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Prioritised list of biomarkers, matrices and analytical methods for the 1st prioritisation round of substances

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Contributors

Table 1: List of partners contributing to this deliverable 9.2 sorted by group of chemical substances[1].

Chemical group	Inventory	Evaluation
Phthalates and DINCH	Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA; Germany)	IPA
Per- and polyfluoroalkyl substances	University of Las Palmas de Gran Canaria (ULPGC; Spain)	NIPH
Flame retardants	University of Aarhus (Denmark) and ULPGC	NIPH
Bisphenols	ULPGC and University of Antwerp (Belgium)	IPA
Polyaromatic hydrocarbons	ULPGC	ULPGC
Anilines and MOCA	Finnish Institute of Occupational Health	ULPGC
Cadmium	University of Latvia	ULPGC
Chromium	Faculty of Medicine of the University of Lisbon (Portugal)	ULPGC

[1]Further details on persons involved are given in the corresponding sections below.

The Instituto de Salud Carlos III (ISCIII; Spain) and the chemical group leaders (CGLs) are deeply acknowledged for their kind contribution reviewing and giving inputs to the present document. The CGLs are also acknowledged for their input to the inventories.

Abbreviations

List of abbreviations related to analytical methodology:

AAS	atomic absorption spectrometry
APCI	atmospheric chemical ionisation
DRC	dynamic reaction cell
EAAS	electrothermal atomic absorption spectrometry
EBC	exhaled breath condensate
ECD	electron capture detector
ECNI	electron capture negative ion
EDTA	ethylenediaminetetraacetic acid
EI	electron impact
ELISA	enzyme-linked immunosorbent assay
EOF	extractable organic fluorine
ESI	electrospray ionisation
FID	flame ionization detector
FLD	fluorescence detector
GC	gas chromatography
GF-AAS	grafite furnace atomic absorption spectrometry
GPC	gel permeation chromatography
H_2O_2	hydrogen peroxide
HNO_3	nitric acid
HPLC	high pressure liquid chromatography
HR	high resolution
ICP	inductively coupled plasma
IF	inorganic fluorine
IS	internal standard
LC	liquid chromatography
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
LR	low resolution
LW	lipid weight
MAE	microwave assisted extraction

MS	mass spectrometry
MDL	method detection limit
MQL	method quantification limit
NA	not available
PLE	pressurised liquid extraction
QA	quality assurance
QC	quality control
QqQ	triple quadrupole (mass spectrometer)
RBC	red blood cells
SPE	solid phase extraction
TF	total fluorine
TOF	time of flight (mass spectrometer)
TOPA	total oxidisable precursors analysis
UHPLC	ultra high performace liquid chromatography
UVD	ultraviolet detector

Prioritised list of biomarkers, matrices and analytical methods for the 1st prioritisation round of substances

In order to come up with a prioritised list of biomarkers, matrices and analytical methods for the 1st prioritisation round of substances, partners in task 9.1 in collaboration with the chemical group leaders (CGLs) made inventories of available analytical methods as well as suitable exposure biomarkers and matrices. A template to gather information in a harmonised way and according to the criteria established in Deliverable 9.1 was agreed upon (See Deliverable 9.1: Criteria for prioritisation of biomarkers, matrices and analytical methods). Information in the inventories was further evaluated by partners in task 9.1 following guidelines that had been thoroughly discussed and agreed upon. The CGLs were consulted and gave their input on the final draft of the deliverable. The resulting list of suitable pairs of exposure biomarker/matrices as well as analytical methods is presented in Table 2. The substances have been categorised according to the year one scoping documents. Category C substances for which information on analytical methods in human matrices are insufficient or lacking, have been left out of the table. Information on how the inventories were compiled and the evaluations done, with specific comments and needs for methodological improvements for the individual groups of substances, are described in details in the appendix below (for record keeping and transparency). Information in the inventories including references will be available upon request. The inventories and evaluations will be revised and updated if new information is available in year two of HBM4EU.

Table 2: Summary table listing biomarkers, matrices, analytical methods and method detection limit (MDL) suggested for the 1st prioritisation round of substances[1].

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL[2]
Phthalates and DINCH				
Category A				
Di(2-ethylhexyl) phthalate (DEHP)	Mono(2-ethylhexyl) phthalate (MEHP)	Urine (0.3 mL)	LC-MS-MS	0.2 ng/mL
	Mono(2-ethyl-5-hydroxy-hexyl) phthalate (5OH-MEHP, MEHHP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
	Mono(2-ethyl-5-oxo-hexyl) phthalate (5oxo-MEHP, MEOHP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
	Mono(2-ethyl-5-carboxy-pentyl) phthalate (5cx-MEPP, MECPP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
Butyl benzyl phthalate (BBzP)	Mono-benzyl phthalate (MBzP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
Di-n-butyl phthalate (DnBP)	Mono-n-butyl phthalate (MnBP)	Urine (0.3 mL)	LC-MS-MS	0.3 ng/mL

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL[2]
	3-OH-Mono-n-butyl phthalate (OH-MnBP)	Urine (0.3 mL)	LC-MS-MS	0.3 ng/mL
Di-isobutyl phthalate (DiBP)	Mono-isobutyl phthalate (MiBP)	Urine (0.3 mL)	LC-MS-MS	0.3 ng/mL
	2-OH-Mono-iso-butylphthalate (OH-MiBP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
Diethyl phthalate (DEP)	Mono-ethyl phthalate (MEP)	Urine (0.3 mL)	LC-MS-MS	0.2 ng/mL
Category B				
Di-isononyl phthalate (DiNP)	7-OH-(Mono-methyl-octyl) phthalate (OH-MiNP, MHNP)	Urine (0.3 mL)	LC-MS-MS	0.3 ng/mL
	7-Oxo-(Mono-methyl-octyl) phthalate (oxo-MiNP, MONP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
	7-Carboxy-(mono-methyl-heptyl) phthalate (cx-MiNP, MCOP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
Di-isodecyl phthalate (DiDP) (all C10 phthalates including DPHP)	6-OH-Mono-propyl-heptyl phthalate (OH-MiDP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
	6-Oxo-Mono-propyl-heptyl phthalate (oxo-MiDP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
	Mono(2,7-methyl-7-carboxy-heptyl) phthalate (cx-MiDP, MCNP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
Di-n-octyl phthalate (DnOP)	Mono-n-octyl phthalate (MnOP, MOP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
Dimethyl phthalate (DMP)	Mono-methyl phthalate (MMP)	Urine (0.3 mL)	LC-MS-MS	0.3 ng/mL
Di-n-pentyl phthalate (DnPeP)	Mono-n-pentyl phthalate (MnPeP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
Dicyclohexyl phthalate (DCHP)	Mono-cyclo-hexyl phthalate (MCHP)	Urine (0.3 mL)	LC-MS-MS	0.1 ng/mL
Di(2-propylheptyl) phthalate (DPHP)	6-OH-Mono-propyl-heptyl phthalate (OH-MPHP)	Urine (1 mL)	GC-MS-MS	0.1 ng/mL
	6-Oxo-Mono-propyl-heptyl phthalate (oxo-MPHP)	Urine (1 mL)	GC-MS-MS	0.08 ng/mL

