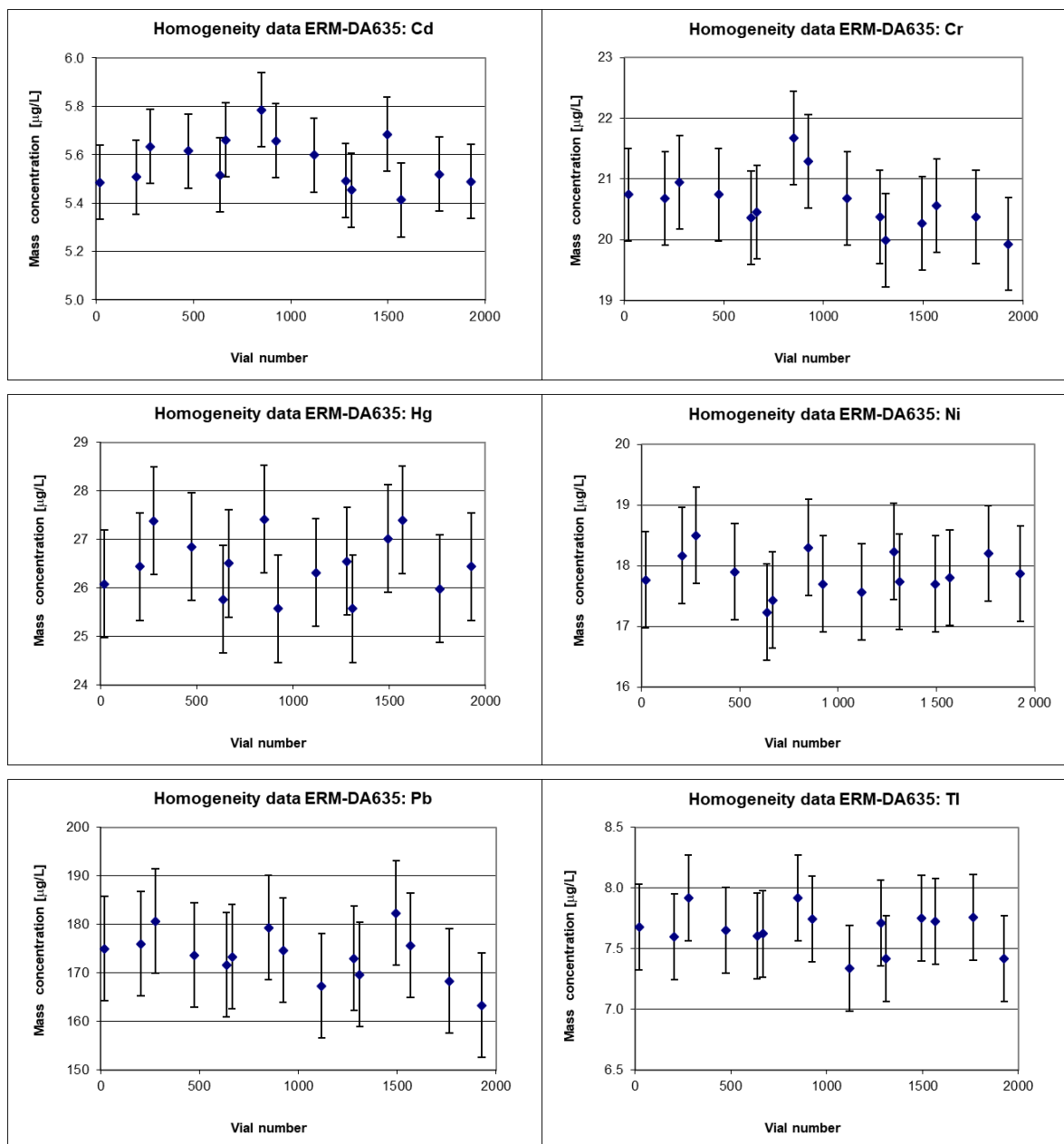
Annexes

Annex 1. Results of the homogeneity measurements

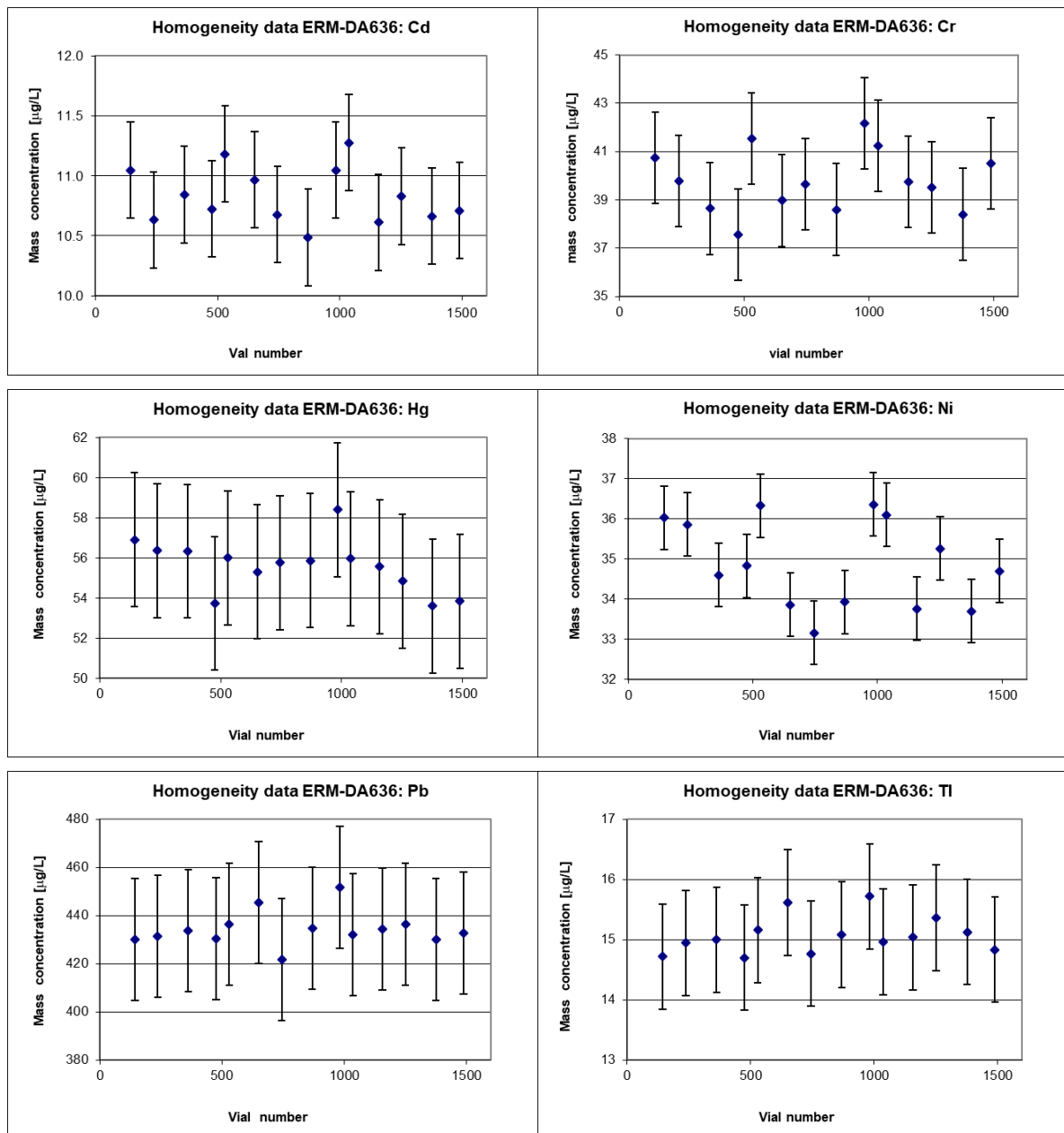
Shown are the averages per unit. The error bars correspond to the 95 % confidence interval of the average per unit and are based on the within-unit standard deviation as calculated by one-way ANOVA



Source: JRC



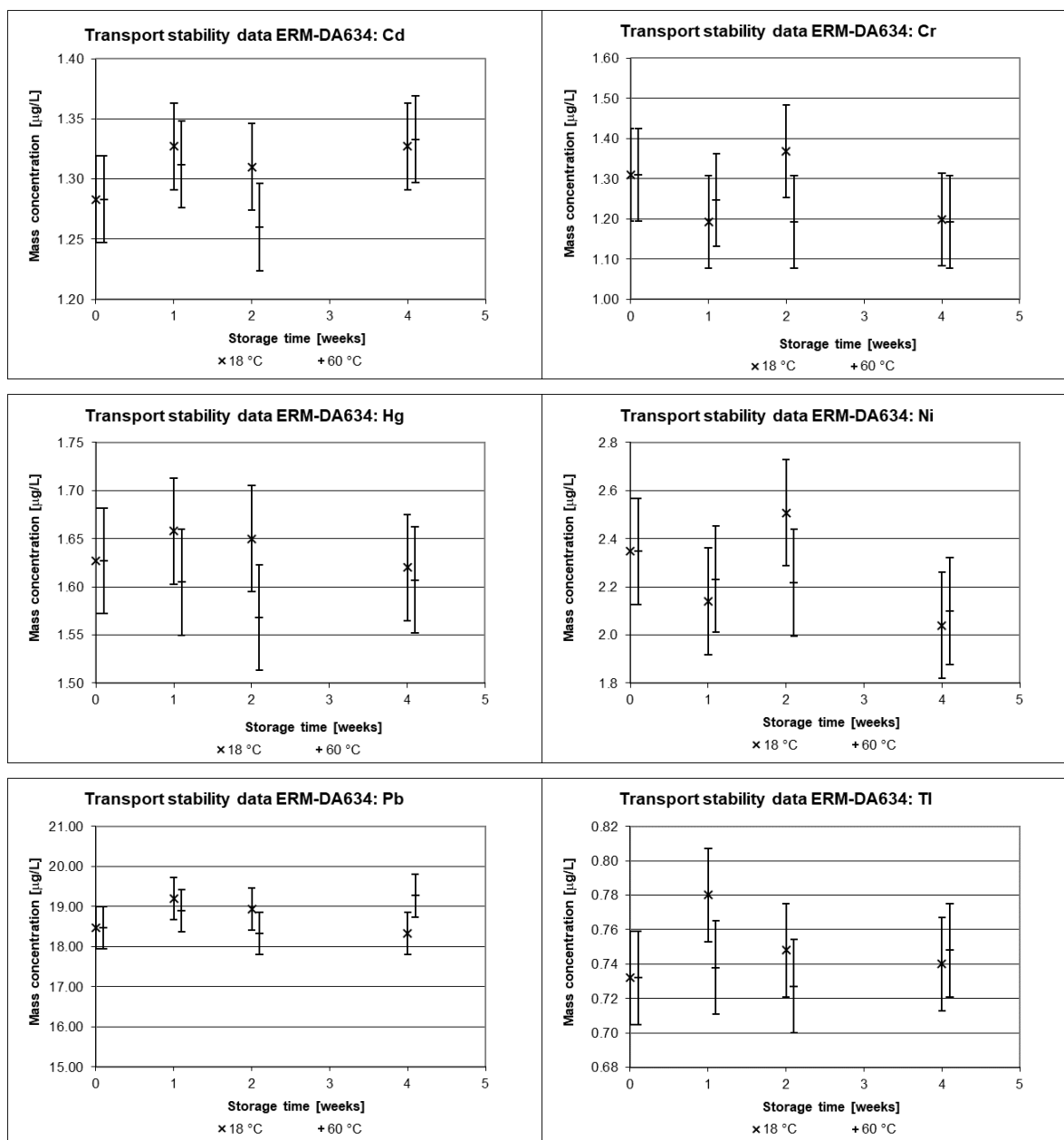
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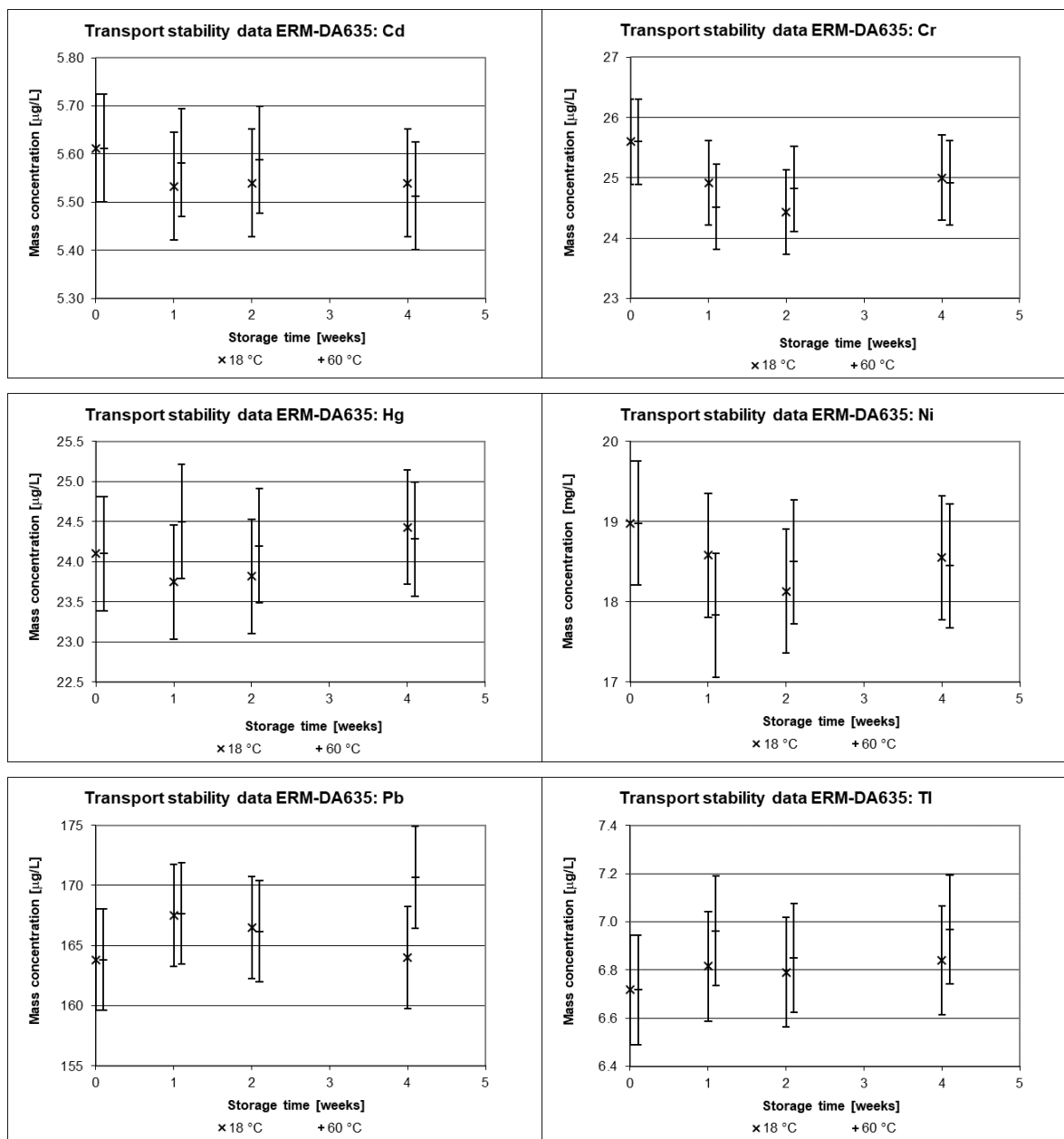
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Annex 2. Results of the transport stability measurements

