2nd HBM4EU Training School 2018

Nijmegen

HBM4EU

science and policy for a healthy future

HUMAN-BIOMONITORING: FROM EXPOSURE BIOMARKER IDENTIFICATION TO POPULATION STUDIES – BASIC PRINCIPLES IN MATRIX/BIOMARKER SELECTION

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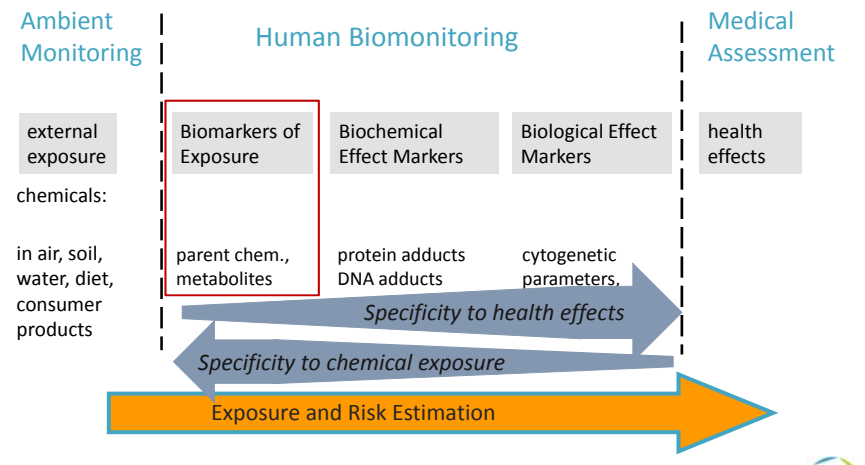
Exposure Assessment (in the exposure-disease continuum)



adapted from: Biological Monitoring- Prospects in Occupational and Environmental Medicine
Angerer and Weiss (eds.), Wiley-VCH, Weinheim, 1. Edition (2002)

2

Biomarkers of Exposure

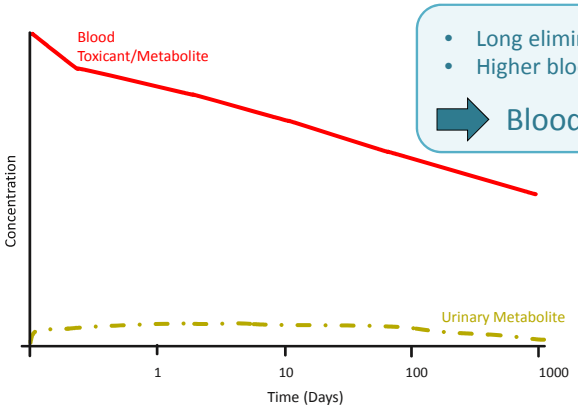
Biomarkers of Exposure parent chem., metabolites	- specific to target compound (exposure) ability to capture all: - sources of exposure (known and unknown) - routes of exposure (oral, inhalative, dermal)

… defined by:

- Metabolism
- Toxikokinetics
  - Matrix (urine, blood, etc.)
  - Specificity
  - Contamination

3

Toxikokinetics: *persistent chemicals*



- Long elimination half-times
- Higher blood levels relative to urine

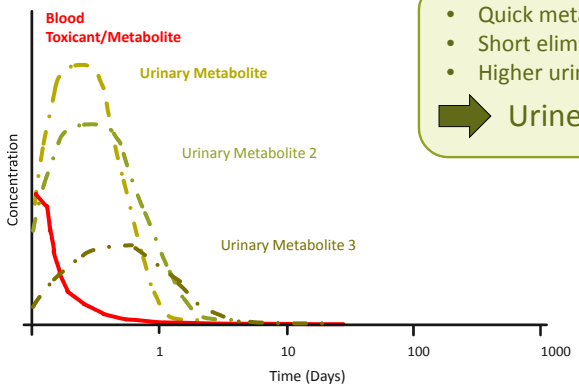
➡ Blood is preferred matix

Needham and Sexton. JEAEE 10: 611-629 (2000)
Henderson et al. Crit Rev Toxicol 20: 65-82 (1989)

taken from ISEA/ISES Wesolowski Award Presentation, Paris 2006:
Biomonitoring: An Integral Part of Exposure Analysis
Larry L. Needham, Ph.D., National Center for Environmental Health, Centers for Disease Control and Prevention

4

Toxikokinetics: *non-persistent chemicals*



- Quick metabolisation
- Short elimination half-times
- Higher urine levels relative to blood

➡ Urine is preferred matrix

Needham and Sexton. JEAEE 10: 611-629 (2000)
Henderson et al. Crit Rev Toxicol 20: 65-82 (1989)

