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)

Bundesministerium für Umwelt, Naturschutz, Bau und Reaktorsicherheit

Umwelt Bundesamt

VCI

25 Jahre Responsible Care in Deutschland

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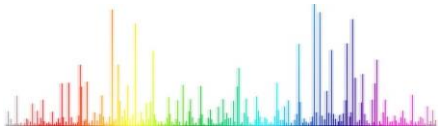
HBM PROJECT - STEPWISE APPROACH

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



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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

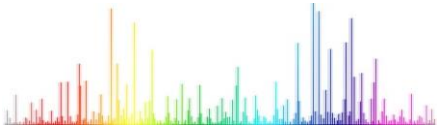
Presented here:

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

matrix: urine

4. Hexabromocyclododecane (HBCDD)

matrix: blood



Further method developments:

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

matrix: blood

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

matrix: urine

DI-2-PROPYLHEPTYL-PHTHALATE (DPHP)

Reasons for nomination:

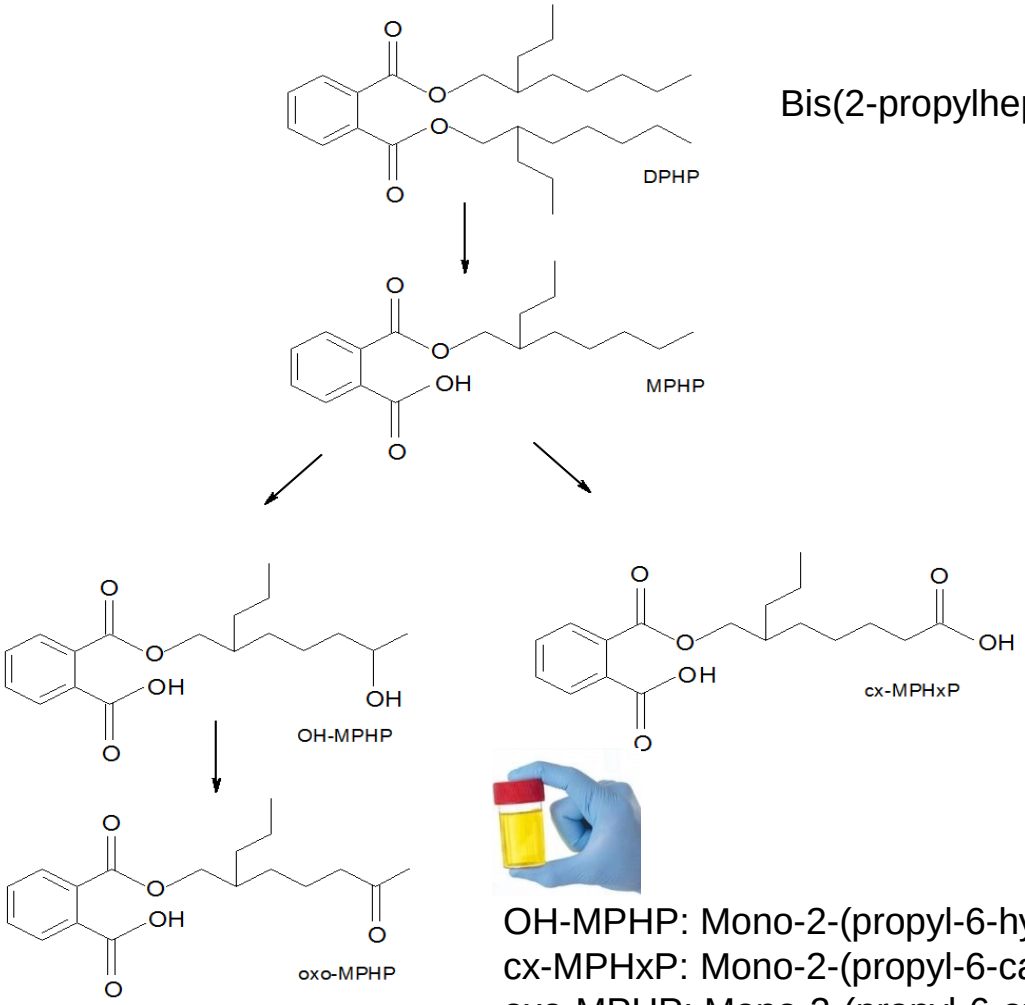
- New introduced substitute for endocrine active phthalates
- used as a plasticizer for technical PVC and VC application

- 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:

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

Study design:

