IPA
Institute for Prevention and Occupational Medicine
of the German Social Accident Insurance
Institute of the Ruhr-Universität Bochum

# 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)

| Ambient Monitoring | | Medical Assessment |
|---|---|---|
| external exposure<br>chemicals:<br>in air, soil, water, diet, consumer products | model calculations, including<br>- **various sources of exposure** (known and unknown)<br>- **routes of exposure** (oral, inhalative, dermal) | health effects |

**Exposure and Risk Estimation**

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

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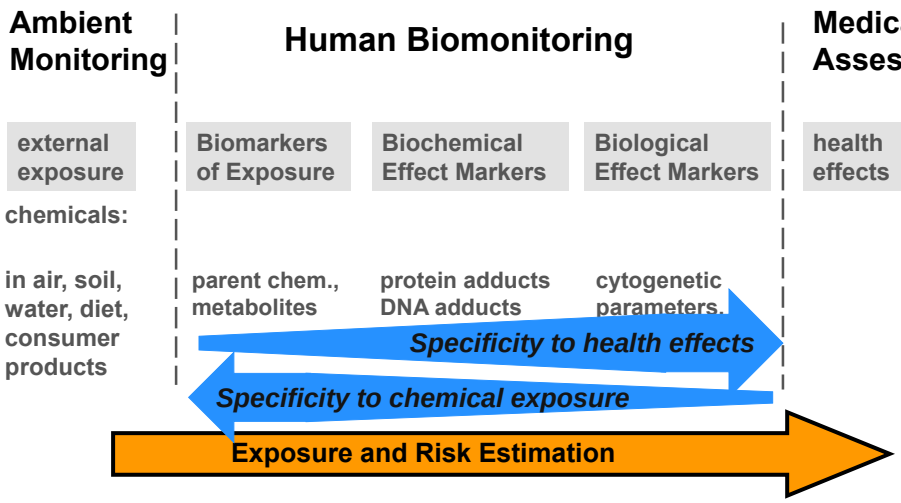
## Exposure Assessment (in the exposure-disease continuum)




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

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## Biomarkers of Exposure

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

## … defined by:

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

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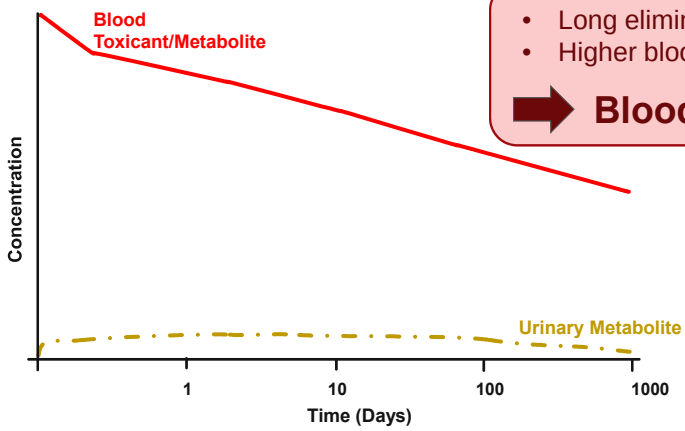
## Toxikokinetics: *persistent* chemicals




- 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

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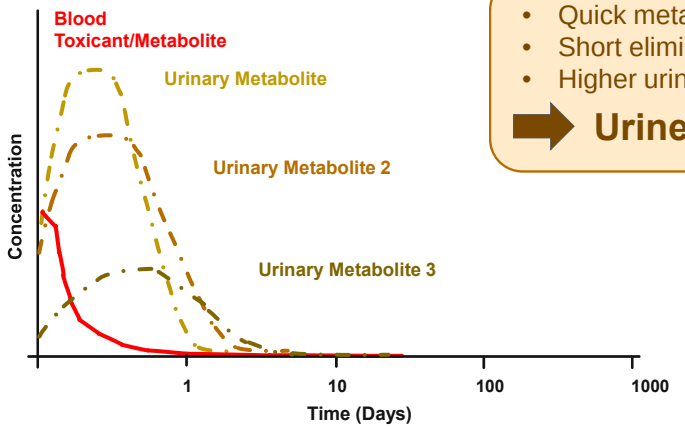
## Toxikokinetics: *non-persistent* chemicals




