Advanced mass spectrometry in biomarker discovery for emerging contaminants

Assessing human exposure

Toxicological Centre
University of Antwerp

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Exposome
Centre of Excellence
University of Antwerp

Outline

- Investigating external exposure

- In vitro identification of biomarkers - to assess internal exposure

- Applications in human samples

Target analysis of biological samples

Suspect screening of biological samples

Investigating external exposure

INTAKE

Food

Air

Dust

Consumer Products

Use:

- **target analysis**
- **suspect/non-target screening**
- **a combination of the two**

Food and Chemical Toxicology 100 (2017) 1–7

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Dietary intake of phosphorus flame retardants (PFRs) using Swedish food market basket estimations

Giulia Poma [a, *], Anders Glynn [b], Govindan Malarvannan [a], Adrian Covaci [a, **], Per Ola Darnerud [b, ***]

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	TCEP	TPHP	EHDPHP	TDCIPP	TCIPP[a]
RfD values (ng/kg bw/day)	22,000	70,000	–	15,000	80,000
food groups					
Cereals	57	77	955	87	282
Pastries	12	36	448	12	39
Meat	21	97	136	39	15
Fish	4	28	112	13	3
Dairy, fluid	41	24	137	56	20
Dairy, solid	11	11	79	11	7
Eggs	2	2	25	4	3
Fats/oils	44	213	228	44	33
Vegetables	81	13	55	72	36
Fruits	21	17	109	67	25
Potatoes	14	23	44	36	21
Sugar/sweets	28	31	466	92	25
Beverages	70	78	472	269	63
Total (ng/day)	*406*	*650*	*3266*	*802*	*572*
Total[b] (ng/kg bw/day)	*6.0*	*9.7*	*48.6*	*11.9*	*8.5*

Target-food

Universiteit Antwerp

Suspect-dust

In-house **database HECHIER**
(~3000 compounds)
Keywords: phthalates, terephthalates, adipates, sebacates, citrates, trimellitates, phosphates, carboxylic & dicarboxylic acids, pesticides (pyrethroids, neonicotinoids)

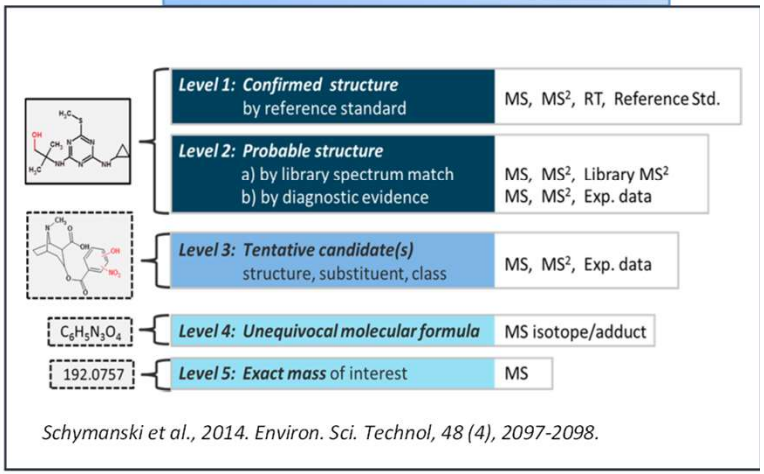
Data files Analysis
(Agilent Mass Hunter Qualitative Analysis software)
Find by formula tool, Limitations derived from QC, CEF files creation

Mass Profiler Professional software
Filtering/Features (compounds) Lists Generation

ESI+; 348

ESI-; 726

Level of Confidence L.o.C.



Schymanski et al., 2014. Environ. Sci. Technol, 48 (4), 2097-2098.

Prioritization Lists (ESI+, ESI-)
frequency in Ds, FBLKs, BLKs, & L.o.C.

Selection of compounds for further investigation
L.o.C, type of application, theoretical bioaccessibility

Suspect - dust

Newly identified compounds in dust

Chemosphere 263 (2021) 127817

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Suspect screening analysis in house dust from Belgium using high resolution mass spectrometry; prioritization list and newly identified chemicals

Christina Christia*, Giulia Poma, Noelia Caballero-Casero, Adrian Covaci**

Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610, Wilrijk, Antwerp, Belgium

Information on newly identified compounds in Flemish dust.

Compound Name (Cas Num)	Acronym	Trade Names	Formula	ESI	RT (min)	Monoisotopic Mass	Precursor Ion	Adduct	Mass Diff. (ppm)	Isotopic pattern Match Score %	Product Ions		DF % (W/S)	LogK$_{ow}$	Ba	Application
											CID 10 V	CID 30 V				
Tributyl trimellitate (1726-23-4)	TBTM	DIPLAST TM 4, Morflex TM 540	$C_{21}H_{30}O_6$	+	18.7	378.2041	379.2116	[M-H]+	−0.13	99.95	305.135, 249.074, 193.012, 57.070	249.0742, 193.0124, 57.0704	8/24	5.92	0.61	Plasticizer, additive in adhesives or printing inks, nail coatings, nail coatings removers
Bis (3,5,5-trimethylhexyl) phosphate (7153-98-2)	Bis-3,5,5-TMHPh	n.a.	$C_{18}H_{39}PO_4$	+	14.8	350.2585	351.2623	[M-H]+	0.52	94.08	98.9839, 71.0856, 57.0699	98.9833, 71.0850, 57.0698	32/8	6.8	0.44	Chemical blowing agent in polyurethane foams
Iso-octyl 2-phenoxy ethyl terephthalate (72512-75-5)	IOPhET	n.a.	$C_{24}H_{30}O_5$	+	18.6	398.2094	399.21161	[M-H]+	1.19	95.23	305.1750, 261.1468, 149.0205	305.1778, 261.1507, 149.0255, 121.0288, 65.0414	52/4	6.76	0.44	Additive in adhesives
Dimethyl Azelate (1732-10-1)	DMA	n.a.	$C_{11}H_{20}O_4$	+	12.2	216.1361	217.1436	[M-H]+	−0.2	99.73	125.0950, 97.1005, 83.0860, 55.0545	55.0546, 125.0927	28/56	2.86	0.8	Additive in materials of food packaging, active ingredient in pesticide products
Dimethyl sebacate (106-79-6)	DMS	n.a.	$C_{12}H_{22}O_4$	+	13.8	230.1518	231.1596	[M-H]+	−2.29	99.95	199.1334, 171.1365, 139.1114, 69.0705, 55.0502	69.0703, 55.0547	12/20	3.35	0.8	Additive in paints, fillers, adhesives, pesticides and ingredient used in automotive industry
Dipropylene glycol dibenzoate (27138-31-4)	DiPGDB	VELSIFLEX® 328, Velsiflex 342, Finsolv® PG-22	$C_{20}H_{22}O_5$	+	14.9	342.1467	343.1544	[M-H]+	−1.26	99.88	163.0755, 105.0338, 77.0395	163.0753, 105.0340, 77.0393	40/48	3.9	0.8	Additive in adhesives, building construction materials, paints, manufacture of furniture, personal care products
3,5-Di-*tert*-butyl-4-hydroxy benzaldehyde (1620-98-0)	BHT-CHO	n.a.	$C_{15}H_{22}O_2$	−	14.3	234.162	233.1547	[M-H]-	−1.25	99.8	217.1226, 59.0147	217.1221, 59.0147, 133.0656	8/28	4.2	0.8	Additive chemical in toys, drug product or related with the manufacturing of drugs

Target - dust

Environmental Research 197 (2021) 111193

Contents lists available at ScienceDirect

Environmental Research

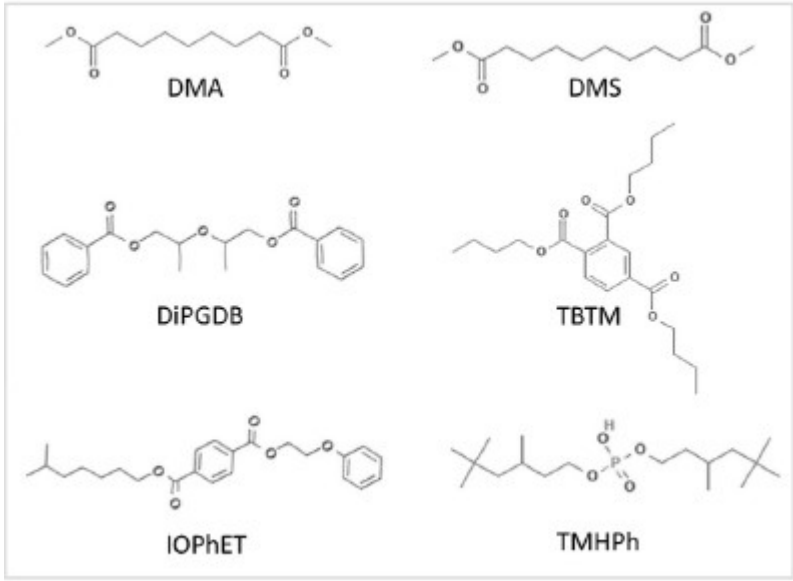
journal homepage: www.elsevier.com/locate/envres

From suspect screening to target analysis: Occurrence of six newly identified compounds in indoor dust from Belgium*

Christina Christia *, Giulia Poma, Noelia Caballero-Casero [1], Adrian Covaci **

Descriptive statistics for 50 paired dust samples from Flemish region (Belgium).

