EXPOSURE TO ARSENIC IN RELATION WITH DNA DAMAGE IN FLEMISH ADOLESCENTS

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CONCEPT OF HUMAN BIOMONITORING (HBM)

- Complex relation between environmental pollution and human health
  - Presence of chemicals: reference values in three age groups
  - Monitoring hotspots
    - Elevated environmental exposure
    - Belonging to specific social classes, with specific dietary habits

**FLEHSII (2007-2011): 606 adolescents**

**FLEHSIII (2012-2016): 408 adolescents**
Two classes of biomarkers can be measured: biomarkers of exposure and biomarkers of effect.

**Environmental monitoring**
- Air
- Dust
- Consumer products
- Water
- Soil

**Biomarkers of exposure**
- Heavy metals in blood
- Phthalates in urine
- BPA in urine
- Cotinine in urine
- ...

**Biomarkers of effect**
- Hormone levels
- DNA damage
- Gene expression
- Epigenetic changes
- ...

**Adverse health effects**
- Asthma
- Cancer
- Puberty
- IQ / behavior
- Birth weight
# EXPOSURE TO ARSENIC

## Sources of exposure

- Contaminated drinking-water and food  
  (e.g. seafood, poultry, cereals, rice, apple juice)
- Industrial and environmental processes  
  (e.g. mining and smelting of metal ores, antifungal wood preservatives, alloying agent, pesticides)
- Tobacco

## Health effects

- **Acute**: vomiting, abdominal pain and diarrhoea
- **Long-term**: skin lesions, cancer (e.g. skin, lung, bladder), developmental effects, neurotoxicity, diabetes and cardiovascular disease

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*WHO, factsheet ‘Arsenic’, December 2012*
WHAT HAPPENS TO ARSENIC IN THE BODY?

Arsenic is excreted in urine in several chemical forms:

- **organic arsenic species**: arsenobetaine and arsenecholine
- **species associated with exposure to and metabolism of iAs**: arsenous and arsenic acid (together, iAs)
- **through methylation reactions**: monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)

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S.M. Hays et al./Regulatory Toxicology and Pharmacology 58 (2010) 1-9
ASSESSMENT OF THE GENOTOXIC POTENTIAL

**COMET ASSAY**

**MICRONUCLEUS TEST**

**8-HYDROXYDEOXYGUANOSINE (8-OHDG)**

Formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) by oxygen radical.

DIFFERENT EXPOSURE CONDITIONS

In vivo animal experiments

In vitro experiments

Occupational exposure

Arsenic (As) is a known human carcinogen; however, very little is known about the health consequences of occupational exposure to As. In the present study, we assessed the genotoxic damage in the blood cells and in the buccal cells of south Indian glass factory workers who are occupationally exposed to As. The As content in the whole blood of 200 workers and 165 controls was evaluated with inductively coupled plasma mass spectrometry. Blood leukocytes from the subjects were monitored for the level of DNA damage using the Comet assay (mean comet tail length); buccal cells were used to determine the frequency of micronuclei (MN). The mean As concentration was significantly higher in the workers (56.76 µg/L) than in the controls (11.74 µg/L) (P < 0.05). After assay conducted with formamidopyrimidine glycosylase (FPG) digestion to detect oxidative DNA damage indicated that oxidative lesions were present in leukocytes from both the exposed and control groups, but the levels of damage were significantly higher among the workers. Incubation of the cells in culture resulted in a significant reduction in the levels of DNA damage, especially among leukocytes from the workers, suggesting that the DNA damage was subject to repair. Our findings indicate that copper smelter workers have increased levels of DNA damage in somatic cells, suggesting a potential health risk for the workers. Although i-As was present in air samples from the smelters and in urine samples from workers, no clear association could be made between i-As exposure and the DNA damage. Environ. Mol. Mutagen. 46:81–87, 2005. © 2005 Wiley-Liss, Inc.

Key words: occupational exposure; arsenic; DNA assay

Key words: arsenic; occupational exposure; human lymphocytes; Comet assay; urine
Are current levels of arsenic (As) in Flemish adolescents associated with oxidative stress biomarkers or biomarkers of DNA damage?
MATERIAL AND METHODS

Study population:
» 408 adolescents (14-15 y.o.)

Individual exposure to As:
» total As in whole blood
» toxic As metabolites in urine:
  » AsIII, AsV, MMA, DMA
  » sum = toxic relevant As (TRA)
» non-toxic, organic compound arsenobetaine

DNA damage:
» alkaline comet assay in whole blood
» micronucleus test in whole blood
» 8-OHdG in urine

Statistics: multiple linear regression models
» adjusted for gender, age, smoking status and statistically significant (p<0.05) covariates
» AsIII was categorised due to the large number of values below the quantification limit (LOQ)
### DESCRIPTIVE STATISTICS