- 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

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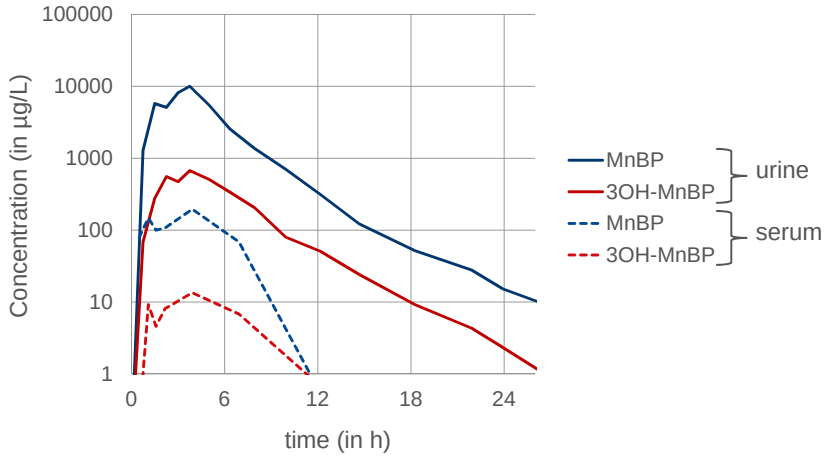
## Toxikokinetics: *n-butyl phthalate*

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



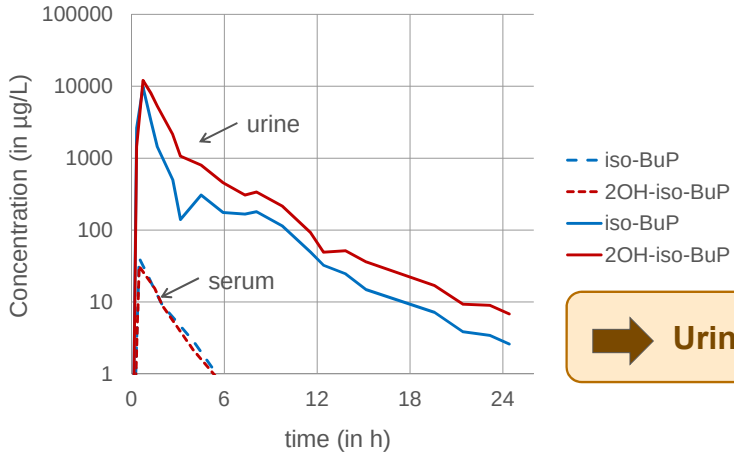

Koch et al (2012) Arch Tox 86, 1829-1839.
Lorber and Koch (2013) Environ Int 59, 469–477.





## Toxikokinetics: *iso-butyl paraben*

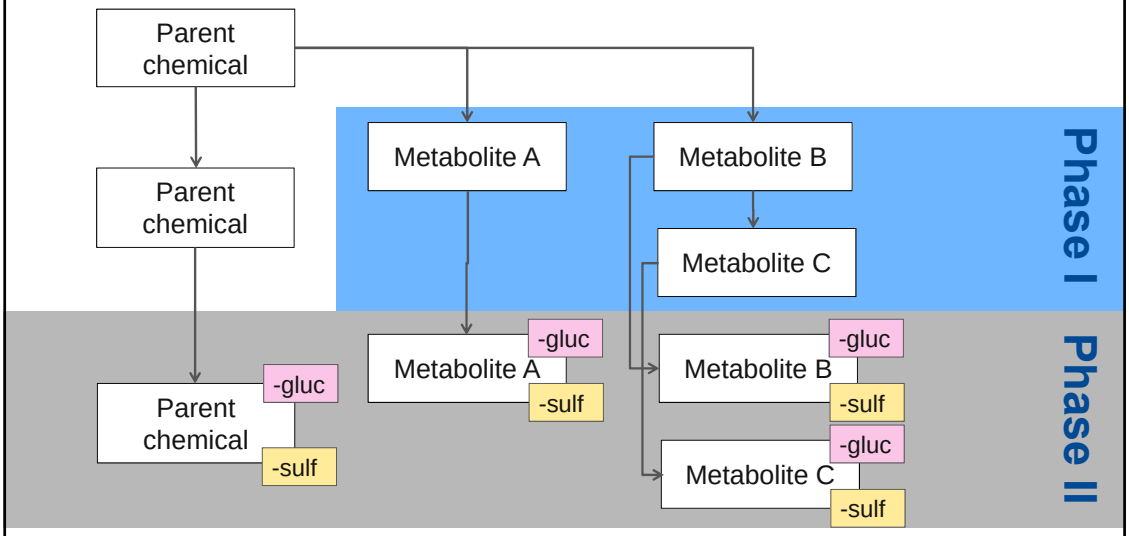
5mg D4-iso-butyl paraben (oral, one human volunteer)