Targeted Analyte	Mean (μg/g)	SD	Median (μg/g)	Min (μg/g)	Max (μg/g)	DF %	mLOQ (μg/g)
Winter (n = 25)							
DMA	0.01	0.01	0.01	0.01	0.05	16	0.03
DMS	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	0.09
DiPGDB	16	23	6.2	0.56	89	100	0.08
TBTM	0.67	2.5	0.04	0.02	12	64	0.03
IOPhET	0.01	0.01	2E-03	2E-03	0.03	48	4E-03
TMHPh	0.27	0.36	0.06	0.02	1.2	68	0.03
Summer (n = 25)							
DMA	0.05	0.03	0.04	0.02	0.15	60	0.03
DMS	0.02	0.03	0.01	0.01	0.12	8	0.09
DiPGDB	13	26	5.4	0.83	129	100	0.08
TBTM	0.14	0.36	0.01	0.01	1.7	48	0.03
IOPhET	2E-03	4E-03	6E-04	6E-04	0.02	16	4E-03
TMHPh	0.12	0.24	0.01	0.01	1.1	36	0.03



Target - dust

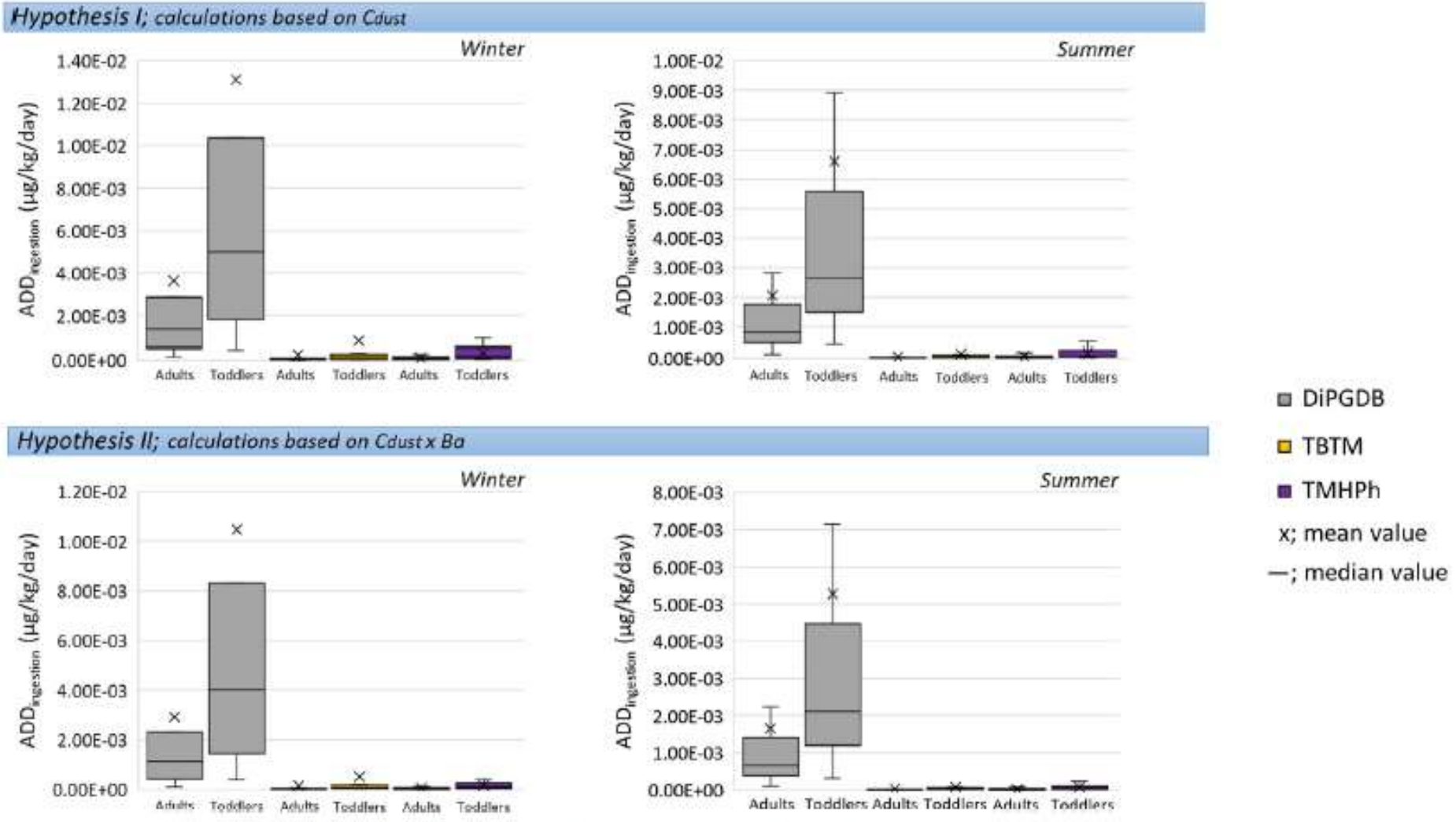


Fig. 5. $ADD_{ingestion}$ (µg/kg/day) calculated for adults and toddlers per season.

In vitro identification of biomarkers

- to assess internal exposure

INTAKE

METABOLISM

Food

Air

External exposure

BIOMONITORING

Serum/Blood

Dust

Consumer Products

Urine

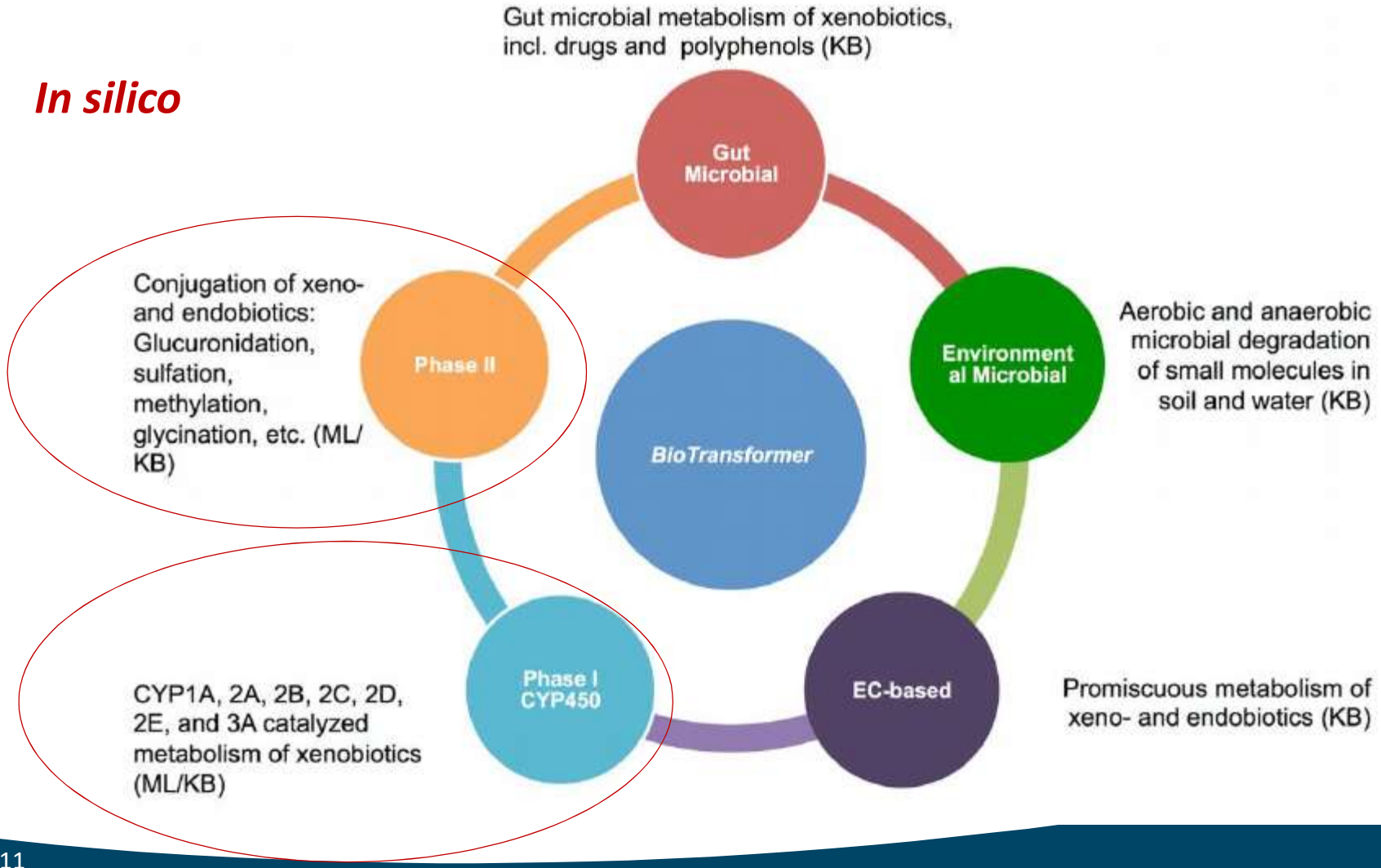
Need to measure the right biomarkers !