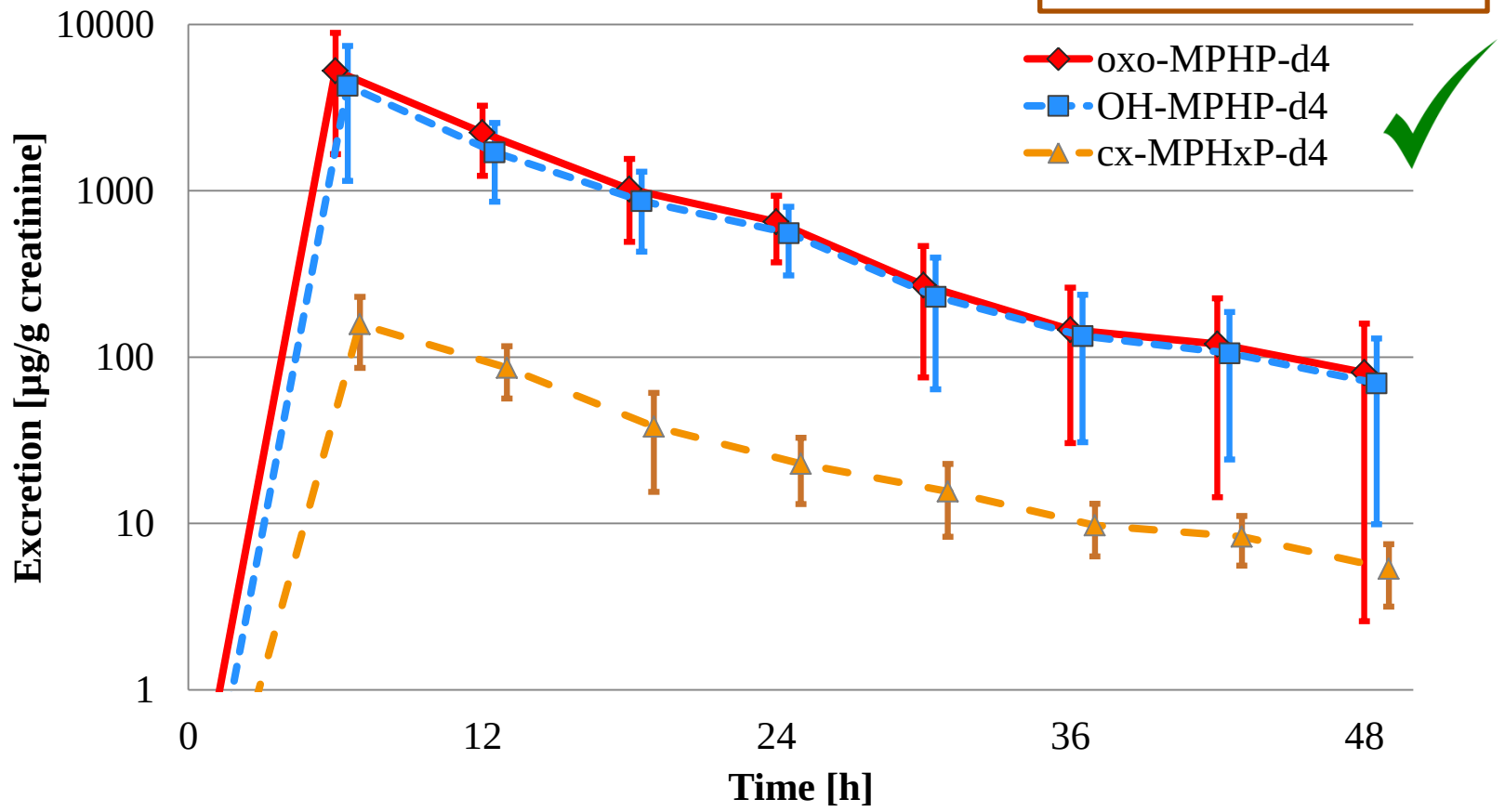
- 5 male volunteers
- Oral intake of about 50 mg D4-DPHP
- Urine collected during the first 48 hours after intake in intervals
- Analysis of DPHP metabolites

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

DPHP - ELIMINATION KINETICS

DPHP-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 %



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DPHP – DETECTION: LC-MS/MS VS GC-HRMS

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

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!

