NEW SPECIFIC AND SENSITIVE BIOMONITORING METHODS FOR CHEMICALS OF EMERGING HEALTH RELEVANCE

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THE GERMAN HUMAN BIOMONITORING PROJECT

Aim of the project is the development of biomonitoring methods for selected substances for which by now no suitable analytical method exists. Up to 50 new methods from 2010 – 2020!

Cooperation project between

BMUB (The German Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety),

UBA (The German Federal Environment Agency)

and

VCI (The German Chemical Industry Association)
HBM PROJECT - STEPWISE APPROACH

1. Selection of chemical – expert group / steering group / BMUB, UBA, VCI
   - Substance:
     - which might be taken up by the population in larger amounts
     - which could be relevant to human health
     - for which no (suitable) biomonitoring method exists
   - Examples: plasticizers, foam components, flame retardants, antioxidants, UV filters

2. Development of analytical method – analytical lab / expert group / VCI
   - search for new specific markers (based on animal or human kinetic study; analogy to similar substances)
   - urine as the preferred matrix (compared to blood)
   - detection by LC/MS/MS or (HR)-GC/MS with
     - lowest possible limit of quantification
     - minimum blood / urine volume
   - application to ca. 40 persons to get a first hint of possible background level
   - report with detailed method description; DFG-approval: experimental investigation from another lab according DFG-requirements
   - publications in peer-reviewed journal; presentations on scientific congresses
HBM PROJECT - STEPWISE APPROACH

3. Application of new developed biomonitoring method in population – UBA
   • biomonitoring performed in the framework of
     ➢ The German Environmental Survey (GerES)
     ➢ Environmental Specimen Bank (ESB)
     ➢ Others

4. Derivation of HBM value - German HBM Commission / UBA
   • To evaluate body burden measured
HBM-PROJECT - METHOD DEVELOPMENTS
BY CURRENTA BIOMONITORING

Presented here:

1. Di(2-propylheptyl)phthalate (DPHP)
2. 2-Mercaptobenzothiazole (MBT)
3. 3,5-Di-tert-butyl-4-hydroxytoluene (BHT)
4. Hexabromocyclododecane (HBCDD)

matrix: urine

5. 4,4´-Methylene diphenyl diisocyanate (MDI)
6. 2,4- / 2,6-Toluene diisocyanates (TDI)

matrix: blood

Further method developments:

7. 4-Nonylphenol (NP)
8. 4-tert-Octylphenol (OP)
9. 3-(4-Methylbenzylidene)camphor (4-MBC)
10. Mesamoll
11. Climbazole

classification

matrix: urine

matrix: blood
DI-2-PROPYLHEPTYL-PHTHALATE (DPHP)

Reasons for nomination:
• New introduced substitute for endocrine active phthalates
• used as a plasticizer for technical PVC and VC application
• No specific biomonitoring method available

Aim:
• Environmental Specimen Bank: point of entry
• population monitoring: presence of substance although only technical use
DPHP – POSTULATED METABOLISM

Bis(2-propylheptyl)phthalate

OH-MPHP: Mono-2-(propyl-6-hydroxy-heptyl)-phthalate

cx-MPHxP: Mono-2-(propyl-6-carboxy-hexyl)-phthalate

oxo-MPHP: Mono-2-(propyl-6-oxoheptyl)-phthalate

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DPHP-HUMAN ELIMINATION KINETIC STUDY

Aim:

1. To examine if Cx-MPHxP, OH-MPHP and Oxo-MPHP are the main DPHP-metabolites in humans; to verify postulated metabolism
2. To get information about the elimination kinetics of DPHP in humans as well as the conversion factors

Study design:
- 5 male volunteers
- Oral intake of about 50 mg D4-DPHP
- Urine collected during the first 48 hours after intake in intervals
- Analysis of DPHP metabolites

Performed by Holger Koch at IPA
approved by Ethical commission University of Bochum
(registration number 4022-11, 24.5.2011)
DHPH - ELIMINATION KINETICS

DHPH-metabolites are eliminated within 48 h
24 % of applied oral dose is found

Conversion factors:
Oxo-MPHP: 12.6 ± 3.9 %
OH-MPHP: 9.9 ± 3.5 %
Cx-MPHxP: 0.4 ± 0.1 %

Excretion [µg/g creatinine]

Time [h]

0 12 24 36 48

Excretion [µg/g creatinine]