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL[2]
	Mono(2,7-methyl-7-carboxy-heptyl) phthalate (cx-MPHP)	Urine (1 mL)	GC-MS-MS	0.05 ng/mL
Di-isononyl cyclohexane-1,2-dicarboxylate (DINCH)	cyclohexane-1,2-dicarboxylate-mono-(7-carboxylate-4-methyl)heptyl ester (cx-MINCH, MCOCH)	Urine (0.3 mL)	LC-MS-MS	0.03 ng/mL
	cyclohexane-1,2-dicarboxylate-mono-(7-hydroxy-4-methyl)octyl ester (OH-MINCH, MHNCH)	Urine (0.3 mL)	LC-MS-MS	0.03 ng/mL
	cyclohexane-1,2-dicarboxylate-mono-(7-oxo-4-methyl)octyl ester (oxo-MINCH, MONCH)	Urine (0.3 mL)	LC-MS-MS	0.02 ng/mL
Category C				
Di-n-hexyl phthalate (DnHexP)	Mono-n-hexyl phthalate (suspected) (MnHxP)	Urine (0.1 mL)	LC-MS-MS	0.2 ng/mL
Per- and polyfluoroalkyl substances (PFAS)				
Category A				
Perfluorobutanoic acid (PFBA)	PFBA	Serum (0.15 mL)	LC-MS-MS	0.003 ng/mL
Perfluoropentanoic acid (PFPeA)	PFPeA	Serum (0.15 mL)	LC-MS-MS	0.008 ng/mL
Perfluorohexanoix acid (PFHxA)	PFHxA	Serum (0.15 mL)	LC-MS-MS	0.007 ng/mL
Perfluoroheptanoic acid (PFHpA)	PFHpA	Serum (0.15 mL)	LC-MS-MS	0.009 ng/mL
Perfluorooctanoic acid (PFOA)	PFOA	Serum (0.15 mL)	LC-MS-MS	0.006 ng/mL
Perfluorononanoic acid (PFNA)	PFNA	Serum (0.15 mL)	LC-MS-MS	0.004 ng/mL
Perfluorodecanoic acid (PFDA)	PFDA	Serum (0.15 mL)	LC-MS-MS	0.002 ng/mL
Perfluoroundecanoic acid (PFUnDA)	PFUnDA	Serum (0.15 mL)	LC-MS-MS	0.006 ng/mL

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL[2]
Perfluorododecanoic acid (PFDoDA)	PFDoDA	Serum (0.15 mL)	LC-MS-MS	0.007 ng/mL
Perfluorotridecanoic acid (PFTrDA)	PFTrDA	Serum (0.15 mL)	LC-MS-MS	0.004 ng/mL
Perfluorotetradecanoic acid (PFTeDA)	PFTeDA	Serum (0.15 mL)	LC-MS-MS	0.05 ng/mL
Perfluorobutane sulfonic acid (PFBS)	PFBS	Serum (0.15 mL)	LC-MS-MS	0.009 ng/mL
Perfluorohexane sulfonic acid (PFHxS)	PFHxS	Serum (0.15 mL)	LC-MS-MS	0.007 ng/mL
Perfluoroheptane sulfonic acid (PFHpS)	PFHpS	Serum (0.15 mL)	LC-MS-MS	0.01 ng/mL
Perfluorooctane sulfonic acid (PFOS)	PFOS	Serum (0.15 mL)	LC-MS-MS	0.003 ng/mL
Perfluorodecane sulfonic acid (PFDS)	PFDS	Serum (0.15 mL)	LC-MS-MS	0.05 ng/mL
Category B				
Perfluoro-1-octaperfluoro-1-octanesulphonamide (FOSA)	FOSA	Serum (0.15 mL)	LC-MS-MS	0.02 ng/mL
N-Ethylperfluoro-1-octanesulphonamide (N-EtFOSA)	N-EtFOSA	Serum (0.15 mL)	LC-MS-MS	0.009 ng/mL
N-Ethyl-perfluorooctane sulphonamidoethanol (EtFOSE)	EtFOSE	Serum (0.20 mL)	LC-MS-MS	0.4 ng/mL
N-Methylperfluoro-1 octanesulphonamide (N-MeFOSA)	N-MeFOSA	Serum (0.15 mL)	LC-MS-MS	0.009 ng/mL
N-ethyl-perfluorooctane sulfonamidoacetate (EtFOSAA)	EtFOSAA	Serum (1 g)	LC-MS-MS	0.002 ng/g
Category C				
6:2 polyfluoroalkyl phosphoric acid diesters (6:2 diPAP)	6:2 diPAP	Serum (0.05 mL)	LC-MS-MS	0.018 ng/mL

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL[2]
8:2 polyfluoroalkyl phosphoric acid diesters (8:2 diPAP)	8:2 diPAP	Serum (0.05 mL)	LC-MS-MS	0.009 ng/mL
6:2 polyfluoroalkyl phosphoric acid monoesters (6:2 monoPAP)	6:2 mono PAP	Serum (0.05 mL)	LC-MS-MS	0.09 ng/mL
8:2 polyfluoroalkyl phosphoric acid monoesters (8:2 monoPAP)	8:2 monoPAP	Serum (0.05 mL)	LC-MS-MS	0.045 ng/mL
Perfluorohexylphosphonic acid (PFHxPA)	PFHxPA	Serum (0.05 mL)	LC-MS-MS	0.045 ng/mL
Perfluorooctylphosphonic acid (PFOPA)	PFOPA	Serum (0.05 mL)	LC-MS-MS	0.009 ng/mL
Perfluorodecylphosphonic acid (PFDPA)	PFDPA	Serum (0.05 mL)	LC-MS-MS	0.009 ng/mL
Ammonium 4,8-dioxa-3H-perfluorononanoate (ADONA)	ADONA	Serum (0.20 mL)	LC-MS-MS	0.2 ng/mL (LOQ)
Flame retardants (FRs)				
Category A				
Polybrominated diphenylethers (PBDEs, 7 in total)	PBDEs[3]	Serum (2 mL)	GC-LRMS	0.0007 - 0.002 ng/mL
Hexabromocyclododecane (HBCDs, α, β, γ isomers)	HBCDs	Serum (1 mL)	LC-MS-MS	0.002-0.005 ng/mL
Category B				
PFRs (4 in total)	Diester metabolites[4]	Urine (1 mL)	LC-MS-MS	0.04 – 0.2 ng/mL
PFRs (4 in total)	Diester metabolites[5]	Urine (0.01 mL)	LC-HRMS	0.1 - 0.6 ng/mL
Tetrabromobisphenol A (TBBPA)	TBBPA	Serum (1 mL)	LC-MS-MS	0.008 ng/mL
Hexabromobenzene (HBB)	HBB	Serum (2 mL)	GC-LRMS	0.0003 ng/mL
Pentabromoethylbenzene (PBEB)	PBEB	Serum (5 mL)	GC-LRMS	0.0023 ng/mL