**Urine is preferred matrix**

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

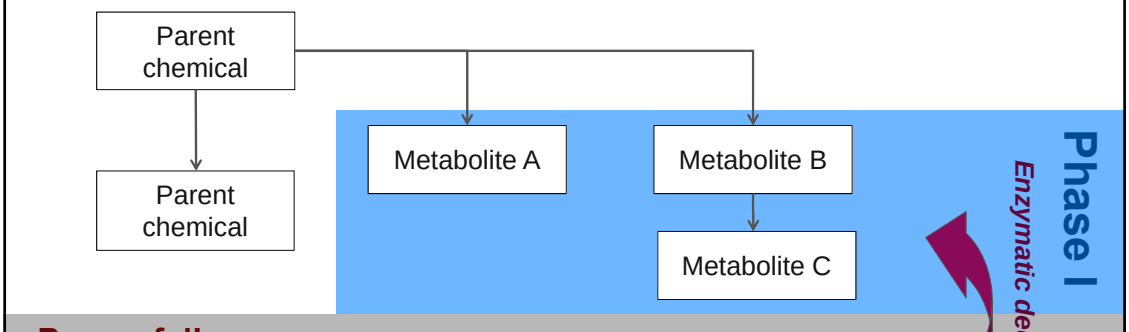
## Metabolism








## Metabolism

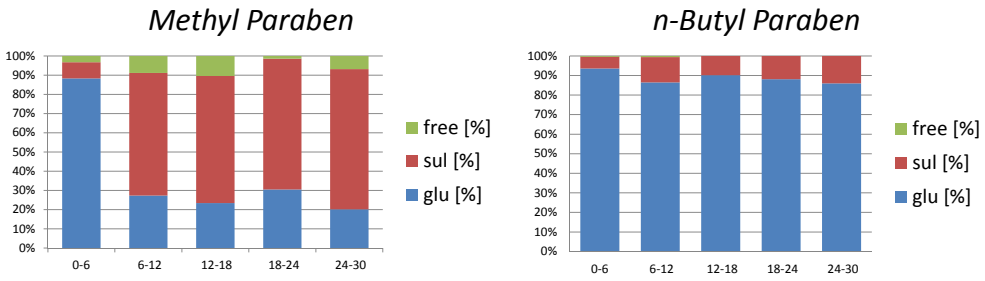



**Be careful!**

- 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 !***

## Conjugate Distribution (Parabens)




➡ 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)

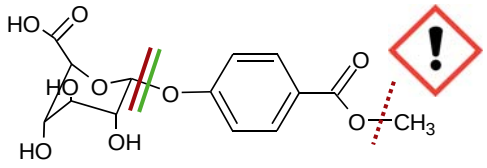
Moos et al (2016) Arch Tox 90(11), 2699-2709; and unpublished data



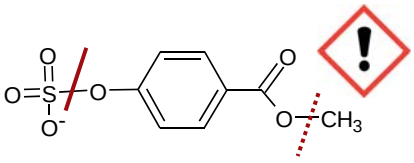


## Conjugates (Parabens)

*β-Glucuronidase pure (E. coli K 12)*




*Methyl paraben glucuronide*




*Methyl paraben sulfate*

*β-Glucuronidase/aryl sulfatase (from Helix pomatia)*

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

## Biomarkers: non-persistent chemicals

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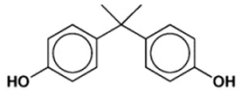
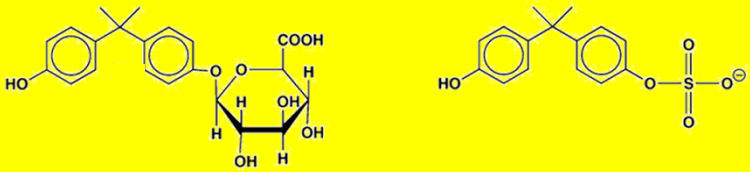
### Conjugates (Bisphenol A)