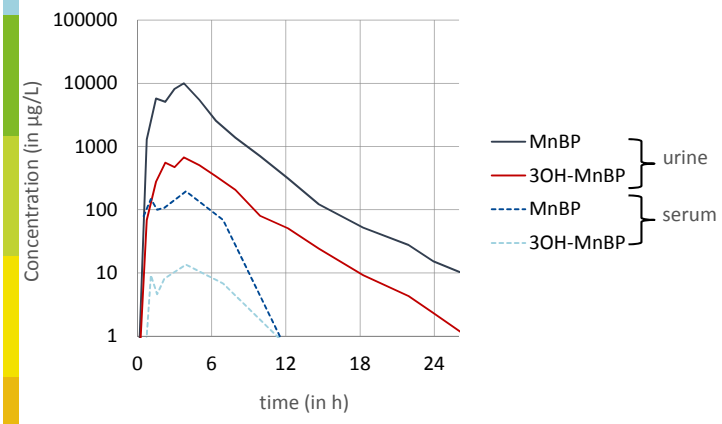
taken from ISEA/ISES Wesolowski Award Presentation, Paris 2006:
Biomonitoring: An Integral Part of Exposure Analysis
Larry L. Needham, Ph.D., National Center for Environmental Health, Centers for Disease Control and Prevention

5

Biomarkers: non-persistent chemicals

Toxikokinetics: *n-butyl phthalate*

5mg D4-DnBuP (oral, one human volunteer)



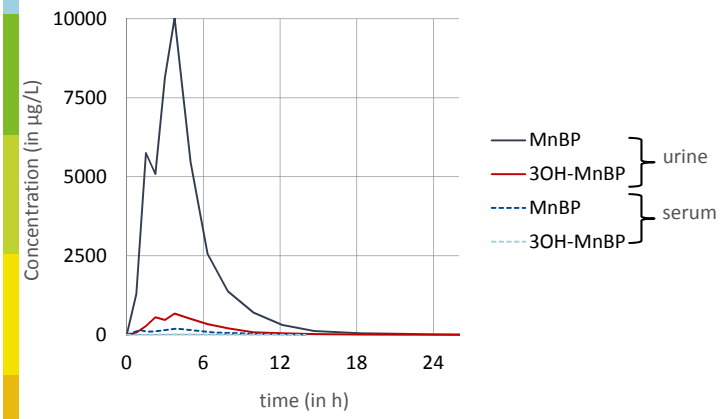
Koch et al (2012) Arch Tox 86, 1829-1839.
Lorber and Koch (2013) Environ Int 59, 469–477.

6

Biomarkers: non-persistent chemicals

Toxikokinetics: *n-butyl phthalate*

5mg D4-DnBuP (oral, one human volunteer)



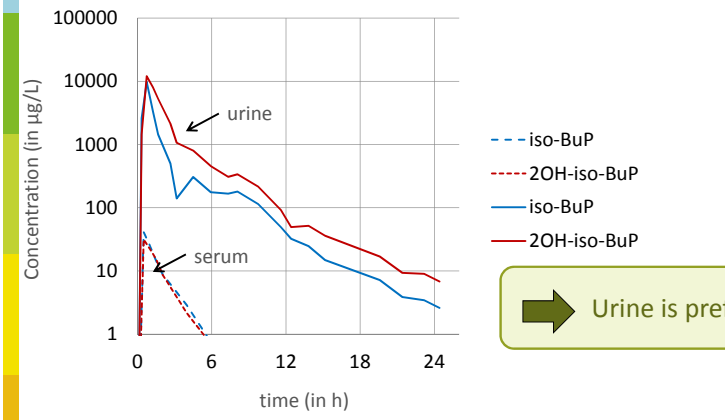
Koch et al (2012) Arch Tox 86, 1829-1839.
Lorber and Koch (2013) Environ Int 59, 469–477.

7

Biomarkers: non-persistent chemicals

Toxikokinetics: *iso-butyl paraben*

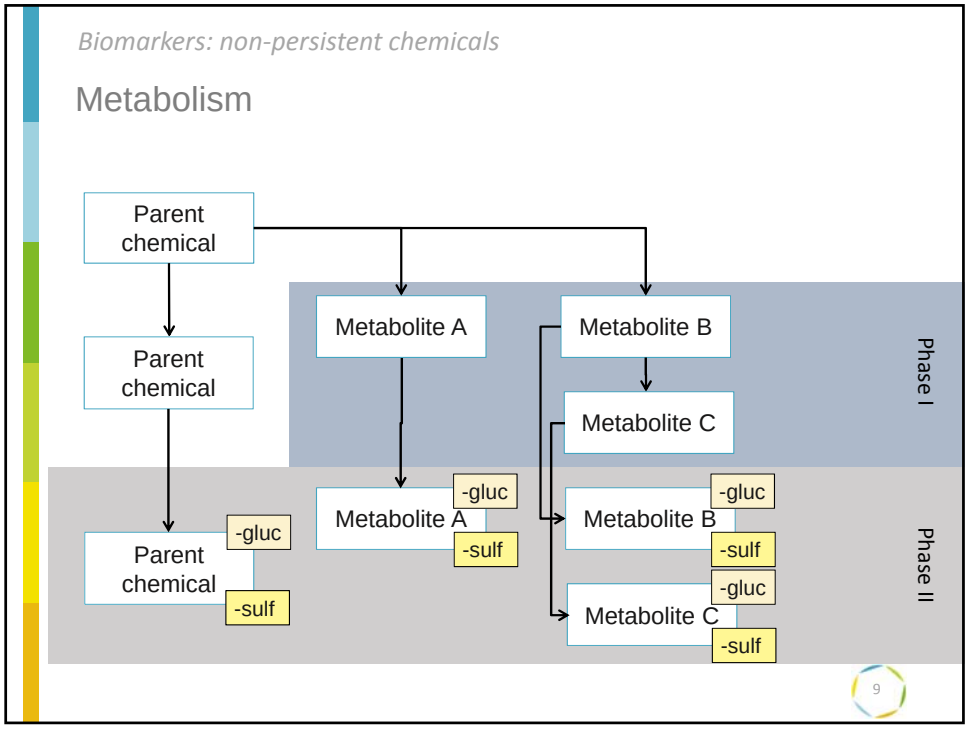
5mg D4-iso-butyl paraben (oral, one human volunteer)