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL[2]
1,2 bis(2,4,6-tribromophenoxy)ethane (BTBPE)	BTBPE	Serum (2 mL)	GC-LRMS	0.0023 ng/mL
Pentabromotoluene (PBT)	PBT	Serum (5 mL)	GC-LRMS	0.0016 ng/mL
Dechlorane Plus (DP)	DP	Serum (2 mL)	GC-LRMS	0.001 – 0.002 ng/mL
Decabromodiphenylethane (DBDPE)	DBDPE	Serum (2 mL)	GC-LRMS	0.02 ng/mL
Hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO)	HCDBCO	Serum (2 mL)	GC-LRMS	5.4 ng/g lw
2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB)	EH-TBB	Serum (2 mL)	GC-MS-MS	0.4 ng/g lw
bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (BH-TEBP)	BEH-TEBP	Serum (3 mL)	GC-MS-MS	0.2 ng/g lw
Octabromotrimethylphenylindane (OBIND)	OBIND	Serum (2 mL)	GC-MS-MS	1.5 ng/g lw
2,3,5,6-tetrabromo-p-xylene (TBX)	TBX	Breast milk (8-10 mL)	GC-HRMS	0.001 ng/g lw
Tetrabromoethylcyclohexane (TBECH)	TBECH	Breast milk (8-10 mL)	GC-HRMS	1.8 ng/g lw
Category C				
2,4-Dibromophenol	Derivatised FR	Serum (2-3 mL)	GC-LRMS	5 ng/g lw
	2,4-Dibromophenol	Breast milk (15 mL)	LC-MS-MS	0.2 ng/mL
2,4,6-Tribromophenol	Derivatised FR	Serum (1 mL)	GC-MS-MS	0.05 ng/mL
	2,4,6-Tribromophenol	Breast milk (15 mL)	LC-MS-MS	0.03 ng/mL
Pentabromophenol	Derivatised FR	Serum (2-3 mL)	GC-LRMS	0.5 ng/g lw
	Pentabromophenol	Breast milk (15 mL)	LC-MS-MS	0.03 ng/mL
Dechlorane 603 (Dec603)	Dec603	Serum (2 mL)	GC-LRMS	0.00064 ng/mL
Dechlorane 602 (Dec602)	Dec602	Serum (2 mL)	GC-HRMS	0.02 ng/g lw

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL[2]
Dechlorane 604 (Dec604)	Dec604	Serum (2 mL)	GC-LRMS	3.6 ng/g lw
Bisphenols (BPs)				
Category A				
Bisphenol A (BPA)	BPA	Urine (0.5 mL)	LC-MS-MS	0.02 ng/mL
Category B				
Bisphenol S (BPS)	BPS	Urine (NA)	LC-MS-MS	0.03 ng/mL
Bisphenol F (BPF)	BPF	Urine (NA)	LC-MS-MS	0.06 ng/mL
Polyaromatic hydrocarbons (PAHs)				
Category B				
Naphthalene (NAPH)	1-, 2-hydroxynaphthalene	Urine (0.5 - 2 mL)	LC-MS-MS	0.01 – 0.04 ng/mL
Fluorene (FLUO)	2-hydroxyfluorene (2-FLUO)	Urine (0.5 - 2 mL)	LC-MS-MS	0.01 – 0.04 ng/mL
Phenanthrene (PHE)	2-, 3-, 4-, 9-hydroxyphenanthrene	Urine (0.5 - 2 mL)	LC-MS-MS	0.01 – 0.04 ng/mL
Pyrene (PYR)	1-hydroxypyrene (1-PYR)	Urine (0.5 - 2 mL)	LC-MS-MS	0.01 – 0.04 ng/mL
Chrysene (CRY)	1-, 6-hydroxychrysene (1-CRY)	Urine (0.5 - 2 mL)	LC-MS-MS	0.01 – 0.04 ng/mL
Benzo[c]-phenanthrene (BcPh)	3-hydroxybenzo[c]-phenanthrene (3-BCP)	Urine (0.5 - 2 mL)	LC-MS-MS	0.01 – 0.04 ng/mL
Benz[a]anthracene (BaA)	1-hydroxybenz[a]anthracene (1-BaA)	Urine (0.5 - 2 mL)	LC-MS-MS	0.01 – 0.04 ng/mL
Acenaphthene (ACE)	ACE	Serum (1 mL)	GC-MS-MS	0.03 ng/mL
Acenaphthylene (ACY)	ACY	Serum (1 mL)	GC-MS-MS	0.03 ng/mL
Anthracene (AN)	AN	Serum (1 mL)	GC-MS-MS	0.03 ng/mL
Benz[a]anthracene (BaA)	BaA	Serum (1 mL)	GC-MS-MS	0.06 ng/mL
Benzo[a]pyrene (BaP)	BaP	Serum (1 mL)	GC-MS-MS	0.06 ng/mL
Benzo[c]phenanthrene (BcPh)	BcPh	Serum (1 mL)	GC-MS-MS	0.05 ng/mL
Benzo[b]fluoranthene (BbFA)	BbFA	Serum (1 mL)	GC-MS-MS	0.06 ng/mL

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL[2]
Benzo[c]fluorene (BcFL)	BcFL	Serum (1 mL)	GC-MS-MS	0.06 ng/mL
Benzo[g,h,i]perylene (BghiP)	BghiP	Serum (1 mL)	GC-MS-MS	0.1 ng/mL
Benzo[j]fluoranthene (BjFA)	BjFA	Serum (1 mL)	GC-MS-MS	0.06 ng/mL
Benzo[k]fluoranthene (BkFA)	BkFA	Serum (1 mL)	GC-MS-MS	0.06 ng/mL
Chrysene (CRY)	CRY	Serum (1 mL)	GC-MS-MS	0.06 ng/mL
Cyclopenta[c,d]pyrene (CPP)	CPP	Serum (1 mL)	GC-MS-MS	0.06 ng/mL
Dibenzo[a,h]anthracene (DBahA)	DBahA	Serum (1 mL)	GC-MS-MS	0.05 ng/mL
Dibenzo[a,h]anthracene (DBahA)	DBahA	Serum (1 mL)	GC-MS-MS	0.1 ng/mL
Dibenzo[a,e]pyrene (DBaeP)	DBaeP	Serum (1 mL)	GC-MS-MS	0.3 ng/mL
Dibenzo[a,i]pyrene (DBaiP)	DBaiP	Serum (1 mL)	GC-MS-MS	0.3 ng/mL
Dibenzo[a,l]pyrene (DBalP)	DBalP	Serum (1 mL)	GC-MS-MS	0.3 ng/mL
Fluoranthene (FLA)	FLA	Serum (1 mL)	GC-MS-MS	0.06 ng/mL
Fluorene (FLUO)	FLUO	Serum (1 mL)	GC-MS-MS	0.03 ng/mL
Indeno[1,2,3-cd]pyrene (IP)	IP	Serum (1 mL)	GC-MS-MS	0.1 ng/mL
Naphtalene (NAPH)	NAPH	Serum (1 mL)	GC-MS-MS	0.03 ng/mL
Phenanthrene (PHE)	PHE	Serum (1 mL)	GC-MS-MS	0.03 ng/mL
Pyrene (PYR)	PYR	Serum (1 mL)	GC-MS-MS	0.06 ng/mL
Anilines and MOCA				
Category A				
4,4-methylenedianiline (MDA)	MDA	Urine (1 mL)	LC-MS-MS	0.01 – 0.1 ng/mL
4,4'-methylenebis(2-chloroaniline) (MOCA)	MOCA	Urine (1 mL)	LC-MS-MS	1 ng/mL
	N-acetyl 4,4'-methylenebis (2-chloroaniline) (acetyl-MOCA)	Urine (1 mL)	LC-MS-MS	0.03 ng/mL