Metabolite simulation

- Rat liver S9 metabolism (OECD Toolbox)
- BioTransformer

- Meteor
- MetaSense

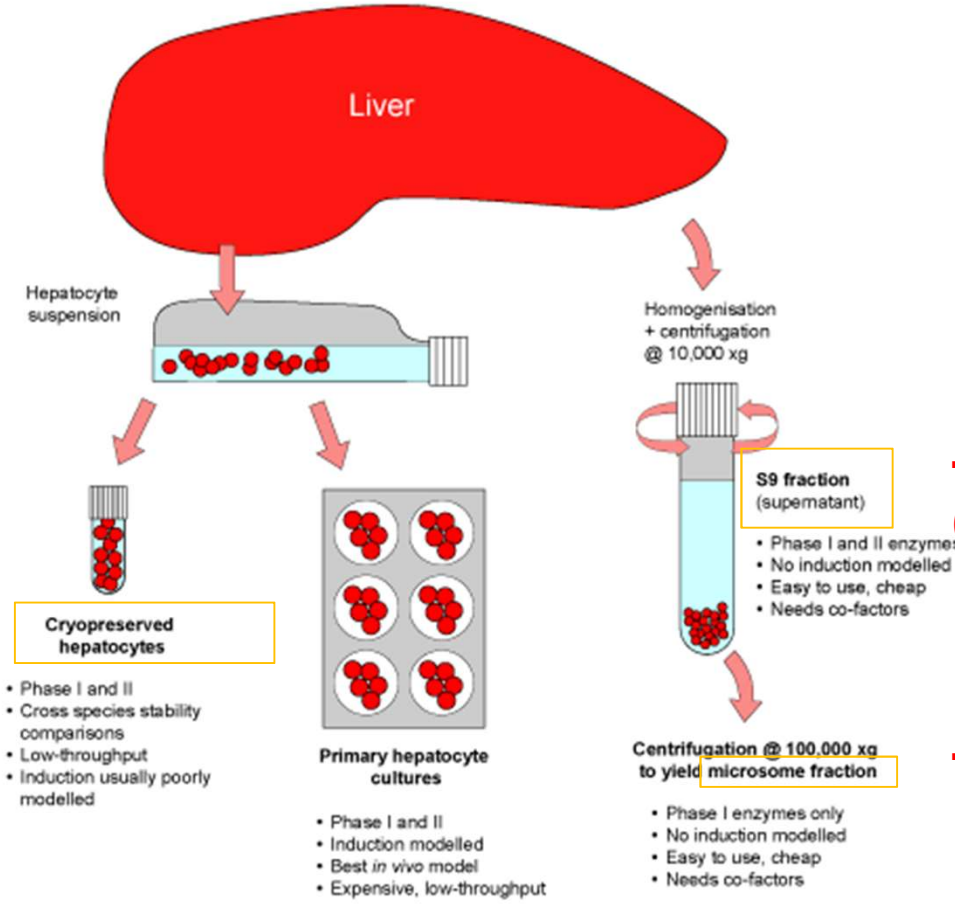
In silico



- identify best to monitor biomarkers in human body

(using in vitro testing with human liver microsomes or liver cells)

In vitro



- HepG2
- HepaRG

- S9 (cytosol)

- HLM

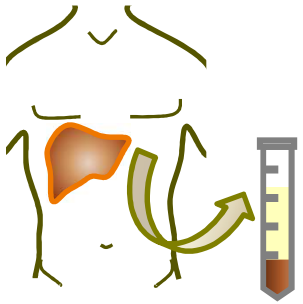
Identification of metabolites

1. Generate a **metabolite database by using specialized software**

(e.g., *Meteor, Biotransformer*)

2. Perform ***in vitro*** **incubations with microsomes** (CYP, UGT) **or**

S9 fractions (SULT)



3. **Analysis by LC-QTOFMS**

suspect screening

+

non-target screening

Threshold (5-10 ppm)

Suspect or Non-target screening?