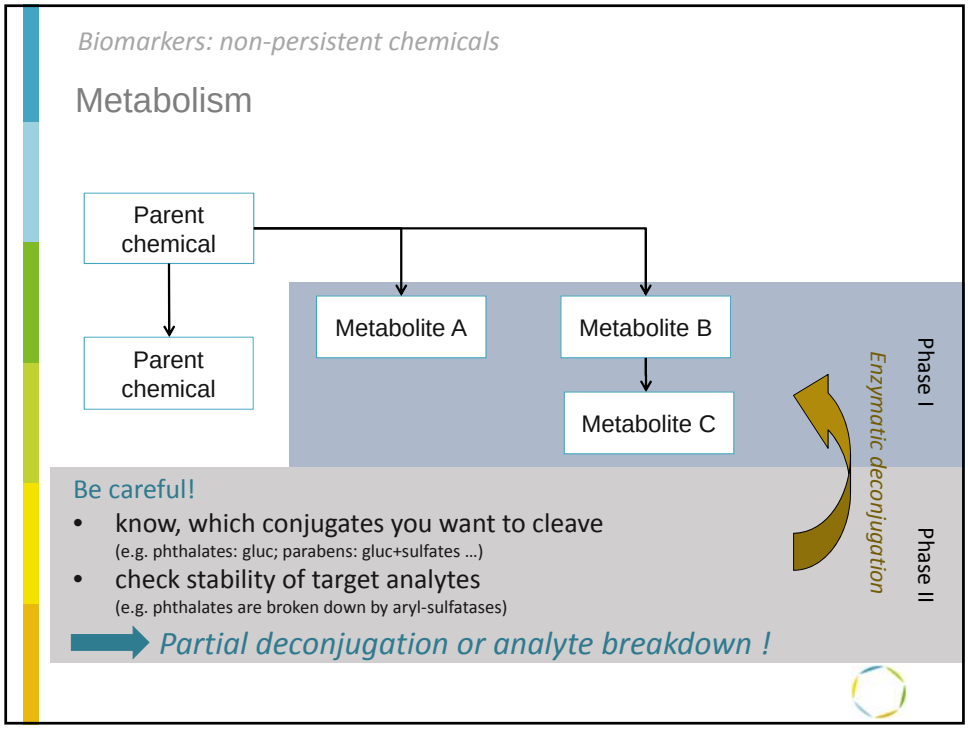


Urine is preferred matrix

Moos et al (2016) Arch Tox 90(11), 2699-2709
Serum data in preparation

8

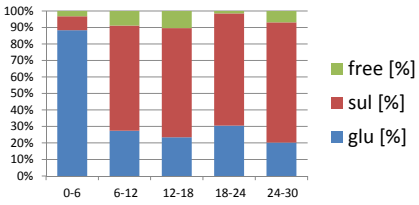

Biomarkers: non-persistent chemicals
Metabolism
Parent chemical
Parent chemical
Parent chemical
-gluc
-sulf
Metabolite A
Metabolite B
Metabolite C
Metabolite A
-gluc
-sulf
Metabolite B
-gluc
-sulf
Metabolite C
-gluc
-sulf
Phase I
Phase II
9


Biomarkers: non-persistent chemicals
Metabolism
Parent chemical
Parent chemical
Metabolite A
Metabolite B
Metabolite C
Phase I
Phase II
Enzymatic deconjugation
Be careful!
• know, which conjugates you want to cleave
(e.g. phthalates: gluc; parabens: gluc+sulfates …)
• check stability of target analytes
(e.g. phthalates are broken down by aryl-sulfatases)
Partial deconjugation or analyte breakdown !

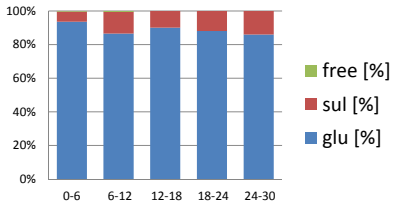
Biomarkers: non-persistent chemicals

Conjugate Distribution (Parabens)

Methyl Paraben



n-Butyl Paraben



→ to transform all conjugates into *free phase I metabolites* *Gluc-sulf-enzyme* (like HP2) is needed

→ Gluc-sulf-enzymes cleave ester-bonds of phthalates;
Phthalates/substitutes need pure Gluc-enzyme (like E.coli K12)

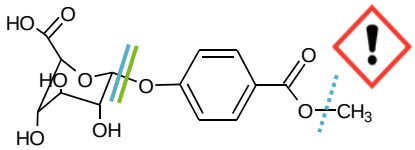
Moos et al (2016) Arch Tox 90(11), 2699-2709; and unpublished data