- 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)*

## Biomarkers: non-persistent chemicals

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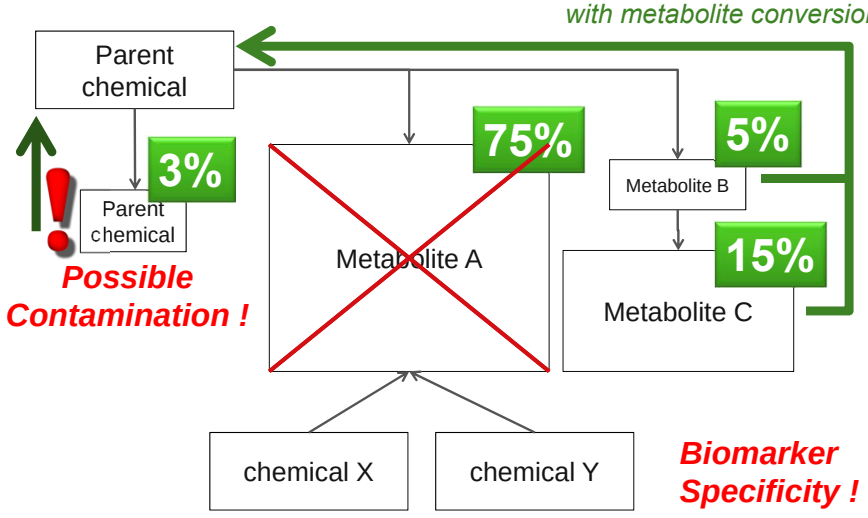
### Metabolism

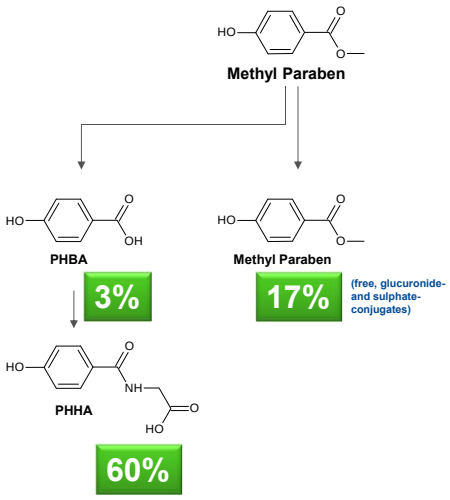
*Back-calculation of the dose with metabolite conversion factors ($F_{ue}$)*




***Possible Contamination !***

***Biomarker Specificity !***

## **Metabolism** (example parabens)

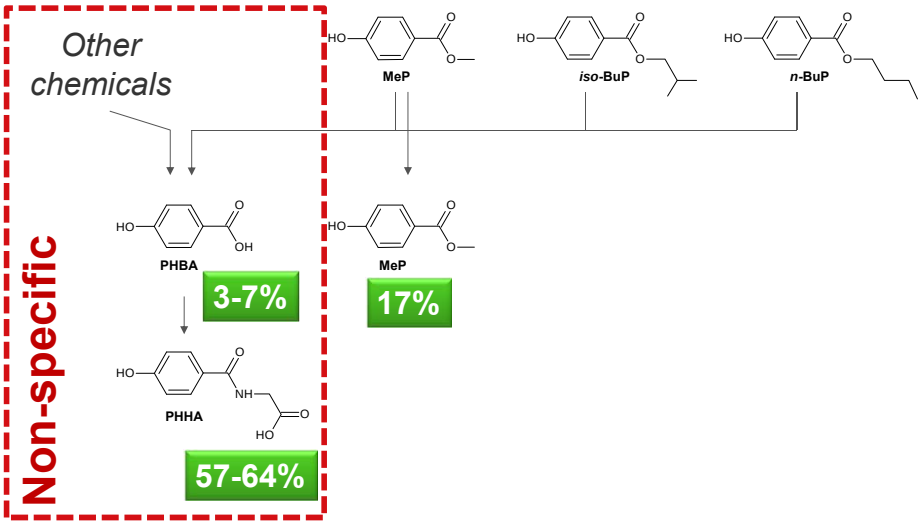



Moos et al (2016) Arch Tox 90(11), 2699-2709





## **Metabolism** (example parabens)




Moos et al (2016) Arch Tox 90(11), 2699-2709
* Longnecker et al. (2013) Environ Res 126, 211-214. (2013).