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL[2]
Category B				
Aniline	Aniline / p-aminophenol	Urine (1 mL)	LC-MS-MS	0.1 ng/mL
o-, p-toluidine	o-, p-toluidine	Urine (1 mL)	LC-MS-MS	0.25 ng/mL
Category C				
o,m,p-Fluoroaniline (o,m,p-FA)	o,m,p-FA	Urine (1 mL)	GC-MS	0.6 ng/mL
3-Trifluormethoxyaniline (3TFMA)	3TFMA	Urine (1 mL)	GC-MS	0.6 ng/mL
4-Trifluormethoxyaniline (4TFMA)	4TFMA	Urine (1 mL)	GC-MS	0.6 ng/mL
4-Ethylaniline (EtA)	EtA	Urine (1 mL)	GC-MS	0.6 ng/mL
o,m,p-Chloroaniline (o,m,p-ChA)	o,m,p-ChA	Urine (1 mL)	GC-MS	1.2 ng/mL
2,4-Dichloroaniline (2,4DChA)	2,4DChA	Urine (1 mL)	GC-MS	1 ng/mL
2,5-Dichloroaniline (2,5DChA)	2,5DChA	Urine (1 mL)	GC-MS	1 ng/mL
2,3-Dichloroaniline (2,3DChA)	2,3DChA	Urine (1 mL)	GC-MS	0.7 ng/mL
3,5-Dichloroaniline (3,5DChA)	3,5DChA	Urine (1 mL)	GC-MS	0.7 ng/mL
3,4-Dichloroaniline (3,4DChA)	3,4DChA	Urine (1 mL)	GC-MS	1.6 ng/mL
4-Isopropylaniline (IPA)	IPA	Urine (1 mL)	GC-MS	0.7 ng/mL
2,6-Diisopropylaniline (DIPA)	DIPA	Urine (1 mL)	GC-MS	0.5 ng/mL
2,6-Diaminotoluene (6TDA)	6TDA	Urine (1 mL)	LC-MS-MS	0.01 – 0.1 ng/mL
2,4-Diaminotoluene (4TDA)	4TDA	Urine (1 mL)	LC-MS-MS	0.01 – 0.1 ng/mL
3-Aminobiphenyl (3ABPH)	3ABPH	Urine (1 mL)	LC-MS-MS	0.05 ng/mL
4-Aminobiphenyl (4ABPH)	4ABPH	Urine (1 mL)	LC-MS-MS	0.05 ng/mL

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL[2]
4-Methyl-m-phenylenediamine (PDA)	PDA	Urine (1 mL)	LC-MS-MS	0.01 – 0.1 ng/mL
Cadmium (Cd)				
Category A				
Cd	Cd	Whole blood (0.5 mL)	ICP-MS	0.05 – 0.2 ng/mL
		Urine (1 mL)	ICP-DRC-MS[6]	0.03 – 0.09 ng/mL
Chromium (Cr)				
Category C				
Cr	Cr (VI)	Red blood cells (erythrocytes)	ICP-MS	0.01 ng/mL
	Cr (VI) and (III)	Exhaled breath condensate (EBC)	AAS	0.002 and 0.007 ng/mL

[1]Sample intake, technique and MDL were extracted from the inventory and additional publications, which are mentioned in the corresponding sections. Some compounds from the scoping document (Category C) could not be included in the present Table due to lack of published analytical methods.
[2]These concentrations are values obtained after applying an on/off-line sample pretreatment (if needed) with acceptable QA/QC results.
[3]BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183.
[4]Bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), Diphenyl phosphate (DPHP), Bis(2-chloroethyl) phosphate (BCEP), bis(1-chloro-2-propyl) phosphate (BCIPP).
[5]Bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), Diphenyl phosphate (DPHP), Di-n-butyl phosphate (DnBP), bis(2-butoxyethyl) phosphate (BBOEP).
[6]Inductively coupled plasma dynamic reaction cell mass spectrometry
lw lipid weight
NA not available

Appendix

1 Inventory of available analytical methods, suitable exposure biomarkers and matrices

1.1 Process for compilation of the inventories

A template for the inventory was provided by the Norwegian Institute of Public Health (NIPH) and was approved by all partners in task 9.1. The inventory covered the following analytical parameters and information:

- CAS number
- Compound Category (A, B and C)
- Biomarker commercially available
- Matrix
- Storage
- Reported sample intake
- Sample preparation (extraction, clean-up, etc)
- Technique/method (GC-MS, LC-MS, injection volume, ionisation mode, etc)
- Internal standard
- Method detection limit (MDL)
- Recovery
- Accuracy
- Inter- and intra-precision
- Blank contamination
- Matrix effect
- Quality assurance (inter-laboratory comparisons, certified material, etc)
- Method analysis time
- Laboratory accreditation
- Health based guidance values
- Method throughput
- Study sample size
- Method used for larger epidemiological studies
- References

The inventory of the prioritised substances for year one (Phthalates and DINCH, Per- and polyfluoroalkyl substances, Flame retardants, Bisphenols, Polyaromatic hydrocarbons, Anilines and MOCA, Cadmium, and Chromium) was undertaken by partners in WP 9 and Chemical Group Leaders (CGLs). The complete inventories (excel files) are not part of this document, but will be available upon request.

1.2 Description of inventories

For transparency and record keeping, descriptions of how the inventories were compiled by the corresponding partners and CGLs are presented below (as received). The years covered in the bibliographic search varies for the different substances. This is because the year the method development started varies, as well as the technological development of relevant analytical instrumentation.

1.2.1 Phthalates and DINCH

Description of the bibliographic search: The inventory of methods for analyses of phthalate and DINCH metabolites represents an expert summary of the current state of the art in terms of analytical methodologies and phthalates/DINCH (metabolites) covered. A bibliographic Web of Science- and PubMed-search about the most common methods for analyses of phthalate metabolites and DINCH on the basis of the most recent publications for methods and epidemiological data was done. Based upon the most recent publications (2015-2017) a second search was performed to find the original method papers of the remaining articles from the first search. Focusing on published articles from Sweden, Denmark, Norway, Belgium, Czech Republic, Greece, Austria, Germany was included but also from America and Canada (from both the National Health Surveys). Work from Asia, South America and Australia was not included. Furthermore, the result of the search is not all-encompassing and we restricted the search to urine as preferred matrix and laboratories with known experience in HBM. Some papers did not provide method data suitable for the inventory and therefore were excluded. Currently, methods of ten laboratories for phthalates and four laboratories for DINCH are listed in the inventory.

One lab additionally provided a method for analyses in nails. Ten laboratories for phthalates and four laboratories for DINCH are listed in the inventory.

Remarks: We added one more column for the enzyme used for deconjugation of the glucuronides because the type of enzyme used is very important. Some glucuronidases (such as that from *Helix pomatia*) break down the ester bonds of the phthalates. The best choice would be the *E. coli* K12 glucuronidase (pure beta-glucuronidase activity). Two labs did not specify the enzyme used, and one lab used the *Helixa pomatia* enzyme (with both glucuronidase and arylsulfatase activities).