11

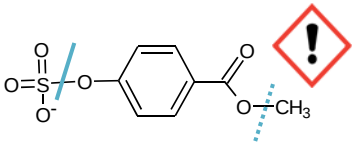
Biomarkers: non-persistent chemicals

Conjugates (Parabens)

ß-Glucuronidase pure (E. coli K 12)



Methyl paraben glucuronide



Methyl paraben sulfate

ß-Glucuronidase/aryl sulfatase (from Helix pomatia)

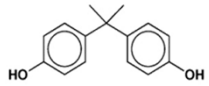
→ *Carefully test/adapt enzymatic conditions: concentration, temperature, time, pH*

12

Biomarkers: non-persistent chemicals

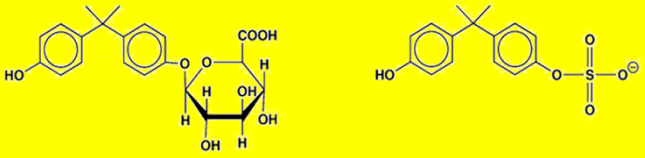
Conjugates (Bisphenol A)

- fast Glucuronidation/Sulfatation (close to 100%)
- fast Elimination in Urine



detection of high free BPA (>10%) indicates external contamination!

< 24 h

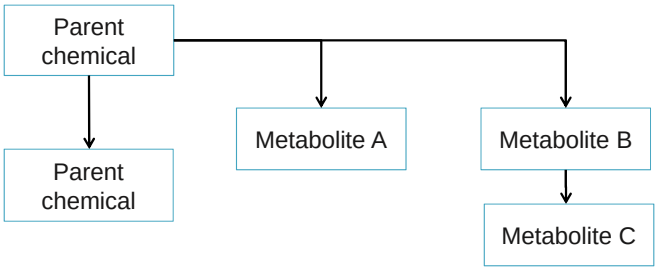


Dekant W, Völkel W.
Toxicol Appl Pharmacol 228(1):114-34 (2008)