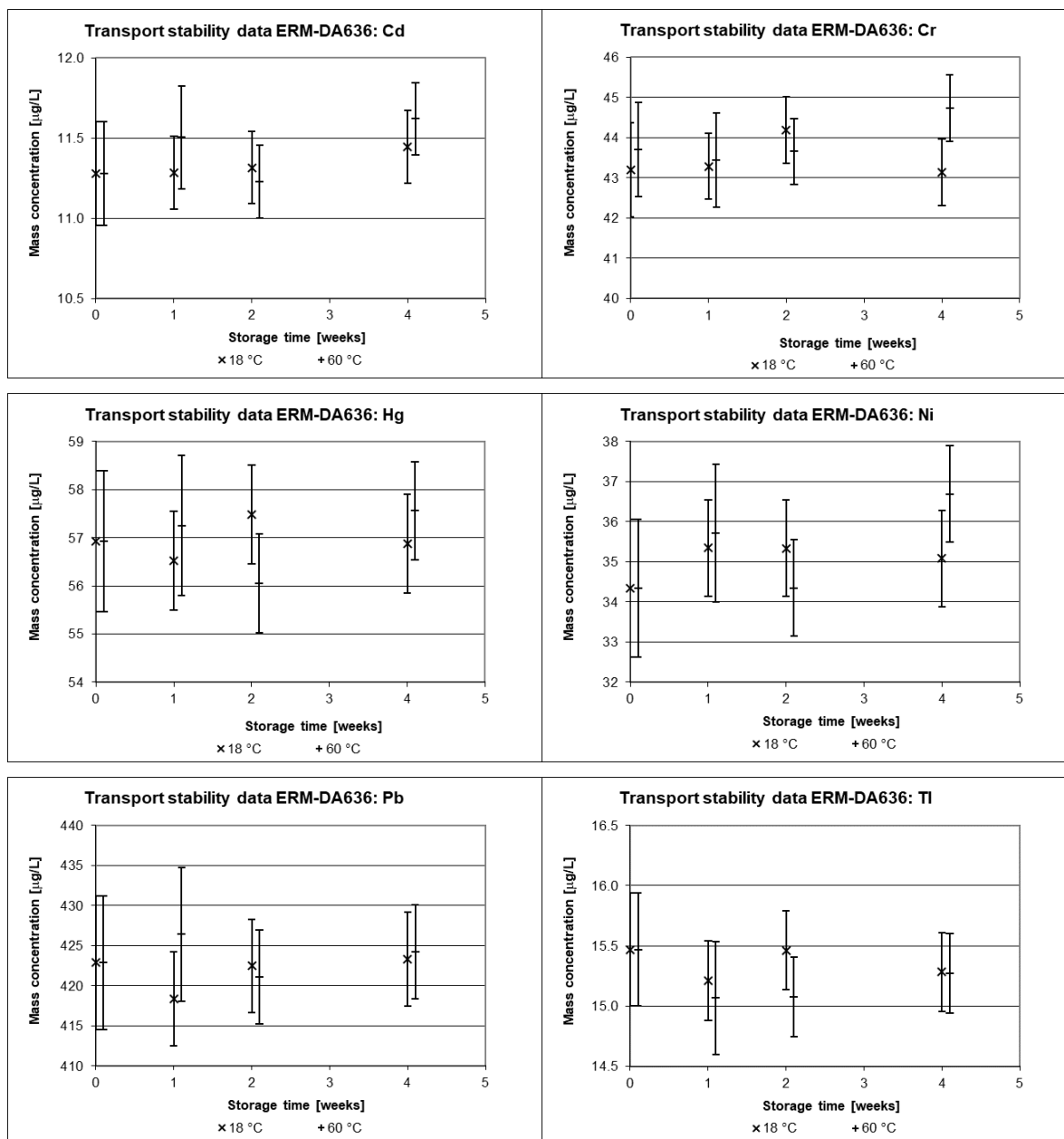
Shown are the averages per time point. Error bars correspond to the confidence interval of the means per time point, calculated using the standard deviation of all measurements. The data for 18 °C and 60 °C were separated graphically.



Source: JRC



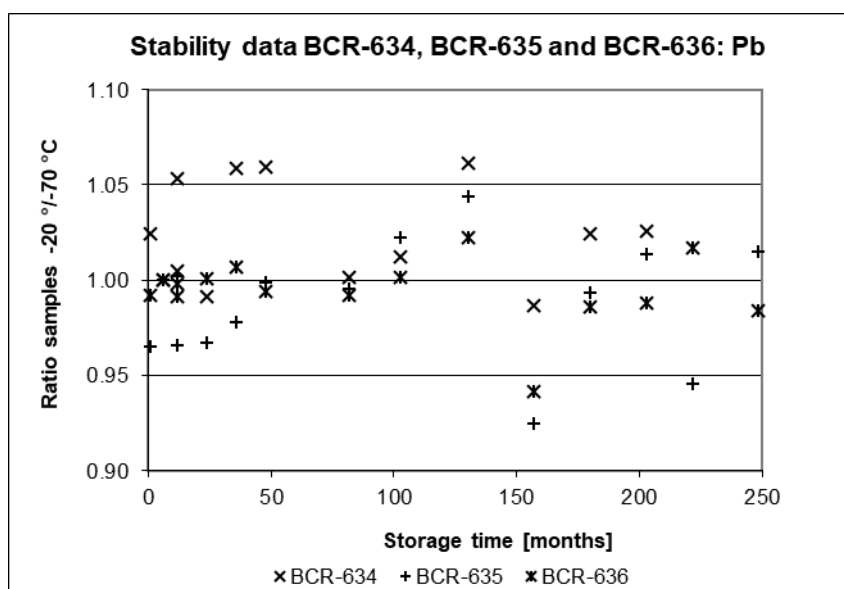
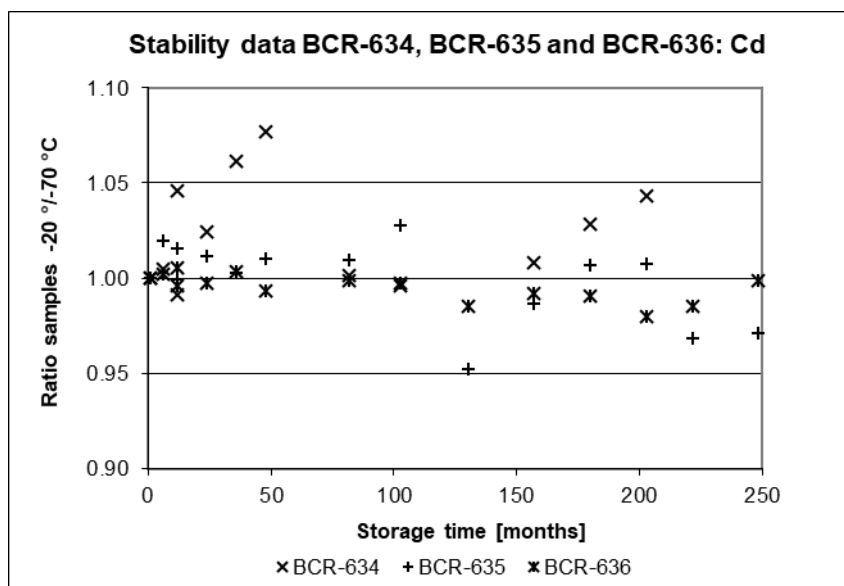
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Source: JRC

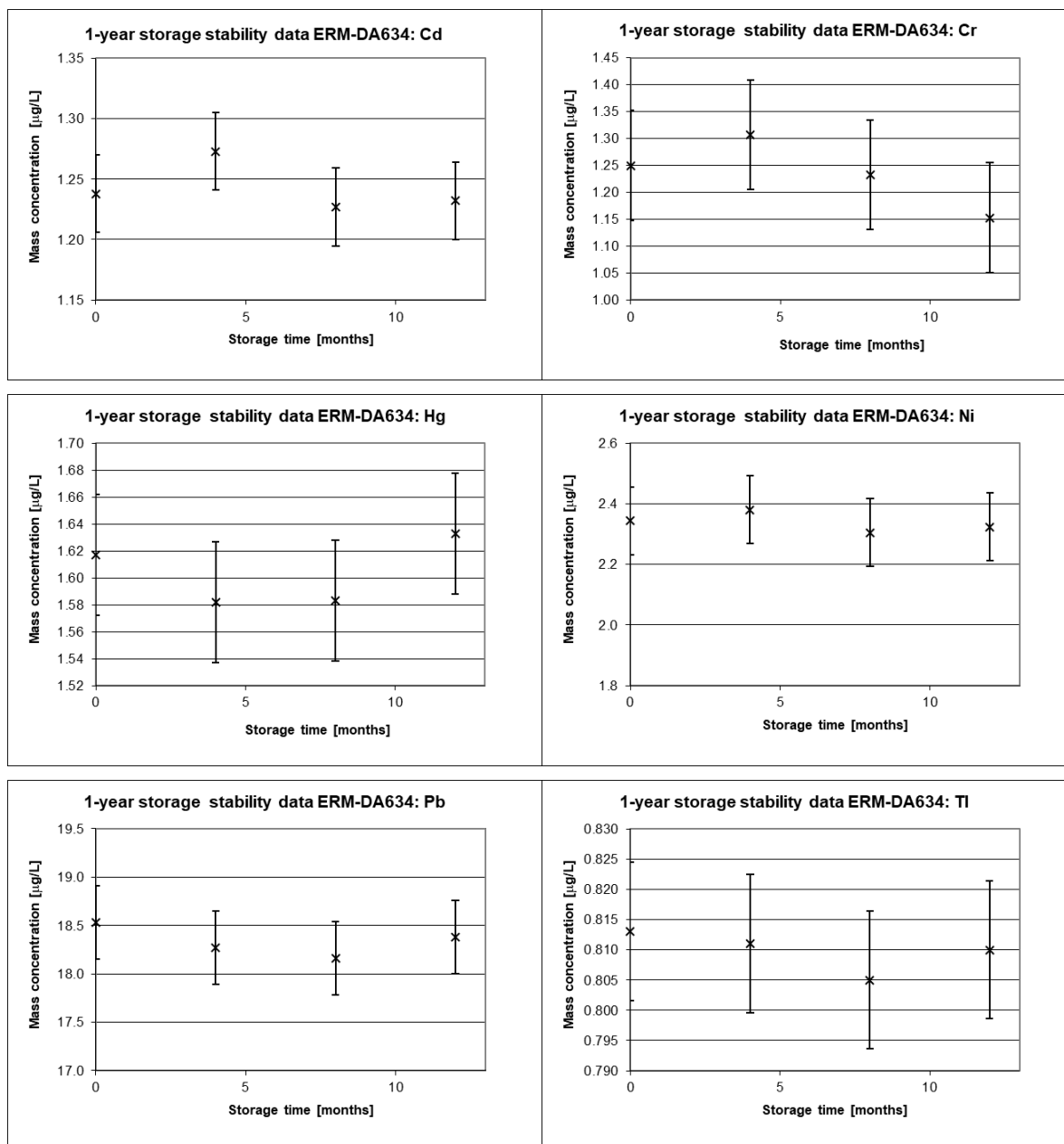
Annex 3. Results of the storage stability measurements

Data of the stability monitoring of BCR-634, BCR-635 and BCR-636. Shown are the ratios of results from units stored at -20 °C to samples stored at -70 °C. Each data point is usually an average of six results.