## Biomarkers: non-persistent chemicals

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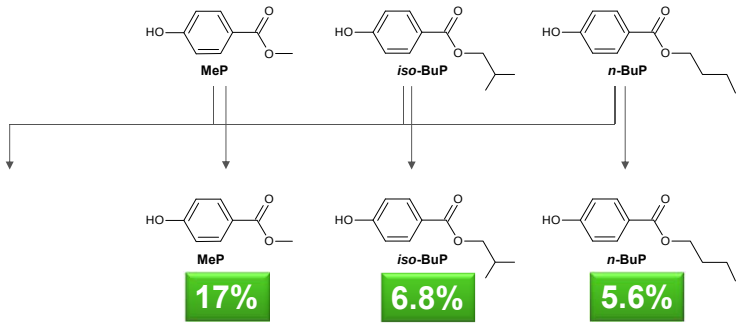
# **Metabolism** (example parabens)




**Parent parabens as biomarkers:**

- Different excretion fractions
- Rather low excretion fractions
- Contamination control!
- Preservative in biobanked samples*

Moos et al (2016) Arch Tox 90(11), 2699-2709
* Longnecker et al. (2013) Environ Res 126, 211-214. (2013).



## Biomarkers: non-persistent chemicals

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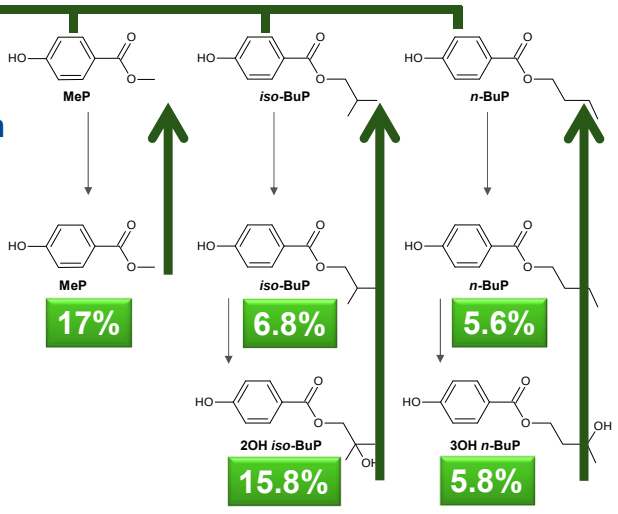
# **Metabolism** (example parabens)

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




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

## Biomarkers: non-persistent chemicals

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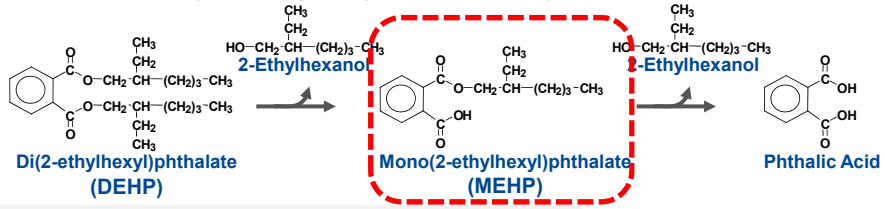
# **Metabolism** (example phthalates)




| 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.*



## Biomarkers: non-persistent chemicals

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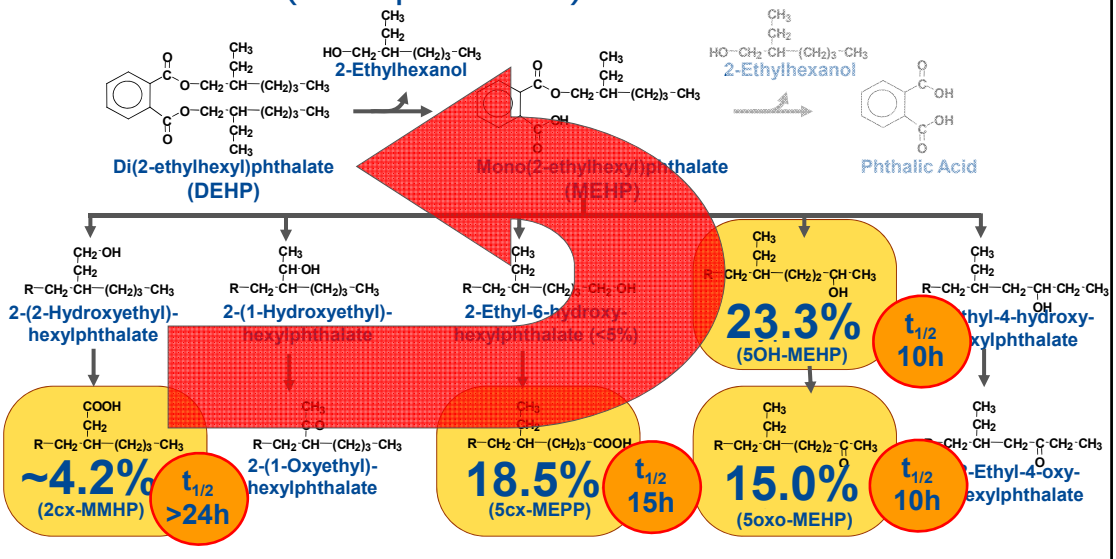
# **Metabolism** (example DEHP)




