Estimating uncertainty and quality of lead determination in blood in the occupation exposure

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Occupational exposure to lead

- presents a significant hazard in the onset of serious intoxication. Potentially high levels of lead may still occur in the
  - lead smelting
  - refining industries
  - battery manufacturing plants
  - steel welding or cutting operations.

Lead is a potent, systemic poison that causes unknown harm once absorbed by body

Occupational exposure to lead

- Significant portion of the lead that is inhaled or ingested, gets into blood stream [3]. 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 [3].

Lit. [3] *ATSDR toxicological profile for lead. Draft.* Agency for Toxic Substances and Disease Registry, Atlanta, 2005.

Occupational exposure to lead

- Chronic overexposure to lead may result in severe damage to blood-forming, nervous, urinary and reproductive systems [4], kidney disease.

- Lit. [4] *Environmental health criteria 165: Inorganic lead.* World Health Organization, Geneva, 1995.

Occupational exposure to lead

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

Slovak regulation limit

- In compliance with, Slovak regulation limit the concentration of lead in blood referred to as harmful in occupation exposure is 700 μg/L.
- Medical examination is recommended if concentration of lead in blood exceeds the level of 400 μg/L.
- The government order No 355/2006, Coll., on the protection of workers from the risks related to exposure to chemicals factors at work

Analytical method

- After venipuncture, blood samples were collected in plastic 2.7 mL Li-Heparin sample tubes (SARSTEDT, Monovette) which contained EDTA as anticoagulant [5].
- 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% $NH_4H_2PO_4$, in 0,4% TRITON - 100 p.a.
- 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.

- Lit.: Morton, S.: **Lead in whole blood**. *Atom. Spectr., 2000, 12, 5, 24-28.*

Analytical method

- 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.

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

Uncertainty estimate

- 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 [10], 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.

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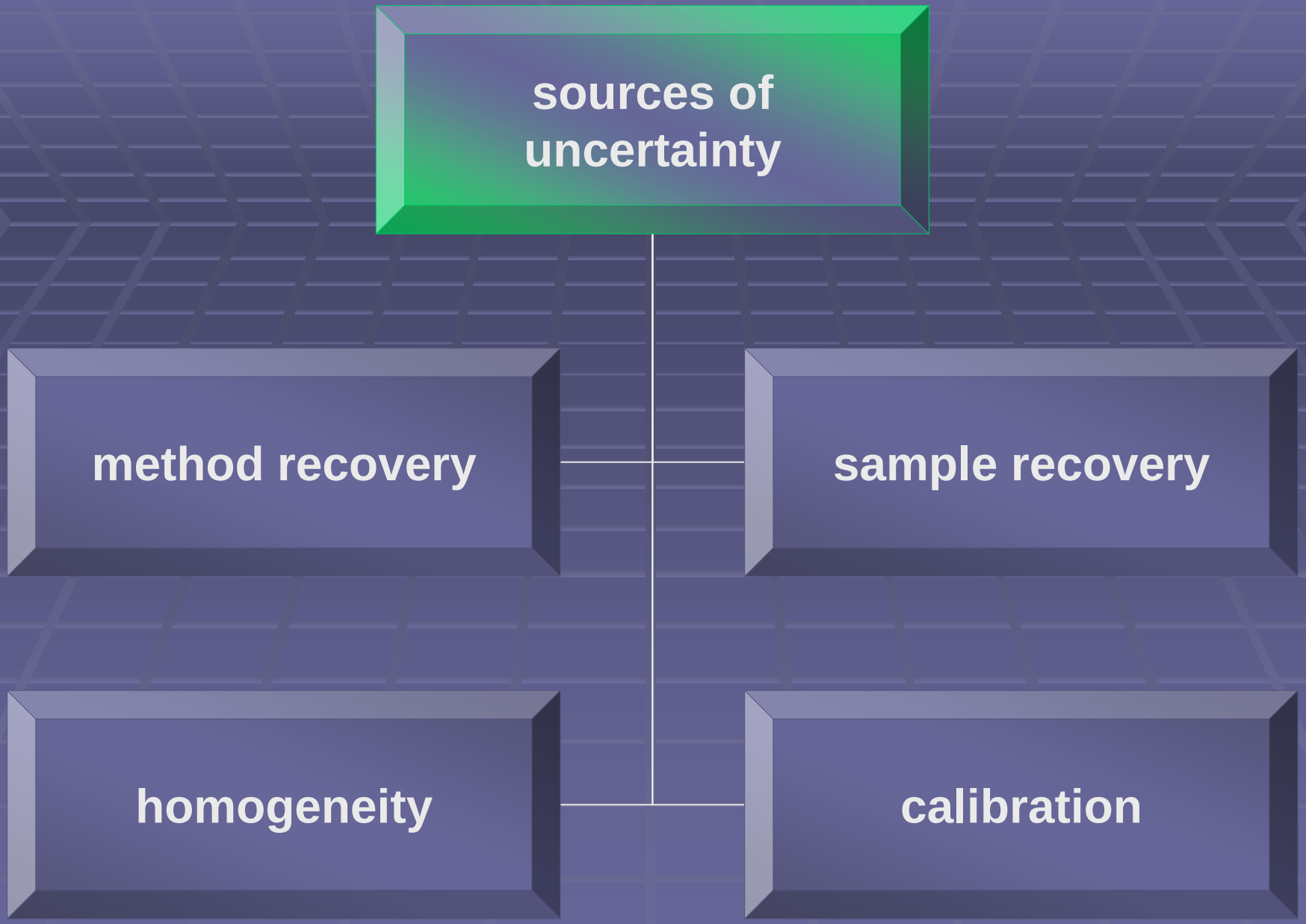
Lit. [10] - *STN EN ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories*. SÚTN, Bratislava, 2005



