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ESTIMATING UNCERTAINTY AND QUALITY OF LEAD DETERMINANTION IN HUMAN BLOOD

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Abstract: Estimating uncertainty and quality of lead determination in blood.
The method for the direct determination of lead by AAS in whole human blood is presented. The previous experience and validation data are suggested as sources of performance information. The method recovery, sample recovery, homogeneity, precision and calibration were included to estimate measurement uncertainty compliant with ISO/IEC 17025 : 2005. The results of measurements using this method have uncertainty (52-23)% in the working range (70 – 700) $\mu g.l^{-1}$.

Keywords: blood, lead, uncertainty, biomonitoring, human exposure to Pb

1. INTRODUCTION

Exposure to lead presents a significant hazard in the onset of serious intoxication. There still are certain jobs, hobbies and foods that are more likely to be associated with lead.

Potentially high levels of lead may occur in the lead smelting and refining industries, battery manufacturing plants, steel welding or cutting operations. Lead is a potent, systemic poison that causes unknown harm once absorbed by body (NIOSH, 2005).

Significant portion of the lead that is inhaled or ingested, gets into blood stream (ATSDR, 2005). Once in blood stream, lead circulates throughout body and stored in various organs and body tissues. Some of this lead is quickly filtered out of body and excreted, but some remains in the blood and other tissues. When exposure to lead continues, the amount stored in body increases if absorption of lead is higher than excretion (ATSDR, 2005).

Chronic overexposure to lead may result in severe damage to blood-forming, nervous, urinary and reproductive systems, kidney disease (WHO, 1995).
Lead can also affect muscular and digestive systems (Križáni, Andráš & Danáková, 2007).

The ideal biomarker of lead exposure is a measurement of total lead body burden. Biomarkers of exposure in practical use are measurements of total levels in tissues or body fluids, such as blood bone, urine or hair.

Big advantage of blood lead levels monitoring is that blood lead levels can rise quickly. With frequent monitoring of blood lead levels, dangerous concentrations can be quickly identified, and further exposure of such vulnerable human beings can be avoided. A blood lead level over 250 $\mu g.l^{-1}$ shows that substantial exposure to lead is occurring. There is also increasing evidence that health effects may occur at this blood lead level.

In the Slovak Republic, it does not exist relevant legislation regarding concentration of blood lead level as a result of environmental exposure.

In compliance with Slovak occupational legislation (The government order No 355/2006), the concentration of lead in blood 700 $\mu g.l^{-1}$ is referred to be harmful. Medical examination is recommended if concentration of lead in blood exceeds the level of 400 $\mu g.l^{-1}$.

The result of measurement is unacceptable and may even be misleading if the quality of the method is not declared. Laboratories that are authorised with the respect to analytical methods shall continuously document the quality of this method. Moreover, all results is supposed to be estimated in the range, within which the true value lies. Under STN EN ISO/IEC 17025, testing laboratory shall have and shall apply procedures for estimating uncertainty of measurement. In severe cases a reasonable estimation shall be based on knowledge of method performance and on the measurement scope and shall make use of previous experience and validation data.

2. EXPERIMENTAL

The method for the direct determination of lead by AAS in whole human blood is presented (Morton, 2000). A mixed matrix modifier solution containing nitric acid, ammonium dihydrogen phosphate and triton was used for preparation venous blood samples.

After venipuncture, blood samples were collected in plastic 2.7 ml Li-Heparin sample tubes (SARSTEDT, Monovette) which contained EDTA as anticoagulant (Liang, 1991). 200 µl portions of blood sample were mixed with 1200 µl of the mixed matrix modifier solution. Mixed matrix modifier solution (0,2 % HNO_3, p.a. and 0,5% $NH_4H_2PO_4$, Suprapure (Merck) in 0,4% TRITON - 100 p.a. SERVA (FENBIOCHEMICA)) was prepared by mixing of 10 ml of 0,5% HNO_3 + 0,125g $NH_4H_2PO_4$ + 5 cm^3 of 2% TRITON-X, filled with deionized water in 25 ml volumetric flask. Blood test samples were left at rest for 5 minutes and were centrifuged at 3000 rpm within 6 min. Samples were poured into measuring vial. The blank sample was prepared from the matrix modifier solution.

The Perkin Elmer 4100 ZL atomic absorption spectrometer with transversely

heated graphite furnace atomiser with Zeeman background correction and lead hollow cathode lamp at 283.3 nm were used for all analyses. The peak area was applied for evaluation of lead response. Temperature set for the Pb determination in whole blood is given in Table 1.

