

# Workpackage 6

## “Utility and sensitivity of biomarkers”

Marek Jakubowski & Danuta Ligocka,  
Nofer Institute of Occupational Medicine,  
91-348 Lodz , 8 Teresy St., Poland.

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Marek Jakubowski, Nofer Institute of Occupational  
Medicine,  
91-348 Lodz , 8 Teresy St., Poland.  
e-mail: [majakub@imp.lodz.pl](mailto:majakub@imp.lodz.pl)

Only **the biomarkers of exposure** for which there is sufficient analytical experience, in terms of **validated analytical methods**, are currently regarded as suitable to develop and test out a harmonized approach to HBM at the European level.

The basic scenario will cover: lead in blood, cadmium and cotinine in urine, and methylmercury in scalp hair.

# Lead

Lead is a well known neurotoxic metal. Impaired neurodevelopment in children is the most critical health effect.

## PROSPECTIVE AND CROSS-SECTIONAL STUDIES

→ negative associations between PbB and psychometric intelligence in children

## CROSS-SECTIONAL AND PROSPECTIVE STUDIES

→ Pb-B levels below 250  $\mu\text{g/l}$ , the size of apparent IQ effect (at ages 4 and above) is a deficit of 0÷5 points (on a scale of 100, SD=15) for each 100  $\mu\text{g/l}$  increment in Pb-B level, with a likely apparent effect size of 1 – 3 points.

1991 – CDC recommended the Pb-B for children should be **below 100  $\mu\text{g/l}$**

2000 – The WHO Air Quality Guidelines for Europe recommended that at least **98%** of the population exposed in the general environment should have **Pb-B below 100  $\mu\text{g/l}$** , and the **median** blood lead level should not exceed **54  $\mu\text{g/l}$** .

However, the results of a recent study imply that in children, the effects of environmental lead exposure can occur at levels below **100 $\mu\text{g/l}$**

## Children, Low level blood lead below 100 $\mu\text{g/l}$

1. At ages 3 and 5 years  $\rightarrow$  the related declines in IQ are greater than at higher levels

2. Children whose maximal Pb-B remained **below 100  $\mu\text{g/l}$** , IQ declined by **7.4 points** as the lifetime average Pb-B increased from 10 to 100  $\mu\text{g/l}$

3. Recent findings  $\rightarrow$  a possible impairment of the neuropsychological functions and lead-related **deficit in colour vision**.

## Women

In **pregnancy** maternal lead transfer follows a pattern similar to that for calcium without any barrier at the placental level.

Particularly during **the last part of pregnancy** and the lactation period when **maternal Pb-B** increases by **25–100%**.

This increase derives from the further mobilization of lead from bones. **Pb-B in infants** is mainly the expression of **skeletal lead stores**.

In 2006 the Scientific Committee on Neurotoxicology and Psychophysiology and the Scientific Committee on the Toxicology of Metals of the ICOH resolved that current exposure standards for lead urgently need to be reduced.

For children, the action level, which triggers community prevention efforts to reduce exposure sources, should be immediately reduced to a Pb-B concentration of 50 µg/l.

Also for female industrial workers of reproductive age, the standard for Pb-B should be reduced to the lowest obtainable, preferably to 50 µg/l.

In environmental exposure to lead, the health effects can be related to the blood lead levels (Pb-B).

Reports on the geometric mean values in different European countries revealed that in women and children, Pb-B levels are within the range of 10–30  $\mu\text{g}/\text{l}$  considered as a „baseline” of minimal anthropogenic origin.

The PbB levels are **log normal distributed**, then even at relatively low geometric mean values, a proportion of the population will have PbB **above 100  $\mu\text{g/l}$**

- at a geometric mean PbB level of **36  $\mu\text{g/l}$** ,  **$\sim 1.5\%$**  of the population will have Pb-B above 100  $\mu\text{g/l}$ ,
- at a geometric mean PbB level of **60  $\mu\text{g/l}$** ,  **$\sim 6\%$**  of the population will have Pb-B above 100  $\mu\text{g/l}$ .