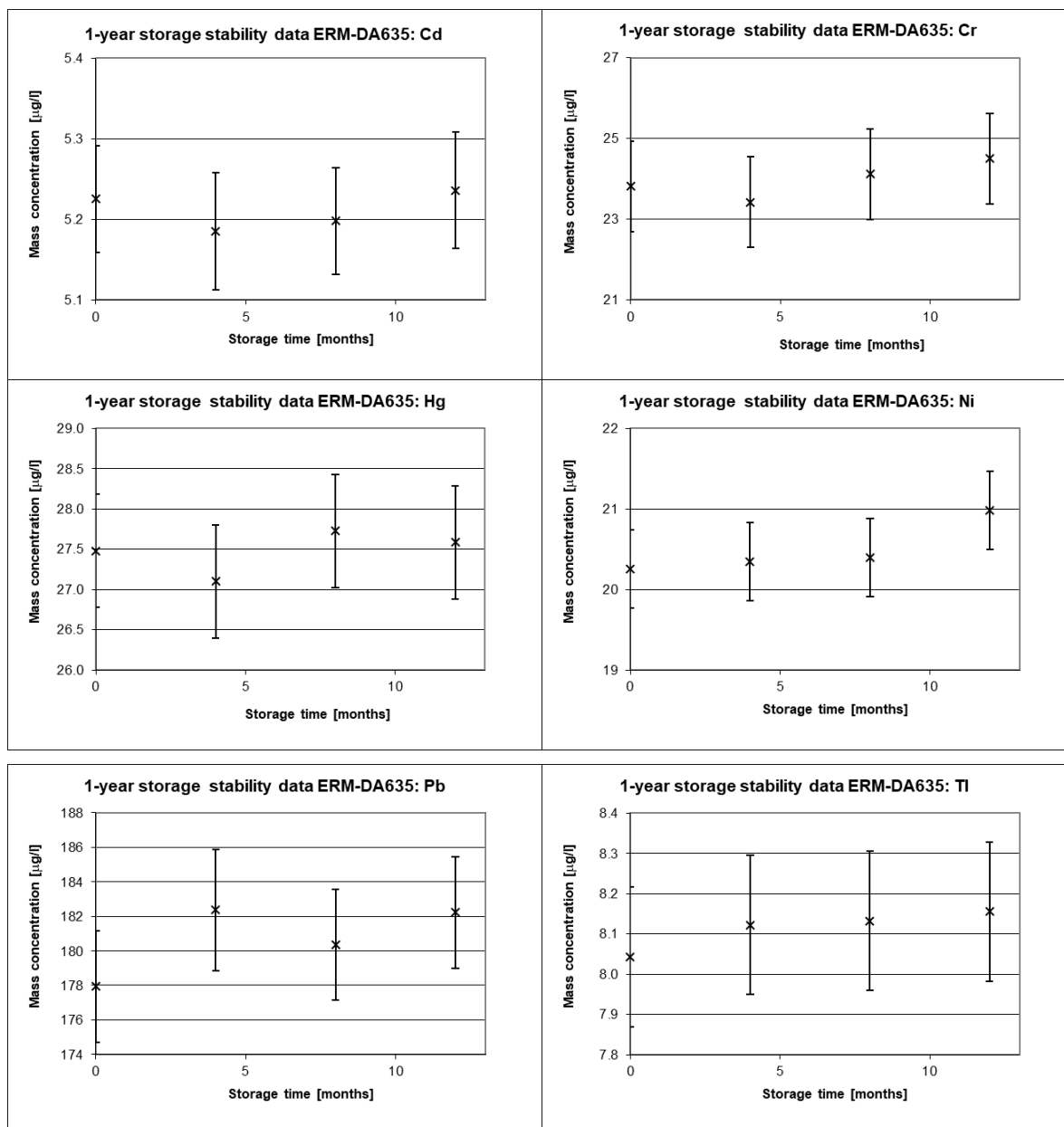


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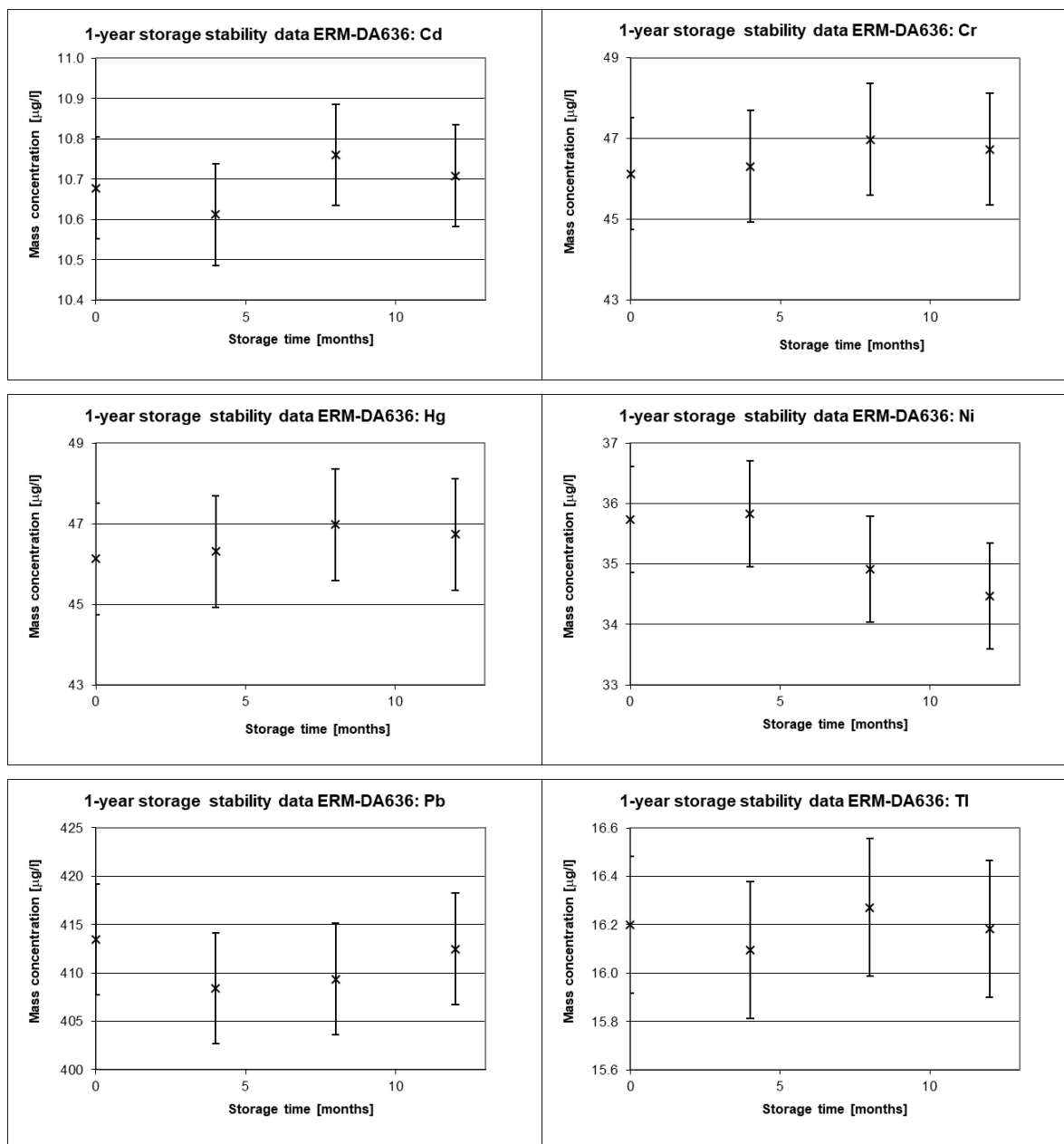
Data of the 1-year study of ERM-DA634, ERM-DA635 and ERM-DA636. Shown are the averages per time point. Error bars correspond to the confidence interval of the means per time point, calculated using the standard deviation of all measurements.



Source: JRC



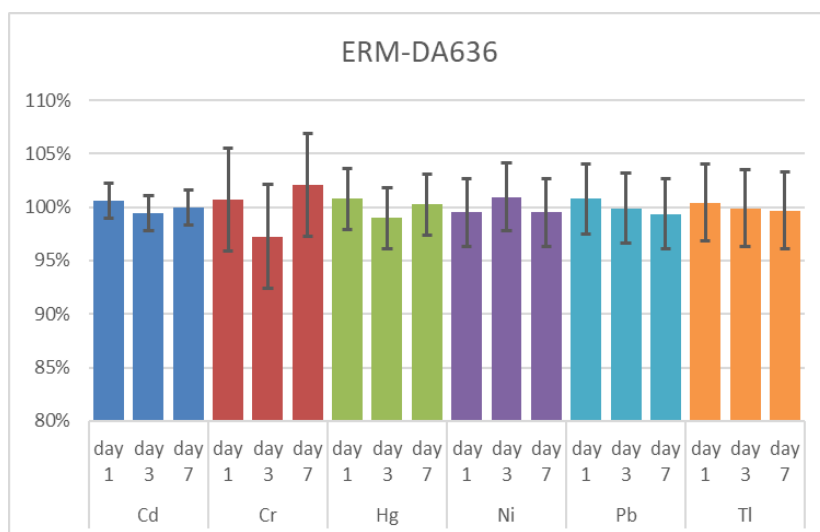
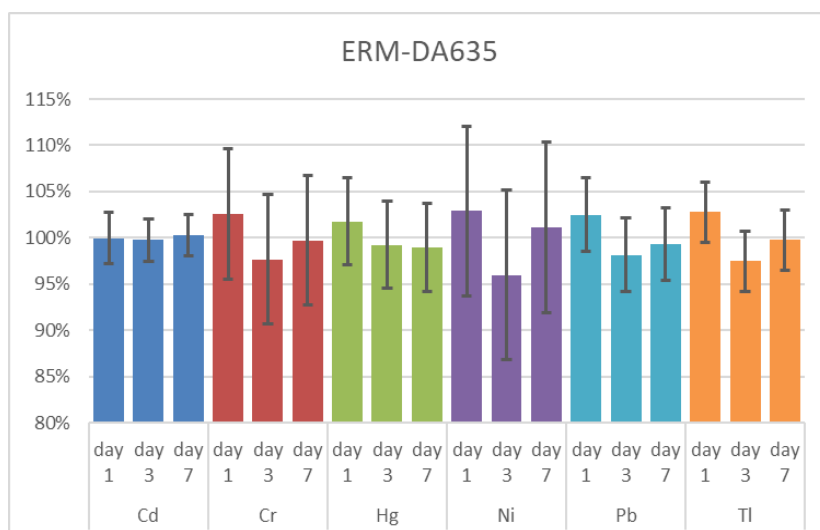
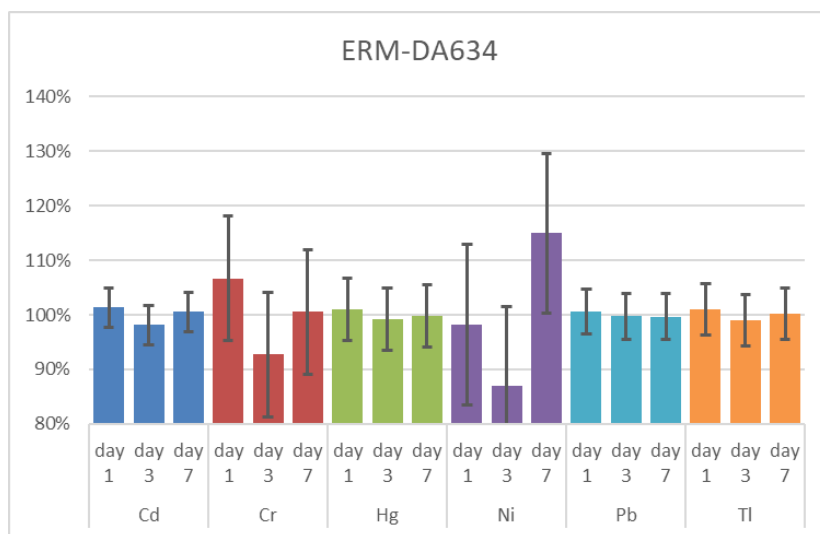
Source: JRC



Source: JRC

Annex 4: Results of the measurements of the repeated use study.

Shown are the averages of the three results per day as percentage of the average over all days. The error bars correspond to the 95 % confidence interval using the within-group standard deviation as obtained by one-way ANOVA.



Source: JRC

Annex 5. Summary of methods used in the characterisation study

Method information is reported as given by the laboratories

Dataset	Sample preparation	Elements	Quantification	Calibration
D01	Sample intake 500 mg Closed vessel, microwave-assisted digestion with HNO ₃	Cd, Cr, Hg, Ni, Pb, Tl	Sector field ICP-MS, ThermoScientific ELEMENT XR; resolution >300 (Cd, Hg, Pb, Tl) and >4000 (Cr, Ni) Masses used: 111, 114 (Cd), 52, 53 (Cr), 199, 200, 201 (Hg), 60, 62 (Ni), 206, 207, 208 (Pb), 203, 205 (Tl)	External calibration Single element standard solutions from Agilent; traceable to the SI via NIST CRMs
D02	Sample intake 300 mg Microwave pressure digestion: 0.3 g sample + 0.5 mL 65 % HNO ₃ ; keep for 20 min at 240 °C; dilution to 5 mL	Cd, Cr, Hg, Ni, Pb, Tl	ICP-triple quadrupole MS Agilent 8800; resolution approx. 0.7 amu for both quadrupoles Masses measured: 114 (Cd), 53 (Cr), 202 (Hg), 60 (Ni), 208 (Pb), 205 (Tl)	External calibration Hg: NIST SRM 3133 Multi-element standard solution Supelco TraceCert Mix 1; traceable to the SI via NIST CRMs
D03	Sample intake: 2.5 mL Pooling of 3 reconstituted samples. Microwave pressure digestion according to EN 13805:2014 with 2.5 mL sample + 2 mL 67-69 % HNO ₃ + 2 mL 30 % H ₂ O ₂	Cd, Cr, Ni, Pb, Tl Hg	ICP-single quadrupole MS Perkin Elmer NexION 350D; resolution 0.7 amu ; Masses measured: 114 (Cd), 52 (Cr), 60 (Ni), 208+206+207(Pb), 205 (Tl) AAS (DMA) Analytik Jena Mercur Duo Plus; wavelength 253.7 nm	External calibration with multi-element standard solution made from ThermoScientific Single element solutions; each traceable to the SI via NIST CRMs