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DPHP – APPLICATION: PRESTUDY OF GerES V IN 2013 (N = 51)

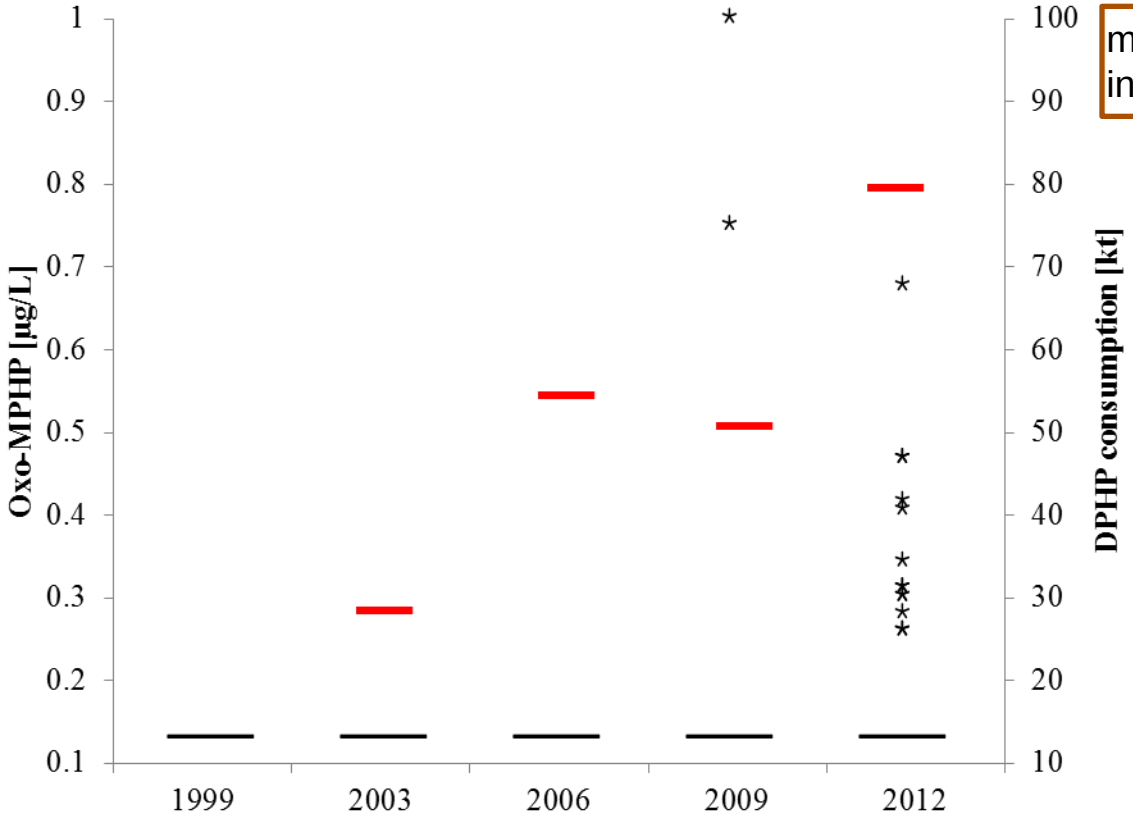
Analyte	N > LOQ	Range (µg/l)	Mean (µg/l)	Median (µg/l)	90th Percentil (µg/l)
Cx-MPHxP	0	0.075*	0.075*	0.075*	0.075*
OH-MPHP	20	0.15*- 3.81	0.507	0.150*	1.113
Oxo-MPHP	17	0.125*- 3.27	0.404	0.125*	0.921

* = 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

	OH-MPHP		oxo-MPHP	
Year	% > LOQ	Maximum	% > LOQ	Maximum
1999	0	< LOQ	0	< LOQ
2003	0	< LOQ	0	< LOQ
2006	0	< LOQ	0	< LOQ
2009	3.3	0.64	3.3	0.96
2012	3.3	0.36	21.7	0.65

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2-MERCAPTOBENZOTHIAZOLE (MBT)

Reasons for nomination:

- Substance which might be taken up by the population in larger amounts
  - Vulcanization accelarator especially in rubber products
  - Mostly used for car tires