Cognitive effects in children are associated with Pb levels in blood of about 100–150  $\mu\text{g/l}$ ,

The recently published data provide strong evidence that they can occur at Pb-B levels below 100  $\mu\text{g/l}$ .

There may be no threshold for these effects.

In general, blood lead levels have been decreasing over the last few decades, mainly due to the elimination of leaded petrol.

However, elevated exposures can still occur due to local sources.

# Cadmium

## Critical effects

**Kidneys** and **bones** – in the chronic environmental exposure.

- increased urinary excretion of **low molecular weight proteins** (as a result of proximal tubular cell damage)
- an increased risk of **osteoporosis**.

## Source of cadmium exposure

**Food** (above 90% of total intake in non-smokers)  
**soil** and **dust** (in heavily contaminated areas)

# Kidneys

The relation between cadmium exposure and tubular and glomerular function in women with cadmium in blood 0.38 µg/l, urine 0.67 µg/g creatinine → effects on renal tubules (indicated by increased levels of human complex-forming protein and NAG in urine).

Tubular renal effects occurred at lower cadmium levels the effects were relatively moderate, they may represent early signs of adverse effects, affecting large segments of the population

## Bones

Environmental and occupational exposure to cadmium the dose-response relationship was found between cadmium dose and osteoporosis.

The OR for men was 2.2 (95% CI, 1.0–4.8) for the dose of 0.5–3  $\mu\text{g Cd/g creatinine}$  and 5.3 (2.0–14) for the highest dose (>3  $\mu\text{g Cd/g creatinine}$ ) compared with the lowest dose level (< 0.5  $\mu\text{g Cd/g creatinine}$ ).

For women, the OR was 1.8 (0.65–5.3) for the dose of 0.5–3  $\mu\text{g Cd/g creatinine}$

## Biomarkers of exposure

Cadmium concentration in urine (Cd -U) is mainly influenced by the body burden and it is proportional to cadmium concentration in healthy kidney.

The geometric mean Cd-U concentrations in non-smokers amounted to 0.15-0.20 µg/g creat.

## Reference values

In Germany (2003), the P50 and P95 values of Cd-U (n=4740, adults 18–69 years) amounted to 0.22 and 0.96  $\mu\text{g}/\text{g creat.}$  (0.20 and 0.76 in non-smokers ; 0.29 and 1.0 in smokers).

The proposed reference value is 0.8  $\mu\text{g}/\text{g creat.}$

In the Czech Republic (2006), the mean Cd-U level amounted to 0.31  $\mu\text{g}/\text{g creat.}$  and the proposed reference value ( P 95) is 1.2  $\mu\text{g}/\text{g creat.}$

## Trends of exposure

for cadmium, mercury and lead:

!!! Annual decreases of  $\sim 6\%$  – for Ery-Pb & EryHg levels.

!!! Cd, the **decline Ery-Cd** → in smokers

Not decrement of cadmium body burden in non-smokers over the last decade

!!! **Environmental sources** of Cd have **not changed significantly**

The **margin of safety** between the present dietary daily intake of cadmium and the level of intake which can bring about **health effects** is **very narrow**.

Highly exposed subpopulations, this margin may even be **non-existing**. Population groups at risk include the **elderly, diabetics** and **smokers**.

**Women** may be at an increased risk because they **absorb more cadmium** than men due to the lower iron stores.

Therefore, monitoring of cadmium levels in urine is highly recommended.

# Methylmercury

## Critical effects

**Adults** – differ both quantitatively and qualitatively from the effects observed after **prenatal** or, possibly, **postnatal exposure**.