D04	<p>Cd, Pb: Sample intake 800 mg</p> <p>Digestion in a closed microwave oven (Multiwave5000, ANTON PAAR) with 4 mL HNO₃ (69 %, V/) (25 min ramp time to 190 °C, 20 min at 190 °C).</p> <p>Hg: Sample intake 150 µL.</p> <p>Aliquots introduced directly into an AMA254 mercury analyser: combustion at 750°C under oxygen, followed by atomic absorption analysis (DMA) after amalgamation on a gold amalgam.</p>	<p>Cd, Pb</p> <p>Hg</p>	<p>ICP-QMS with collision cell (Thermo Fisher iCAP Q ICP-MS) in kinetic energy discrimination mode at a He flow of 5 mL/min</p> <p>Masses measured: 110, 111 (Cd), 206, 208 (Pb) in low resolution mode</p> <p>AAS (DMA) Altac AMA 254, wavelength 253.7 nm</p>	<p>Double isotope-dilution mass spectrometry. Two CRMs are used to determine the spike concentrations: Supelco Tracecert CRMs (traceable to the SI via NIST CRM) and digestion high purity metal (99.999 % and 99.9999 % purity, respectively).</p> <p>3 standard additions per sample corresponding to 0.5, 1 and 2 times the measured concentration.</p> <p>Calibration with standard from SCP Science, directly traceable to NIST SRM 3133</p>
D05	<p>Samples diluted 1:50 with a solution containing 0.7 mmol/l NH₄OH, 0.01 mmol/l EDTA, 0.07 % (v/v) Triton X-100 in ultrapure water. Addition of internal standard</p>	<p>Cd, Cr, Hg, Ni, Pb, Tl</p>	<p>ICP- triple quadrupole MS, Agilent 8900MS Agilent 8900</p> <p>Masses measured: 114 (Cd), 52 (Cr), 60 (Ni), 202 (Hg), 205 (Tl), 208 (Pb)</p>	<p>Standard addition with single element standards from Agilent, SCP Science, Inorganic Ventures produced by digestion of pure elements. Values traceable to the SI by the gravimetric preparation confirmed by comparison with NIST CRMs (Cd, Ni, Pb, Tl), direct comparison with NIST CRM (Cr) or a combination of EDTA assay/direct comparison with NIST CRM.</p> <p>Correctness of the calibration confirmed by analysing "SeronormTM Trace Elements Whole Blood" and "ClinChek[®] Whole Blood Controls"</p>

D06	<p>Sample intake 2000 mg (Hg), 250 mg (all others)</p> <p>Cd, Cr, Ni, Pb, Tl: samples diluted 20-fold with a solution containing 2 % v/v 1-butanol, 0.5 g/L EDTA, 0.05 % v/v Triton X-100 and 1 % v/v NH₄OH.</p> <p>Hg: 1 % L-Cysteine, 1 % NaCl, 45 % NaOH and octanol added. Inorganic Hg reduced with SnCl₂; then organic Hg reduced with mixture of SnCl₂/CdCl₂. Total Hg calculated sum of inorganic and organic mercury.</p>	<p>Cd, Cr, Ni, Pb, Tl</p> <p>Hg</p>	<p>ICP- triple quadrupole MS Agilent 8800; resolution approx. 0.7 amu for both quadrupoles Sc, Ge, Y, In and Bi used as internal standards Masses used: 111 (Cd), 52, 53 (Cr), 60 (Ni), 206, 207, 208 (Pb), 203, 205 (Tl)</p> <p>CV-AAS Perkin-Elmer AAnalyst 800; 0.7 nm slit width</p>	<p>External calibration</p> <p>Cd, Cr, Ni, Pb, Tl: multielement or single element (Tl) standards from Romil Ltd or Inorganic Ventures;</p> <p>Traceability for Ni via NIST CRMs SRM 3136, 928</p> <p>Hg: Inorganic Hg from SigmaAldrich; organic Hg from Alfa Aesar; traceable to the SI via NIST CRMs SRM 728</p>
D07	<p>Sample intake 400 µL (Cd, Pb) or 200 µL (Cr)</p> <p>Addition of 700 µL HNO₃ and 1 mL 0.1 % Triton-X-100; centrifugation. The supernatant is measured.</p>	Cd, Cr, Pb	<p>ET-AAS (Perkin Elmer PinAAcle 900T)</p> <p>Wavelengths used: 228.80 nm (Cd), 357.87 nm (Cr), 283.31 (Pb)</p> <p>Slit width 0.7 nm</p>	<p>External calibration with matrix-matched multi-element standard solution from Recipe (lyophilised whole blood). The values are traceable to the SI through a combination of results from at least two different methods derived from laboratories accredited to ISO/IEC 17025 or ISO 15189</p> <p>Checked with control samples from Recipe</p>
D08	<p>Sample intake 150 mg (Hg), 500 mg (all other elements ERM-DA634) 200 mg (all other elements ERM-DA635 and ERM-DA636))</p> <p>Cd, Cr, Ni, Pb, Tl: Digestion with Digestion with HNO₃:H₂O₂:H₂O 2:1:3 at ambient pressure (Digiprep blok digestion)</p> <p>Hg: Direct measurement; sample intake 150 mg</p>	<p>Cd, Cr, Ni, Pb, Tl:</p> <p>Hg</p>	<p>ICP- single quadrupole MS (Thermo iCAP)</p> <p>Masses measured: 114 (Cd), 52 (Cr), 60 (Ni), 206,207, 208 (Pb), 203 (Tl),</p> <p>AAS (DMA) Milestone DMA-80); wavelength 253.7 nm</p>	<p>External calibration</p> <p>Cd, Cr, Ni, Pb, Tl: Multi element standard solution from Astasol Analytica. Traceable to the SI via NIST CRMs, 3108, 3112a, 3136, 928, 3128 and 3158</p> <p>Hg: Mono element standard from CPA Chem: Production from high purity metal and checked using NIST and BAM CRMs.</p>

Source: JRC

Annex 6. Results of the Characterisation study

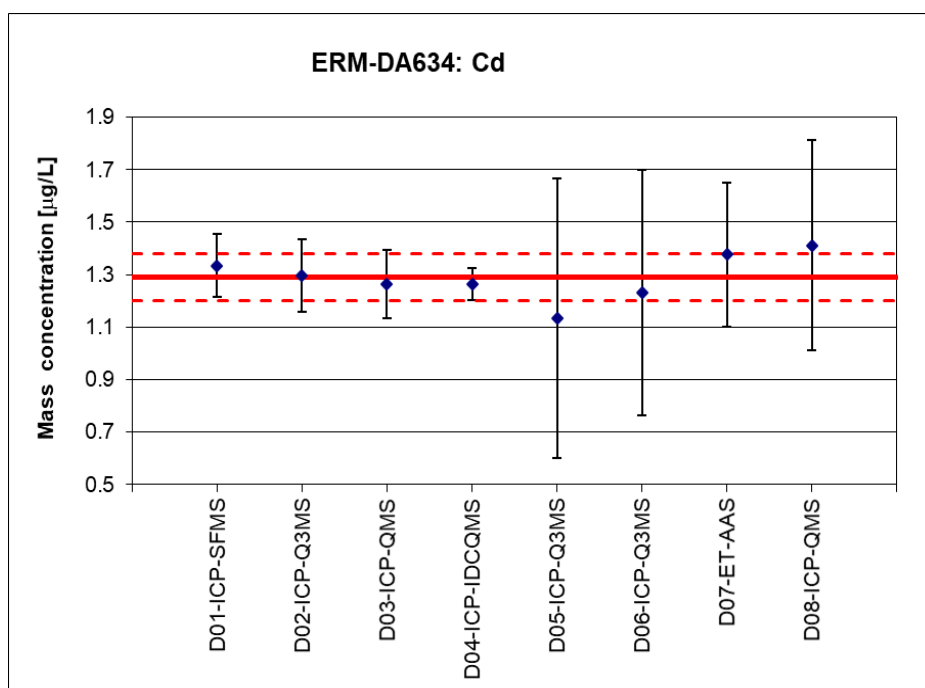
Cadmium

Table 6.1. Data from the characterisation for Cd, ERM-DA634. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	1.4	1.28	1.29	1.38	1.32	1.34	1.34	0.12 µg/L
D02-ICP-Q3MS	1.316	1.345	1.273	1.27	1.261	1.315	1.30	0.14 µg/L
D03-ICP-QMS	1.29	1.28	1.25	1.22	1.29	1.25	1.26	0.13 µg/L
D04-ICP-IDCQMS	1.27	1.28	1.26	1.33	1.23	1.22	1.27	0.06 µg/L
D05-ICP-Q3MS	1	1.1	1.3	1.3	1.1	1	1.13	47 %
D06-ICP-Q3MS	1.07	1.15	1.41	1.44	1.13	1.18	1.23	38 %
D07-ET-AAS	1.42	1.41	1.48	1.39	1.23	1.33	1.38	20 %
D08-ICP-QMS	1.24	1.42	1.44	1.37	1.37	1.63	1.41	0.4 µg/L

Source: JRC

Figure 6.1. Characterisation data for Cd, ERM-DA634, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively.



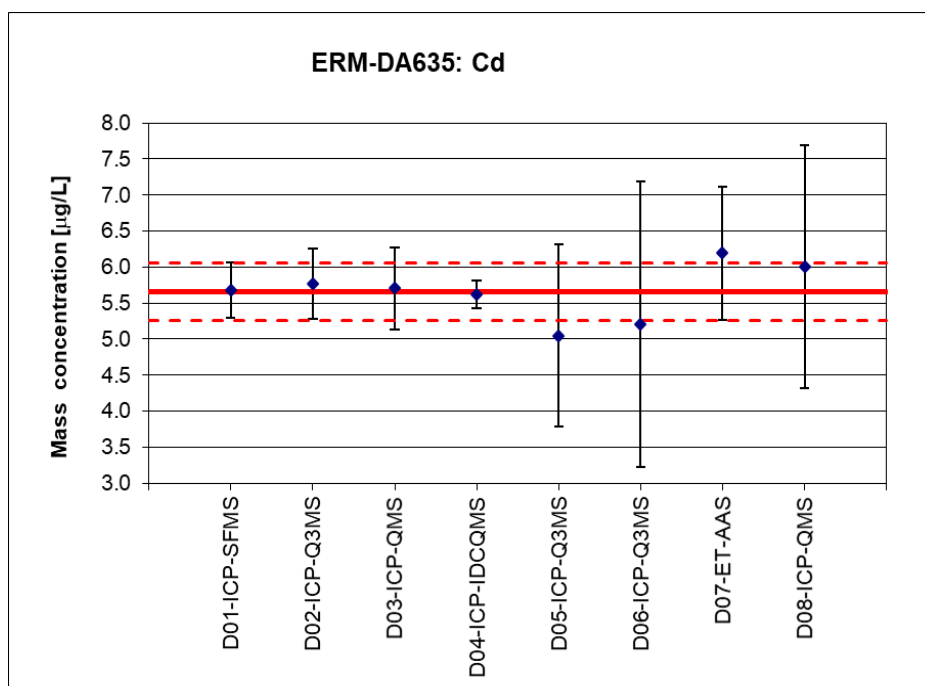
Source: JRC

Table 6.2. Data from the characterisation for Cd, ERM-DA635. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	5.68	5.67	5.63	5.57	5.73	5.81	5.68	0.38 µg/L
D02-ICP-Q3MS	5.81	5.9	5.81	5.76	5.81	5.54	5.77	0.49 µg/L
D03-ICP-QMS	5.79	5.65	5.8	5.68	5.7	5.61	5.71	0.57 µg/L
D04-ICP-IDCQMS	5.74	5.61	5.68	5.65	5.64	5.42	5.62	0.19 µg/L
D05-ICP-Q3MS	5.0	4.8	5.5	5.6	4.7	4.7	5.05	25 %
D06-ICP-Q3MS	5.26	5.18	5.26	5.18	5.39	4.99	5.21	38 %
D07-ET-AAS	6.31	6.28	6.13	6.4	6.06	5.98	6.19	15 %
D08-ICP-QMS	6.13	5.78	6.07	5.9	6.17	5.98	6.01	1.68 µg/L

Source: JRC

Figure 6.2. Characterisation data for Cd, ERM-DA635, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively.



Source: JRC

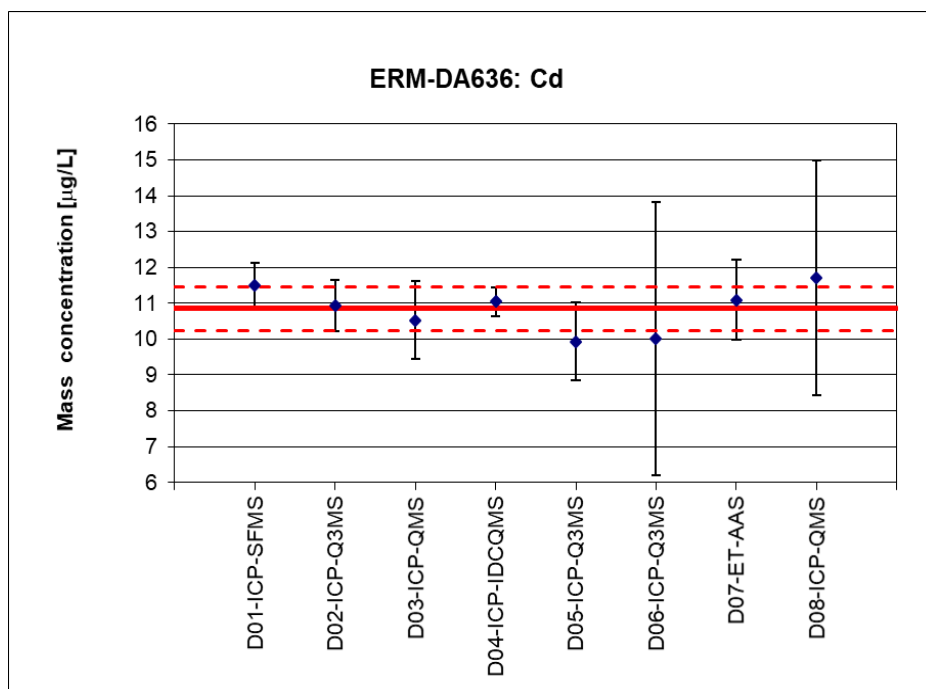
Table 6.3. Data from the characterisation for Cd, ERM-DA636. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	11.4	11.5	11.2	11.4	11.9	11.6	11.50	0.64 µg/L
D02-ICP-Q3MS	11.22*	10.56	11.41	10.29	11.2	11.17	10.93	0.72 µg/L
D03-ICP-QMS	10.5	10.7	10.1	10.5	10.8	10.6	10.53	1.1 µg/L
D04-ICP-IDCQMS	10.9	11.1	11.1	11.0	11.2	11.0	11.05	0.4 µg/L
D05-ICP-Q3MS	9.8	9.7	10.6	10.9	9.5	9.1	9.93	11 %
D06-ICP-Q3MS	10.1	9.7	10.15	9.94	10.21*	10.17	10.01	38 %
D07-ET-AAS	11.01	11	11.14	11.13	10.97	11.31	11.10	10 %
D08-ICP-QMS	11.34	11.35	11.63	12.01	12.17	11.81	11.72	3.28 µg/L

*: result not used as the unit was in the part of a rising trend in the filling volume

Source: JRC

Figure 6.3. Characterisation data for Cd, ERM-DA636, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively.