Suspect = Identification of predicted (expected) metabolites
→ Known unknowns

Non-target = Identification of new, not predicted metabolites
→ Unknown unknowns

Agilent Technologies MZmine 2 R

Suspect screening

Subscription software

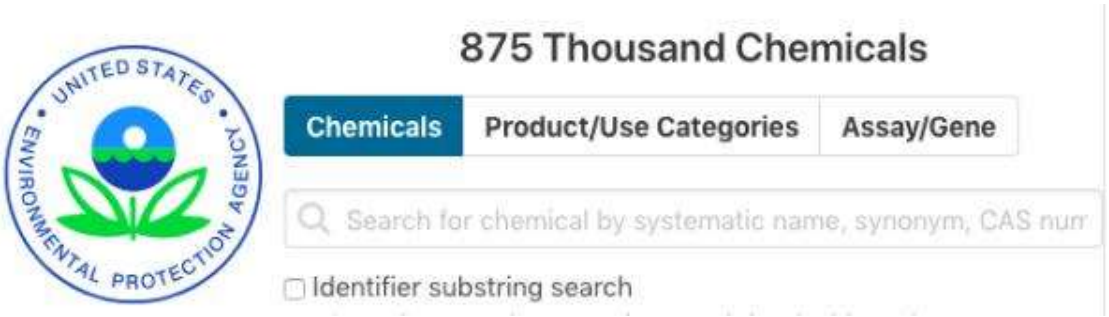
Meteor nexus

Free access

Biotransformer.ca

ChemSpider

CompTox Chemicals Dashboard



Database

= List of chemicals of interest

- Name
- Chemical formula
- Monoisotopic mass
- Retention time
- MS/MS spectra

Non-target screening

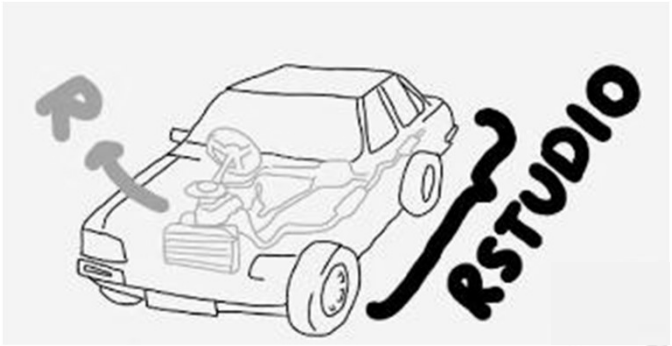
Open Source Workflow

MZmine 2

Find all features of interest
in samples

R Studio

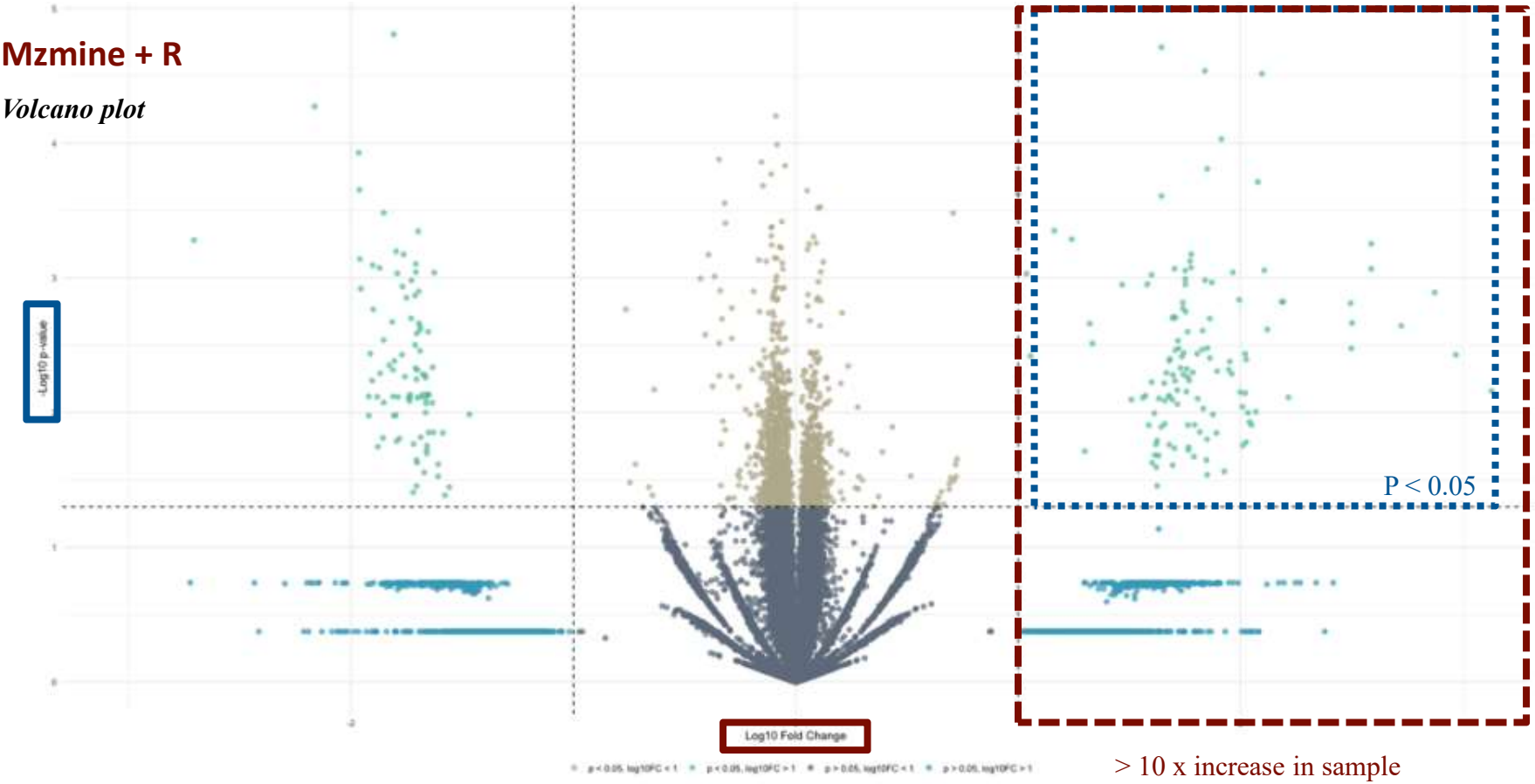
Prioritization of features



MS-DIAL

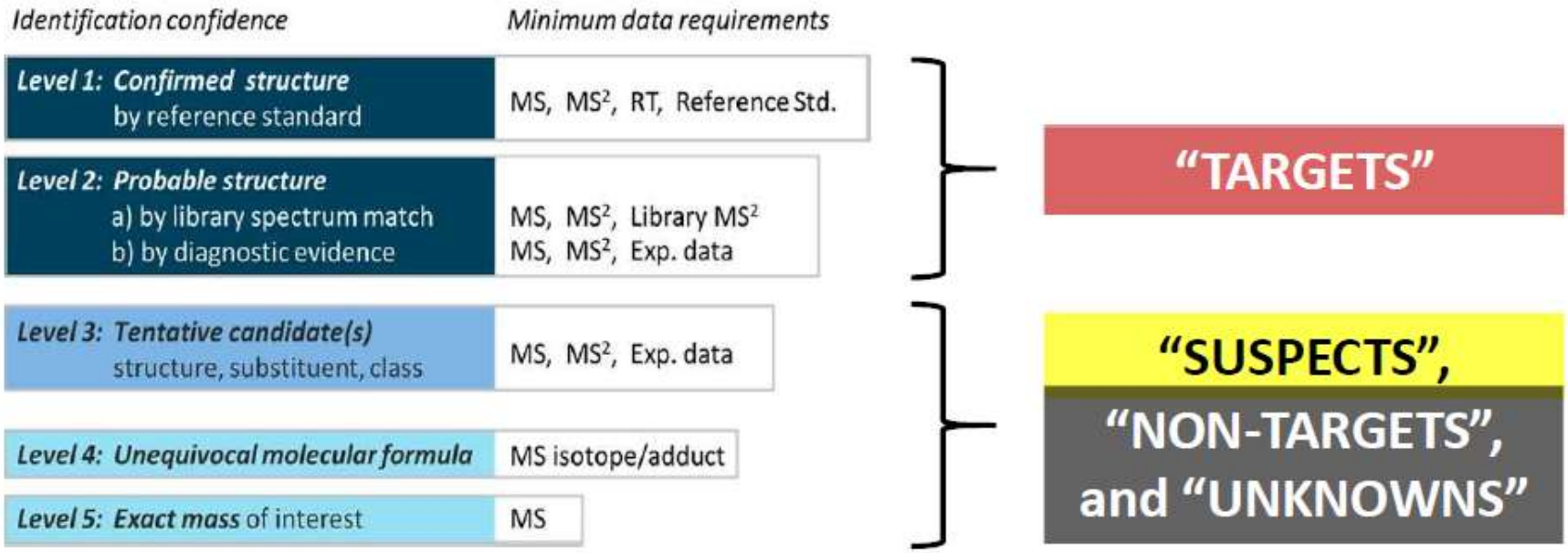
Mzmine + R

Volcano plot



> 10 x increase in sample

Identification confidence



Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J. Environ Sci Technol 2014, 48, 2097-2098

Correct annotation is the key to communicate confidence

Identification of metabolites

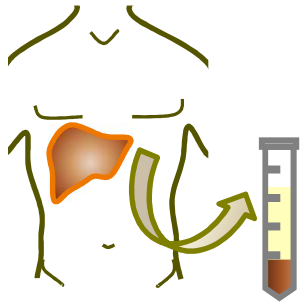
New metabolites identified by untargeted screening are added to the original database

1. Generate a **metabolite database by using specialized software**

(e.g., *Meteor, Biotransformer*)

2. Perform ***in vitro*** **incubations with microsomes** (CYP, UGT) **or**

S9 fractions (SULT)



3. Analysis by LC-QTOFMS

suspect screening

+

non-target screening

Threshold (5-10 ppm)

4. Confirmation: MS/MS experiments

+

Formation of metabolites by

- increasing incubation time, compound and protein concentration

+

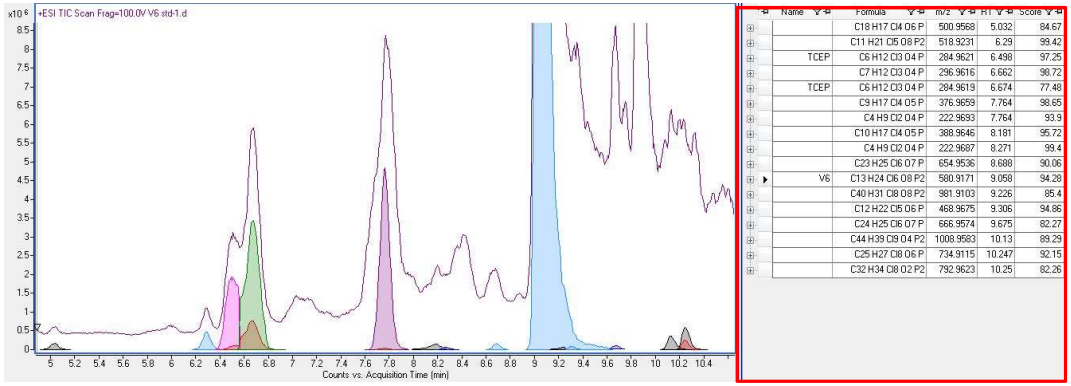
- injection of authentic standards

- Propose a metabolism pathway

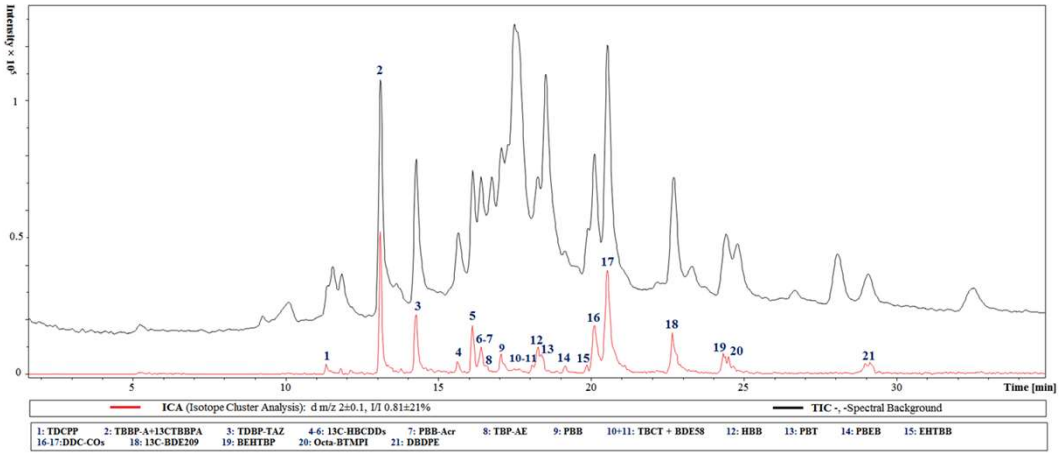
- Propose suitable candidate biomarkers

How to facilitate the detection of halogenated chemicals?

- Mass defect filtering:
  - based on the particularity that halogens have a negative mass defect and that halogenated FRs contain > 3 halogen atoms;
  - output: extracted compounds



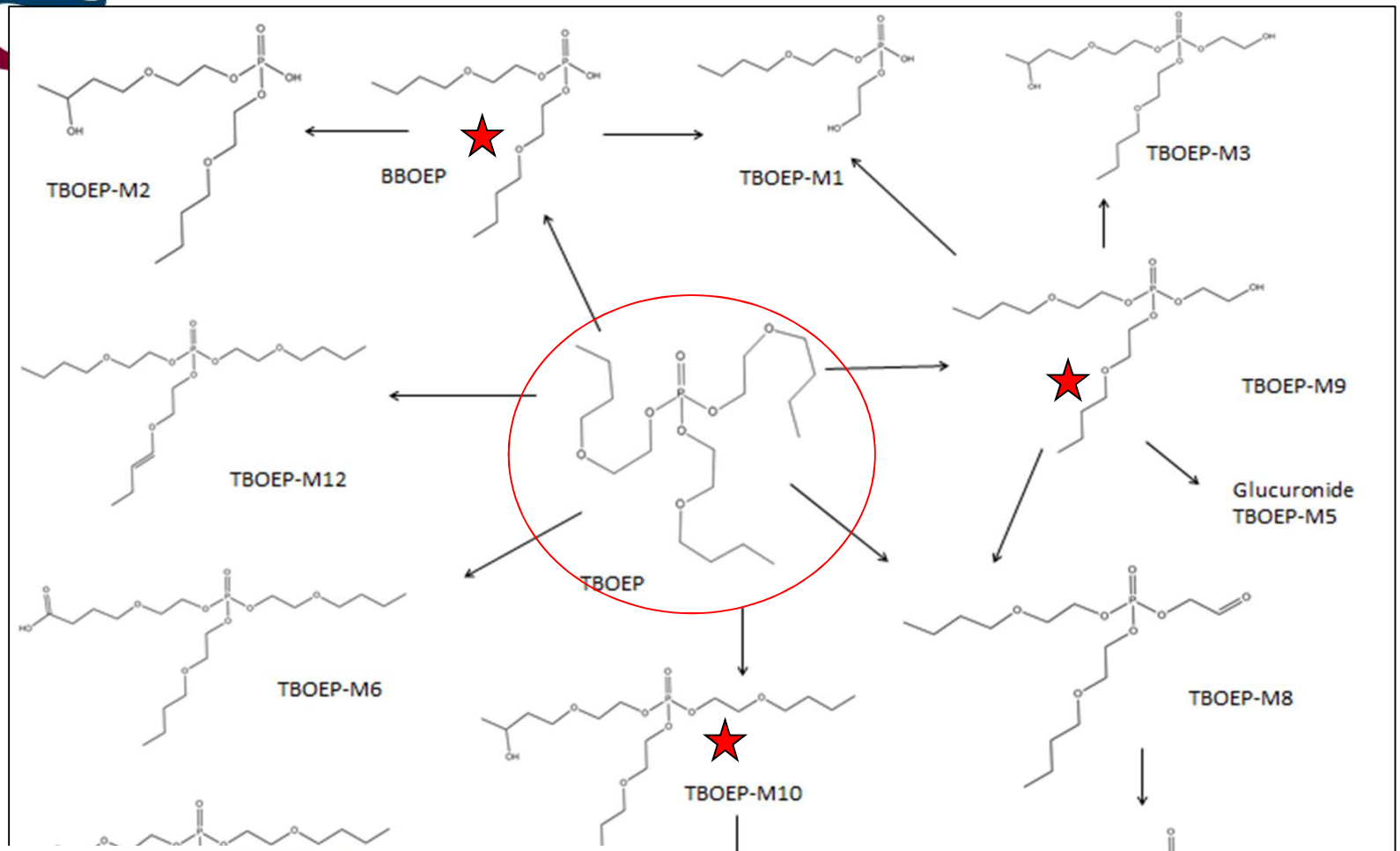
- Isotope cluster analysis:
  - based on the exact mass difference and intensity between two isotopomers;
  - output: extracted chromatogram