The critical organ is the **nervous system**

1. developmental neurologic abnormalities in human **infants**,

2. paraesthesia in **adults**

3. Prenatal exposure was reported to cause psychomotor retardation in **infants**

The benchmark dose, (endpoints onset of walking and talking, neurologic scores, mental symptoms, and seizures) 11  $\mu\text{g/g}$  in maternal hair, equivalent to maternal blood level of 44  $\mu\text{g/l}$  or daily intake of 1.1  $\mu\text{g/kg/day}$ .

The same for methylmercury-associated delays on evoked potential latencies of approximately 5% BMDL 10  $\mu\text{g/g}$  maternal hair

The US National Academy of Sciences Committee on the Toxicological Effects of Methylmercury, applying an **uncertainty factor of 10**, arrived at the value of about **1  $\mu\text{g/g}$  mercury** in maternal hair.

**TDI of 0.1  $\mu\text{g/kg/day}$** , the USEPA current RfD, would result in such level

## Biological indicators of exposure

About 90% of mercury found in red blood cells was in the form of MeHg. THg in plasma was associated with both IHg and MeHg.

THg in RBC and hair are suitable proxies for MeHg exposure.

THg in urine is a suitable proxy for IHg exposure

The present **background level of Hg-H**

- low fish consumption of low fish methyl mercury concentration amount to 0.25  $\mu\text{g/g}$  – 0.8  $\mu\text{g/g}$  in,
- 0.06  $\mu\text{g/g}$  in non-fish eating

Hair T-Hg and blood MeHg increased with increasing total fish consumption

Hg-H levels ranged from 1.6  $\mu\text{g/g}$  (1 fish meal/week) to 5.2  $\mu\text{g/g}$  (4 fish meals/week)

In fishermen from Madeira (Portugal) and their families, levels of 38.9  $\mu\text{g/g}$  in men and 10.4  $\mu\text{g/g}$  in women.

The average total fish consumption – 4 times/week → T-Hg in hair 0.70  $\mu\text{g/g}$ )

MeHg in blood 1.7  $\mu\text{g/l}$  (0.30–14  $\mu\text{g/l}$ ,  $r_s = 0.78$ ,  $p < 0.001$ ).

High fish consumption can result in hair mercury levels several times higher than the  $1 \mu\text{g/g}$  recommended by the US Academy of Sciences.

## Cotinine

Environmental tobacco smoke (ETS) – complex mixture of thousands of compounds.

Inhalation of tobacco smoke is the main source of nicotine exposure for the general population.

Cigarettes contain about 1.5% nicotine by weight.

In the United States, nicotine concentrations in the homes where smokers dwell ranged from less than  $1 \mu\text{g}/\text{m}^3$  to over  $10 \mu\text{g}/\text{m}^3$ .

WHO has estimated that some 9 –13% of all cancer cases can be attributed to ETS in a non-smoking population (50 % exposed to ETS).

In infants, the proportion of lower respiratory illness attributed to ETS exposure – 15–26%, (35% of the mothers smoke at home)

Those estimates, when applied to the European population, will result in approximately 3 000–4 500 cancer cases per year among adults, and between 300 000 and 550 000 episodes of lower respiratory illness per year in infants

Up to 92 % of the nicotine delivered in smoke is absorbed from the lungs into the blood stream.

**Cotinine** is a metabolite of nicotine and is currently regarded as **the best biomarker** in active smokers and in nonsmokers exposed to environmental tobacco smoke.

For cotinine, the half-life in blood plasma is about 16 hours

**Cotinine** can be measured in serum, urine, saliva, and hair.

Three biomarkers ( Pb-B, Cd-U and MeHg-H) have health-based guidance values and risk associations for adverse health outcomes.

1. Methods of determination
2. reference materials
3. external quality assurance system

are available for all four presented parameters.

## Development and validation of biomarkers

Most chemical substances which are the focus of discussion on environmental health worldwide can be measured with HBM (e.g. the CDC program).

However, there is a need for a larger number of validated biomarkers that would allow for an accurate risk assessment and clear risk communication.

The validation efforts aim at harmonizing analysis and data interpretation.

