

# Analysis of total As and As species in urine samples and Cd and Pb in blood samples of adolescents living near metal processing plants (BioBro)

## METHODOLOGICAL INFORMATION

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## 1. Introduction

This report describes the methodology used for the analysis of total As and As species in urine samples and Cd and Pb in blood samples of adolescents living near metal processing plants (Project BioBro 2022-2023).

Urine and blood samples were collected by ISSeP and transferred for analysis to the trace element laboratory of Sciensano. Samples were kept at  $-20^{\circ}\text{C}$  until analysis.

Statistical treatment of the data and calculation of reference values ( $\text{RV}_{95}$ ) was done according to the document 'Méthodologie d'élaboration des valeurs de référence dans le cadre du projet BMH-Wal 1' issued by ISSeP. These results are summarized per element in separate reports. Raw data were transferred to ISSeP in excel files.

## 2. Materials and Methods

### 2.1. Standards and chemicals

Nitric acid (Suprapur, SpA 67-69%,) was purchased from Romil (UK), and  $\text{H}_2\text{O}_2$  (30%, pergydrol, pro analyse) from Merck (Germany). Arsenic standards to prepare a multi species solution of 1000 mg/kg used for quantification of As and As species ( $\text{As}_3$ ,  $\text{As}_5$ , DMA, MA, AB) was purchased from Analytika (Prague, Czech Republic), Sigma (Merck, Germany) and Inorganic Ventures (Instrument Solutions, The Netherlands). A multi-element standard solution of 1000 mg/kg used for quantification of Cd and Pb was purchased from Analytika (Prague, Czech Republic). A multi-elemental  $5 \mu\text{g L}^{-1}$  tuning solution (Spectropure, Arlington, USA) or  $1 \mu\text{g L}^{-1}$  tuning solution (Thermo Fisher Scientific, Belgium) was used for tuning the ICP-MS or IC-ICP-MS respectively. Water used in this study was home produced doubly distilled water (Aquatron, Cole-Parmer, UK).

### 2.2. Determination of total As by ICP-MS

After thawing, urine samples were centrifugated (10 min at 1500 g) and supernatant was diluted and acidified to a final dilution factor of 10 (final at 4%  $\text{HNO}_3$ ). Sample solutions were stored at  $4^{\circ}\text{C}$  until ICP-MS analysis.

Total As concentrations of the urine samples were determined by ICP-MS (Agilent 8800), with O<sub>2</sub> as a reaction gas on mass 91. Quantification was carried out using a matrix matched external calibration of the linear type (range 0.005 – 10 µg L<sup>-1</sup>).

### 2.3. Determination of As species by IC-ICP-MS

For determination of As species in the urine samples, the above mentioned supernatant was diluted to a final dilution factor of 5 in Milli-Q H<sub>2</sub>O with phenylarsonic acid (PAA) added as an internal standard (final concentration 1 µg L<sup>-1</sup>). Sample solutions were stored at 4°C until ICP-MS analysis. As species (As<sub>3</sub>, As<sub>5</sub>, DMA, MA, AB) concentrations of the urine samples were determined by IC-ICP-MS (Dionex ICS-6000 + ICAP RQ; Thermo Fisher Scientific, Belgium), with He as collision gas. For chromatographic separation of the As species, an anion exchange column was used (IonPac AG7 (4x50mm; PN 035394) + IonPac AS7 (4x250mm; PN 035393), Thermo Fisher Scientific). The mobile phase consisted of ammonium carbonate (100 mM + 3%MeOH with pH of 10.3) in a gradient with Milli-Q water, for a run time of 14 minutes to obtain the maximum separation between the different peaks. Quantification was carried out using a matrix matched external calibration of the linear type (range 0.2 – 5 µg L<sup>-1</sup>), including 1,0 µg/L PAA.

Interspecies conversion between As<sub>3</sub> and As<sub>5</sub> might occur due to the long storage of the urine samples and aqueous dilution to minimize manipulation of the urine composition during sample preparation (Verdon et al., 2009). For this reason, further calculations/statistics were performed with the sum of both inorganic As species.

### 2.4. Determination of Cd and Pb by ICP-MS

After thawing, ± 1 ml of blood was digested with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> in a DigiPREP heating block system (SCP SCIENCE, Quebec, Canada). Further sample preparation included dilution of the digested samples to a final dilution factor of 12.5. Sample solutions were stored at 4°C until ICP-MS analysis. Total Cd and Pb concentrations of the blood samples were determined by ICP-MS (ICAP RQ; Thermo Fisher Scientific, Belgium), with He as collision gas. Cd was measured on mass 111 (and confirmed on mass 114), Pb was measured on mass 208 (and confirmed on mass 206). Quantification was carried out using an external calibration of the linear type. Calibration standards were in the range 0.005 – 10 µg L<sup>-1</sup> for Cd and 0.05-10 µg L<sup>-1</sup> for Pb and prepared by making the appropriate dilutions of the multi element mother stock.

