Is measuring new chemicals in human samples easy?

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History of FLEHS biomonitoring cycles

FLEHS – I (2002 – 2006)

PCBs
+
OCPs
+
Dioxins
+
Heavy metals

FLEHS – II (2007 – 2011)

Same as FLEHS – I
+
BFRs
+
BPA and Triclosan
+
Phthalate metabolites
+

FLEHS – III (2012 – 2016)

Same as FLEHS – II
+
More metabolites of current-use pesticides
+
MeHg in hair

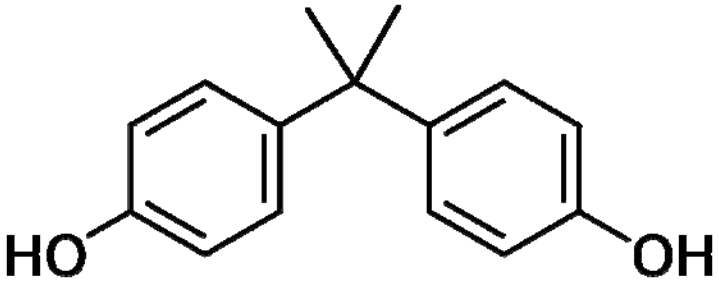
FLEHS – IV (2017 – 2021)

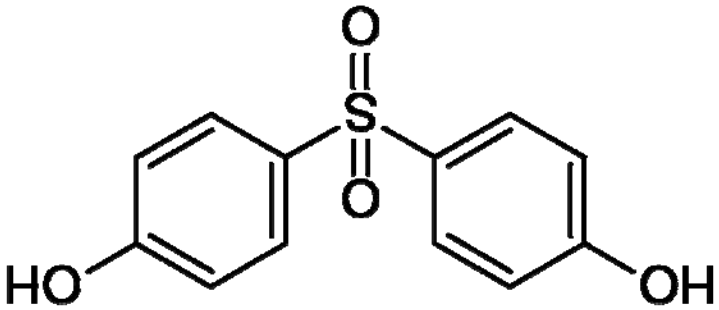
Same as FLEHS – III
+
PFR metabolites
+
BPA, BPS and other bisphenols
+
Phthalate alternatives (DINCH)
+
Additional PFASs

Emerging chemicals

Emerging contaminants?

- Introduced as **substitutes** for toxic chemicals





Bisphenol A

BPA applied in plastics, beverage cans, thermal paper, …

Banned in baby bottles, limited in food contact materials

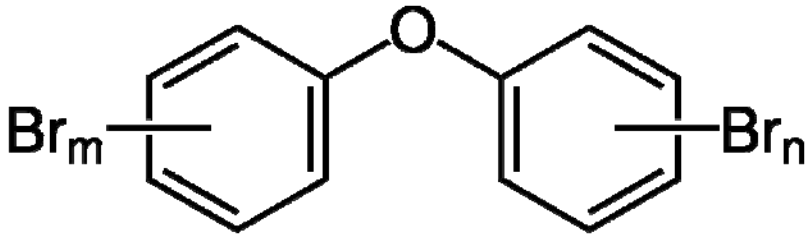
Bisphenol S

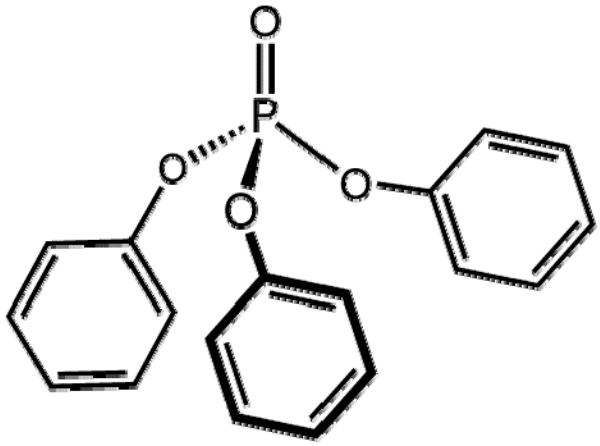
BPS and others (e.g. BPF) found in similar applications

Equally endocrine disruptive?

Emerging contaminants?