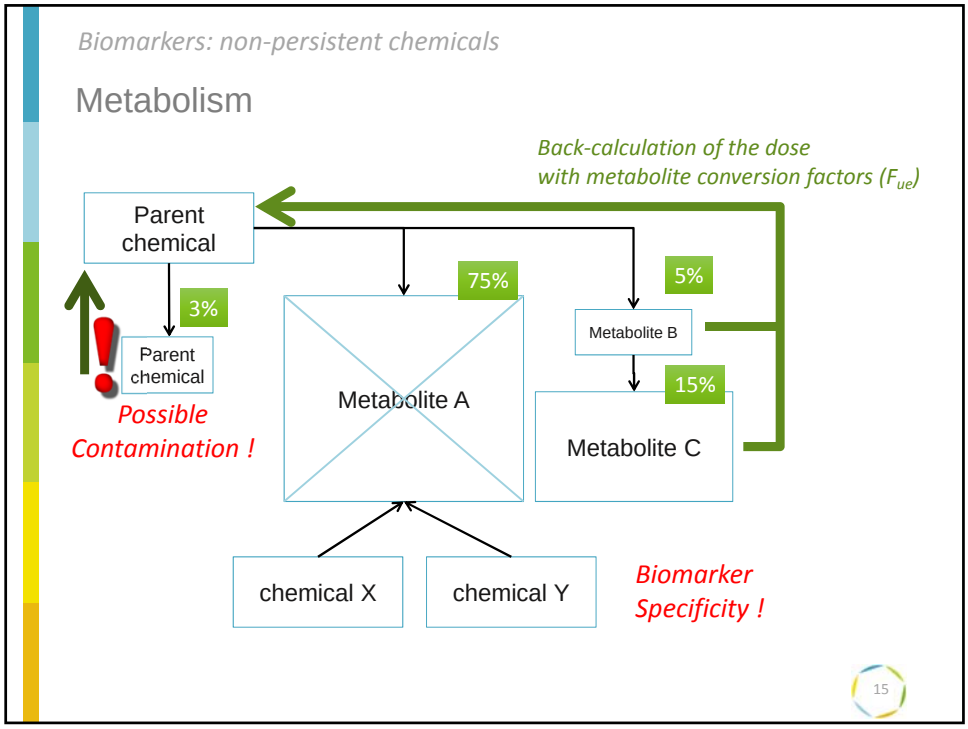
13

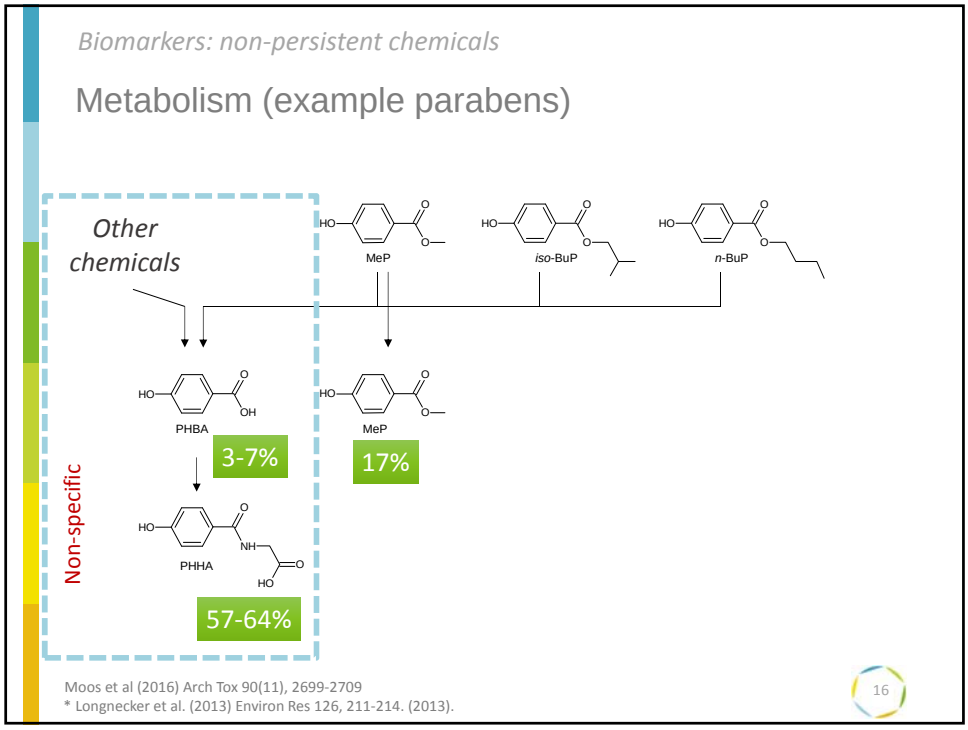
Biomarkers: non-persistent chemicals

Metabolism



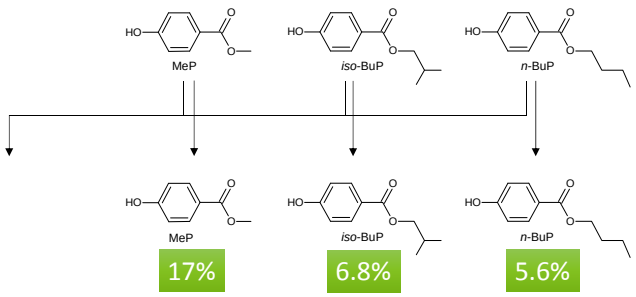
14


Biomarkers: non-persistent chemicals
Metabolism
Back-calculation of the dose
with metabolite conversion factors (F_{ue})
Parent chemical
3%
Parent chemical
Possible Contamination !
75%
Metabolite A
5%
Metabolite B
15%
Metabolite C
chemical X
chemical Y
Biomarker Specificity !
15


Biomarkers: non-persistent chemicals
Metabolism (example parabens)
Other chemicals
MeP
iso-BuP
n-BuP
PHBA
3-7%
MeP
17%
Non-specific
PHHA
57-64%
Moos et al (2016) Arch Tox 90(11), 2699-2709
* Longnecker et al. (2013) Environ Res 126, 211-214. (2013).
16

Biomarkers: non-persistent chemicals

Metabolism (example parabens)



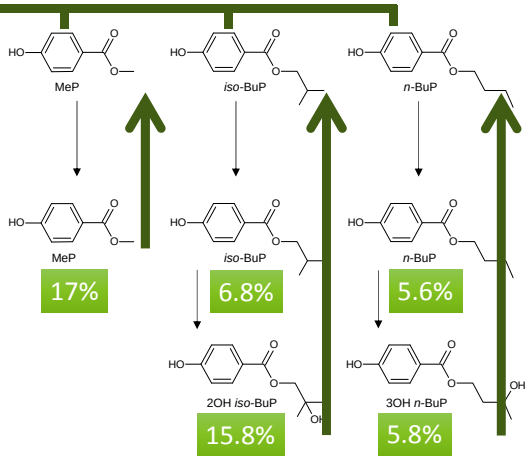
Parent parabens as biomarkers:
- Different excretion fractions
- Rather low excretion fractions
- Contamination control!
- Preservative in biobanked samples*

17

Biomarkers: non-persistent chemicals

Metabolism (example parabens)

- dose extrapolation (daily intakes)
- risk assessment (comparison to health benchmarks, HQ/HI)

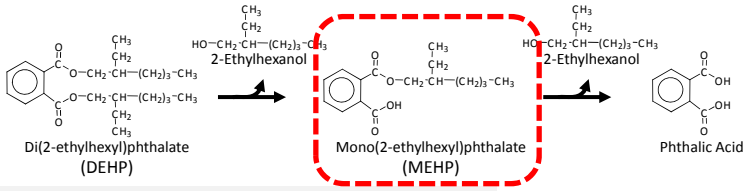


Moos et al (2016) Arch Tox 90(11), 2699-2709

18

Biomarkers: non-persistent chemicals

Metabolism (example phthalates)



Chain length	Phthalate	Monoester-Metabolite	$f_{ue\text{-}pm}$
2	DEP	MEP	~80%
4	DnBP	MnBP	84%
4	DiBP	MiBP	71%
6	BBzP	MBzP	73%
8	DEHP	MEHP	6%
9	DiNP	MiNP	1%
10	DIDP/DPHP	MiDP/MPHP	<1%

Pre-Analytical / Analytical
Contamination !