#### Table: Geometric mean, minimum-maximum range (P25-P75) for biomarkers of arsenic exposure and DNA damage

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Unit</th>
<th>Number</th>
<th>Geometric mean</th>
<th>Range (min.-max.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As in blood</td>
<td>μg/L</td>
<td>406</td>
<td>0,78</td>
<td>0,13 – 11,3</td>
</tr>
<tr>
<td>Arsenobetaine in urine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>μg/L</td>
<td>404</td>
<td>2,24</td>
<td>0,04 – 259</td>
</tr>
<tr>
<td>AsV in urine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>μg/L</td>
<td>407</td>
<td>0,18</td>
<td>0,03 – 2,74</td>
</tr>
<tr>
<td>MMA in urine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>μg/L</td>
<td>407</td>
<td>0,57</td>
<td>0,05 – 4,49</td>
</tr>
<tr>
<td>DMA in urine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>μg/L</td>
<td>407</td>
<td>3,45</td>
<td>0,06 – 60,2</td>
</tr>
<tr>
<td>TRA in urine&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>μg/L</td>
<td>407</td>
<td>4,69</td>
<td>0,49 – 63,0</td>
</tr>
<tr>
<td>Comet assay</td>
<td>% DNA migration</td>
<td>386</td>
<td>1,17</td>
<td>0 – 4,8</td>
</tr>
<tr>
<td>8-OHdG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>μg/L</td>
<td>407</td>
<td>13,4</td>
<td>1,85 – 55,3</td>
</tr>
<tr>
<td>Micronucleus test</td>
<td># MN/1000 binuclear cells</td>
<td>376</td>
<td>1,6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0 – 7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Correction for specific gravity.

<sup>b</sup>TRA = sum arsenic III, arsenic V, MMA and DMA

<sup>c</sup>The arithmetic mean was calculated for the micronucleus test.

#### Table: Arsenic III as a categorical variable

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Unit</th>
<th>Number</th>
<th>LOQ&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Category</th>
<th>Number (proportion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AsIII in urine</td>
<td>μg/L</td>
<td>407</td>
<td>0,1 μg/L</td>
<td>&lt;0,1 μg/L</td>
<td>227 (56%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0,1 – 0,3 μg/L</td>
<td>103 (25%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt; 0,3 μg/L</td>
<td>77 (19%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>LOQ: limit of quantification
A Biomonitoring Equivalent (BE) is defined as the concentration of a chemical in blood or urine that corresponds to an allowable exposure guidance value, such as a reference dose (RfD) or tolerable daily intake (TDI), considered safe by regulatory agencies.

- important tool for interpreting population-based biomonitoring data
- rely on existing chemical risk assessments developed by EPA and similar government agencies
- low to high priority for “risk assessment follow-up”
  - keeping exposures at acceptable levels
### RELATION BETWEEN ARSENIC AND DNA DAMAGE MEASURED BY THE COMET ASSAY

#### Table: Significant exposure-response relationships for the comet assay

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Confounders</th>
<th>Covariates</th>
<th>Estimate* (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AsIII</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat2 vs. &lt; LOQ</td>
<td>Gender Age Smoking status</td>
<td>Education BMI Average sunshine last 7 days Average PM$_{2.5}$ last 7 days</td>
<td>-0.081 (-0.310; 0.149) 0.333 (-0.061; 0.605) 0.090 (-0.014; 0.166)</td>
<td>0.49 0.02 0.02</td>
</tr>
<tr>
<td>Cat3 vs. &lt; LOQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMA$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$MMA was corrected for specific gravity.

95% CI: confidence interval; LOQ: limit of quantification

![Relation between MMA and comet assay](relation_mma_comet.png)
# Relation Between Arsenic and 8-OHdG in Urine

## Table: Significant exposure-response relationships for 8-OHdG in urine (µg/L)\(^a\)

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Confounders</th>
<th>Covariates</th>
<th>Estimate* (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AsIII</td>
<td></td>
<td>Education</td>
<td>1.216 (1.114; 1.326)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Age</td>
<td>1.231 (1.107; 1.369)</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Cat2 vs. &lt; LOQ</td>
<td>Average UV last 2 days</td>
<td>1.074 (1.014; 1.137)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Cat3 vs. &lt; LOQ</td>
<td>Smoking status</td>
<td>1.154 (1.105;1.205)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AsV(^a)</td>
<td></td>
<td>Education</td>
<td>1.074 (1.014; 1.137)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Age</td>
<td>1.154 (1.105;1.205)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Smoking status</td>
<td>1.099 (1.041;1.160)</td>
<td>0.0007</td>
</tr>
<tr>
<td>MMA(^a)</td>
<td></td>
<td></td>
<td>1.131 (1.073; 1.192)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DMA(^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRA(^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Correction for specific gravity.

95% CI: confidence interval; LOQ: limit of quantification
NO SIGNIFICANT ASSOCIATIONS WERE FOUND WITH THE MICRONUCLEUS TEST
Seafood is the main source of organic arsenic compounds. Most fish and shellfish have high tissue levels of arsenobetaine, a non-toxic arsenical that in humans is excreted unchanged via the kidneys contributing to total urine arsenic.

* p < 0.001
CONCLUSION

Association of arsenic with oxidative stress and DNA damage

» chronic exposure to low levels of toxic inorganic As
  » 82% of the adolescents had TRA concentration < BE

» transient DNA damage?
  » significant associations with the comet assay and concentrations 8-OHdG
  » no significant associations with the micronucleus test

» concentrations of organic, non-toxic As metabolite arsenobetaine can be linked to fish consumption based on questionnaires
ACKNOWLEDGEMENTS

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» PIH (Provincial Institute of Hygiene)
» AML (Algemeen Medisch Laboratorium)

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THANK YOU!

THANK YOU FOR LISTENING!

QUESTIONS