- Generally **less persistent**



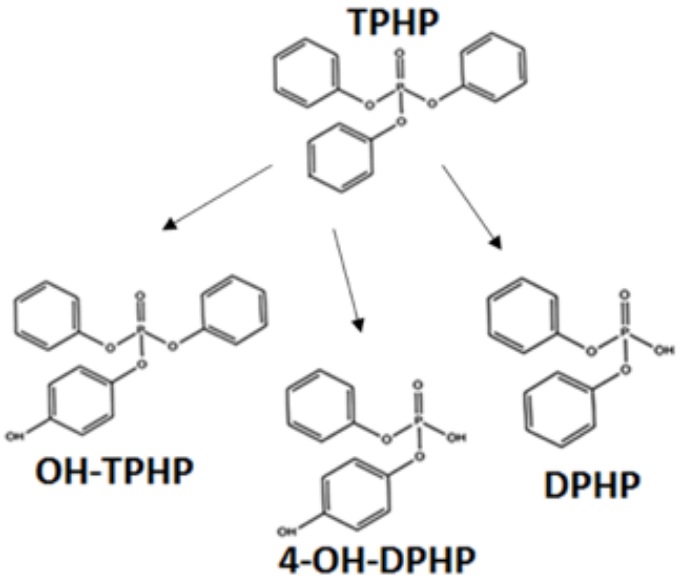


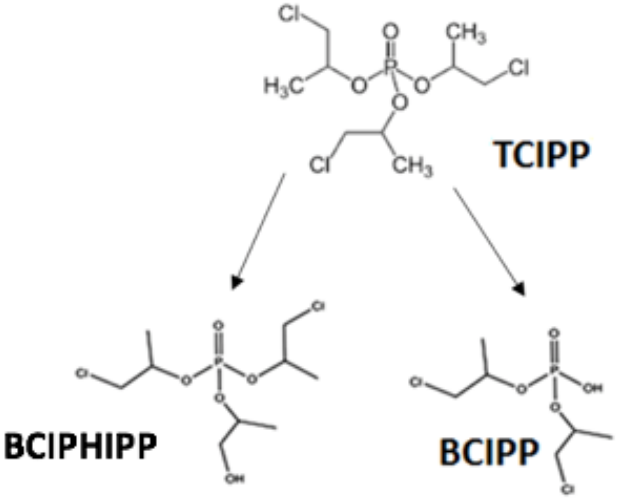
PBDEs

very hydrophobic, high lipid solubility, bioaccumulation

PFRs

more hydrophylic, metabolites easily detected in urine





Emerging contaminants?

Which metabolite is the best target?

Need to **properly identify** biomarkers of emerging contaminants

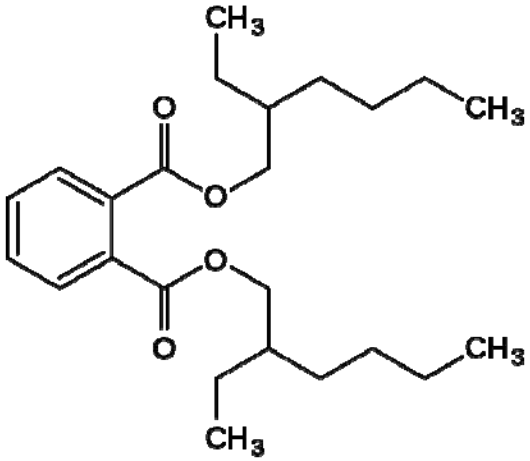
Check the **suitability** of *in vitro* metabolites in *in vivo* situations