Table 1. The Furnace AAS programme

Step	Temp, °C	Ramp Time	Hold Time	Internal Flow	Read Step
1	110	1	60	250	
2	140	10	40	250	
3	1000	10	10	250	
4	1800	0	5	0	X
5	2400	1	2	250	

The blood sample with low lead was used for the method of standard additions for calibration. Lead intermediate standard of 100 $mg.l^{-1}$ and 1 $mg.l^{-1}$ were prepared by adjusting of stock standard solution of 1.000 $g.l^{-1}$ (the Slovak Institute of Metrology, Bratislava). Then there were 10, 20, 50, 75, 100 $\mu g.l^{-1}$ of 1 $mg.l^{-1}$ standard solution diluted with 0.5% HNO_3 in 10 ml volumetric flask. This corresponded to 70, 140, 350, 525, 700 $\mu g.l^{-1}$ Pb.

3. RESULTS AND DISCUSSION

3.1. Uncertainty estimate

The method shall have uncertainty estimated especially in the case if the observed concentration is compared with reference value. Under regulation limit, the concentration of lead in blood referred to as harmful in occupational exposure is 700 $\mu g.l^{-1}$. Medical examination is recommended if the concentration of lead in blood exceeds the level 400 $\mu g.l^{-1}$.

In principle the development of a comprehensive mathematical model describing the test procedure can be impractical. Factors such as diffusion between matrix modifier solution and sample solution, temperature, the use of volumetric flasks, centrifuge, operation on AAS and process of calibration contribute to the uncertainty. Rigorous identification and statistical quantification can be long lasting and non-effective. Therefore, the sources of uncertainty were identified in accordance with Armishaw´s estimating measurement uncertainty in the practical application of measurement uncertainty of toluene measurement in water (Armishaw, 2003). Armishaw identified method recovery, sample recovery, precision, homogeneity and calibration as sources of uncertainty in GC-MSD measurement. All these components were calculated using the AAS method of lead determination in blood sample, measured within validation procedure.

Quantitative measurement in atomic absorption (Atomic Absorption Laboratory Benchtop, 1992), used in the method of addition calibration, are based on an equation *(1)*:

$$C = -K_1 \cdot A \qquad (1)$$

where C is a concentration added to an aliquot of sample, A is a difference between the absorbance for the aliquot with added standard and the absorbance measured for the sample. The final sample concentration is calculated by multiplying the slope ($-K_1$) times the absorbance of the sample. The least square technique is used to determine the K_1 coefficient when two or more standards are used for calibration. Method of standard addition is used on the first sample and then group of samples, having a similar matrix is analysed. The concentrations of the remaining samples are determined from the calibration curve, generated with the first sample.

The effect of uncertainty components can modify (Mocák et al, 1998) the equation *(1)*

$$C = -K_1 \cdot A \cdot f_{rm} \cdot f_{rs} \cdot f_{\mathrm{hom}} \cdot f_{std} \qquad (2)$$

where f_{rm} – recovery method, f_{rs} – recovery sample, f_{hom} – homogeneity, f_{st} – preparation of the standards. There is a condition when the method is under statistical control in the definition range and the uncertainty of the method is the standard deviation of the normal distribution (σ_y) at a given true value and must be constant in the definition range of the method.

Combined standard uncertainty for the model above is given by the equation *(3*(Armishaw, 2003)*)*:

$$\frac{u_{(c)}}{C} = \sqrt{\left(\frac{u_{rm}}{rm}\right)^2 + \left(\frac{u_{rs}}{rs}\right)^2 + \left(\frac{u_{dup}}{dup}\right)^2 + \left(\frac{u_{std}}{std}\right)^2} \qquad (3)$$

Method recovery uncertainty (u_{rm}) - A series of seven spiked blood samples at concentration (100 – 700) $\mu g.l^{-1}$ of lead equidistantly covered definition range was selected to estimate method recovery uncertainty. The spiked blood samples went through the whole analytical procedure and thus represent many particular contributions in course of sample preparation. Least square regression analysis was used to estimate the standard deviation of predicted values, standard deviation - s_x obtained 16.17 $\mu g.l^{-1}$. This value is considered to be uncertainty of the method recovery (u_{rm}) calculated at the concentration 400 $\mu g.l^{-1}$ in the centre of linear regression.

Sample recovery uncertainty (u_{rs}) – fresh-prepared matrix blood control samples spiked with 100 $\mu g.l^{-1}$ of lead were analysed with each series of measured sample. The obtained average recovery was 97.6% and standard deviation 4.2 % (n = 13 control sample).

Sample homogeneity uncertainty (u_{dup}) - six blood samples selected at random were analysed in duplicates. Variability between duplicates was normalised to the mean ratio of duplicates according to equations *(4)*:

$$\frac{A}{(A+B)/2} \qquad \frac{B}{(A+B)/2} \qquad (4)$$

A, B are concentrations of each duplicate. The average ratio of duplicate series is 1.0 and the standard deviation of ratio series is 0.106.

Calibration standard uncertainty (u_{std}) - the lead standard of purity 100.02±0.19% was used for the calibration. The rectangular distribution modifies the uncertainty to the value of 0.19/√3 = 0.11%.