Universiteit Antwerpen

Ionas et al., J. Mass Spectrom, 2015

TBOEP metabolism



Toxicology and Applied Pharmacology 284 (2015) 246–253

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

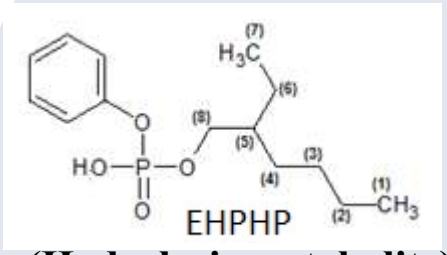
2015

journal homepage: www.elsevier.com/locate/ytaap

In vitro biotransformation of tris(2-butoxyethyl) phosphate (TBOEP) in human liver and serum

Nele Van den Eede [a,*], Claudio Erratico [a], Vassiliki Exarchou [b], Walid Maho [a], Hugo Neels [a], Adrian Covaci [a,*]

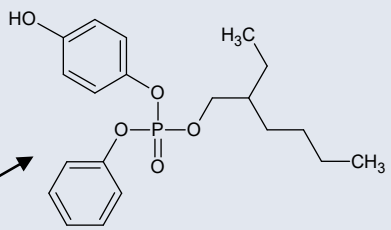
EHDPHP metabolism



(Hydrolysis metabolite)

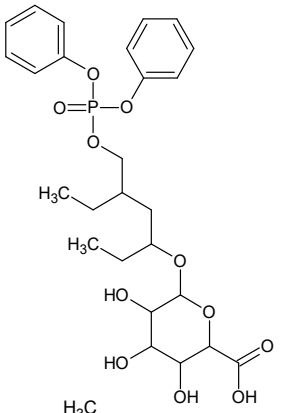
Phase-I

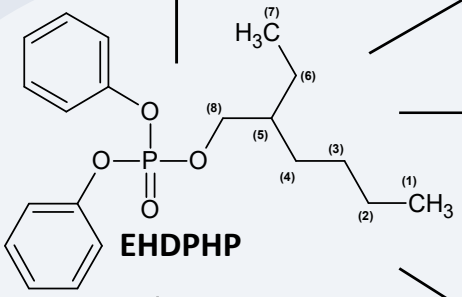
Phase-II



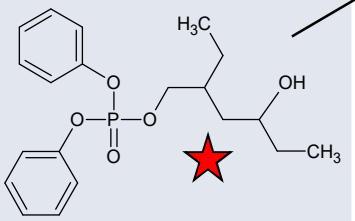
Monohydroxylated metabolites with HO- in the phenyl group (HO9)

Phase-II glucuronide-conjugates (GLUC1-8)

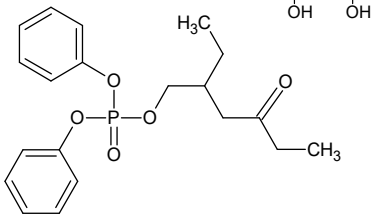




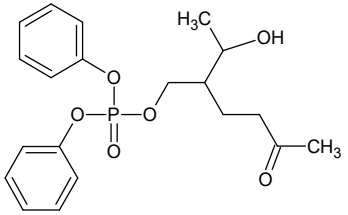
EHDPHP



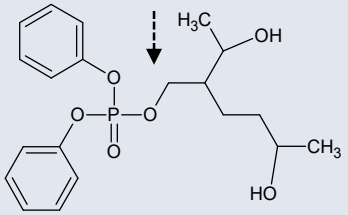
Monohydroxylated metabolites with HO- in the alkyl chain (HO1-8)



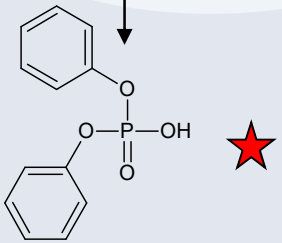
Metabolites with one ketone group (O1 and O2)



Metabolites with one OH- and one ketone group (OOH1-OOH5)

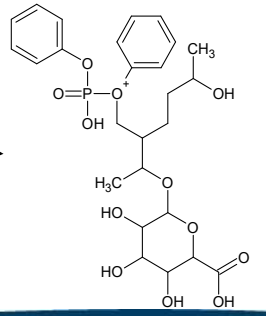


Dihydroxylated metabolites (di-HO1-10)



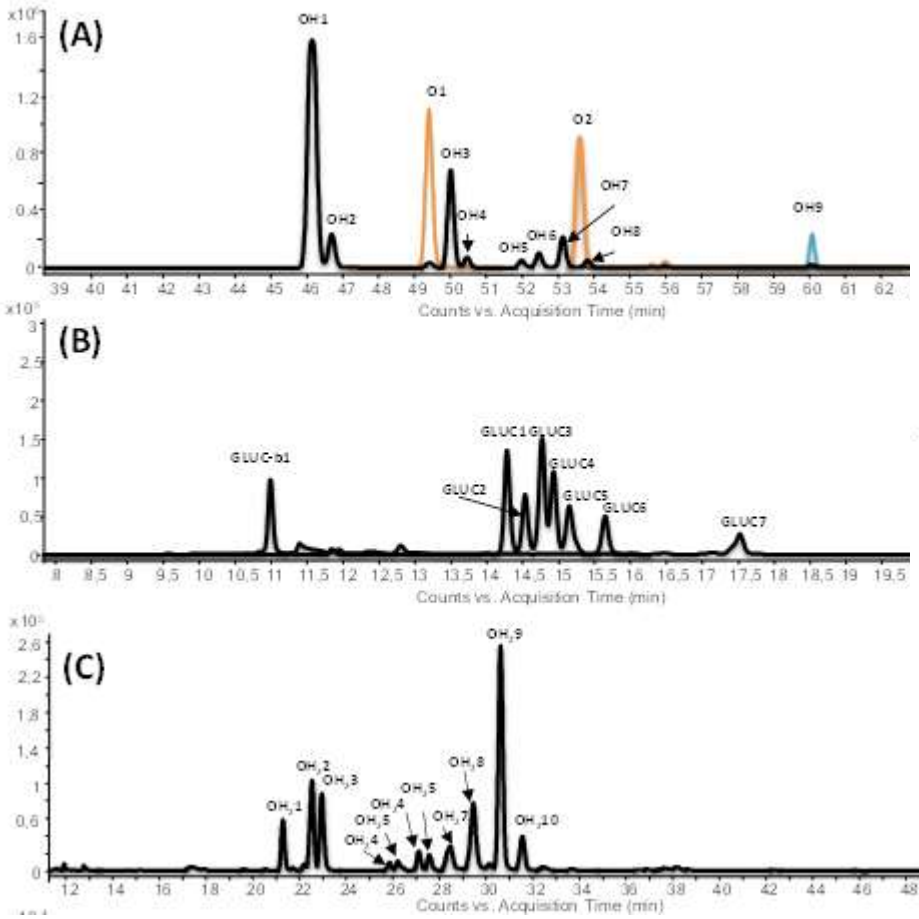
DPHP
(Hydrolysis metabolite)

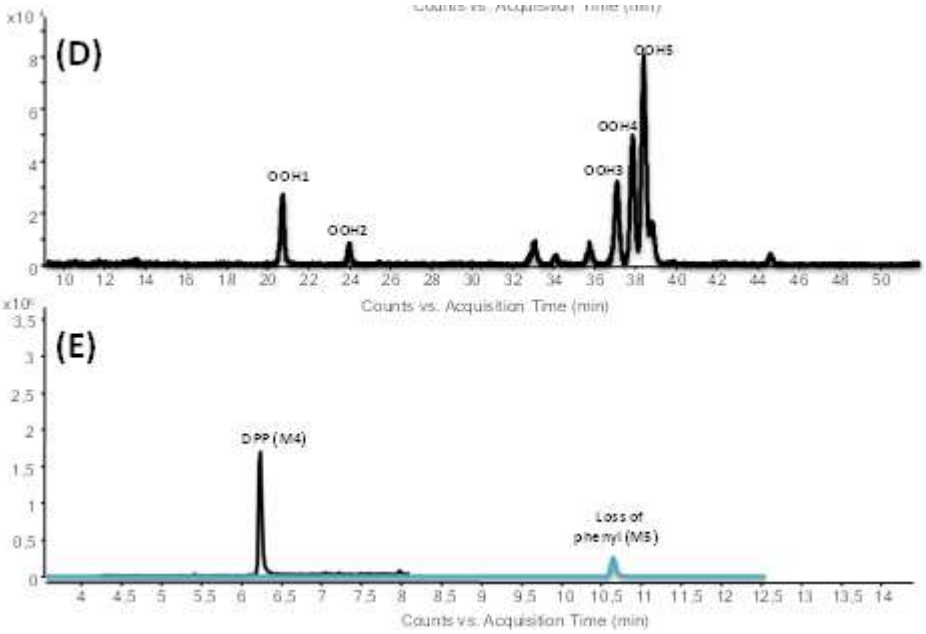
Phase-II glucuronide-conjugates (GLUCb1)