Source: JRC

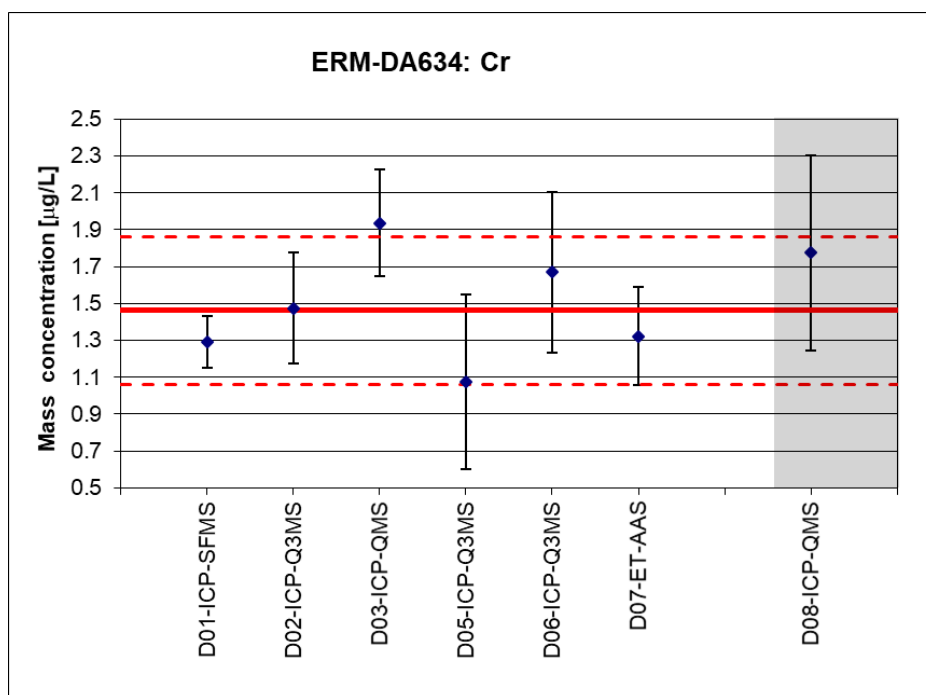
Chromium

Table 6.4. Data from the characterisation for Cr, ERM-DA634. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	1.29	1.24	1.26	1.33	1.3	1.33	1.29	0.14 µg/L
D02-ICP-Q3MS	1.7	1.75	1.1	1.93	1.08	1.29	1.48	0.3 µg/L
D03-ICP-QMS	1.78	1.9	1.91	1.94	2.01	2.08	1.94	0.29 µg/L
D05-ICP-Q3MS	1.2	0.95	1.1	1.2	1	1	1.08	44 %
D06-ICP-Q3MS	1.61	1.9	1.72	1.62	1.41	1.76	1.67	26 %
D07-ET-AAS	1.14	2.32	1.2	1.13	1.18	0.97	1.32	20 %
<i>Results not used for value assignment</i>								
D08-ICP-QMS	1.09	2.73	1.27	0.3	3.01	2.25	1.78	0.53 µg/L

Source: JRC

Figure 6.4. Characterisation data for Cr, DA634 bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively. Results in the shaded area were no used for the calculation of the certified value and uncertainty.



Source: JRC

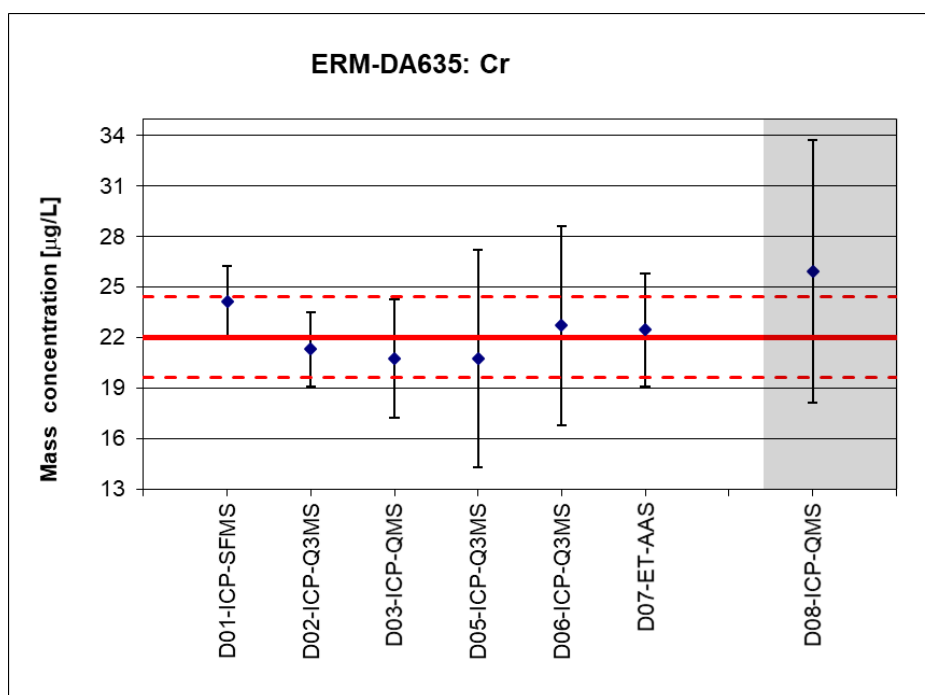
Table 6.5. Data from the characterisation for Cr, ERM-DA635. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	24.1	22.6	24.9	24	24.7	24.5	24.13	2.1 µg/L
D02-ICP-Q3MS	22.7	22.9	20.8	21.7	20.1	19.7	21.32	2.2 µg/L
D03-ICP-QMS	20.6	20.4	19.2	21.3	22.3	36.2*	20.76	3.5 µg/L
D05-ICP-Q3MS	21.3	21	20.1	20.4	21	20.8	20.77	31 %
D06-ICP-Q3MS	23.63	23.12	21.84	22.24	22.9	22.68	22.74	26 %
D07-ET-AAS	23.7	23.6	21.5	22.3	21.8	21.9	22.47	15 %
<i>Results not used for value assignment</i>								
D08-ICP-QMS	27.24	30.5	26.38	28.28	27.47	15.78	25.94	7.78 µg/L

*: Result not used (contamination)

Source: JRC

Figure 6.4. Characterisation data for Cr, DA635, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively. Results in the shaded area were no used for the calculation of the certified value and uncertainty.



Source: JRC

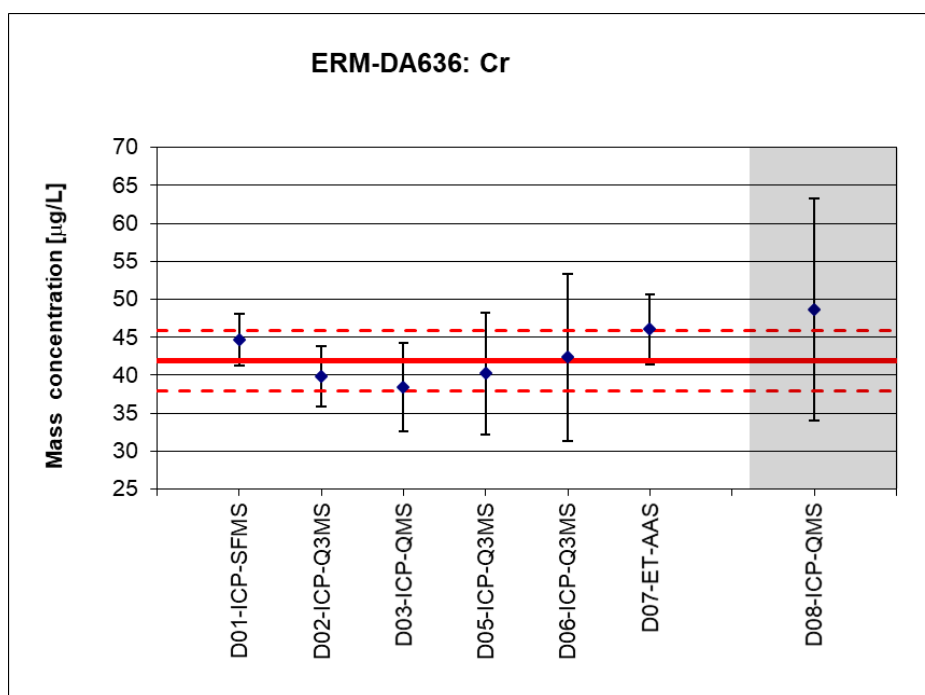
Table 6.6. Data from the characterisation for Cr, ERM-DA636. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	45.5	44.8	44.4	43.2	46.1	44	44.67	3.4 µg/L
D02-ICP-Q3MS	42*	40	39.7	42.1	39.5	37.9	39.84	4 µg/L
D03-ICP-QMS	36.6	38.3	39.7	38.8	37.8	39.2	38.40	5.8 µg/L
D05-ICP-Q3MS	41	41.3	38.5	39	40.4	41.2	40.23	20 %
D06-ICP-Q3MS	43	42.29	41.22	42.46	43.14*	42.81	42.36	26 %
D07-ET-AAS	50.2	48.1	48.7	46.2	41.4	41.9	46.08	10 %
<i>Results not used for value assignment</i>								
D08-ICP-QMS	48.78	43.11	56.37	57.49	41.56	44.94	48.71	14.61 µg/L

*: result not used as the unit was in the part of a rising trend in the filling volume

Source: JRC

Figure 6.6. Characterisation data for Cr, ERM-DA636, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively. Results in the shaded area were no used for the calculation of the certified value and uncertainty



Source: JRC

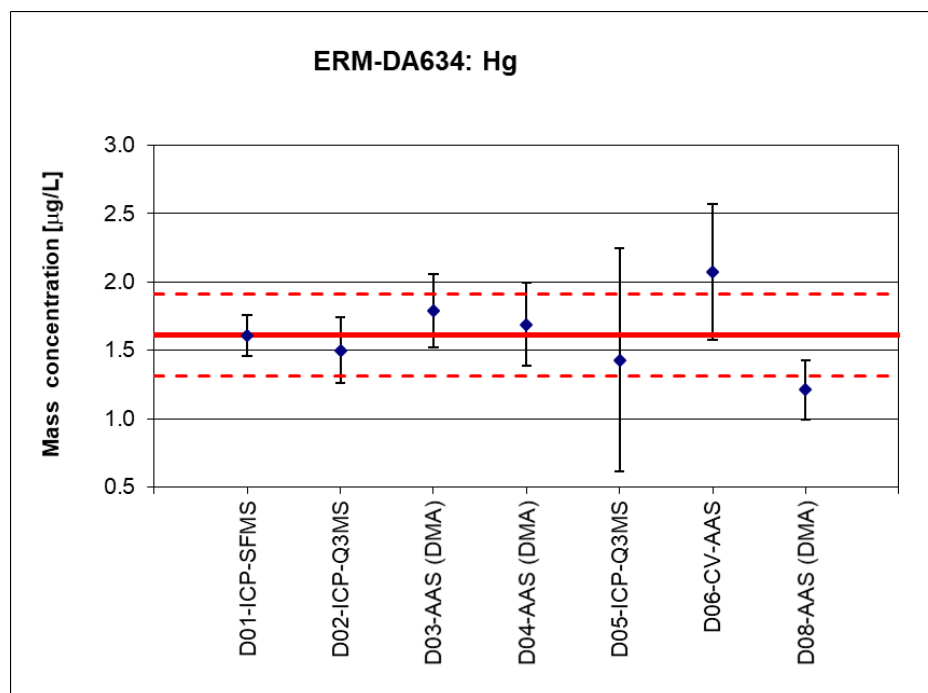
Mercury

Table 6.7. Data from the characterisation for Hg, ERM-DA634. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	1.54	1.61	1.65	1.59	1.58	1.7	1.61	0.15 µg/L
D02-ICP-Q3MS	1.59	1.56	1.46	1.43	1.49	1.49	1.50	0.24 µg/L
D03- AAS (DMA)	1.84	1.85	1.85	1.91	1.67	1.63	1.79	0.27 µg/L
D04- AAS (DMA)	1.73	1.59	1.55	1.67	1.81	1.76	1.69	0.30 µg/L
D05-ICP-Q3MS	1.4	1.4	1.3	1.2	1.6	1.7	1.43	57 %
D06- CV-AAS	1.76	1.47	3.12	2.5	1.56	1.98	2.07	24 %
D08- AAS (DMA)	1.22	1.15	1.14	1.2	1.29	1.25	1.21	0.22 µg/L

Source: JRC

Figure 6.7. Characterisation data for Hg, ERM-DA634, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively.