Personal Care Products

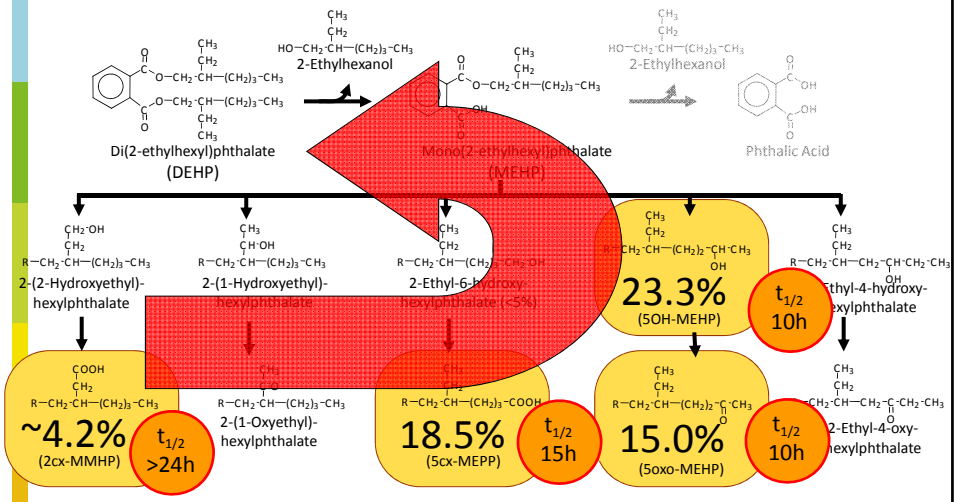
Plasticizers

Koch et al. (2004) Arch Toxicol 78, 123-130.
Koch et al. (2005) Arch Toxicol 79, 367-376.

19

Biomarkers: non-persistent chemicals

Metabolism (example DEHP)



Koch et al. (2004) Arch Toxicol 78, 123-130.
Koch et al. (2005) Arch Toxicol 79, 367-376.

20

Biomarkers: non-persistent chemicals

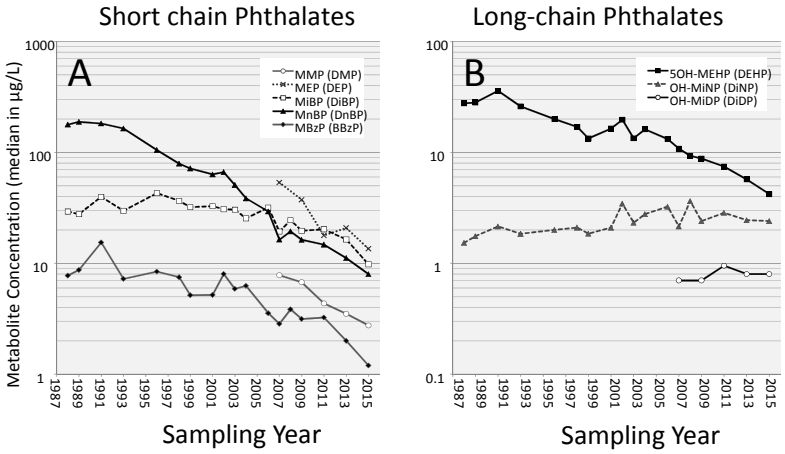
Biomarkers of exposure for phthalates

Chain length	Phthalate	Monoester Metabolite	$f_{ue\text{-}pm}$	Secondary metabolite	$f_{ue\text{-}pm}$	reference
2	DEP	MEP	80%			*estimated*
4	DnBP	MnBP	84%			*Koch et al. (2012), Anderson et al. (2001)*
				OH-MnBP	7%	
4	DiBP	MiBP	71%			*Koch et al. (2012) Anderson et al. (2001)*
				OH-MiBP	20%	
6	BBzP	MBzP	73%			*Anderson et al. (2001)*
8	DEHP	MEHP	6%			*Koch et al. (2005), Anderson et al. (2011), Kessler et al. (2012)*
				5OH-MEHP	23%	
				5oxo-MEHP	15%	
				5cx-MEPP	18%	
9	DiNP	MiNP	1%			*Koch et al. (2007), Anderson et al. (2011)*
				OH-MiNP	18%	
				oxo-MiNP	10%	
				cx-MiNP	9%	
10	DPHP /DiDP			OH-MPHP	12%	*Schütze et al. (2014), Wittassek and Angerer (2008)*
				oxo-MPHP	14%	
				cx-MPHP	0.5%	

21

Biomarkers: non-persistent chemicals

Phthalates time trends (Germany)