The substances in question include: phthalates and PAHs, organotin compounds, inorganic arsenic, brominated flame retardants ( BFR) and perfluorooctane sulfonate (PFOS).

In addition, the use of saliva as a matrix in HBM will be assessed.

## Phthalates and PAH

Phthalates are among the most abundant environmental contaminants worldwide.

The EU has classified three phthalates: di-(2-ethylhexyl) phthalate (DEHP), di-n-butylphthalate (DnBP) and benzylbutyl phthalate (BBP), as toxic to reproduction.

## Biomarkers of exposure

HBM has been proven to be an excellent tool for human phthalate exposure assessment in several studies, mainly from Germany and the USA.

Biomonitoring data show that the general population is ubiquitously exposed to phthalates.

The parameter spectrum has been increasing, implementing the **secondary, oxidized metabolites** which are not prone to contamination and have longer half-lives than the simple monoesters.

# PAHs

Because of their carcinogenic properties and ubiquity, PAHs are an important environmental–health problem of worldwide concern.

## Biomarkers of exposure

- the internal PAH exposure – the urinary metabolite **1-hydroxypyrene** (1HP) as a biomarker of pyrene.
- **Monohydroxylated phenantrenes** (OH–Phen), metabolites of phenantrene, are also used as suitable biomarkers for PAH–exposure.
- Recently, the Human Biomonitoring Commission of the German Federal Environmental Agency published a **reference value** (background burden of the non–smoking general population) of **0.5 µg hydroxypyrene /l urine**.

## Brominated flame retardants (BFR) – HBM of PBDEs

Flame retardants have a wide range of applications in today's consumer products, textiles, electronic etc. Polybrominated diphenyl ethers (PBDEs) are one of the main groups of BFR.

Due to the large production volume, lack of regulations, and their persistence, PBDEs are now ubiquitous environmental pollutants. They have been identified in various environmental media, as well as in human tissues.

PBDEs levels are increasing in the environment and biota at an exponential rate. In Swedish human milk, PBDE concentrations have increased from 1972 to 1997 with redoubling every 5 years, but samples from 1998 and later showed decreasing levels.

In experimental studies, PBDEs have had low acute toxicity but in repeated exposure they could produce thyroid hormon disruption, developmental neurotoxicity, and some changes in fetal development

## Biomarkers of exposure

Human samples such as serum/plasma, breast milk, and adipose tissue have been used for biomarker studies to assess the extent of human exposure to PBDEs.

Breast milk levels 200 ng/g fat 3 – 4 ng/g fat

The following PBDE–congeners are considered to be most important for human biomonitoring purposes: BDE–47, 99, 100, 153, and 209.

## Perfluorooctanoic acid/Perfluorooctane sulfonate (PFOA/PFOS)

tendency to bioaccumulation and biomagnification.

Organic fluorine has been found in the serum of all human populations studied. PFOA was the principal organic fluorine compound in human serum because it has a long biological half-life.

## Organotins

Toxicity data indicate that human exposures to these compound may impair the immune, nervous and reproductive systems.

Inorganic arsenic is a known human carcinogen. Investigation of the arsenate/arsenite/MMA,DMA will be carried out by assessing the levels of these arsenic compounds in urine. The use of saliva as an alternative non-invasive matrix will also be studied.

## Saliva as a matrix for HBM

Saliva is a promising non-invasive matrix for human biomonitoring. The saliva content of chemicals, chemical residues or metabolites can be used as an indicator of exposure.

Non-invasive matrixes have an obvious advantage in large-scale biomonitoring programs because the samples are easy to collect, process and store, plus the ethical obstacles are greatly reduced.

However, the use of saliva in environmental analysis is still in its infancy and the predictive value needs to be established.

The perspective of pilot project gives an opportunity to compare the levels of contaminants in saliva with those in other matrixes like blood, urine and hair in the same individuals.

We do hope that in spite of the complexity of the problem, the implementation of HBM on the European scale will start soon.

Thank you for your attention