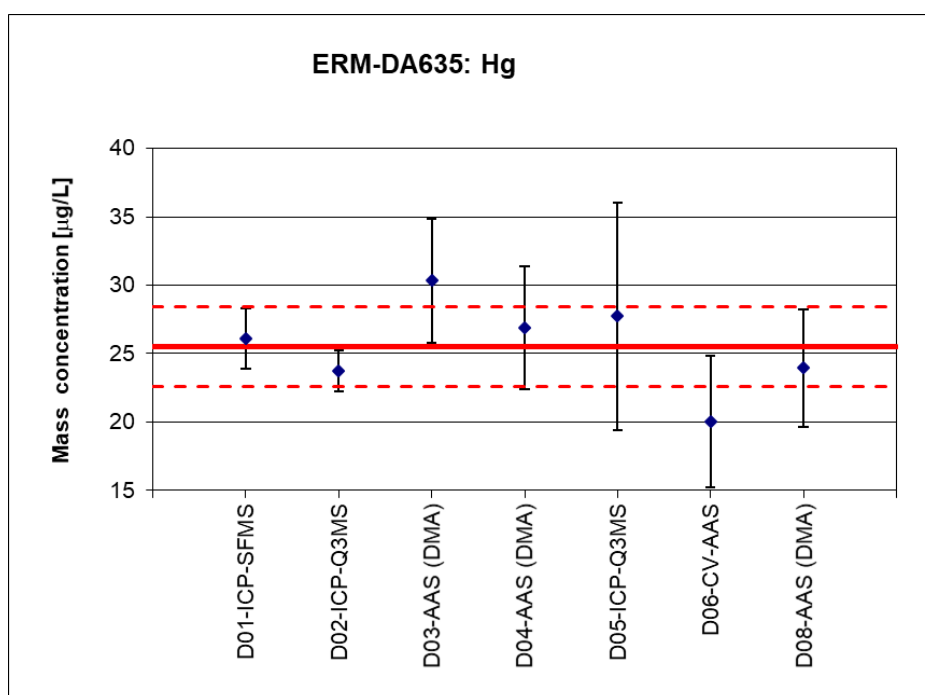
Source: JRC

Table 6.8. Data from the characterisation for Hg, ERM-DA635. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	26.4	25.6	26.0	25.9	26.3	26.3	26.08	2.2 µg/L
D02-ICP-Q3MS	23.5	24.1	23.6	23.6	23.7	23.7	23.70	1.5 µg/L
D03- AAS (DMA)	30.6	31	30.9	31.1	29.4	28.9	30.32	4.5 µg/L
D04- AAS (DMA)	27.9	26.5	27.5	26.7	26.2	26.4	26.87	4.5 µg/L
D05-ICP-Q3MS	27.9	27.7	26.1	26.1	29.3	29.2	27.72	30 %
D06-CV-AAS	18.7	16.36	18.58	20.04	24.1	22.54	20.05	24 %
D08- AAS (DMA)	24.65	23.3	23.06	23.71	24.59	24.25	23.93	4.31 µg/L

Source: JRC

Figure 6.8. Characterisation data for Hg, ERM-DA635, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively.



Source: JRC

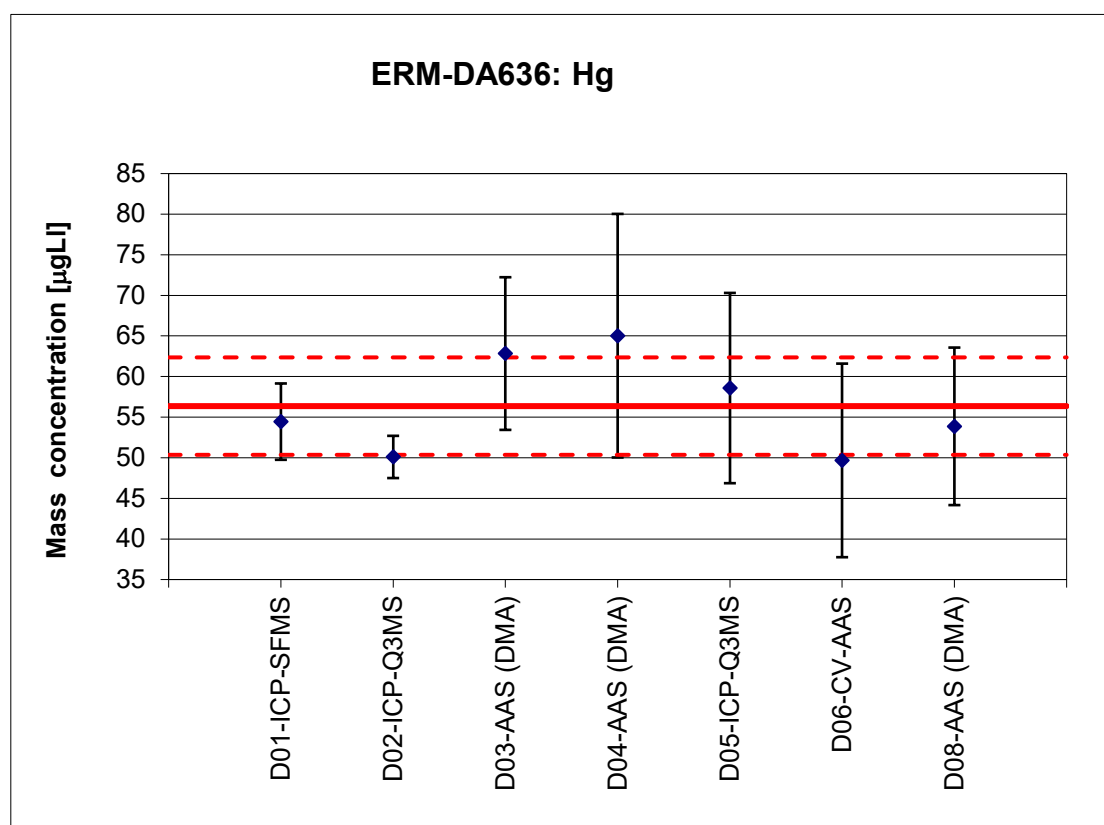
Table 6.9. Data from the characterisation for Hg, ERM-DA636. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	54.9	54.1	55.3	53.8	53.9	54.7	54.45	4.7 µg/L
D02-ICP-Q3MS	51.5*	47.4	48.8	54	50.4	49.9	50.10	2.6 µg/L
D03- AAS (DMA)	59.7	60.9	66	65	59.6	65.8	62.83	9.4 µg/L
D04- AAS (DMA)	66.9	62.0	63.9	65.5	65.3	66.6	65.03	15 µg/L
D05-ICP-Q3MS	59.6	58.6	54.5	54.2	61.7	62.9	58.58	20 %
D06-CV-AAS	49.05	46.42	51.79	48.43	49.76*	52.68	49.67	24 %
D08- AAS (DMA)	54.86	55.02	53.86	51.77	54.19	53.52	53.87	9.7 µg/L

*: result not used as the unit was in the part of a rising trend in the filling volume

Source: JRC

Figure 6.9. Characterisation data for Hg, ERM-DA636, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively.



Source: JRC

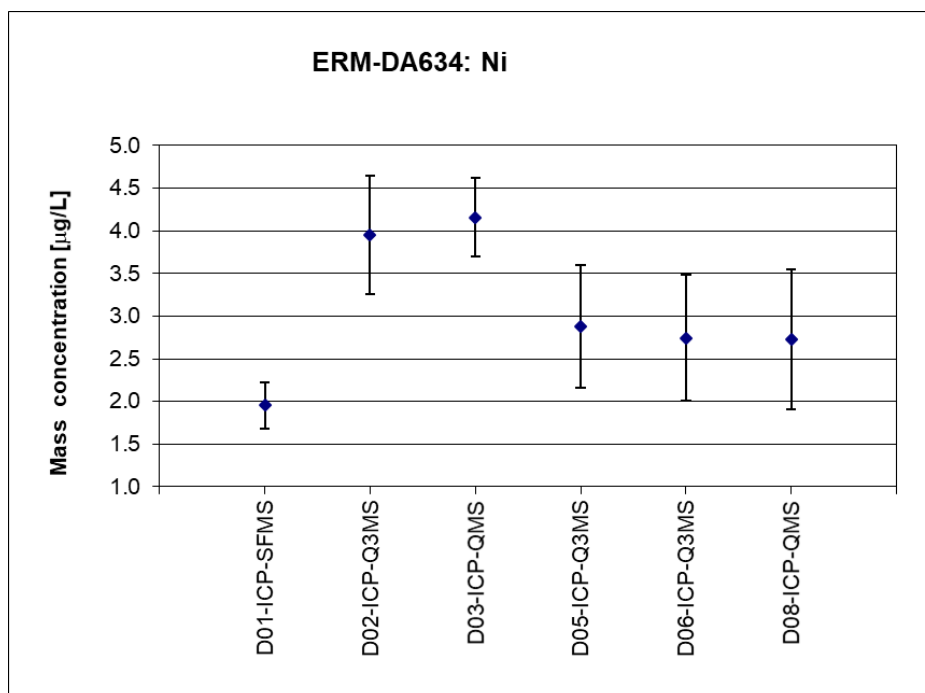
Nickel

Table 6.10. Data from the characterisation for Ni, ERM-DA634. The Ni mass concentration is not certified in ERM-DA634. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	2.17	1.82	1.98	2.03	1.72	2.01	1.96	0.27 µg/L
D02-ICP-Q3MS	3.35	3.06	3.77	5.33	3.7	4.5	3.95	0.69 µg/L
D03-ICP-QMS	3.28	3.29	3.51	4.55	4.4	5.9	4.16	0.46 µg/L
D05-ICP-Q3MS	2.8	2.4	2.6	3	3.3	3.2	2.88	25 %
D06-ICP-Q3MS	2.96	3.07	2.45	2.49	2.7	2.8	2.75	27 %
D08-ICP-QMS	3.57	2.15	3.33	2.31	2.33	2.67	2.73	0.82 µg/L

Source: JRC

Figure 6.10. Characterisation data for Ni, ERM-DA634. The Ni mass concentration is not certified in ERM-DA634.



Source: JRC

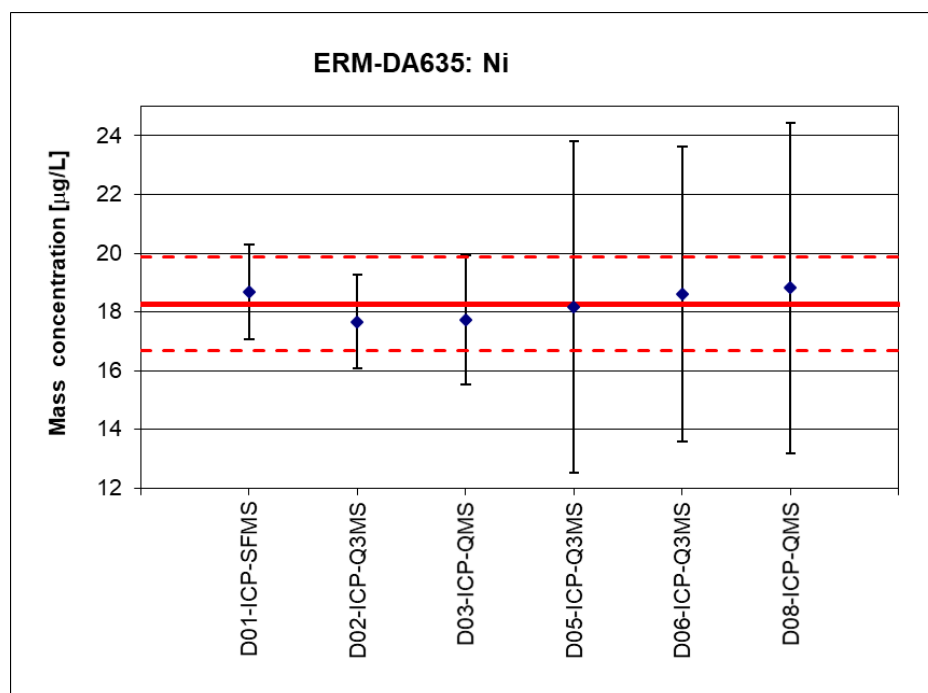
Table 6.11. Data from the characterisation for Ni, ERM-DA635. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	18.5	18.9	18.2	18.6	18.9	19	18.68	1.6 µg/L
D02-ICP-Q3MS	17.7	18.2	17.7	17.5	18.1	16.8	17.67	1.6 µg/L
D03-ICP-QMS	17.4	17.5	17.9	18.1	17.7	31.9*	17.72	2.2 µg/L
D05-ICP-Q3MS	19.0	17.7	17.5	17.8	18.7	18.3	18.17	31 %
D06-ICP-Q3MS	17.96	18.25	19.59	17.92	18.52	19.34	18.60	27 %
D08-ICP-QMS	20.95	19.55	15.31	18.88	20.3	17.88	18.81	5.64 µg/L

*: Result not used (contamination)

Source: JRC

Figure 6.11. Characterisation data for Ni, ERM-DA635, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively.