Ballesteros et al., Toxicol Lett, 2014

EHDPHP metabolism





Toxicology Letters 232 (2015) 203–212

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Universiteit Antwerpen

In vitro metabolism of 2-ethylhexyldiphenyl phosphate (EHDPHP) by human liver microsomes

Ana Ballesteros-Gómez[a,b,*], Claudio A. Erratico[a], Nele Van den Eede[a], Alin C. Ionas[a], Pim E.G. Leonards[b], Adrian Covaci[a]

EHDPHP

60 morning urine samples from A-TEAM cohort

ng/mL	DPHP	EHPHP	5-HO-EHDPHP
min	0.09	<MLQ	<MLQ
max	8.55	3.46	1.30
median	0.78	<MLQ	0.06
DF (%)	**100**	**33**	**98**

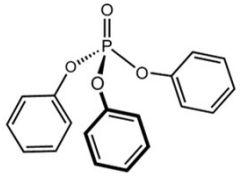
Hydrolysis products

Oxidative metabolite

Xu et al., Environ Int 2019

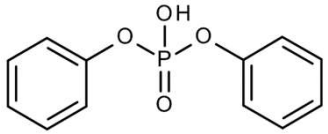
Potential biomarkers of exposure

- Formation of **dialkyl/aryl phosphates** (DAPs)



Triphenyl phosphate (TPHP)

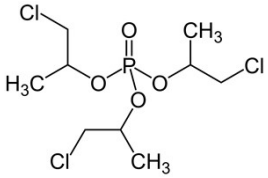
Phase I



Diphenyl phosphate (DPHP)

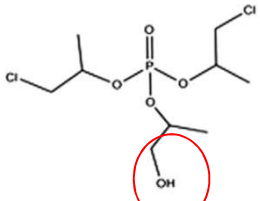
Direct excretion in urine

- Formation of **hydroxylated metabolites** (HO-PFRs)



Tris (1-chloro-2-propyl) phosphate (TCIPP)

Phase I



1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP)

Phase II

Candidate biomarkers

Excreted as glucuronide-conjugates in urine

Van den Eede et al., (2013) Toxicol. Lett.

Summary

Parent	Hydrolysis metabolite	Oxidative metabolite
TDCIPP	BDCIPP	-
TCIPP	BCIPP	TCIPP-OH
TBOEP	BBOEP	TBOEP-OH Desbutyl-TBOEP
TPHP	DPHP	HO-TPHP
EHDPHP	EHPHP DPHP	5-HO-EHDPHP
RDP	DPHP	HO-RDP, di-OH-RDP
BDP	DPHP	HO-BDP, others
V6	Several	Several

Specific metabolite Non-specific metabolite

Target analysis of biological samples

In vivo application

Parent compound	Biomarkers in urine
EHDPHP	5-HO-EHDPHP
	EHPHP
TBOEP	BBOEP
	TBOEP-OH
	BBOEHEP
TCIPP	BCIPP
	BCIPHIPP
TPHP	DPHP
	4-HO-DPHP
	3-HO-TPHP
	4-HO-TPHP
TDCIPP	BDCIPP
TBP	DNBP
TCEP	TCEP

- **2 mL** urine sample spiked with IS
- Deconjugation with β-glucuronidase

- SPE with **Bond-Elut C18**
- Washing with H2O
- Elution with 3 mL of MeOH

- Separation on **Biphenyl column**
- LC-MS/MS analysis on Agilent 6460

Bastiaensen et al., Anal. Bioanal. Chem., 2018

QA/QC measures – target analysis

- **QA/QC activities**

- **Participation in 4 rounds of Interlab comparison organised within HBM4EU (within WP9)**
  - passed each time for DPHP, BDCIPP and BCIPP

- **Participation since 2018 in the Canadian AMAP interlab scheme**
  - Passed each round for DPHP and BDCIPP

- **Established indicative values for PFR metabolites in SRM 3673 (organics in human urine)**

Chemosphere 236 (2019) 124348

Contents lists available at ScienceDirect

Chemosphere 2019

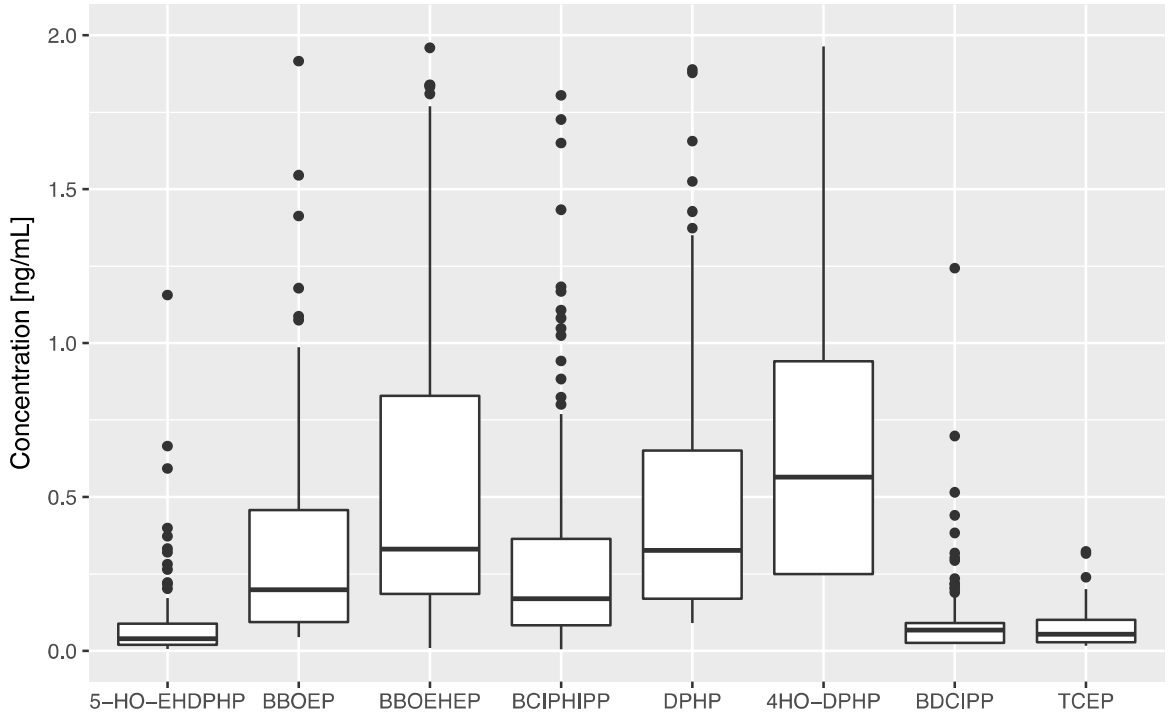
journal homepage: www.elsevier.com/locate/chemosphere

Towards establishing indicative values for metabolites of organophosphate ester contaminants in human urine

Michiel Bastiaensen [a], Nele Van den Eede [a], Guanyong Su [b], Robert J. Letcher [c], Heather M. Stapleton [d], Adrian Covaci [a, *]

Japan: Hokkaido study

PFR metabolites in Japanese children (in ng/mL)



- 8 out of 14 metabolites frequently detected
- Median concentrations < 1 ng/mL
- Increased exposure to TBOEP, specific to Japan

Araki et al., Environ Int 2018

Main OPEs in food : TPHP, TCIPP and EHDPHP

Main OPE metabolites in urine : DPHP, BCIPHIPP and EHPHP

Belgium

EHPHP – particular signature in the Belgian exposure

(other factors, such as dust ingestion might also contribute to this)

Environment International 147 (2021) 106368

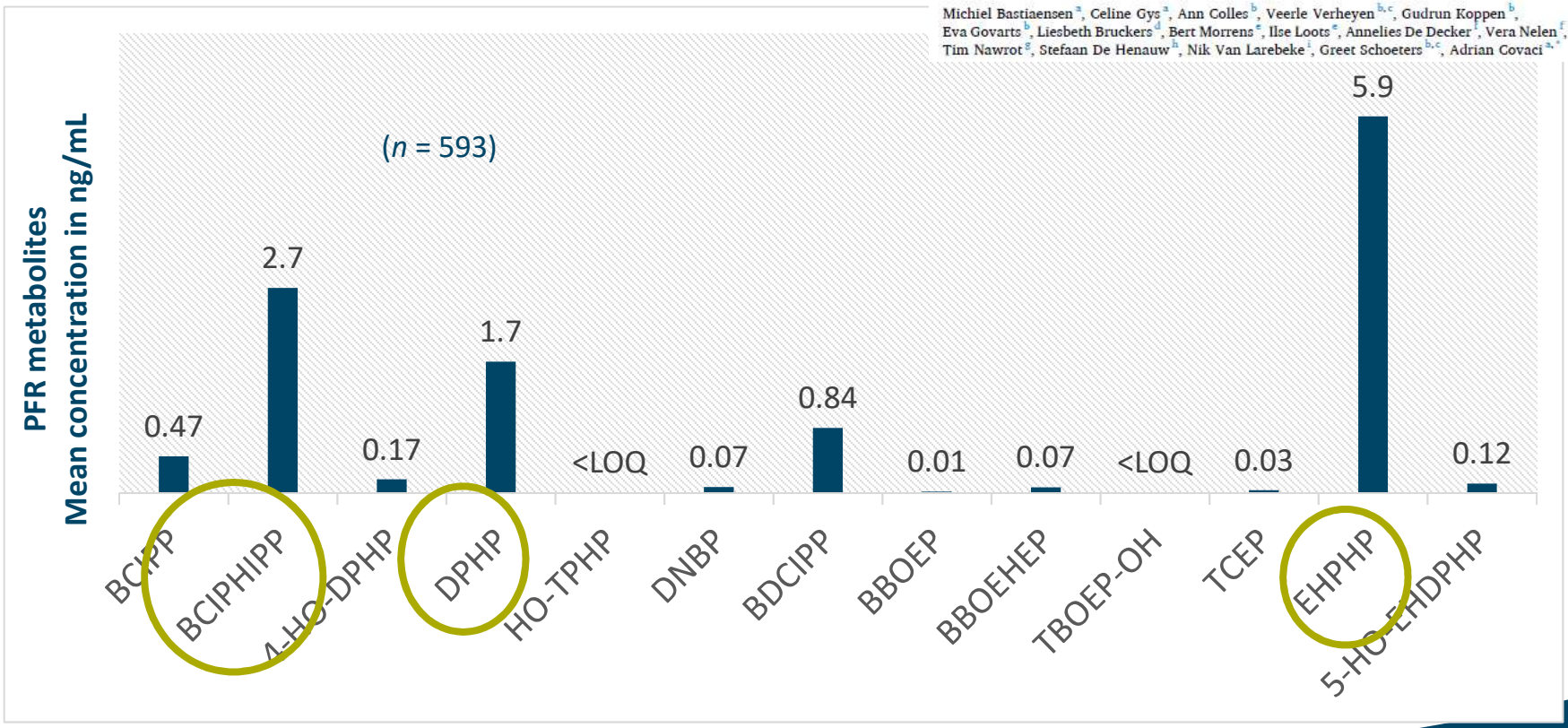
Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Exposure levels, determinants and risk assessment of organophosphate flame retardants and plasticizers in adolescents (14–15 years) from the Flemish Environment and Health Study