We added additional columns for a more detailed report of recoveries for up to 3 spiking levels.

In our view, the column for method throughput as a criterion for method selection seemed not to be very helpful. Instead, we would suggest providing the number of samples that could be analysed in the labs (e.g. per week; including sample work-up and actual measurement). This point should be included in the lab questionnaire.

Contribution of the corresponding CGLs: Yes.

Author/s: Holger Koch, Monika Kasper-Sonnenberg, Daniel Bury.

Affiliation/s: Institute for Prevention and Occupational Medicine of the German Accident Insurance (IPA), Bochum, Germany.

1.2.2 Per- and polyfluoroalkyl substances

Description of the bibliographic search: Type of journals (field): analytical journals, toxicology journals, environmental science journals, pharmacology journals, biochemical research methods journals, etc.

Websites: Sciencedirect, Scopus, Web of science, Pubmed.

Matrix: Serum and other matrices hair, nails, breast milk.

Years: 2008-2017.

Total number of articles reviewed and compiled: 45 (reviewed) – 11 (included in the inventory)

Remarks: We selected analytical methods based on triple quadrupole mass spectrometry, or on Time of Flight coupled to a quadrupole, because of their high sensitivity and compound specificity.

We focused on fully validated methods, which were tested in (large) series of real samples, at least for compounds in categories A and B. This was not possible for compounds in Category C, for many of which even unpublished but fully validated methods were taken into account.

We did not consider methods based on immunological techniques, because of their low compound specificity, and the possible of cross reactivity.

When several references used similar methodologies (“duplicate” methods), we selected the one that provided the largest amount of data related to validation as the first criterion, and if there were still similarities, we selected the one that was published in the journal with the highest impact index (Journal Citation Reports).

Contribution of the corresponding CGLs: No.

Author/s: Octavio Pérez Luzardo & Ana Gonzalez Antuña.

Affiliation/s: ULPGC, Canary Islands, Spain.

Additional papers considered during evaluation and used in table 2:

Poothong S, Lundanes E, Thomsen C, Haug LS. Anal Chim Acta. 2017, 957:10.

Kuklenyik Z, Needham LL, Calafat AM. Anal Chem 2005, 77:6085

Gebbink WA, Glynn A, Berger U.Environ Pollut. 2015, 199:166

Fromme H, Wöckner M, Roscher E, Völkel W. Int J Hyg Environ Health. 2017, 220:455

1.2.3 Flame retardants

1.2.3.1 Halogenated flame retardants

The group of flame retardants was split into phosphorous and halogenated flame retardants, agreed at an internal meeting. The inventory of the phosphorous flame retardants was prepared by Octavio Luis Pérez Luzardo (ULPGC, Spain).

Description of the bibliographic search:

1. Web of Science, search terms “flame retardant human”, “novel flame retardant human”, “dechlorane plus human”, moving backwards in time until 2012.
2. References in this primary list of articles.
3. Input from CGLs.

Total number of articles reviewed and compiled:

Number of articles reviewed: 35.

Number of articles included in the inventory: 25 (+ 3 articles pointed out by the CGL).

Additional six articles on halogenated flame retardants were included in the inventory template when it was circulated.

Remarks: I used the template which had been provided by NIPH. Blank cells mean that no information was available. The parameter “Method analysis time” was only included when provided in the paper, no re-calculation from temperature programmes was done.

“PBDEs” were considered one compound/biomarker in the template. Separation into BDE-209 and lower brominated flame retardants could be relevant from an analytical chemistry point of view.

Contribution of the corresponding CGLs: Yes.

Author/s: Katrin Vorkamp.

Affiliation/s: Aarhus University, Denmark.

1.2.3.2 Organophosphorous flame retardants (PFRs)

Within the group of flame retardants, we focused in organophosphorous flame retardants, and the team in Aarhus University, Denmark (Katrin Vorkamp) in the rest of compounds.

Description of the bibliographic search: Type of journals (field): analytical journals, toxicology journals, environmental science journals, pharmacology journals, biochemical research methods journals, etc.

Websites: Sciencedirect, Scopus, Web of science, Pubmed.

Matrix: serum, hair, nails, urine, breast milk.

Years: 2011-2017.

Total number of articles reviewed and compiled: 32 (reviewed) – 9 (included in the inventory)

Remarks: Within this group the bibliography is still scarce, so we have not limited the bibliographic search only to methods used in human samples. All methods selected are based on triple quadrupole mass spectrometry, either coupled to gas chromatography (for parent compounds) or high performance liquid chromatography (for metabolites used as biomarkers in urine).

Where possible, methods that have been tested on large series of human samples or, in their absence, on similar matrices (blood and urine) from other species have been selected.

We avoided including duplicated methods. We selected the most complete reference, this is that reference that allowed us to complete the maximum number of cells in the template provided.

Contribution of the corresponding CGLs: No.

Author/s: Octavio Pérez Luzardo & Ana Gonzalez Antuña.

Affiliation/s: ULPGC, Canary Islands, Spain.

1.2.4 Bisphenols

1.2.4.1 Bisphenols (specifically BPS and BPF)

Description of the bibliographic search: Type of journals (field): analytical journals, toxicology journals, environmental science journals, pharmacology journals, biochemical research methods journals.

Websites: Scopus, Web of science, Pubmed.

Matrix: Plasma, serum, blood, urine, breast milk, food samples, paper products.

Years: 2007-2017.

Total number of articles reviewed and compiled: 46 (reviewed) – 12 (included in the inventory)

Remarks: Focus on having a complete inventory that describes the relevant characteristics of all included methodologies. Only methodologies that are able to detect/quantify BPS and/or BPF (among other bisphenols) were considered and categorised per compound.

Methodologies included are all based on mass spectrometry due to its versatility, number of publications, analytical features and its presence in routine laboratories around the world.

We avoided including duplicated methods. We selected the most complete reference; this is the reference that allowed us to complete the maximum number of cells in the inventory.

Most methodologies are applied on biological matrices, but some on food samples and paper products are also included.

Since this inventory serves a European biomonitoring project, the country where the methodology was developed, is also described.

Contribution of the corresponding CGLs: No.

Author/s: Celine Gys, Govindan Malarvannan, Adrian Covaci.

Affiliation/s: University of Antwerp, Antwerp, Belgium.

1.2.4.2 Bisphenol A

Description of the bibliographic search: Type of journals (field): analytical journals, toxicology journals, environmental science journals, pharmacology journals, biochemical research methods journals.

Websites: Sciencedirect, Scopus, Web of science, Pubmed.

Matrix: Plasma, serum, blood, urine, breast milk, adipose tissue, saliva.

Years: 2005-2017.

Total number of articles reviewed and compiled: 67 (reviewed) – 15 (included in the inventory)

Remarks: We selected methods based on liquid chromatography coupled to triple quadrupole mass spectrometry, because the majority of the most updated references employ this methodology.

We focused in fully validated methods, which have been tested in wide series of human samples, especially urine, but also serum or plasma, breast milk, and other matrices of secondary interest for biomonitoring studies.