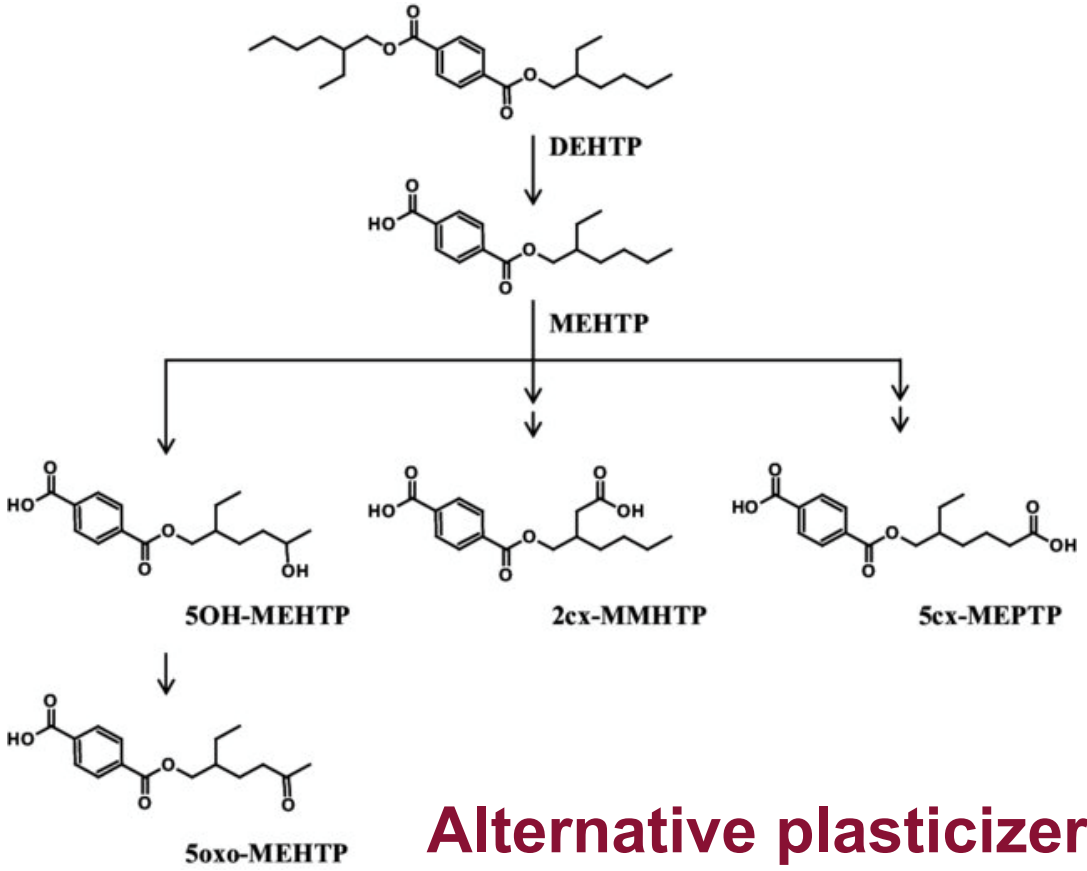
Learn more about **toxicokinetic** properties

Van den Eede et al., Toxicol. Appl. Pharmacol., 2015
Van den Eede et al., J. Appl. Toxicol., 2016

Emerging contaminants?

- Less **toxic?**





Phthalate esters such as DEHP, DINP, …

Alternative plasticizers such as DINCH, DEHTP, …

Emerging contaminants?

1. Introduced as substitutes for toxic persistent chemicals
2. Generally less persistent
3. Less toxic?

Emerging contaminants
and / or
Contaminants of emerging concern

→ Development of new targeted methods

→ Quality control through HBM4EU

HOWEVER, not all target analytes included in ringtests (ICI)

Emerging chemicals included in FLEHS IV

Class	Chemicals & Biomarkers	Target	Matrix
Pesticides	Glyphosate	metabolite	Urine
Plasticizers	Bisphenols	parents	Urine
	Phthalates & Alternative plasticizers	metabolites	Urine
Flame retardants	Organophosphate flame retardants (PFRs)	metabolites	Urine
Water-repellents	Additional perfluoralkylated compounds (PFASs)	parents	Serum

Aims FLEHS IV Emerging Chemicals

I. ***'Measure and report levels of internal exposure'***
   - *Establish reference values in FLEHS population*
   - *Establish time trends*

II. ***'Relate biomonitoring data with where and how people live (i.e. eco-behaviour: food, indoor)***
   - *Gain more knowledge on potential determinants of exposure*

III. ***'Link with relevant health effects'***
   - *Measured through biomarkers of effects*

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Phthalate metabolites
+
PFOS, PFOA
+
OP pesticides
+
PAH metabolite
+
Benzene metabolite
+
P-DCB
+
Musk compounds
+
Parabens

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+
Non-target screening leading to discovery of new/unknown compounds

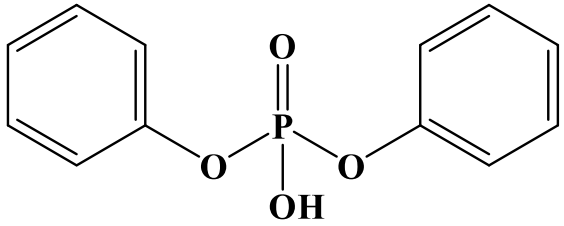
Traditional targeted biomonitoring for exposure