Lit.: Armishaw, P.: **Estimating measurement uncertainty in the afternoon. A case study in the practical application of measurement uncertainty**. *Accred. Qual. Assur., 2003, 8, 218-224*.

Combined standard uncertainty

$$\frac{u_{(y)}}{y} = \sqrt{\left(\frac{u_{(a)}}{a}\right)^2 + \left(\frac{u_{(b)}}{b}\right)^2 + \left(\frac{u_{(c)}}{c}\right)^2 + \ldots}$$

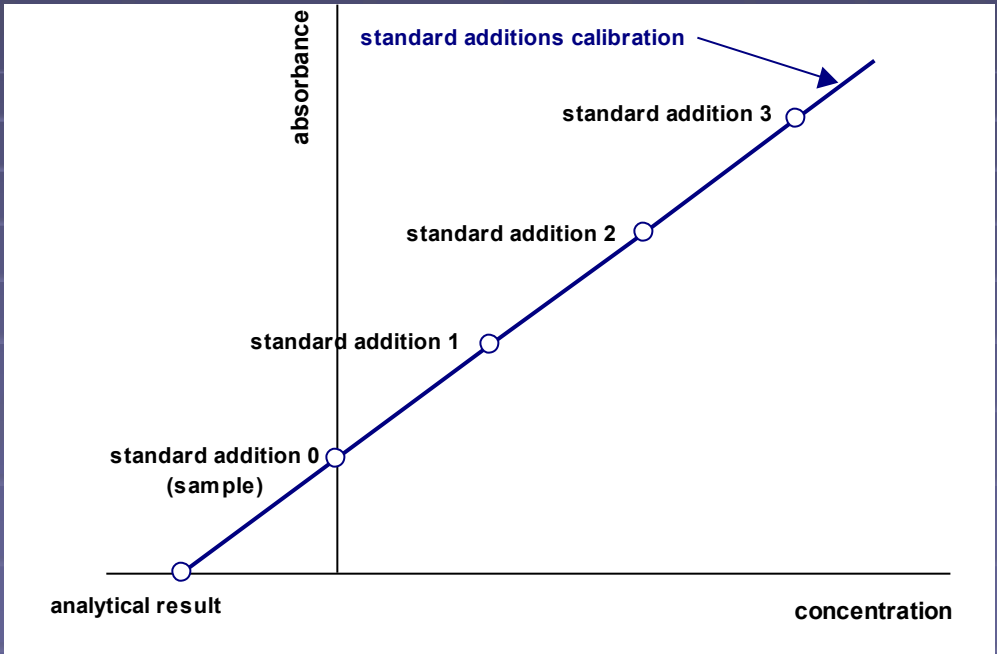
$$\frac{u_{(c)}}{C} = \sqrt{\left(\frac{u_{rm}}{rm}\right)^2 + \left(\frac{u_{rs}}{rs}\right)^2 + \left(\frac{u_{dup}}{dup}\right)^2 + \left(\frac{u_{std}}{std}\right)^2}$$

Lit.: Mocák, J., Kordík, D., Máriássy, M., Vyskočil, L. : **Vyjadrovanie neistôt pri meraní**. In: *Súčasné trendy pri vyhodnocovaní analytických metód a postupov. CHEMIS, Bratislava, 1996, 26-38.*