## 2.5. Quality control

Each analytical batch included internal quality control measures such as two procedure blanks and a reagent blank as a monitor for possible cross-contamination, a QC standard check every 20 samples to allow verification of potential instrument drift and, a reference material (Seronom-level 1 and 2 or NIST 2996) to assess trueness and day to day variations. A series of acceptance criteria were applied to each batch, including calibration blank value  $\leq \text{LOQ}/2$ , procedure blank  $\leq \text{LOQ}$  and drift  $\leq 10\%$ .

## 2.6. Performance characteristics

*Limit of Detection - Limit of Quantification* – The LOQ of the method is the lowest level that can be determined with an acceptable performance. The LOQ for total As was calculated as 3.3 times the Limit of Detection (LOD = 3 times the standard deviation of 10 blanc samples or 10 pseudoblanc (low spiked) samples for speciation). This resulted in LOQ values in the matrix (urine) of  $0.5 \mu\text{g L}^{-1}$  for both total As and As species (corresponding LOD of  $0.15 \mu\text{g L}^{-1}$ ), and in the matrix blood of  $0.09 \mu\text{g L}^{-1}$  for Cd (corresponding LOD of  $0.0.027 \mu\text{g L}^{-1}$ ) and  $2 \mu\text{g L}^{-1}$  for Pb (corresponding LOD of  $0.61 \mu\text{g L}^{-1}$ ); after taking into account the dilution factor of the samples.

*Trueness* – Trueness is a theoretical concept expressing how close the mean of infinite number of results produced by the method is to a reference value. It can be assessed in practice by calculating the relative recovery compared to the reference value (in %). The reference value used in this study is the concentrations of the analyte cited on the certificate of the reference material used. The results are given in Table 1, reference material was added in each measurement and an average trueness was calculated.

CRM or PT samples	Analyte	Reference value ( $\pm \text{MU}$ ) $\mu\text{g L}^{-1}$	Measured value ( $\pm \text{SD}$ ) $\mu\text{g L}^{-1}$	Trueness
Seronom-L1 (urine)	Total As	$97 \pm 19$	$100 \pm 4$	103%
Seronom-L2 (urine)	Total As	$198 \pm 25$	$208 \pm 10$	105%
Equas 67 2A (urine)	Total As	$211 \pm 32$	$225 \pm 6$	107%
NIST 2669 L1 (urine)	AB	$12.4 \pm 1.9$	$15.9 \pm 0.4$	128%
	DMA	$3.47 \pm 0.41$	$4.37 \pm 0.09$	126%
	MA	$1.87 \pm 0.39$	$1.95 \pm 0.10$	104%

	As3+As5	3.88 ± 0.32	3.74 ± 0.40	96%
Equas 62 8A (urine)	AB	12.52 ± 3.63	13.53 ± 0.43	108%
	DMA	1.61 ± 0.48	1.65 ± 0.09	103%
	MA	1.24 ± 0.36	1.34 ± 0.09	108%
	As3+As5	0.65 ± 0.21	0.75 ± 0.16	115%
Seronom L1 (blood)	Cd	0.28 ± 0.06	0.31 ± 0.02	112%
	Pb	10.0 ± 2.0	11.5 ± 0.3	115%
Seronom L2 (blood)	Cd	5.1 ± 1.0	5.5 ± 0.4	107%
	Pb	303 ± 31	292 ± 62	96%
Equas 66 7A (blood)	Cd	0.27 ± 0.09	0.24 ± 0.02	88%
	Pb	13.28 ± 2.61	14.8 ± 0.4	111%

*Precision* – Repeatability standard deviation ( $s_r$  = within day variation), between-day standard deviation ( $s_d$ ) and intermediate precision standard deviation ( $s_{ip}$ =within lab reproducibility) were determined based on results of the reference material Seronom-L1 and L2 (either urine or blood) for total As, Cd and Pb; and NIST 2669 L1 for As species, that were analyzed together with the samples on different days in independent replicates. The values were calculated via one-way analysis of variance using the equations below:

$$s_r = \sqrt{MSW} \quad (1)$$

$$s_d = \sqrt{\frac{MSB - MSW}{n}} \quad (2)$$

$$s_{ip} = \sqrt{s_r^2 + s_d^2} \quad (3)$$

MSW is the mean squares within days, MSB is the mean squares between days, and n is the number of measurements per day in routine (1 replicate). Relative deviations (expressed in %) were obtained by expressing the corresponding standard deviations as a percentage of the mean measured values.

The repeatability relative standard deviation ( $RSD_r$ ) for As tot, AB, DMA, MA and As3+As5 in urine was respectively 1.2%, 4.5%, 4.4%, 4.0% and 7.2%. The between-day standard deviation

(RSD<sub>d</sub>) respectively 3.0%, 2.0%, 1.5%, 2.6% and 5.5% This resulted in a within lab reproducibility (RSD<sub>ip</sub>) of 3.2%, 5.0%, 4.6%, 4.8% and 9.1 % respectively.

The repeatability relative standard deviation (RSD<sub>r</sub>) for Cd and Pb in blood was respectively 7.6%, and 2.7%. The between-day standard deviation (RSD<sub>d</sub>) respectively 1.6%, and 1.8% This resulted in a within lab reproducibility (RSD<sub>ip</sub>) of 7.8% and 3.2 % respectively.