+	-
Highly **selective**	Suspected chemicals are **less likely** to be captured
Targets often **known chemicals** of toxicological importance	Not always the most important chemicals from a toxicological perspective
Reference data exists for most chemicals	**Limited** to a select group of known chemicals (~ 250)
Allows for specific hypotheses to be tested	Multiple methods required for a large suite of chemicals

from Dennis et al., EHP, 2017

Targeted vs Suspect vs Untargeted

1. Targeted analysis
Standard available (known knowns)



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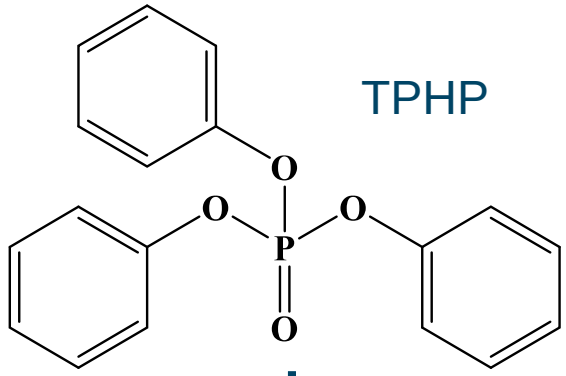
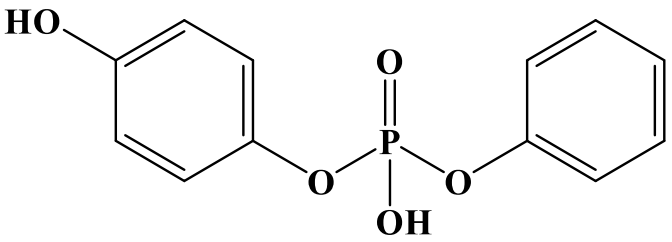




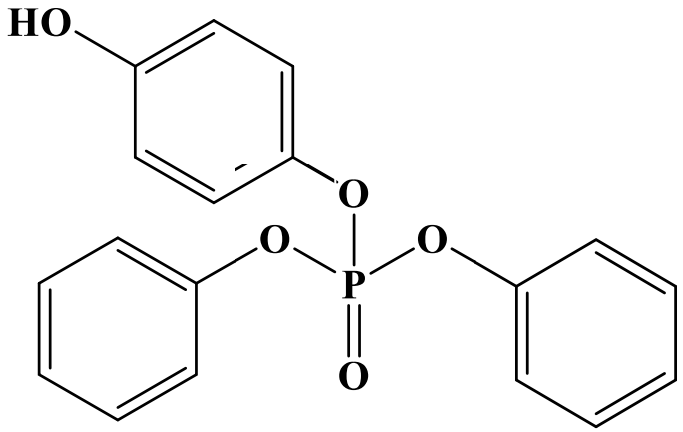
? ? ?

3. Untargeted screening analysis:
Unknown unknowns
No standard
Exact mass
Retention Time

2. Suspect screening analysis:
database with chemical information
(known unknowns)
Standard (?)
Molecular structure
Exact mass & RT

Untargeted screening

- Different instrumentation: LC-QTOF/MS
- Very sensitive mass spec (< 1 ppm, i.e. 3rd decimal)
- Using exact mass, isotope pattern and **fragmentation** pattern to identify ‘features’



Untargeted screening

! 1 Exact mass ≠ 1 Formula ≠ 1 structure !

$C_{10}H_{14}O$

RT	Mass
3.815	150.1037
4.029	150.1043
4.246	150.1046
…	…

Suspect candidates
2-sec-Butylphenol
4-Butylphenol
4-sec-Butylphenol
4-tert-Butylphenol
…

Example from Wang et al., EHP, 2018

Screening approach

+	-
A **large number** of features can be detected (> 10,000)	May not detect chemicals present at low levels
Requires a small amount of sample	**Time consuming** and labor-intensive (i.e. peak picking)
Aims to measure biologically **meaningful** lifetime exposures of health relevance	Semi-quantitative at most
Retrospective data searching	**Less expertise** and QA-QC compared to targeted methods

from Dennis et al., EHP, 2017

Screening within FLEHS IV

- Complementary to classic biomonitoring
- More complete mixture-like assessment
- Database in development through HBM4EU WP16
- Mass spectral library with MS and MS/MS information

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FLEHS IV:

- FLEHS cohort: run optimized method on ~ 50 samples

Take home questions

What will be the approach in the next cycle of FLEHS?

Keep all current targeted measurements?

Focus on other chemicals?

‘New’ emerging contaminants?

Which ones?

Based on what information should they be prioritized?

Expand screening methods?

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HBM4EU

THANK YOU!

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