Source: JRC

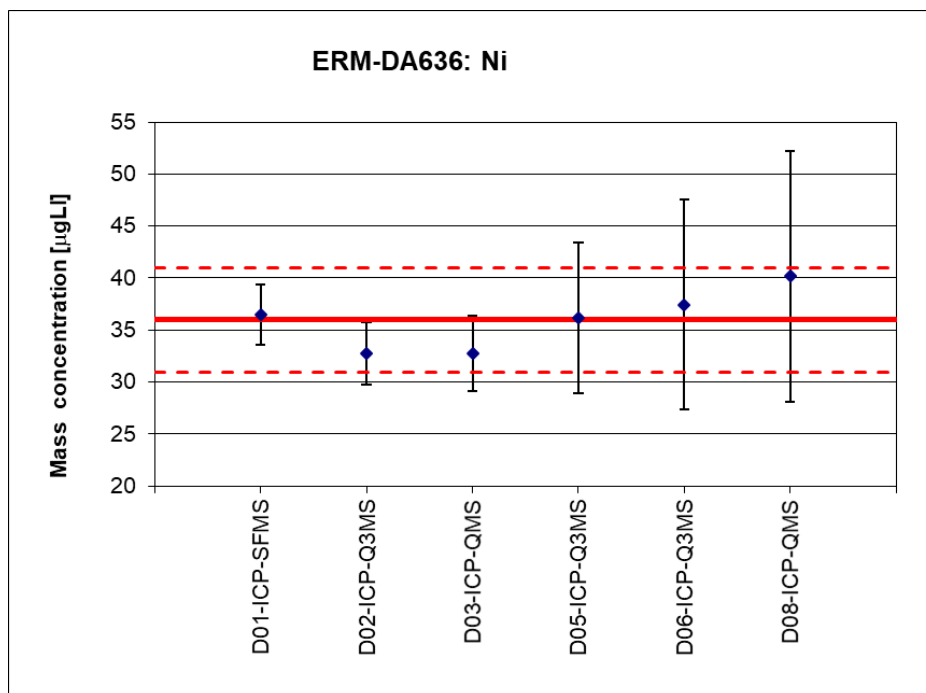
Table 6.12. Data from the characterisation for Ni, ERM-DA636. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	36.4	36.9	36.6	36.2	35.9	36.8	44.67	2.9 µg/L
D02-ICP-Q3MS	35.2*	32.9	33.9	29.5	33	34.4	39.84	3 µg/L
D03-ICP-QMS	32.5	33.8	31	33.4	34.8	31	38.40	3.6 µg/L
D05-ICP-Q3MS	35.2	35.7	35.8	34.4	36.8	39.2	36.18	20 %
D06-ICP-Q3MS	37.56	35.94	37.69	39.15	38.06*	36.89	42.36	27 %
D08-ICP-QMS	51.23	36.82	40.76	38.46	34.56	39.33	46.08	12.06

*: result not used as the unit was in the part of a rising trend in the filling volume

Source: JRC

Figure 6.12. Characterisation data for Ni, ERM-DA636, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively.



Source: JRC

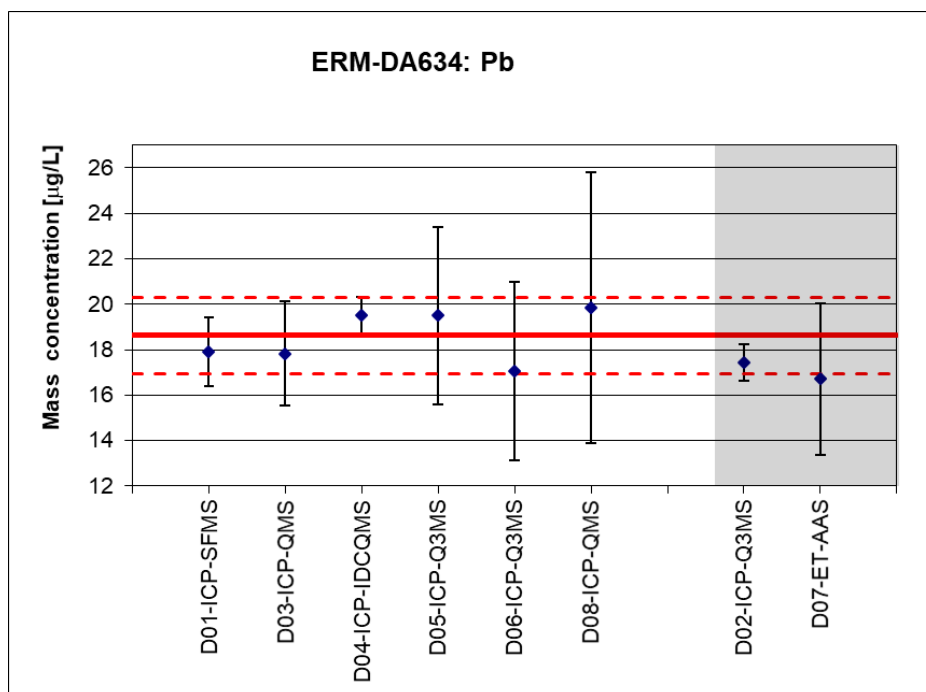
Lead

Table 6.13. Data from the characterisation for Pb, ERM-DA634. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	17.6	16.8	18.8	18.6	18.3	17.4	17.92	1.5 µg/L
D03-ICP-QMS	17.9	17.5	18	17.8	17.9	17.9	17.83	2.3 µg/L
D04-ICP-IDCQMS	19.6	20.3	19.2	18.9	19.3	19.8	19.52	0.8 µg/L
D05-ICP-Q3MS	19.4	19.3	19.9	19.6	19.6	19.2	19.50	20 %
D06-ICP-Q3MS	16.48	16.36	17.22	17.12	17.78	17.48	17.07	23 %
D08-ICP-QMS	19.3	19.93	20.37	18.71	20.59	20.28	19.86	5.96 µg/L
<i>Results not used for value assignment</i>								
D02-ICP-Q3MS	18.27	17.49	17.44	17.3	17.3	16.82	17.4	0.81 µg/L
D07-ET-AAS	11.65	11.25	16.6	19.4	21.76	19.59	16.7	20 %

Source: JRC

Figure 6.13. Characterisation data for Pb, ERM-DA634, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively. Results in the shaded area were no used for the calculation of the certified value and uncertainty.



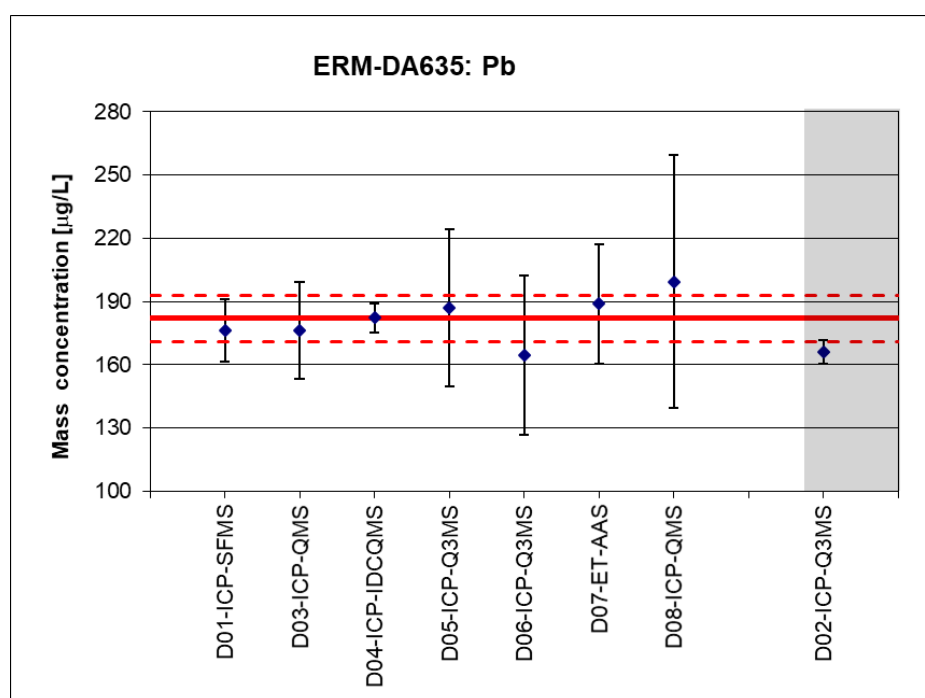
Source: JRC

Table 6.14. Data from the characterisation for Pb, ERM-DA635. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	178	181	177	173	176	172	176.2	15 µg/L
D03-ICP-QMS	178	181	177	173	176	172	176.2	23 µg/L
D04-ICP-IDCQMS	188	182	184	184	181	174	182.2	7 µg/L
D05-ICP-Q3MS	188	186	189	190	185	183	186.8	20 %
D06-ICP-Q3MS	157.5	156.9	165.5	165.3	171.5	170.2	164.5	23 %
D07-ET-AAS	192.7	192.1	190.4	193.2	179.4	184.7	188.8	15 %
D08-ICP-QMS	200.94	202.24	195.23	193.17	204.71	200.28	199.4	59.83 µg/L
<i>Results not used for value assignment</i>								
D02-ICP-Q3MS	170.6	168.2	166	166.8	163.3	160.0	165.8	5.6 µg/L

Source: JRC

Figure 6.14. Characterisation data for Pb, ERM-DA635 bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively. Results in the shaded area were no used for the calculation of the certified value and uncertainty.



Source: JRC

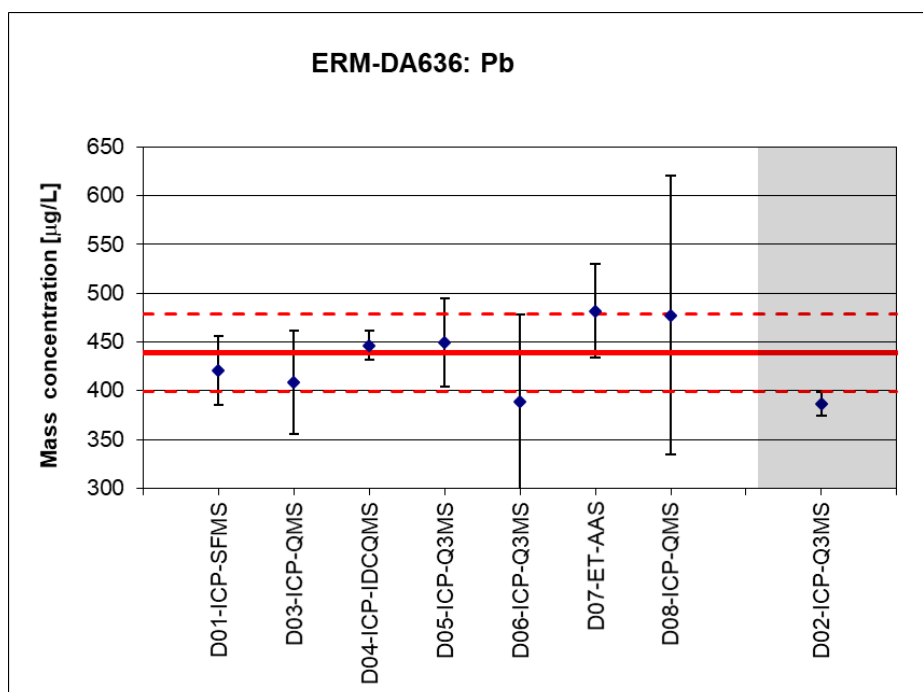
Table 6.15. Data from the characterisation for Pb, ERM-DA636. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	421	427	426	417	412	422	420.8	35 µg/L
D03-ICP-QMS	404	404	413	413	404	412	408.3	53 µg/L
D04-ICP-IDCQMS	445	449	444	446	448	445	446.2	15 µg/L
D05-ICP-Q3MS	448	447	449	452	451	448	449.2	10 %
D06-ICP-Q3MS	367.3	377.3	398	392.9	407.7*	407.5	388.6	23 %
D07-ET-AAS	474.4	480.4	465.2	471.8	489.6	507.6	481.5	10 %
D08-ICP-QMS	479.49	470.79	473.52	471.02	488.82	480.22	477.3	143.19 µg/L
<i>Results not used for value assignment</i>								
D02-ICP-Q3MS	390*	387	395	375	387	388	386.4	12 µg/L

*: result not used as the unit was in the part of a rising trend in the filling volume

Source: JRC

Figure 6.15. Characterisation data for Pb, ERM-DA636, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively. Results in the shaded area were no used for the calculation of the certified value and uncertainty.



Source: JRC

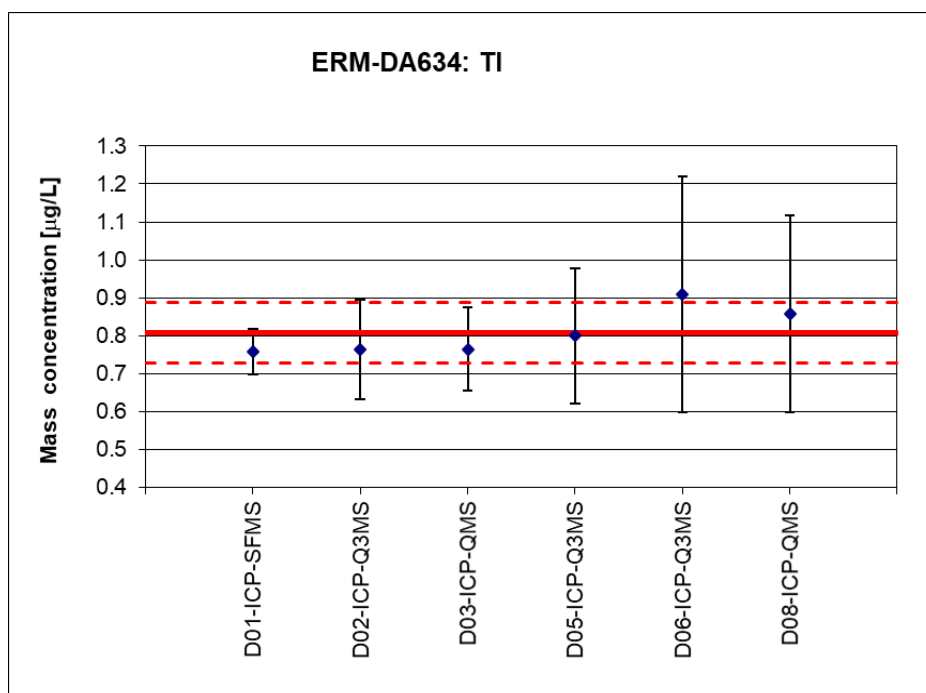
Thallium

Table 6.16. Data from the characterisation for Tl, ERM-DA634. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	0.774	0.778	0.751	0.744	0.759	0.739	0.76	0.06 µg/L
D02-ICP-Q3MS	0.811	0.778	0.736	0.752	0.761	0.749	0.76	0.13 µg/L
D03-ICP-QMS	0.789	0.77	0.744	0.752	0.775	0.762	0.77	0.11 µg/L
D05-ICP-Q3MS	0.8	0.8	0.8	0.8	0.8	0.8	0.80	110 %
D06-ICP-Q3MS	0.85	0.83	0.91	0.9	1.01	0.96	0.91	35 %
D08-ICP-QMS	0.88	0.88	0.93	0.8	0.8	0.86	0.86	0.26 µg/L

Source: JRC

Figure 6.16. Characterisation data for Tl, ERM-DA634 bold lines bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively. Results in the shaded area were no used for the calculation of the certified value and uncertainty.