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

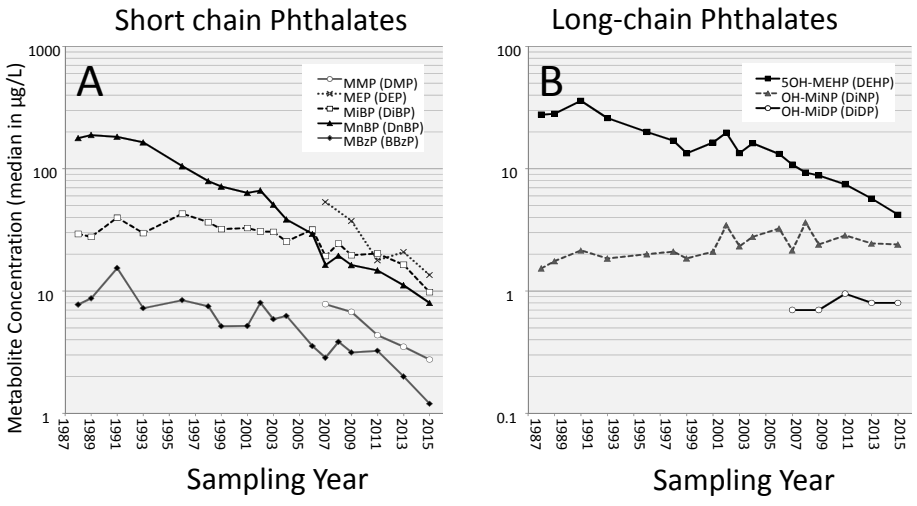
## Biomarkers of exposure for phthalates