Michiel Bastiaensen [a], Celine Gys [a], Ann Colles [b], Veerle Verheyen [b,c], Gudrun Koppen [b], Eva Govarts [b], Liesbeth Bruckers [d], Bert Morrens [e], Ilse Loots [e], Annelies De Decker [f], Vera Nelen [f], Tim Nawrot [g], Stefaan De Henauw [h], Nik Van Larebeke [i], Greet Schoeters [b,c], Adrian Covaci [a,*]



Estimated daily intake for PFRs (FLEHS IV)

$$EDI = \left(\frac{c_{meta} \times V_{urine}}{F_{UE} \times bw}\right) \times \frac{MW_p}{MW_m}$$

EDIs adolescents in ng/kg bw/day (*n* = 582)	**TPHP** based on DPHP	**TDCIPP** based on BDCIPP	**TCIPP** based on BCIPHIPP	**TBOEP** based on BBOEHEP	**EHDPHP** based on sum EHDPHP
F_{ue} (S9)	0.19	0.68	0.28	0.16	0.2
50th per	197.7	14.5	57.4	6.3	567.7
95th per	640.0	119.1	517.5	44.2	2004.2
RfD (ng/kg bw/day)	2.0x10^4	2.0x10^4	1.0x10^4	1.5x10^4	1.5x10^4
% > RfD	0%	0%	**0.17%**	0%	0%
ratio RfD/95th per	31	168	19	340	7

Bastiaensen et al. (2021) *Environ. Int.*

Suspect screening of biological samples

Chemosphere 280 (2021) 130683

Contents lists available at ScienceDirect

Chemosphere

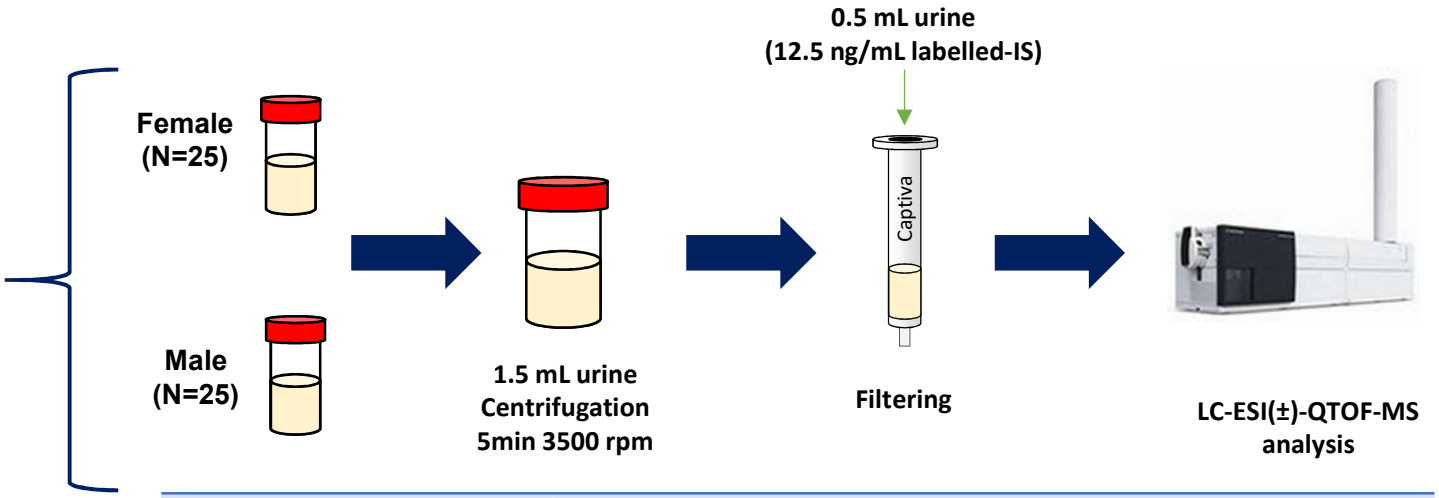
journal homepage: www.elsevier.com/locate/chemosphere

Identification of chemicals of emerging concern in urine of Flemish adolescents using a new suspect screening workflow for LC-QTOF-MS

Noelia Caballero-Casero [a, *, 1], Gabriela Castro [b, 2], Michiel Bastiaensen [a], Celine Gys [a], Nik van Larebeke [c], Greet Schoeters [d, e], Adrian Covaci [a, **]

Sample treatment of urine samples and instrumental analysis

FLEHS IV (2016-2020)
n=600 adolescents



Mobile phase composition	ESI(+): water 0.1%FA (A), MeOH 0.1% FA (B)
	ESI(-): water 1 mM NH4F (A), MeOH 1 mM NH4F (B)
Elution program - Flow rate	Gradient - 0.3 mL/min
Acquisition mode	Data-dependant. Auto MS/MS (by abundance). CE 10, 20, 40 eV
Scan range	100-1500 m/z (MS) – 50-1100m/z (MS/MS)
Data storage	Centroid

Suspect screening data-analysis

***IN-HOUSE* SUSPECT LIST ECs**
10754 Parent & Metabolites Compounds
(Name, IUPAC name, Formula, m/z, SMILES, InChi, InChiKey, CAS, chemical group)

Workflow A

Profinder

- Alignment
- Deconvolution:
  Batch recursive features extraction (small molecules)
  - Mass tolerance (20 ppm for parent ions and 25 ppm for product ions)
  - Match score > 70

Mass Profiler Professional

- Filter by frequency: at least in 1% of the samples in at least 1 condition
- QC on samples
- PCA samples/procedural blanks
  - Pvalue cut off= 0.05
  - Fold change = 10

MassHunter ID Browser

- Database: *in-house suspect list*
  - Spectrum peaks = 5
  - Maximum results = 1
  - Mass tolerance (7.5 ppm for parent ions and 10 ppm for product ions)
  - Mass score > 80
  - Isotope abundance score= 80

MassHunter Qualitative Analysis

- Manual inspection of each identification
- Level of identification based on Schymanski´s scale

MassHunter Qualitative
Find by Formula
(Database containing only IS)

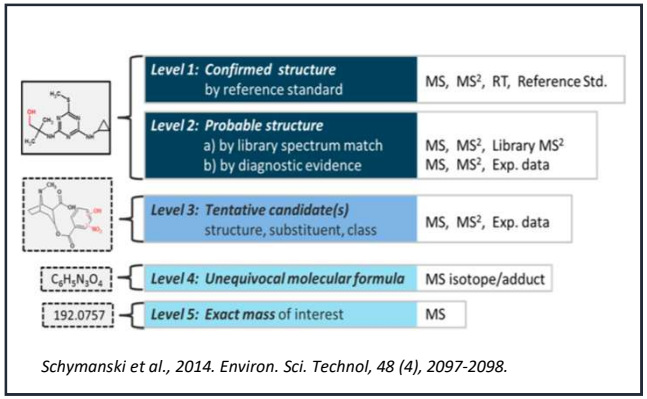
- Δt_{ret}
- Mass accuracy
- Isotopic pattern

Workflow B

European MassBank Database/ MassBank of North America (MoNA)

MS-DIAL

- Alignment
- Batch recursive features extraction (small molecules)
  - Mass tolerance (20 ppm for parent ions and 25 ppm for product ions)
  - Match score > 70
- Database: European MassBank Database/ MassBank of North America (MoNA)
  - Manual inspection of each identification
  - Level of identification based on Schymanski´s scale



QA/QC measures – suspect screening analysis

Trends in Analytical Chemistry 136 (2021) 116201

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Towards harmonised criteria in quality assurance and quality control of suspect and non-target LC-HRMS analytical workflows for screening of emerging contaminants in human biomonitoring

Noelia Caballero-Casero [a, **], Lidia Belova [a], Philippe Vervliet [a], Jean-Philippe Antignac [b], Argelia Castaño [c], Laurent Debrauwer [d, e], Marta Esteban López [c], Carolin Huber [f], Jana Klanova [g], Martin Krauss [f], Arjen Lommen [h], Hans G.J. Mol [h], Herbert Oberacher [i], Olga Pardo [j], Elliott J. Price [g, k], Vera Reinstadler [i], Chiara Maria Vitale [g], Alexander L.N. van Nuijs [a, 1], Adrian Covaci [a, *, 1]