As many methods are based on the same methodology, and therefore could be considered as "duplicated" methods, we made the selection based on two criteria: 1) those methods which allowed us to complete the maximum number of cells in the Excel template that we used internally; and 2) those methods published in the journal of highest impact (JCR).

Contribution of the corresponding CGLs: No.

Author/s: Octavio Pérez Luzardo & Ana Gonzalez Antuña.

Affiliation/s: ULPGC, Canary Islands, Spain.

1.2.5 Polyaromatic hydrocarbons

Description of the bibliographic search: Type of journals (field): analytical journals, toxicology journals, environmental science journals, pharmacology journals, biochemical research methods journals, etc.

Websites: Sciencedirect, Scopus, Web of science, pubmed.

Matrix: whole blood, urine, hair, plasma, breast milk, cord blood.

Years: 2009-2017.

Total number of articles reviewed and compiled: 51 (reviewed) – 16 (included in the inventory)

Remarks: As untransformed polycylic aromatic hydrocarbons are usually separated either by gas chromatography or liquid chromatography both techniques have been considered. For the determination of biomarkers of PAHs exposure, we focused on chromatographic techniques with special emphasis on liquid chromatography. As technique for detection, most updated and sensitive methods are based on simple or triple quadrupole mass spectrometry, and therefore we have focused on this technology, as other methodologies can be considered obsolete, less selective and less sensitive.

For this group there are many references describing similar methodologies, so we selected those which provided more data on validation, those which included the highest number of compounds i multi-residue methods, those that reported the lowest LOQs, and as a final criterion those that were published in high impact journals.

Contribution of the corresponding CGLs: No.

Author/s: Octavio Pérez Luzardo & Ana Gonzalez Antuña.

Affiliation/s: ULPGC, Canary Islands, Spain.

1.2.6 Anilines and MOCA

Description of the bibliographic search: PubMed (years 2000-2016).

Total number of articles reviewed and compiled: 24 (PubMed search – roughly 700 results (anilines)).

Remarks: Documents published elsewhere than peer-reviewed journals: The MAK Collection for Occupational Health and Safety by DFG (Deutsche Forschungsgemeinschaft) (Wiley Online Library); ACGIH (American Conference of Governmental Industrial Hygienists), and SCOEL (the Scientific Committee on Occupational Exposure Limits, European Commission) occupational exposure limits documents.

Methods published before year 2000 not included in the bibliographicsearch.

Methods in articles concerning isocyanate exposure biomarkers excluded.

Data gaps may exist especially in other toluidine methods.

Other anilines or substitute anilines not inventoried.

Contribution of the corresponding CGLs: Yes.

Author/s: Jouni Mikkola.

Affiliation/s: Finnish Institute of Occupational Health (FIOH).

1.2.7 Cadmium

1) Description of the bibliographic search:

Databases used: Scopus, ScienceDirect, Taylor and Francis, Pubmed, Web of Science.

Matrix of samples: whole blood, serum, urine, autopsy tissue, teeth dentine.

Years of literature researched: 2004-2017.

Type of journals: analytical journals, toxicology journals, environmental science journals, biochemical research methods journals, etc.

Total number of articles reviewed and compiled: 45 (reviewed) – 18 (included in the inventory).

Remarks: We tried to add as much information about the sample preparation in the Inventory as it was possible. Most of the methods that do determine direct cadmium levels use ICP-MS and only vary for the matrix and sample preparation steps. For measurements of biomarkers, various ELISA tests are being used in research.

Contribution of the corresponding CGLs: No.

Author/s: Agnese Osīte, Lauma Buša, Arturs Vīksna.

Affiliation/s: University of Latvia, Riga, Latvia.

Additional papers considered during evaluation and used in table 2:

Isotope-dilution ICP-MS for trace element determination and speciation: from a reference method to a routine method.Heumann K.G. Analytical and Bioanalytical Chemistry 378 (2004) 318-319.

Giuseppe Centineo et al. On-line isotope dilution analysis with the 7700 Series ICP-MS: Analysis of trace elements in high matrix samples. Agilent Technologies, Application Note 2011.http://www.chem.agilent.com/Library/applications/5990-9171EN_AppNote_7700x_OIDA.pdf(accessed June 2017).

Eliminating molybdenum oxide interference in urine cadmium biomonitoring using ICP-DRC-MS. Jarrett et al. Journal of Analytical Atomic Spectrometry Volume 23, 2008, Pages 962-967

Stajnko et al, 2017. Low cadmium exposure in males and lactating females–estimation ofbiomarkers Environmental Research 152, 109–119.

Bernard A, 2016. Confusion about cadmium risks: the unrecognised limitations of an extrapolated paradigm. Environ. Health Perspect. 124, 1–5.

2) Description of the bibliographic search: Literature search was done by reviewing the published articles through the databases like NCBI, Science Direct etc. by using key words: cadmium, cadmium exposure, cadmium in urine/blood/bone/autopsy tissues, renal function

biomarkers, A1M, IgG, NAG, KIM, RBP, B2M, metallothioneins etc. We focused on recently published analytical articles (mostly 2016-2017).

Total number of articles reviewed and compiled: 18.

Remarks: search was conducted towards analytical articles.

Contribution of the corresponding CGLs: Yes.

Author/s: Inventory was drafted by Agnese Osite and reviewed by Janja Snoj Tratnik, Darja Mazej, Ingrid Falnoga, Anja Stajnko and Milena Horvat.

Affiliation/s: Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia.

1.2.8 Chromium

Description of the bibliographic search: The first inventory was drafted by Ana Virgolino covering articles published in the years 2010-2017 (12 in total), and Alessandro Alimonti (CGL) completed it by adding 4 new references.

Contribution of the corresponding CGLs: Yes.

Author/s: Ana Patricia Lopes Virgolino[1], Alessandro Alimonti[2].

Affiliation/s:[1] Faculty of Medicine oft he University of Lisbon (FMUL), Portugal; [2]Istituto Superiore di Sanità (ISS), Rome, Italy.

2 Evaluation of available analytical methods, suitable exposure biomarkers and matrices

The objective of the evaluation of the analytical methods was to provide a prioritised list of biomarkers, matrices and analytical methods for the 1st prioritisation round of substances. This was done by comparing a number of state-of-the-art analytical methods, which were suitable for the determination of the pair biomarker/matrix. Information from the inventories and a few other relevant papers provided by the evaluators have been assessed. Expert knowledge from the chemical group leaders and evaluators has also been taken into account. The inventories and evaluations will be revised and updated if new information is available in year two of HBM4EU.

2.1 Process for evaluation

The partners evaluating the information compiled in the inventories (IPA, ULPGC and NIPH) agreed on applying the following guideline:

The first evaluation step of the methods should be based on two parameters:

- Sample intake
- MDL (method detection limit)

In order to rank satisfactory methods with respect to this, a second step should be to assess the performance:

- QA/QC measures at a specified and meaningful spiking level (e.g., precision, accuracy, etc)
- participation in interlab comparison exercises
- use of certified reference materials
- use of in-house control samples

Methods without information on any of the following analytical parameters cannot be assessed properly and will be disregarded:

- Sample intake
- MDL (or Method Quantification Limit, MQL)
- QA/QC measures at a specified spiking level (e.g., precision, accuracy, etc)
- Results from interlaboratory comparison, certified reference material or in-house control samples

The combination of the first and second step will lead to a numeric range for each analytical parameter considered satisfactory, and will represent requirements for a state-of-the -art analytical method for the determination of the pair biomarker/matrix.