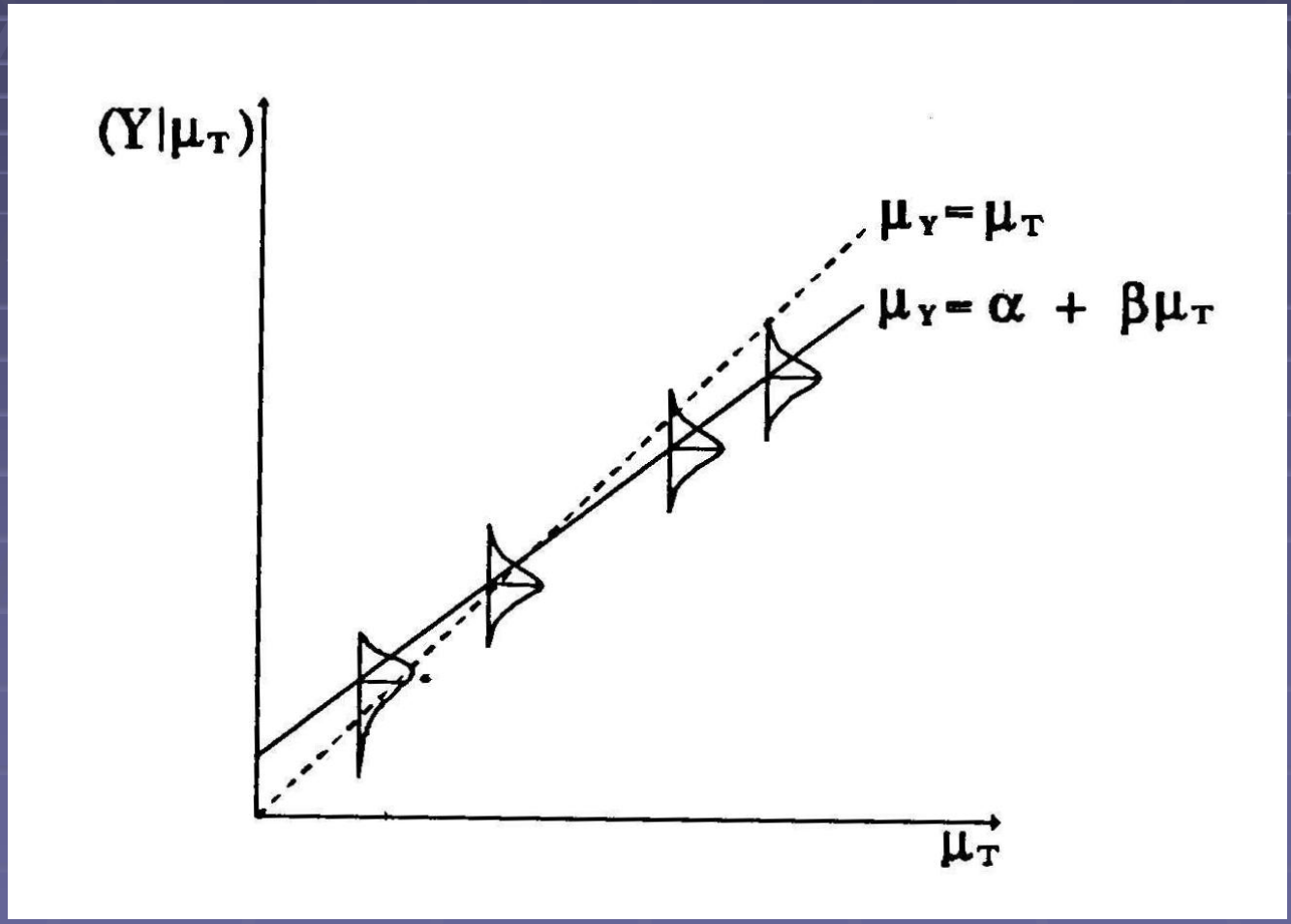
1.Method recovery uncertainty (u_{rm})

- A series of seven spiked blood samples at concentration (100 – 700 μg/L) 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.

Standard addition calibration



Lit.: AMIQAS, User´s Guide, 1. Version. National Institute of Occupational Health, Denmark,
September, 1993



1.Method recovery uncertainty (*urm*)

- Least square regression analysis was used to estimate the standard deviation of predicted values, standard deviation - s_x obtained 16.17 μg/L.
- This value is considered to be uncertainty of the method recovery (u_{rm}) calculated at the concentration 400 μg/L in the centre of linear regression.

2.Sample recovery uncertainty (u_{rs})

- Fresh-prepared matrix blood control samples spiked with 100 μg/L 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)

3.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

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

- *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

4.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

  $0.19/\sqrt{3} = 0.11\%$.