Oberacher et al. Environ Sci Eur (2020) 32:43
https://doi.org/10.1186/s12302-020-00314-9

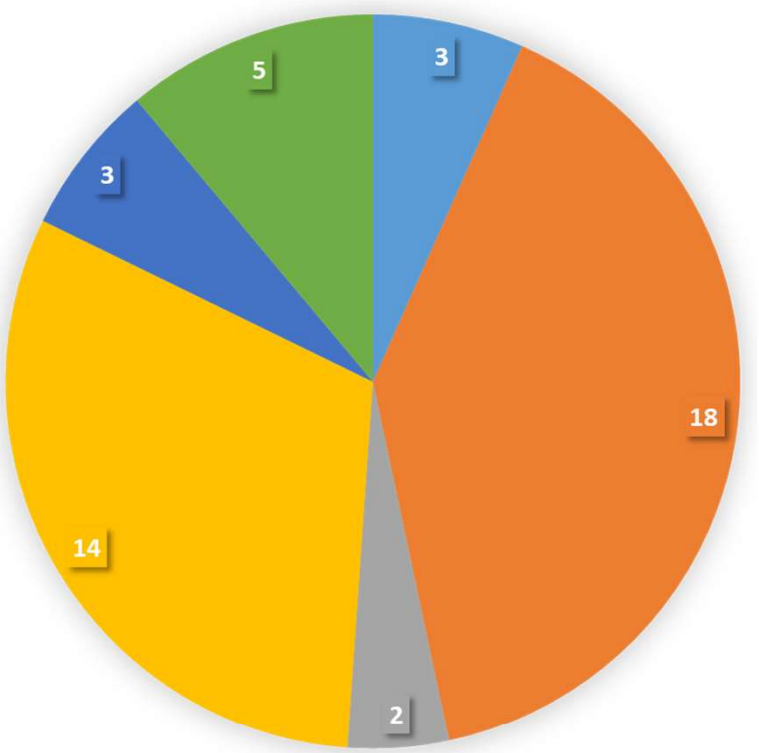
Environmental Sciences Europe

RESEARCH Open Access

A European proposal for quality control and quality assurance of tandem mass spectral libraries

Herbert Oberacher[1*], Michael Sasse[2], Jean-Philippe Antignac[3], Yann Guitton[3], Laurent Debrauwer[4,5], Emilien L. Jamin[4,5], Tobias Schulze[6], Martin Krauss[6], Adrian Covaci[7], Noelia Caballero-Casero[7], Kathleen Rousseau[8], Annelaure Damont[8], François Fenaille[8], Marja Lamoree[9] and Emma L. Schymanski[10*]

45 Identified ECs in urine (Level of identification 1-3)



- Pesticides
- Pharmaceutical and personal care products
- Plasticizers
- UV-filters
- Phenolic antioxidants
- Food additives

Chemosphere 280 (2021) 130683

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Identification of chemicals of emerging concern in urine of Flemish adolescents using a new suspect screening workflow for LC-QTOF-MS

Noelia Caballero-Casero [a, *, 1], Gabriela Castro [b, 2], Michiel Bastiaensen [a], Celine Gys [a], Nik van Larebeke [c], Greet Schoeters [d, e], Adrian Covaci [a, **]

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[d] VITO Health, Flemish Institute for Technological Research (VITO), Boeretang 200, 2400, Mol, Belgium
[e] Department of Biomedical Sciences, University of Antwerp, Universiteitsplein 1, 2610, Wilrijk, Belgium


Chemosphere

Identification of chemicals of emerging concern in urine of Flemish adolescents using a new suspect screening workflow for LC-QTOF-MS

45 Identified ECs in urine (Level of identification 1-3)

Chemical class	Compound	Polarity	MS ready Formula	Level of Identification[a]	DF Total (%)
PPCPs	Methylparaben (MeP)	ESI (−)	$C_8H_8O_3$	L1	10
	Acetylated product of Methylparaben	ESI (+)	$C_{10}H_{11}O_4$	L3	88
	Ethylparaben glucuronide	ESI (+)	$C_{15}H_{18}O_9$	L2	90
	Methylation product of Ethylparaben	ESI (+)	$C_{10}H_{12}O_3$	L3	66
	Oxidation product of Methylparaben	ESI (−)	$C_8H_8O_4$	L3	38
	Glyceramide	ESI (+)	$C_3H_7NO_3$	L3	94
	Oxidation product of Dimethyl sebacate	ESI (−)	$C_{12}H_{22}O_5$	L3	82
	1H-Benzotriazole	ESI (+)	$C_6H_5N_3$	L2	2
	Me-BTR (Tolytriazole)	ESI(+)	$C_7H_7N_3$	L2	4
	Reduction (NH)_xylyltriazole	ESI (−)	$C_{10}H_9N_2$	L3	66
	Benzoic acid	ESI (+)	$C_7H_6O_2$	L2	10
	4-Hydroxybenzoic acid	ESI(+)	$C_7H_6O_3$	L2	4
	Kojic Acid	ESI (+)	$C_6H_6O_4$	L2	2
	Umbelliferon	ESI (+)	$C_9H_6O_3$	L2	2
	3-aminobenzoic acid	ESI (+)	$C_7H_7NO_2$	L2	14
	Lauryl sulfate	ESI (−)	$C_{12}H_{26}O_4S$	L2	52
Plasticizers	Cyclohexyl pentyl phthalate; Phthalic acid	ESI (+)	$C_{19}H_{26}O_4$	L3	78
	Cyclohexyl pentyl phthalate glucuronide; Phthalic acid glucuronide	ESI (+)	$C_{25}H_{34}O_{10}$	L2	72
	Terephthalic acid glucuronide	ESI (+)	$C_{14}H_{14}O_{10}$	L2	90
	Methylation product of Dimethyl terephthalate	ESI (+)	$C_{11}H_{12}O_4$	L3	42
	Methylation product of Terephthalate	ESI (−)	$C_9H_6O_4$	L3	100
	Monopropyl Phthalate (MPrP)	ESI (+)	$C_{11}H_{12}O_4$	L3	38
	Butylene terephthalate glucuronide	ESI (+)	$C_{18}H_{20}O_{10}$	L2	78
	Di-n-nonyl phthalate glucuronide	ESI (+)	$C_{32}H_{50}O_{10}$	L3	42
	Mono-iso-nonyl phthalate glucuronide	ESI (+)	$C_{23}H_{32}O_{10}$	L2	96
	Mono-n-pentyl phthalate glucuronide	ESI (+)	$C_{19}H_{24}O_{10}$	L3	100
	Methylation product of Mono(2-ethyl-5-hydroxyhexyl) phthalate	ESI (+)	$C_{17}H_{24}O_5$	L3	100
	Methylation product of Monobenzyl phthalate	ESI (+)	$C_{16}H_{14}O_4$	L3	90
	Oxidation product of Mono-carboxy-isodecyl phthalate	ESI (+)	$C_{18}H_{24}O_7$	L3	76
	Mono-(5-carboxypentyl) phthalate	ESI (+)	$C_{14}H_{16}O_6$	L3	92
	Mono-hydroxy-isononyl phthalate	ESI (+)	$C_{17}H_{24}O_5$	L3	100
	Bisphenol S (BPS)	ESI (−)	$C_{12}H_{10}O_4S$	L1	18

45 Identified ECs in urine (Level of identification 1-3)


Chemosphere

Identification of chemicals of emerging concern in urine of Flemish adolescents using a new suspect screening workflow for LC-QTOF-MS

Chemical class	Compound	Polarity	MS ready Formula	Level of Identification[a]	DF Total (%)
Antioxidant phenols	Acetylated product of mono-5-carboxy-2-ethylpentyl adipate	ESI (−)	$C_{16}H_{27}O_7$	L3	26
	3,5-di-tert-butyl-4-hydroxy-benzoic acid glucuronide	ESI (−)	$C_{21}H_{30}O_9$	L3	80
UV filters	Trolamine salicylate	ESI (+)	$C_{13}H_{21}NO_6$	L3	52
	Cinoxate glucuronide	ESI (+)	$C_{20}H_{26}O_{10}$	L2	78
	Bis(4-hydroxyphenyl)methanone glucuronide	ESI (−)	$C_{19}H_{18}O_9$	L2	90
Pesticides	Piperonyl butoxide	ESI (+)	$C_{19}H_{30}O_5$	L2	86
	Bacimethrin	ESI (+)	$C_6H_9N_3O_2$	L3	82
	Methothrin glucuronide	ESI (+)	$C_2H_{34}O_9$	L3	58
Food additives	2-Methylindole	ESI (+)	C_9H_9N	L2	6
	Coumarin	ESI (+)	$C_9H_6O_2$	L2	32
	Protocatechuic aldehyde	ESI (−)	$C_7H_6O_3$	L2	14
	Benzeneformic acid	ESI (−)	$C_7H_6O_2$	L2	4
	Dodecylbenzenesulfonic acid	ESI (−)	$C_{18}H_{30}O_3S$	L2	96

[a] Level of identification according to Schymanski et al. (2014). ESI: electrospray ionisation; DF: detection frequency (%).

Quo Vadis?

- Identification of emerging contaminants in relevant samples for human exposure (food, dust, consumer products)
- Identification of biomarkers of emerging contaminants in *in vitro* and *in vivo* situations
- Suitability of *in vitro identified* metabolites in *in vivo* samples

Several running projects at the ToxCenter tackling these topics

Thank you for your attention !!

A·TEAM
ADVANCED TOOLS FOR EXPOSURE ASSESSMENT AND BIOMONITORING

HBM4EU
science and policy for a healthy future

HOKKAIDO UNIVERSITY
Center for Environmental and Health Sciences

INFLAME

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