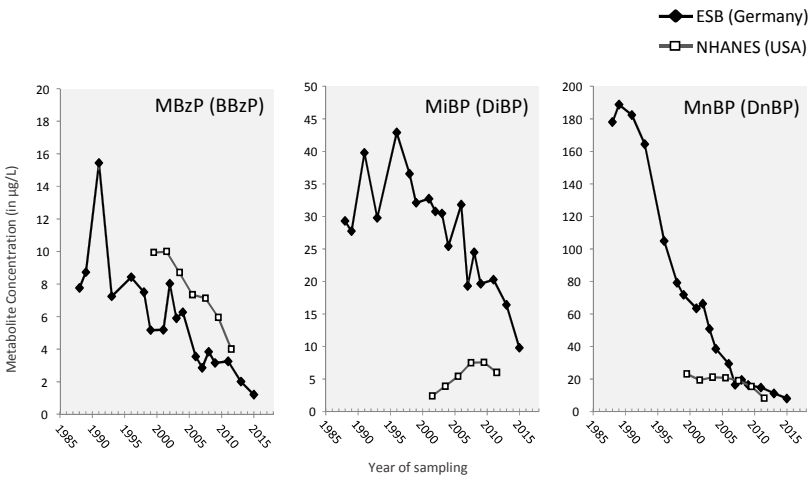


Koch et al (2017) Int J Hyg Environ Health 220: 130–141

22

Biomarkers: non-persistent chemicals

Phthalates: USA vs. Germany



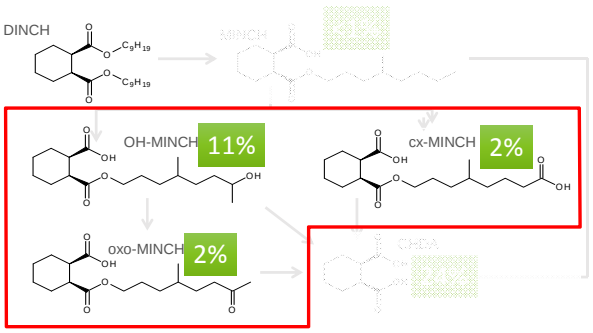
Koch et al (2017) Int J Hyg Environ Health 220: 130–141

23

Biomarkers: non-persistent chemicals

Metabolism (phthalate substitutes)

Hexamoll ® DINCH ®: market introduction in 2002

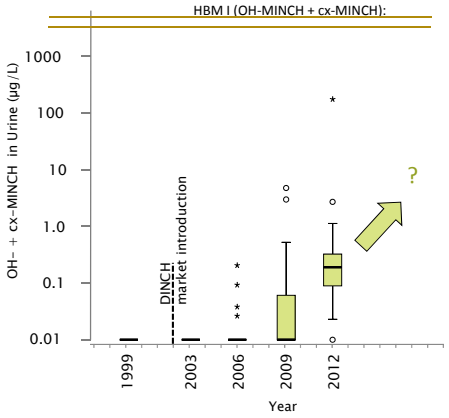


Schütze et al. (2012) J Chrom B. 895-896, 123-130
Koch et al. (2013) Arch Tox 87, 799–806
Schütze et al. (2015) Chemosphere 128, 216–224
Schütze et al. (2017) Arch Tox 91, 179-188

24

Biomarkers: non-persistent chemicals

Hexamoll® DINCH®: Population Study



Exposure Assessment:
- metabolite levels in urine
- daily intake calculation

Risk Assessment:
- Comparison with TDI
- HBM values (UBA)

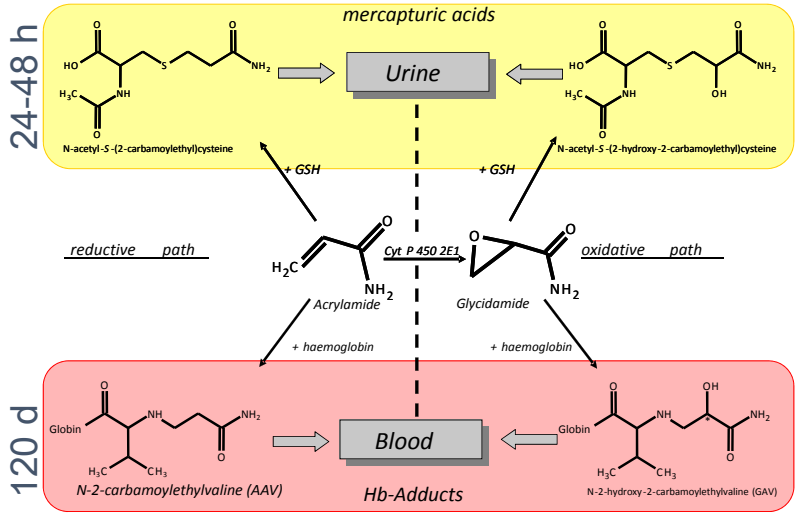
Schütze et al. (2014) Int J Hyg Environ Health 217, 421-426

HBM Commission (2012) Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz 57 (12), 1451-1461.

25

Biomarkers: non-persistent chemicals

Acrylamide



taken with kind permission from: Schettgen et al. and Boettcher et al.

26

Acrylamide:

Correlation Hb-adducts vs. DNA-adducts

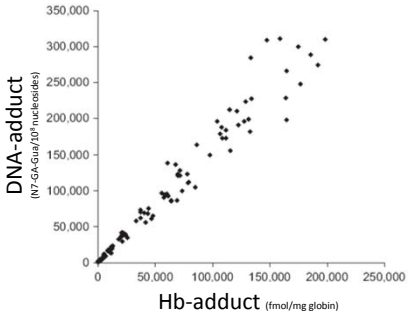


FIG. 4. Relationship between glycidamide DNA adducts in liver and hemoglobin adducts in blood of mice administered acrylamide by gavage for 28 days. Each data point represents one mouse. DNA and hemoglobin adduct levels were quantified as described in the text.