0 10 100 1000 10000

oxo-MPHP-d4
OH-MPHP-d4
cx-MPHxP-d4
Dphp – Detection: LC-MS/MS vs GC-HRMS

<table>
<thead>
<tr>
<th></th>
<th>LC-MS/MS (routine phthalate method)</th>
<th>GC-HRMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found: Sum of metabolites DPHP + DINP + DIDP</td>
<td>Found: Only metabolites DPHP</td>
</tr>
<tr>
<td>Sum of cx</td>
<td>28.3</td>
<td>&lt; 0.15</td>
</tr>
<tr>
<td>Sum of OH</td>
<td>127.7</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>Sum of oxo</td>
<td>16.9</td>
<td>0.25</td>
</tr>
<tr>
<td>Mean (µg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range (µg/L)</td>
<td>2.1 - 99.7</td>
<td>&lt; 0.15</td>
</tr>
<tr>
<td></td>
<td>7.7 - 337</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td></td>
<td>1.1 - 49.2</td>
<td>&lt; 0.25 - 0.72</td>
</tr>
<tr>
<td>Detection rate (%)</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>58</td>
</tr>
</tbody>
</table>

Samples provided by Holger Koch, IPA
Urine of workers exposed to DINP+DIDP

LOQ: cx-MPHxP: 0.15 µg/l urine
OH-MPHP: 0.30 µg/l urine
oxo-MPHP: 0.25 µg/l urine

Valid GC-HRMS method for the selective detection of DPHP metabolites in urine successfully developed!
### DPHP – APPLICATION:
PRESTUDY OF GerES V IN 2013 (N = 51)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N &gt; LOQ</th>
<th>Range (µg/l)</th>
<th>Mean (µg/l)</th>
<th>Median (µg/l)</th>
<th>90th Percentil (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cx-MPHxP</td>
<td>0</td>
<td>0.075*</td>
<td>0.075*</td>
<td>0.075*</td>
<td>0.075*</td>
</tr>
<tr>
<td>OH-MPHP</td>
<td>20</td>
<td>0.15*- 3.81</td>
<td>0.507</td>
<td>0.150*</td>
<td>1.113</td>
</tr>
<tr>
<td>Oxo-MPHP</td>
<td>17</td>
<td>0.125*- 3.27</td>
<td>0.404</td>
<td>0.125*</td>
<td>0.921</td>
</tr>
</tbody>
</table>

* = LOQ/2

Far below HBM-value of 1500 µg/L urine (adults) and 1000 µg/L urine (children)
DPHP – APPLICATION:
GERMAN ENVIRONMENTAL SPECIMEN BANK
1999 - 2012 (N = 300; 60 SAMPLES PER YEAR)

maximum daily DPHP intake: 0.32 µg/kg BW/d
2-MERCAPTOBENZOTHIAZOLE (MBT)

Reasons for nomination:
• Substance which might be taken up by the population in larger amounts
  • Vulcanization accelerator especially in rubber products
  • Mostly used for car tires
• No biomonitoring method available

Aim:
• population monitoring: presence of substance
• Environmental Specimen Bank: presence of substance

HBM-I-values:
• adults: 7000 µg/L urine
• children: 4500 µg/L urine
MBT – POSTULATED METABOLISM

In rats: after oral application 90 % is excreted within 96 hours, mainly as glucuronid

(Fukuoka and Tanaka 1987)

By enzymatic hydrolysis of urine the total amount of MBT is measured.
MBT – METHOD

- Total MBT is measured after enzymatic hydrolysis
- Application of high-pressure liquid chromatography tandem mass spectrometry (HPLC–MS–MS) in positive-electrosprayionization mode (ESI+) using isotope-dilution quantification

- High sample throughput by use of column-switching technique
- LOQ: 1 µg/L; LOD: 0.4 µg/L

Valid LC-MS/MS method for the detection of 2-MBT in urine successfully developed!
1. Results of 40 urine samples of humans not occupationally exposed to MBT:
   - 39 samples < 1 µg/L
   - 1 sample: 10.8 µg/L

2. Results of workers exposed to MBT - 4 urine samples:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N &gt; LOQ</th>
<th>Range (µg/L)</th>
<th>Mean (µg/L)</th>
<th>Median (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“total” MBT</td>
<td>4</td>
<td>567 - 6210</td>
<td>3958</td>
<td>4527</td>
</tr>
<tr>
<td>“free” MBT</td>
<td>3</td>
<td>&lt; 1 - 137</td>
<td>69</td>
<td>70</td>
</tr>
</tbody>
</table>