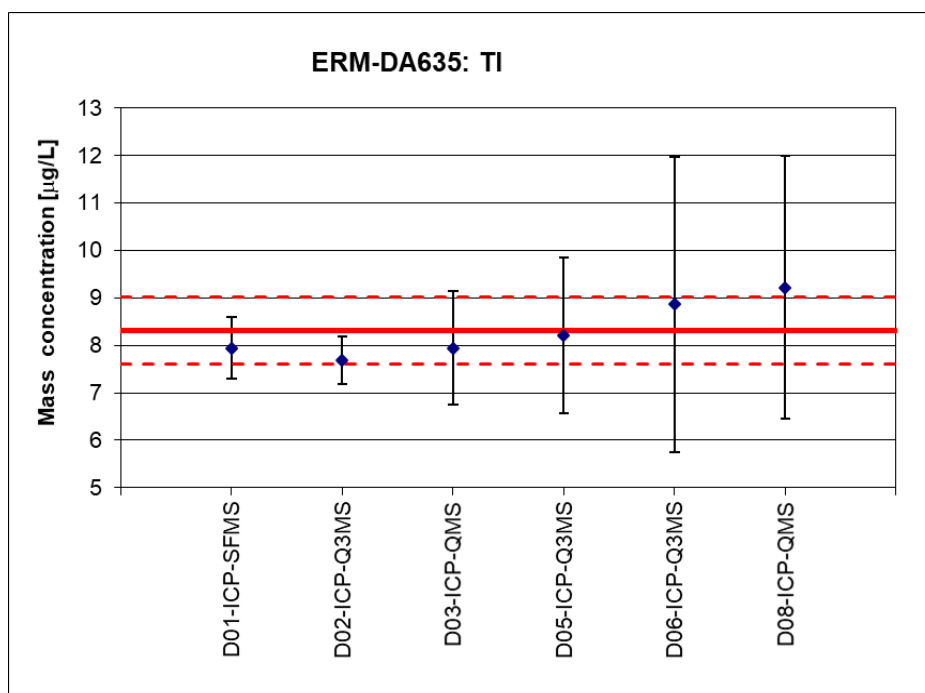
Source: JRC

Table 6.17. Data from the characterisation for TI, ERM-DA635. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	8.13	8.23	7.79	7.65	7.91	7.94	7.94	0.65 µg/L
D02-ICP-Q3MS	7.76	7.71	7.73	7.96	7.51	7.43	7.68	0.5 µg/L
D03-ICP-QMS	8.13	8.23	7.79	7.91	7.94	7.65	7.94	1.2 µg/L
D05-ICP-Q3MS	8.3	8.2	8.2	8.2	8.3	8.1	8.22	20 %
D06-ICP-Q3MS	8.38	8.43	9.2	8.75	9.1	9.3	8.86	35 %
D08-ICP-QMS	9.5	9.53	9.06	8.89	9.34	9.01	9.22	2.77 µg/L

Source: JRC

Figure 6.17. Characterisation data for TI, ERM-DA635, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively.



Source: JRC

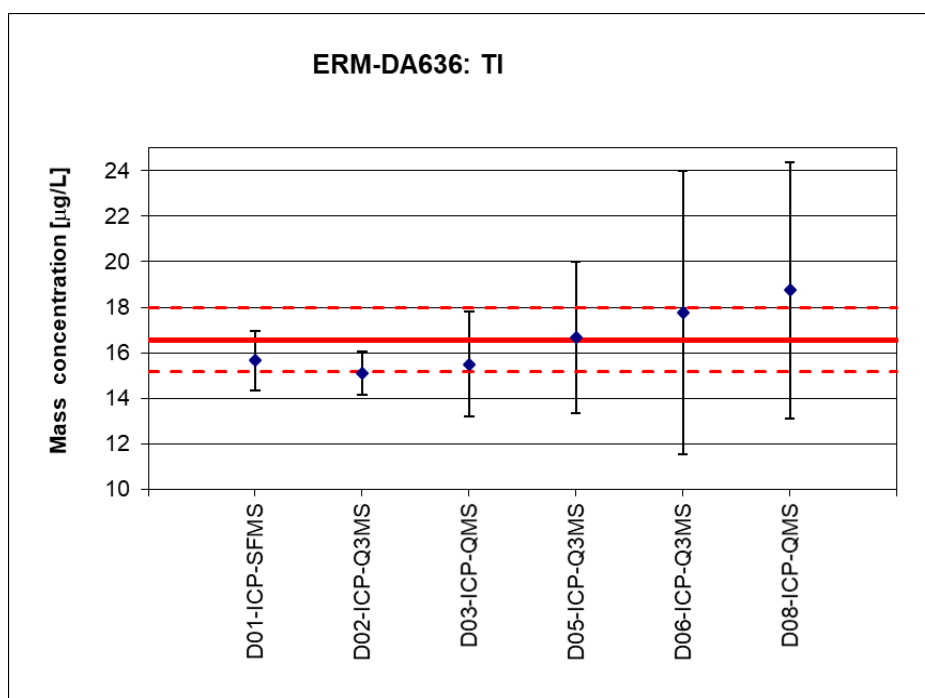
Table 6.18. Data from the characterisation for TI, ERM-DA636. The data are shown as provided by the laboratories.

Dataset code and method	replicate 1 [µg/L]	replicate 2 [µg/L]	replicate 3 [µg/L]	replicate 4 [µg/L]	replicate 5 [µg/L]	replicate 6 [µg/L]	mean [µg/L]	Expanded uncertainty
D01-ICP-SFMS	16.2	15.8	15.7	16.1	15.7	14.5	15.67	1.3 µg/L
D02-ICP-Q3MS	15.15*	14.81	15.58	14.39	15.47	15.2	15.09	0.96 µg/L
D03-ICP-QMS	15.5	15.7	15.4	15.5	15.5	15.4	15.50	2.3 µg/L
D05-ICP-Q3MS	16.7	16.5	16.5	16.7	16.9	16.7	16.67	20 %
D06-ICP-Q3MS	17.03	16.94	18.03	18.34	18.85*	18.51	17.77	35 %
D08-ICP-QMS	18.79	18.51	19	18.05	19.05	19.05	18.74	5.62 µg/L

*: result not used as the unit was in the part of a rising trend in the filling volume

Source: JRC

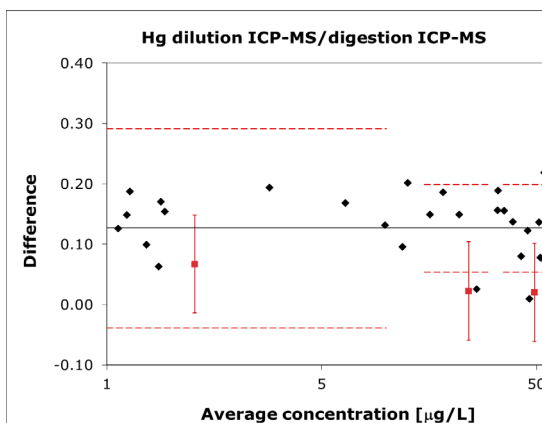
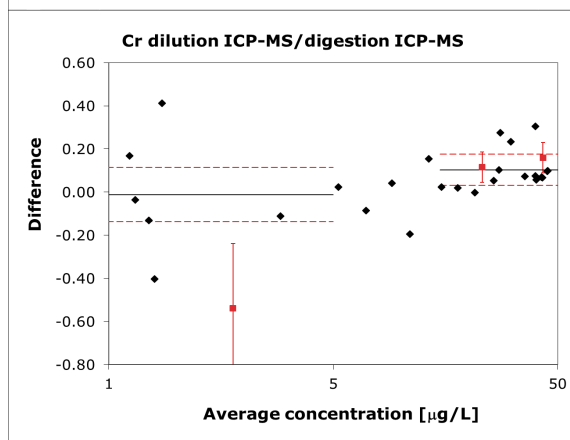
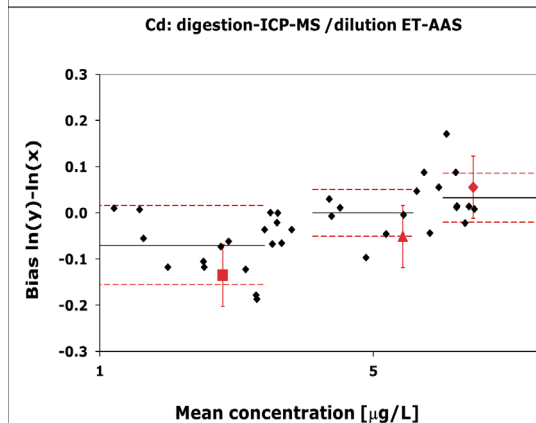
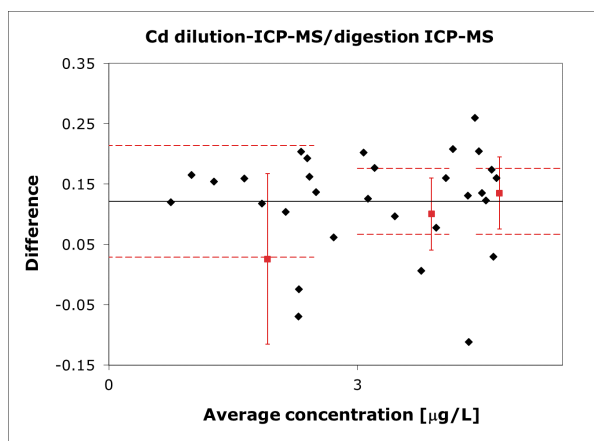
Figure 6.18. Characterisation data for TI, bold red lines and dashed red lines represent the certified value and its expanded uncertainty, respectively.

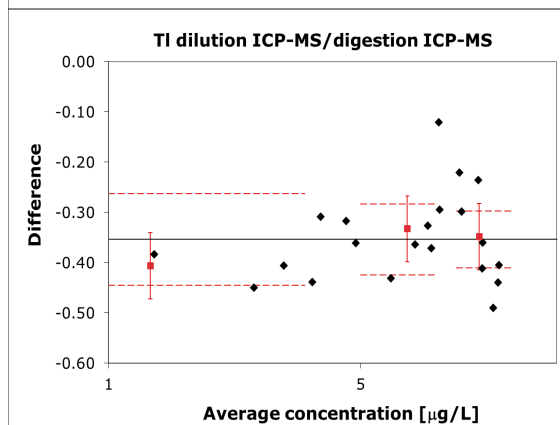
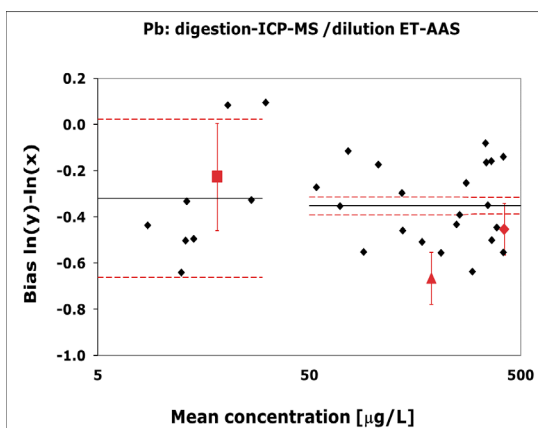
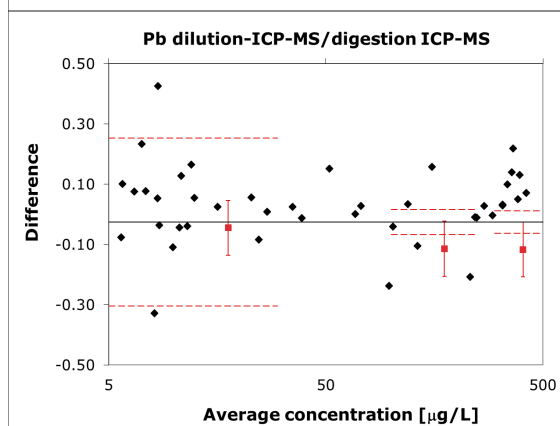
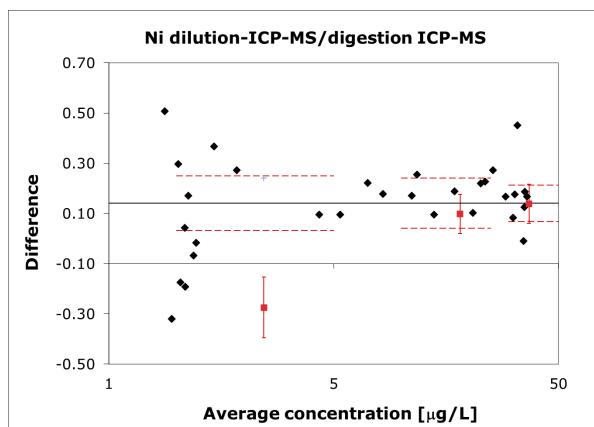


Source: JRC

Annex 7. Results of the commutability study

The graphs show the differences of the natural logarithms of the averages of the two methods of each sample versus the average concentration in $\mu\text{g/L}$. Black diamonds are the fresh samples (spiked and unspiked), red squares correspond to the CRMs. The error bars on the CRMs correspond to the expanded uncertainty of the difference. The dashed red line correspond to the chosen commutability limit.





Source: JRC

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