| 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% | |



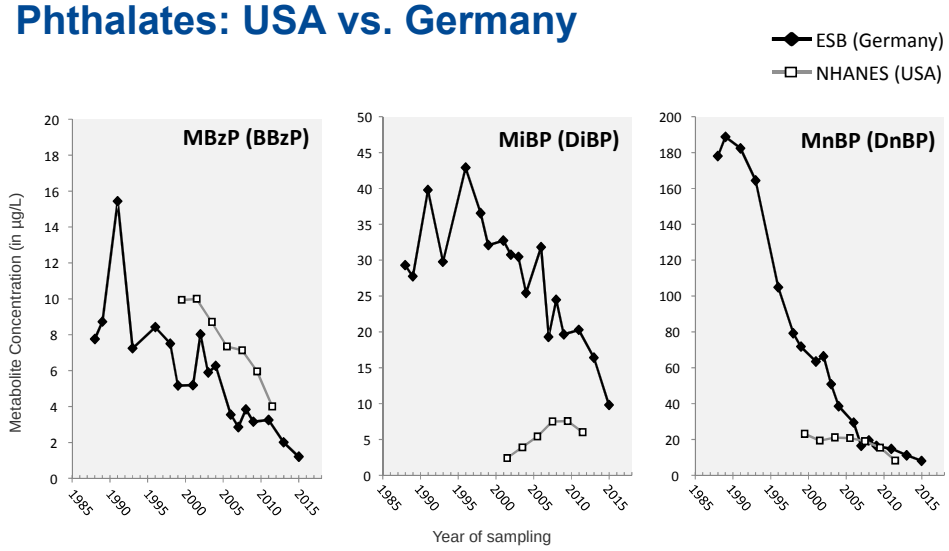


## Phthalates time trends (Germany)




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

## Phthalates: USA vs. Germany



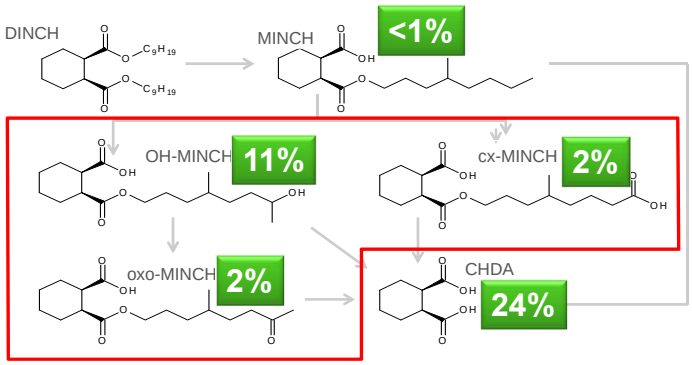

Koch et al (2017) Int J Hyg Environ Health 220: 130–141





## Metabolism (phthalate substitutes)

## Hexamoll ® DINCH ®: market introduction in 2002

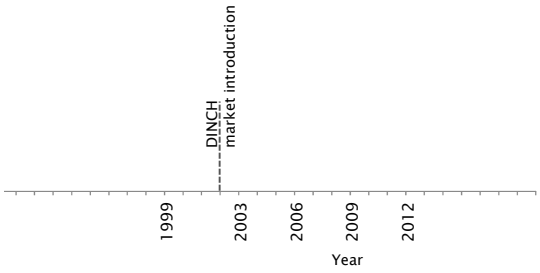



*Schütze et al. (2012) J Chrom B. 895-896, 123-130*
*Koch et al. (2013) Arch Tox 87, 799–806*
*Schütze et al. (2015) Chemosphere 128, 216–224*
*Schütze et al. (2017) Arch Tox 91, 179-188*

## Hexamoll® DINCH®: Population Study

**German Environmental Specimen Bank**:

- 24 h urine samples
- with full info on 24 h urine volume, body weight, etc.