The obtained results of uncertainty calculated are summarized in Table 2.

Table 2. Summary of measured uncertainty contributions

uncertainty contributions:	Value	Standard deviation
method recovery (u_{rm}), µg.L^{-1}	400	16.17
sample recovery (u_{rs}), %	97.6	4.2
sample homogeneity (u_{dup})	1.0	0.106
calibration standard (u_{std}), %	100.02	0.11

The combined standard uncertainty calculated *(5)* is 97 µg.l^{-1}, (cca 24%) when using equation (*3)* for the concentration 400 µg.l^{-1} and the coverage factor k=2.

$$u_{(400)} = 400 \cdot \sqrt{\left(\frac{16.17}{400}\right)^2 + \left(\frac{4.2}{97.6}\right)^2 + \left(\frac{0.106}{1.0}\right)^2 + \left(\frac{0.11}{100.02}\right)^2}$$

$$U = u \cdot k = u_{(400)} \cdot 2 = 97\,\mu g \cdot L^{-1} \qquad (5)$$

The combined standard uncertainties at other concentrations were calculated and the results are given in the Figure 1 where the relative combined uncertainty is plotted versus mentioned concentration range. The relative combined uncertainty decreases from 52% to approximately 23% in the concentration range (70 – 700) µg.L^{-1}. These results are comparable with the Armishaw's conclusion (Armishaw, 2003). High level of relative standard uncertainty at low concentration is connected with high variability at low concentrations.

This fact reflects the ULA (Mocák et al., 1997) computation limit of detection, based on one-sided upper confidence limit of the blank signal, critical value of *t*-distribution, residual standard deviation, s_{yx}, and degrees of freedom *v, v* = n-2. LOD and LOQ computed by this way, were 13 µg.l^{-1} and 38 µg.l^{-1} respectively.

3.2. Exposure assessment

The evaluated method of PbB levels was applied in everyday monitoring of non-occupationally and occupationally Pb-exposured people.

The results of 60 up to date PbB analyses indicate that exposure to lead

continues to be a serious problem in the Slovak industry. The Slovak binding biological exposure index (BEI) value of 700 μg.l^{-1} for workers was exceeded in one case. However, PbB concentrations were higher than the indicative BEI value of 400 μg.l^{-1} in about 37,5 % of employees.

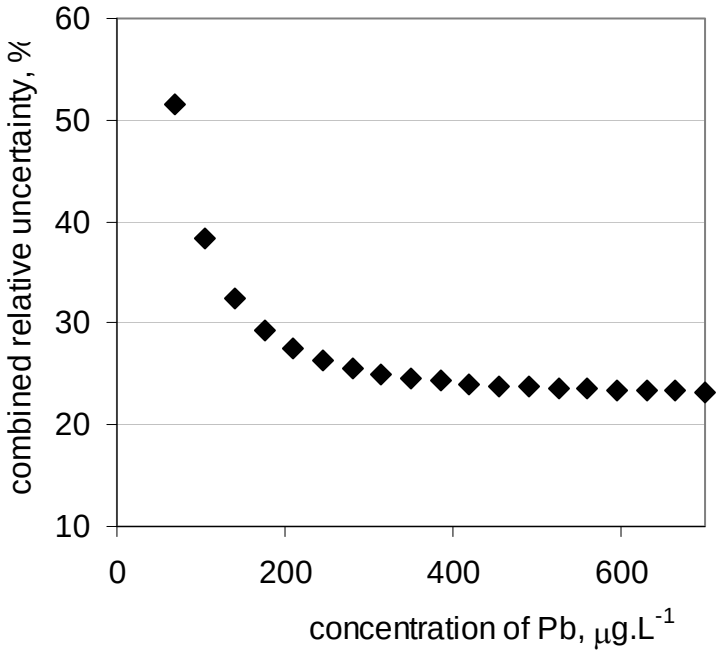

Fig. 1. Relation of combined relative uncertainty and concentration of Pb in blood

Lead is a component of tobacco and tobacco smoke, and smokers often have higher lead blood levels than non-smokers. PbB levels in smokers and non-smokers were analyzed and correlation between tobacco smoke and exposure levels was observed. The arithmetic mean PbB level in smokers was higher (324 μg.l^{-1}) than in non-smokers (198 μg.l^{-1}). The size of the group as well as ignorance of the exact exposure dose, do not enable to postulate explicit conclusions.

It could be considered that the lead hazard is particularly acute in small enterprises and some employees in Slovakia are still at risk to health due to adverse effects from Pb exposure. However, exposure to lead is dependent not only upon the concentrations of lead in workplace air but also upon the personal hygiene and personal habits of the worker.

The necessity of PbB determinations, the improvement of working conditions and the implementation of the health education for workers are the measures to be promptly taken. In order to achieve these goals, a close cooperation between the Authorities of Public Health and the Labour inspectorates as well as the employers are required.

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