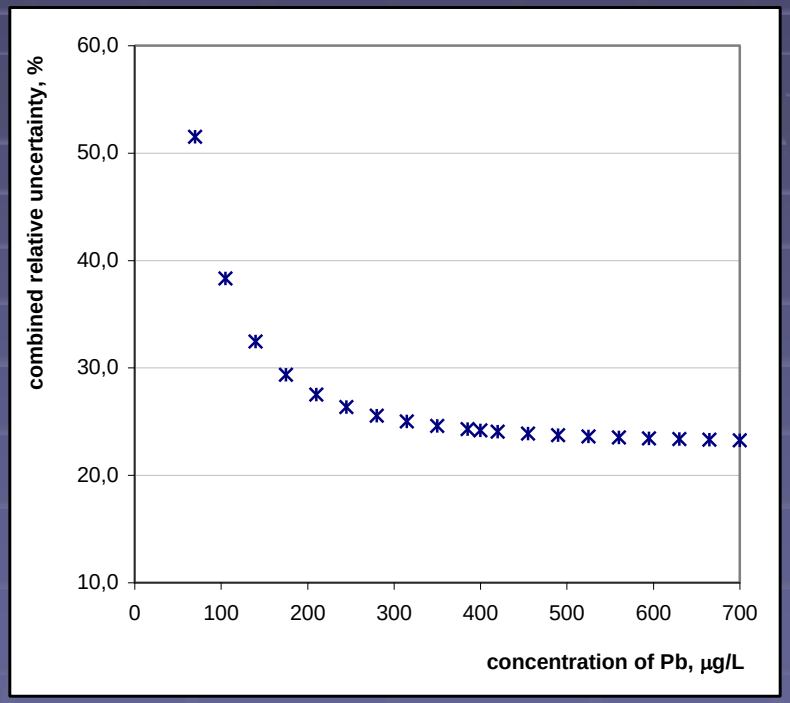
uncertainty contributions:	Value	standard deviation
method recovery (u_{rm}), µg/L	400	16.17
sample recovery (u_{rs}), %	97.6	4.2
sample homogeneity (u_{dup})	1.0	0.106
calibration standard (u_{std}), %	100.02	0.11

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

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

$$U = u \cdot k = u_{(400)} \cdot 2 = 97\ \mu g / L$$

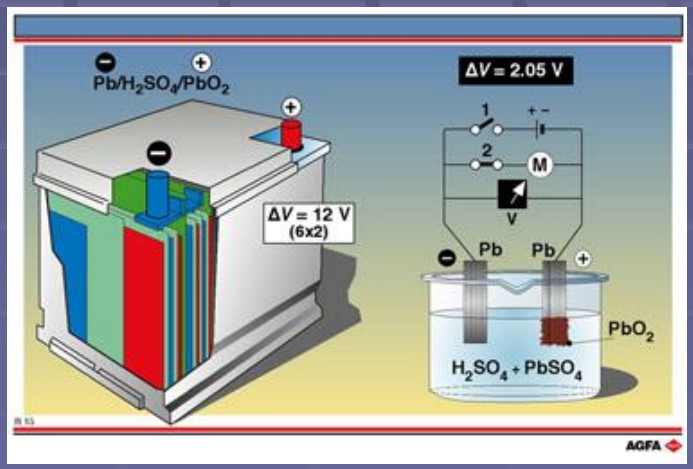
combined standard uncertainties at other concentrations



The relative combined uncertainty decreases from 52% to approximately 23% in the concentration range 70 – 700 µg/L.