Other analytical parameters available in the inventory may also be relevant and used in the evaluation.

2.2 Evaluations

For transparency and record keeping, the evaluations done by the corresponding partners are presented below.

2.2.1 Phthalates and DINCH

2.2.1.1 Phthalate metabolites (Category A and B)

The number of measured metabolites was between 7 and 21. All laboratories (N=10) were able to measure the metabolites from the Category A phthalates (DEHP: MEHP, 5OH-MEHP, 5oxo-MEHP; DEP: MEP; DBzP: MBzP; DiBP: MiBP). Eight labs measured the metabolite from DnBP (MnBP). The oxidised metabolites from DiBP (OH-MiBP) and DnBP (OH-MnBP) were additionally measured in one lab. Six labs measured a fourth metabolite from DEHP (5cx-MEPP).

Category B phthalate metabolites were measured in 8 labs (DMP: MMP - 4 labs; DnOP: MnOP - 5 labs; DnPeP: MnPeP – 2 labs; DiNP: MiNP – 4 labs, OH-MiNP/oxo-MiNP/cx-MiNP – 5 labs; DiDP: MiDP (weak biomarker) – 3 labs, OH-MiDP/oxo-MiDP/cx-MiDP - 2 labs; DCHP: MCHP – 4 labs). The metabolite MCPP (measured in 2 labs) cannot be attributed to a specific parent phthalate because it originates from DnBP, DnOP and other longer chain phthalates.

The maximum number of phthalates, resp. metabolites currently covered by a method is 21 metabolites, representing exposure to 11 phthalates.

For phthalate metabolite analyses, LOD/LOQs are generally between 0.1 and 1.0 µg/L; thus LOQs well below 1 µg/L are achievable today. For most of the biomarkers a sufficient detection of background levels in the general population is possible. For some monoesters, e.g. MnBP, MiBP, MEHP, MiNP, a strict internal and external contamination control is warranted, and LOQs might depend upon blank background levels. Not all methods describe in detail how analytical background contamination was dealt with.

Metabolites of DPHP are quantified together with metabolites of DiDP, because current methodologies by HPLC/UHPLC do not succeed in separating DPHP metabolites from DiDP metabolites because of same molecular masses and similar retention in HPLC. The only way to determine DPHP metabolites separately is by GC-MS (see Gries, W .et al. 2012. J. Chromatography B 908, 128–136.

Short chain phthalates (e.g. DEP, DnBP, DiBP) are generally determined via their simple monoester metabolites, while long chain phthalates are generally determined via their oxidised metabolites (e.g. DEHP, DiNP, DiDP). Simple monoester metabolite analyses is analytically challenging for the long chain phthalates (e.g. MEHP for DEHP) because of external contamination issues and the low metabolite excretion ratio. For the longer phthalates DiNP and DiDP the simple monoesters (MiNP and MiDP) are generally questionable biomarkers of exposure in urine (low excretion factor, prone to contamination).

Additionally challenging is the separation of the isomers. For the short chain phthalates, MiBP and MnBP need to be chromatographically separated. The long chain phthalates, e.g. DiNP and DiDP produce a set of different peaks (because of the isomeric composition of the alkyl side chains) and additionally, different isomers due to different oxidation positions at the alkyl side chain. Experience is needed to identify the various isomers. Quantification needs to be performed by sum integration of the various peaks. For internal standardisation a single isomer derived from the major alkyl

chain isomer is used. Because for these isomers appropriate control material cannot be prepared by spiking, native control material is strongly advised.

Analyses of phthalate metabolites warrant special care in the choice of enzyme used for deconjugation. Some glucuronidases (such as that from Helix pomatia) break down the ester bonds of the phthalates (due to esterase/alkylsulfatase side activities) which can dramatically influence the analytical result. The enzyme of choice is E. coli K12 glucuronidase (pure beta-glucuronidase activity). Seven of the 10 laboratories used E. coli K12 glucuronidase, two did not specify the enzyme used and one lab used the Helix pomatia enzyme.

No information have been found for Di-isopentyl phthalate (DiPeP), Di-C7-11 (linear and branched)-alkyl phthalate and Di(methoxyethyl) phthalate, and these compounds have thus not been added to Table 2.

2.2.1.2 DINCH metabolites (Category B)

Four laboratories reported the analyses of DINCH metabolites. The number of measured metabolites was between 3 and 6, however not all with labelled internal standards. In Germany, a health based biological limit value (HBM-value; German UBA) is derived for the sum of 5OH-MINCH+5cx-MINCH and data for these metabolites is most comprehensive.

The analyses of DINCH metabolites are challenging, because they produce a set of different peaks (because of the isomeric composition of the alkyl side chain) and additionally, different isomers due to different oxidation positions at the alkyl side chain. Experience is needed to identify the various isomers. Quantification needs to be performed by sum integration of the various peaks. For internal standardisation a single isomer derived from the major alkyl chain isomer is used. Because for these isomers appropriate control material cannot be prepared by spiking, native control material is strongly advised. For DINCH metabolites, LOQs around 0.1 µg/L are desirable, because current exposures are rather low (with a tendency to increasing exposures). Not all methods describe in detail how analytical background contamination was dealt with.

Summary: In general, two different methodologies were used for phthalates and DINCH: LC-MS/MS either coupled with online-SPE (LC/LC-MS/MS) or in combination with manual SPE sample clean-up, and UHPLC-MS/MS without sample clean-up. Both methodologies seem equally suitable for phthalate and DINCH biomarker analysis in terms of sample volume needed, LOQs, method runtime, QA/QC measures etc. For DPHP the only way to determine the metabolites separately is by GC-MS.

External commercial quality assessment schemes currently only offer external quality assurance for 7 phthalate metabolites (4 phthalates): 5-OH-MEHP, 5-oxo-MEHP, 5-carboxy-MEPP, MEHP, MnBP, MiBP, MBzP. For DINCH, no external quality assessment scheme is currently available.

Standards and internal standards have become available by commercial providers for most of biomarkers (some very recently).

Author/s: Holger Koch, Monika Kasper-Sonnenberg, Daniel Bury.

Affiliation/s: Institute for Prevention and Occupational Medicine of the German Accident Insurance (IPA), Bochum, Germany.

2.2.2 Per- and polyfluoroalkyl substances

Per- and polyfluoroalkyl substances (PFASs) comprise a very large and diverse group of manmade compounds. The two most frequently studied compound groups are the perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs), which are highly persistent and do not metabolise in the human body. Thus, both PFSAs and PFCAs can be measured unchanged in human samples. On the other hand, a large number of compounds are considered precursors of PFSAs and PFCAs, as these precursors can degrade in the human body to PFSAs and PFCAs. Examples of such substances are fluorotelomer alcohols (FTOHs), polyfluoroalkyl phosphoric acid esters (PAPs), perfluoroalkane sulfonamides (FASAs), N-ethyl perfluoroalkane sulfonamides (EtFASAs) and N-ethylperfluoroalkane sulfonamidoethanols (EtFASEs). Measured levels of PFSAs and PFCAs can thus be a result of direct exposure to PFSAs and PFCAs, but also due to indirect exposure from PFAS precursors. The PFAS precursors do not necessarily degrade completely and their exposure can also be measured. Up to now, most biomonitoring studies have focused on PFCAs and PFSAs. However, as toxicity and exposure to the various compounds differ, it is advantageous to establish methods that are able to determine as many of the single compounds as possible in each sample. In total 16 methods were included in the inventory. The methods included from 3 to 21 substances, which were determined in serum, cord blood, breast milk, hair or nails. In addtition to the methods included in the inventory, information from a few other methods have been included in Table 2, especially for obtaining information on Category B and C substances. The references to these methods are mentioned in section 1.2.2.