- No biomonitoring method available

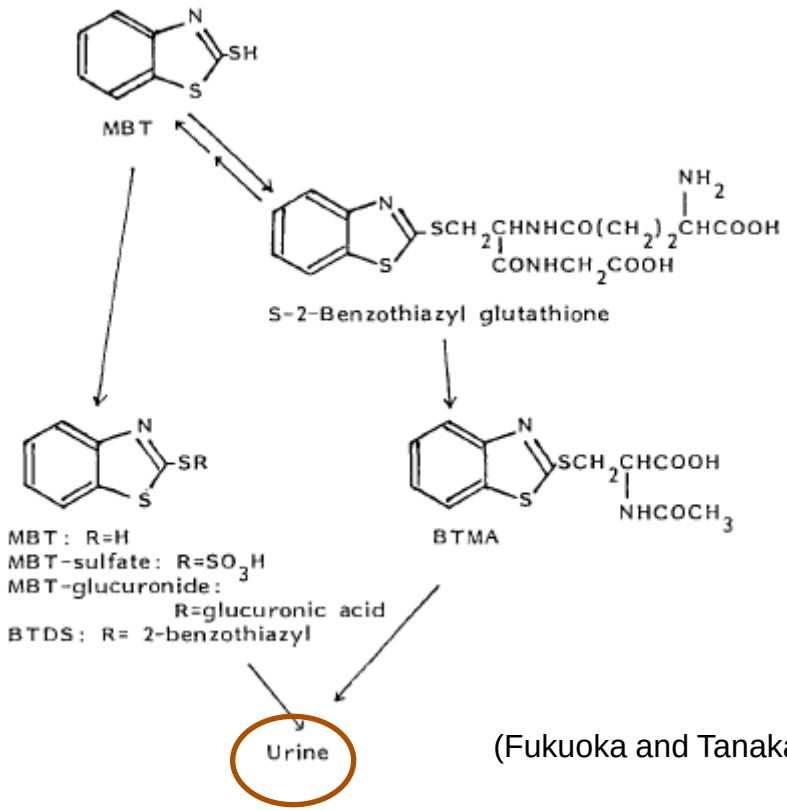
Aim:

- 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



(Fukuoka and Tanaka 1987)

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

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

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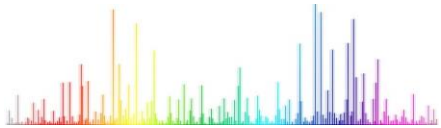
MBT – METHOD

- 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!

2-MBT – APPLICATIONS

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:

Analyte	N > LOQ	Range (µg/L)	Mean (µg/L)	Median (µg/L)
"total" MBT	4	567 - 6210	3958	4527
"free" MBT	3	< 1 - 137	69	70

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)

Analyte	N > LOQ	Range (µg/l)	Mean (µg/l)	Median (µg/l)	90th Percentil (µg/l)
MBT	**10**	**0.6*- 5.95**	**0.975**	**0.6***	**1.74***

* = 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
  - Anitoxidant in food, animal feedstuff, drugs

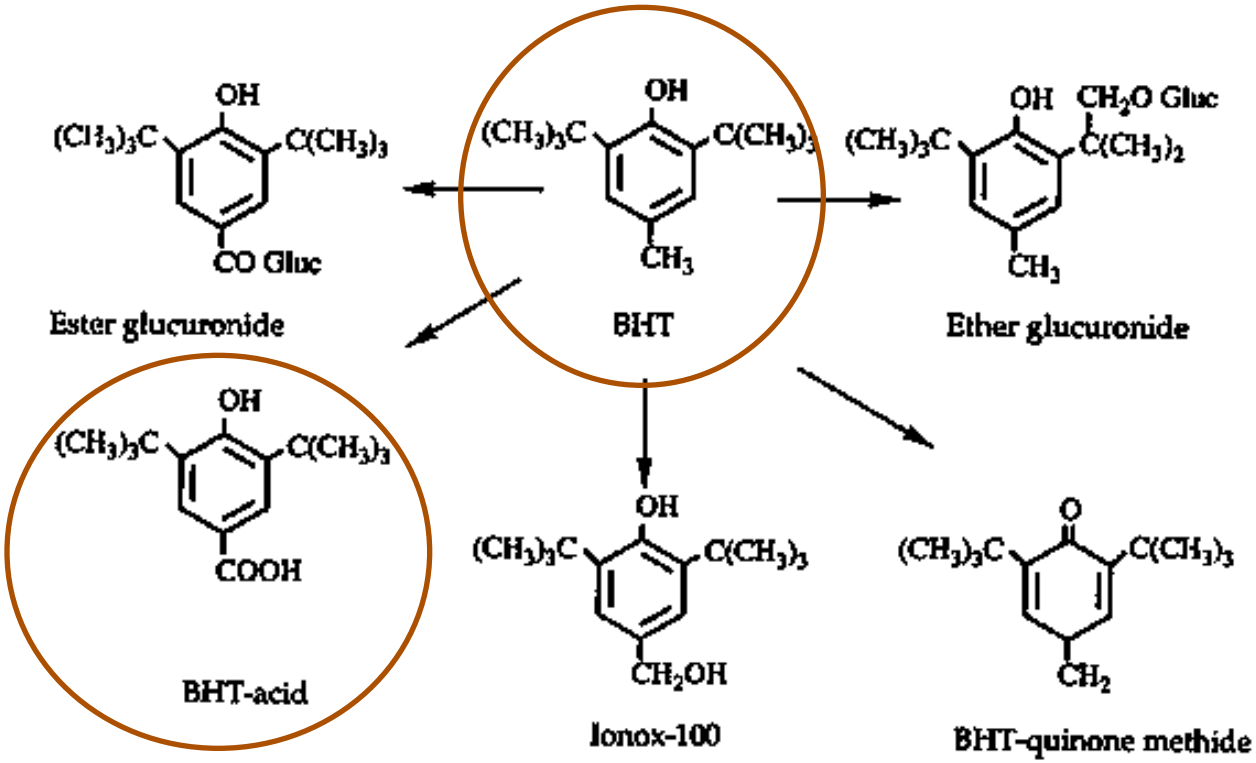
- No specific biomonitoring method available