LOQ: 1 µg/l urine

Far below HBM-value of 7000 µg/L urine
## 2-MBT – APPLICATION: PRESTUDY OF GerES V IN 2013 (N = 51)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N &gt; LOQ</th>
<th>Range (µg/l)</th>
<th>Mean (µg/l)</th>
<th>Median (µg/l)</th>
<th>90th Percentil (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBT</td>
<td>10</td>
<td>0.6*- 5.95</td>
<td>0.975</td>
<td>0.6*</td>
<td>1.74*</td>
</tr>
</tbody>
</table>

* = LOQ/2; LOQ: 1 µg/l urine

Far below HBM-value of 7000 µg/L urine (adults) and 4500 µg/L urine (children)
2,6-DI-TERT-BUTYL-P-CRESOL (BHT)

Reasons for nomination:
- Substance which might be taken up by the population in larger amounts
  - Antioxidant in food, animal feedstuff, drugs
- No specific biomonitoring method available

Aim:
- Population monitoring: presence of substance

HBM-value in progress
Main metabolite (40 % of uptake) is BHT-acid.
BHT-ACID – METHOD

- BHT-acid is measured after enzymatic hydrolysis
- UPLC-MS/MS with 2 D Chromatography
- LOQ: 0.2 µg/l urine; LOD: 0.06 µg/l urine

Valid LC-MS/MS method for the detection of BHT-acid in urine successfully developed!
### BHT - APPLICATIONS

BHT-acid in 72 of 80 urine samples of humans not occupationally exposed to BHT:

1st group:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N &gt; 0.2 µg/l</th>
<th>Range (µg/l)</th>
<th>Mean (µg/l)</th>
<th>Median (µg/l)</th>
<th>90th Percentil (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT-acid</td>
<td>39</td>
<td>0.2 – 3.77</td>
<td>0.82</td>
<td>0.59</td>
<td>1.69</td>
</tr>
</tbody>
</table>

2nd group:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N &gt; 0.2 µg/l</th>
<th>Range (µg/l)</th>
<th>Mean (µg/l)</th>
<th>Median (µg/l)</th>
<th>90th Percentil (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT-acid</td>
<td>33</td>
<td>0.2 - 7.55</td>
<td>1.28</td>
<td>0.83</td>
<td>2.89</td>
</tr>
</tbody>
</table>
HEXABROMOCYCLODODECANE (HBCDD)

Reasons for nomination:
• Substance which might be taken in by the population in larger amounts
• Worldwide found in mother milk; sediment; fish
  • Flame retardant in insulators (polystyrene plastics; electronic devices)
• No specific biomonitoring method available

Aim:
• population monitoring: presence of substance
• Environmental Specimen Bank: point of introduction

HBM-value: 1.6 µg/L plasma
HBCDD – METHOD

- Valid LC-MS/MS method for the detection of α-HBCDD, β-HBCDD and γ-HBCDD in blood plasma successfully developed

α-HBCDD (9-13%)  β-HBCDD (0.5-12%)  γ-HBCDD (72-90%)

- LOQ: 0.10 µg/L plasma; LOD: 0.03 µg/L plasma
### HBCDD - APPLICATIONS

HBCDD-isomers in 90 plasma samples of humans not occupationally exposed

1st group - 48 samples:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N &gt; 0.03 µg/L (LOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-HBCDD</td>
<td>3 (0.031-0.084); 6.3%</td>
</tr>
<tr>
<td>β-HBCDD</td>
<td>0</td>
</tr>
<tr>
<td>γ-HBCDD</td>
<td>0</td>
</tr>
</tbody>
</table>

2nd group - 42 samples:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N &gt; 0.03 µg/L (LOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-HBCDD</td>
<td>3 (0.036-0.053); 7.1%</td>
</tr>
<tr>
<td>β-HBCDD</td>
<td>4 (0.037-0.090); 9.5%</td>
</tr>
<tr>
<td>γ-HBCDD</td>
<td>0</td>
</tr>
</tbody>
</table>

- all values below LOQ 0.10 µg/L
- far below HBM-value of 1.6 µg/L plasma
THANK YOU FOR YOUR ATTENTION!

Biomonitoring Team of Currenta
www.biomonitoring.currenta.de