Zeiger E (2009)
Toxicol Sci. 107(1):247-57.

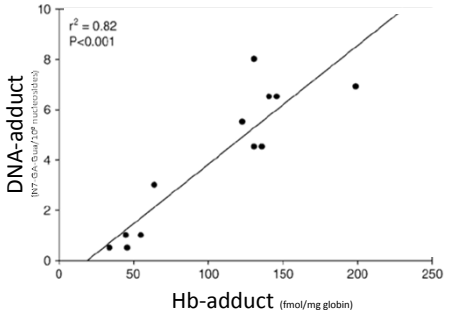


Fig. 6. Correlation of glycidamide-derived hemoglobin and liver DNA adducts in F344 rats and B6C3F$_1$ mice exposed to single dose gavage administration of acrylamide (0.1 mg/kg bw) or an equimolar gavage dose of glycidamide. Individual data points shown represent group mean hemoglobin and DNA adduct values for male and female mice and rats.

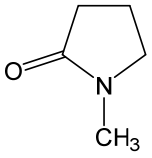
Tareke et al. (2006)
Toxicol Appl Pharamacol 217 (1): 63 - 75.

27

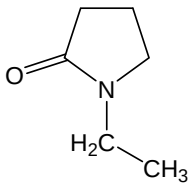
Aprotic Solvents: N-alkyl-pyrrolidones

- **N-alkyl-pyrrolidones are …**
- chemically stable and powerful (organic, aprotic) polar solvents
- excellent, broad–range solvent properties
- water-miscible

N-methyl-
2-pyrrolidone (NMP)



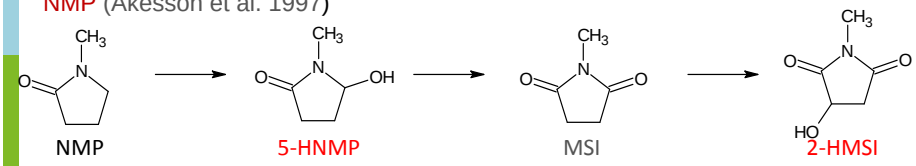
N-ethyl-
2-pyrrolidone (NEP)



28

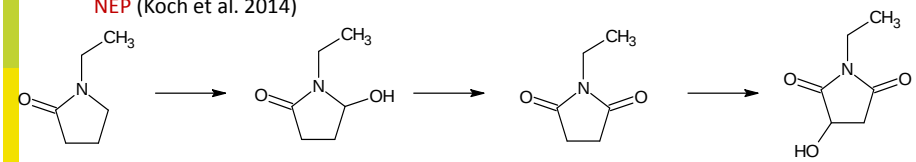
Metabolism / Biomarkers (in urine)

NMP (Akesson et al. 1997)



NMP → 5-HNMP → MSI → 2-HMSI

NEP (Koch et al. 2014)



NEP → 5-HNEP (~ 29.1%, $t_{½}$ = 6.8 h) → ESI → 2-HESI (~ 21.6%, $t_{½}$ = 21.6 h)

Akesson and Paulsson (1997) Occup Environ Med. 54(4), 236-40.
Koch et al. (2014) Arch Tox 88 (4), 893-899.

29

Summary and conclusion

Biomarkers of exposure:

- Persistent chemicals: blood is preferred matrix
- Non-Persistent chemicals: urine is preferred matrix (or Hb)

- Toxikokinetics
- Metabolism
  - Parent chemical – phase I met. – phase II met (conjugates)
  - Metabolite conversion factors
  - Specificity
  - Contamination (parent compounds!)

- Specific Exposure Assessment (comparisons; dose)
- Risk Assessment (TDI; HBM/BE)
- Risk Management

30

Acknowledgement

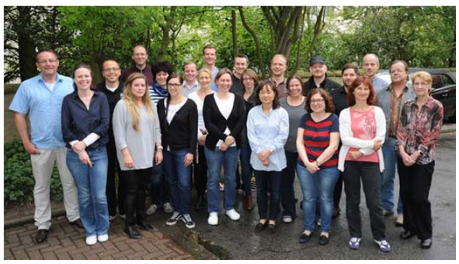
Some of the data shown has been generated within the Cooperation for the enhancement of HBM between the German Ministry for the Environment, the German Environment Agency (UBA), and the German Chemical Industry Association (VCI)

Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety

Umwelt Bundesamt

VCI

IPA:



IPA
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Speaker's information

Within HBM4EU Dr. Koch is leader of task 9.3 (new methods). Since 2006, Dr. Koch is the scientific head of the biomonitoring laboratory at the IPA. He is member of the Human Biomonitoring Commission of the German Environment Agency. Since 2016 Dr. Koch is Editor-in-Chief of the *International Journal of Hygiene and Environmental Health*. He has authored >130 peer-reviewed publications on exposure and risk assessment of occupational and environmental chemicals.

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