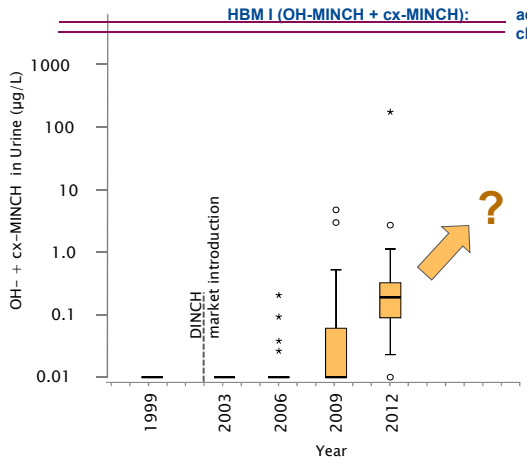



*Schütze et al. (2014) Int J Hyg Environ Health 217, 421-426*





## Hexamoll® DINCH®: Population Study




**Exposure Assessment:**

- metabolite levels in urine
- daily intake calculation

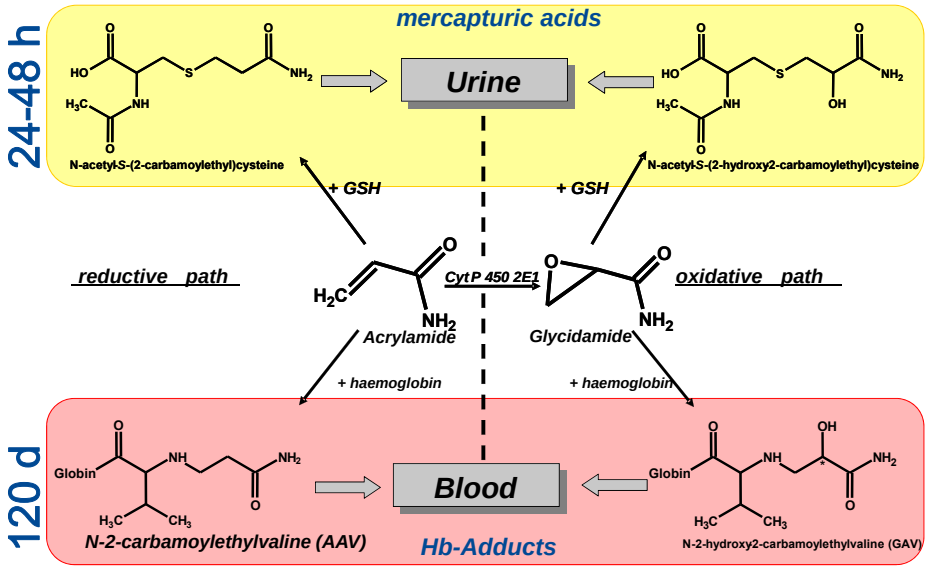
**Risk Assessment:**

- 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.*

## Biomarkers: non-persistent chemicals




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



## Biomarkers: non-persistent chemicals

### Acrylamide: Correlation Hb-adducts vs. DNA-adducts

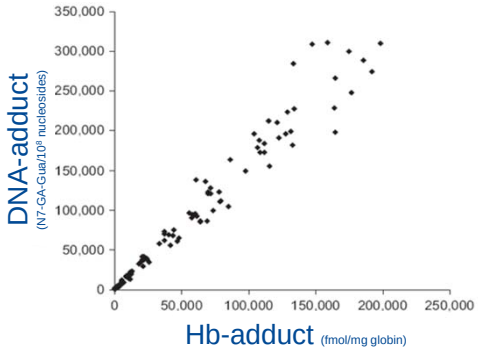



**FIG. 4.** Relationship between glycidamide DNA adducts in liver and hemoglobin adducts in blood of mice administered acrylamide by gavage for 28 days. Each data point represents one mouse. DNA and hemoglobin adduct levels were quantified as described in the text.

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

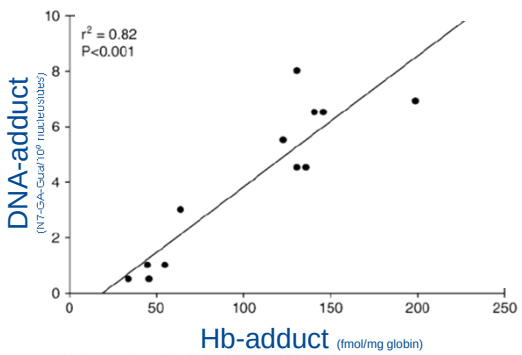


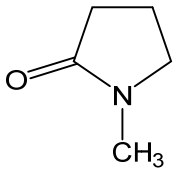

Fig. 6. Correlation of glycidamide-derived hemoglobin and liver DNA adducts in F344 rats and B6C3F$_1$ mice exposed to single dose gavage administration of acrylamide (0.1 mg/kg bw) or an equimolar gavage dose of glycidamide. Individual data points shown represent group mean hemoglobin and DNA adduct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

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

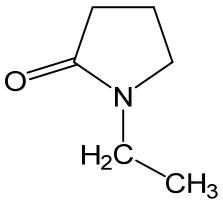
## Aprotic Solvents: N-alkyl-pyrrolidones

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

N-methyl-
2-pyrrolidone (NMP)

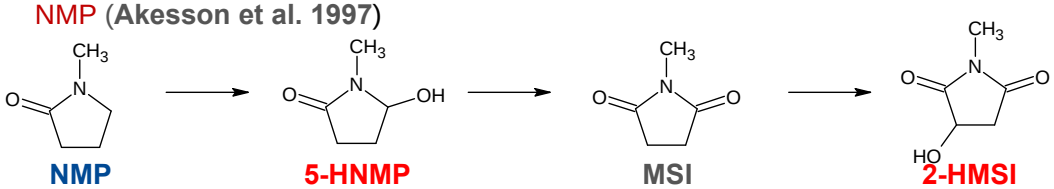


N-ethyl-
2-pyrrolidone (NEP)



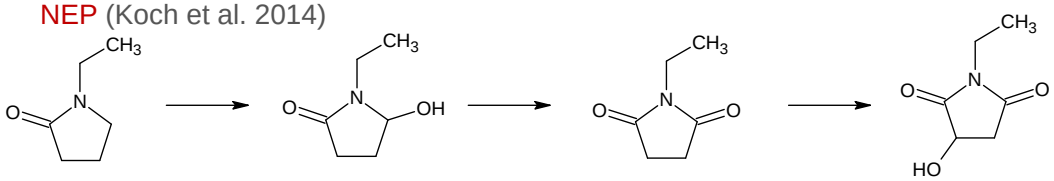
## Metabolism / Biomarkers (in urine)

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.

## 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**

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## Acknowledgement

Some of the work has been performed 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:

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Institute for Prevention and Occupational Medicine
of the German Social Accident Insurance
Institute of the Ruhr-Universität Bochum

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

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