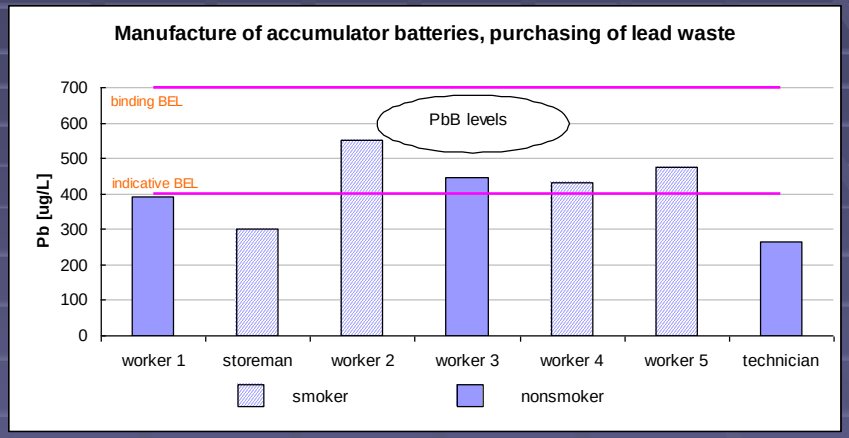
Occupational exposure

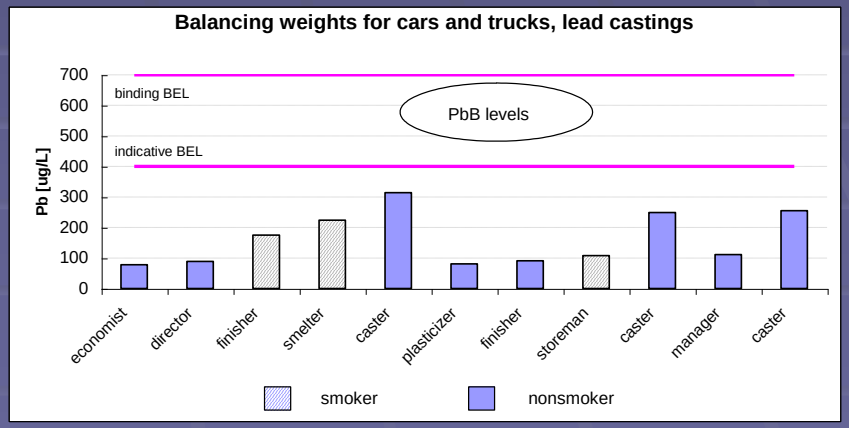
- The lead exposure of 18 employees in 2 enterprises in Slovakia was studied. The determination of blood lead (PbB) levels was performed in a lead battery factory (*n=7*) and at the balancing weights for cars and trucks operation (*n=11*).



Occupational exposure

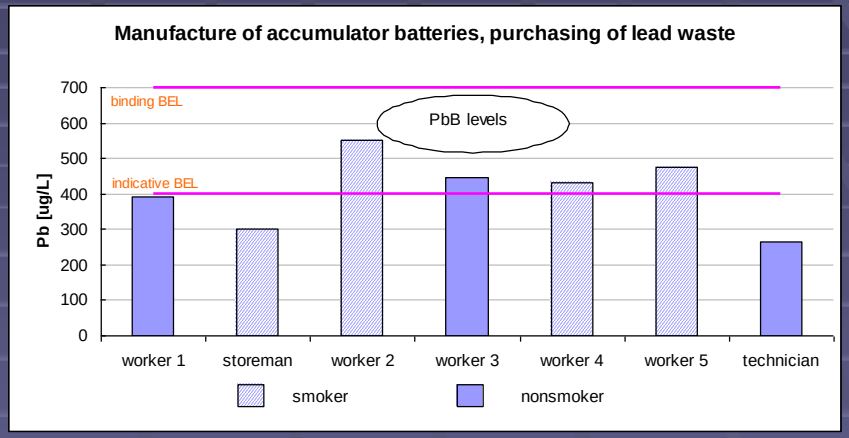
- The arithmetic mean PbB level in the lead battery factory was much higher (409 µg/L) than at the of balancing weights operation (161µg/L).
- PbB levels were significantly increased in workers who directly manipulated with lead accumulator and lead waste in the battery factory.

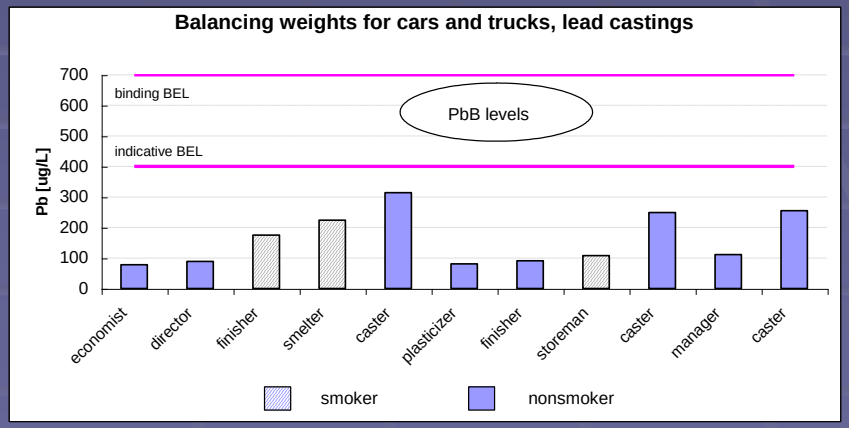




Occupational exposure

- 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) than in non-smokers (198 µg/L).
- The size of the group as well as ignorance of the exact exposure dose, do not enable to postulate explicit conclusions.





Occupational exposure

- 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, occupational 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.

- Ďakujem za pozornosť