Aim:

- population monitoring: presence of substance

HBM-value in progress

BHT – POSTULATED METABOLISM



Food Antioxidants: Technological, Toxicological, and Health Perspectives
Edited by DL Madhavi, SS Deshpande, and DK Salunkhe / Dekker 1996

Main metabolite (40 % of uptake) is BHT-acid.

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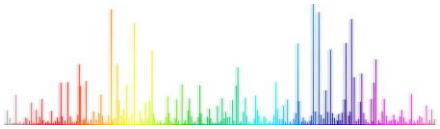
BHT-ACID – METHOD

- 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!

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BHT - APPLICATIONS

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

1st group:

Analyte	N > 0.2 µg/l	Range (µg/l)	Mean (µg/l)	Median (µg/l)	90th Percentil (µg/l)
BHT-acid	39	0.2 – 3.77	0.82	0.59	1.69

2nd group:

Analyte	N > 0.2 µg/l	Range (µg/l)	Mean (µg/l)	Median (µg/l)	90th Percentil (µg/l)
BHT-acid	33	0.2 - 7.55	1.28	0.83	2.89

HEXABROMOCYCLODODECANE (HBCDD)

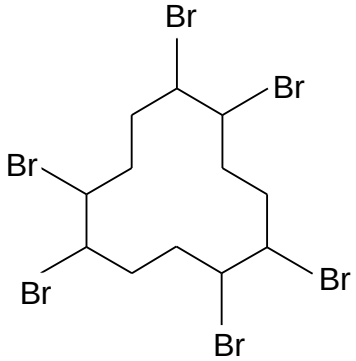
Reasons for nomination:

- 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

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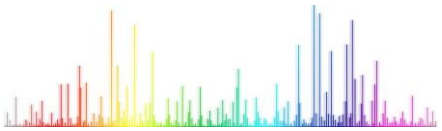
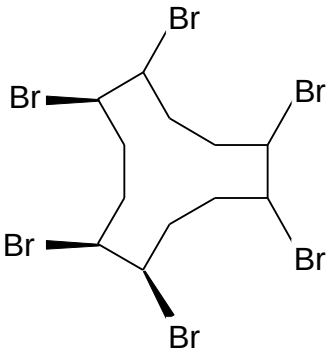
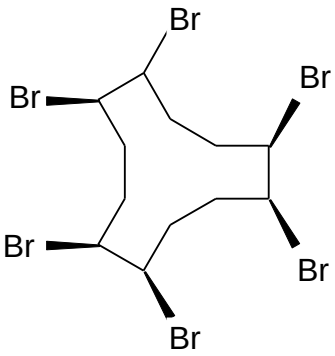
HBCDD – METHOD



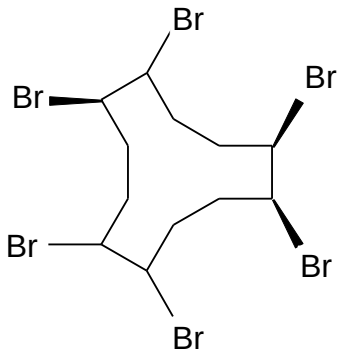
- Valid LC-MS/MS method for the detection of α-HBCDD, β-HBCDD and γ-HBCDD in blood plasma successfully developed



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

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HBCDD - APPLICATIONS

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

1st group - 48 samples:

Analyte	N > 0.03 µg/L (LOD)
α-HBCDD	**3** (0.031- 0.084); 6.3 %
β-HBCDD	**0**
γ-HBCDD	**0**

2nd group - 42 samples:
(from Prof. Fromme, LGL Munich)

Analyte	N > 0.03 µg/L (LOD)
α-HBCDD	**3** (0.036 - 0.053) ; 7.1 %
β-HBCDD	**4** (0.037 - 0.090); 9.5 %
γ-HBCDD	**0**

- 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
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