2.2.2.1 Category A substances

For PFCAs and PFSAs, blood and specifically plasma or serum, has been the most frequently used and preferred matrix. Several methods with satisfactory recoveries, intra- and inter-precision have been established. For the most sensitive methods the MDLs are in the low picogram/mL range and require between 50 and 300µL of blood. Studies have been conducted to obtain information on the relation between different PFCAs and PFSAs in whole blood, plasma and serum, as plasma and serum is preferred due to practical reasons. Blood samples can be obtained from both genders and all age groups, thus being suitable for biomonitoring of general as well as specific populations such as workers. Also, studies on cord blood have been conducted. However, ethical approval for blood collection can sometimes be challenging for the youngest age groups. To overcome this, some few methods have recently been developed for determination of PFCAs and PFSAs from blood spots. This technique, requires no skilled personnel for sample collection and is considered minimal invasive, but there are still some practical issues to overcome to use this technique for routine analyses. Associations between exposure to PFCAs and PFSAs and increased blood levels have been established in both workers and general populations, confirming blood as a suitable matrix for monitoring exposure to these PFASs. Further, in contrast to urine, which reflects short-term exposure, PFAS concentrations in blood reflect long-term exposure, in particular for the persistent PFASs such as PFCAs and PFSAs. Studies on breast milk have been conducted and are of particular interest in cases where exposure to young children through breast-feeding is to be explored. However, PFCA and PFSA determinations in breast milk are more challenging than blood, as the concentrations in breast milk are 1-2 orders of magnitude lower than in blood. Thus, the methods available so far are only capable of measuring the most abundant PFSAs and PFCAs with high detection frequency even though the MDLs are in the same range as for blood. Also for breast milk methods with satisfactory recoveries, intra- and inter-precision have been established. One major limitation for this matrix is that studies on breast milk are feasible only for a limited part of the general population.

The sample preparation methods for both blood and breast milk are in general quite straight forward, including protein precipitation and either on-line and off-line clean-up, and several methods with high sample capacity are available. Previously, challenges related to high levels of PFCAs and PFSAs in procedure blanks have been reported, but this has been sorted out and is no longer considered as a major problem. Standard solutions, including isotope labelled analoges, can be obtained from commercial suppliers for most of the compounds. However, interlaboratory comparisons and certified reference materials are so far only available for the most abundant PFCAs and PFSAs.

Some few methods have been developed for determination of PFSAs and PFCAs in urine, but also urine concentrations are generally much lower than blood concentrations and reflect short-term exposure. So far there is limited information on the relation between PFASs in urine and blood, but urine may be feasable for assessing short-chain PFCAs and PFSAs which have relatively short half-lives compared to long-chain PFCAs and PFSAs such as PFOS and PFOA. However this needs to be further evaluated. A limited number of methods on non-invasive matrices such as hair and nails exists. A sensitive method for PFCAs and PFSAs in hair with MDLs as low as 1 to 4 pg/g has been published. But one major drawback with non-invasive matrices is that it is so far unclear how well the PFCA and PFSA concentrations in these matrices are reflecting the actual exposure. Further, the methods and particularly the sample preparation for nails and hair are more complex and demanding than methods for blood, breast milk and urine. Some very few studies have determined PFCA and PFSA concentrations in other human matrices such as liver and amniotic fluid. However, these studies are usually exploring the distribution of PFASs between various body compartments, and these matrices are generally not suitable for biomonitoring purposes.

All methods included in the inventory used 13C-IS and LC-MS-MS, while there is a lack of methods using HRMS.

2.2.2.2 Category B and C substances

For the Category B and C substances most studies carried out so far have used blood as the sample matrix. However, knowledge on the applicability of blood as well as other human matrices is so far limited.

No information has been found for hexafluoropropylene oxide (HFPO), 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid (HFPO-DA) and perfluoroalkyl ether potassium sulfonate (F-53B), and these compounds have thus not been added to Table 2.

2.2.2.3 Additional methods/tools

As PFASs comprise a very large and rapidly increasing group of compounds, it is challenging to develop methods that cover all important compounds. As an additional tool to explore the proportion of unknown PFASs in samples, methods measuring total fluorine (TF) have been used. Further, by introducing an extraction and/or fractionation step the amount of inorganic fluorine (IF) or exactable organic fluorine (EOF) can be determined. Usually, TF, IF and EOF are measured using combustion ion chromatography. Presently, there is a lack of knowledge on which PFAS precursors are being produced and used. To explore the maximum potential amount of precursors of specific PFASs, a total oxidisable precursors analysis (TOPA) method has been developed. This method is still in the exploratory phase, but may together with the TF method be a complementary tool to gain knowledge on the necessity of expanding methods for PFAS analysis to be used for biomonitoring.

Summary: Most methods so far have been focusing on PFCAs and PFSAs, but recently methods including Category B and to some extent category C substances have been developed. As toxicity and exposure to the various compounds differ, as many PFASs as possible should be measured. LC-MS-MS is the most common technique used for determination of PFASs in any kind of matrix. Generally, the preferred matrix for biomonitoring of PFASs is blood, specifically serum or plasma. However, in specific studies, breast milk may be the desired matrix and sometimes urine may also be a feasible matrix. Sample preparation most commonly includes a protein precipitation step followed by either on-line or off-line clean-up. Several high throughput methods are available.

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2.2.3 Flame retardants

PBDEs (Category A substances) have been evaluated as a group, while Category B and C (i.e., novel or emerging FRs, except PFRs) have been evaluated individually.

2.2.3.1 Category A substances

HBCDs: 3 LC methods (serum and breast milk). All used LC-MS(ESI(-)). The serum methods seemed more sensitive (0.08 ng/g lw using 3-4 g (n=50)) or 2-5pg/mL using 1 mL (n>200)) than the breast milk method (n=50)). Both used 13C-IS, but only 1 mL serum method reported QA/QC parameters (recoveries 87-112% and precision <15%). Breast milk method (15 mL) seemed robust according to the QA/QC results, but did not use 13C-IS. The determination of HBCDs is more advantageous using LC-MS because the isomers α, β and γ can be chromatographically separated and quantified individually.

PBDEs (methods comprising from 3 to 38 congeners, including BDE-209): 23 GC methods in total. Determined in breast milk (2-3 mL), serum (0.5-5 g), cord blood (10 mL), placenta (2 g dry weight (dw)), hair (0.1-3 g) and nails (0.03 g). Most of the methods were developed for serum, where lower MDL were obtained. Most frequent extraction techniques and clean-up were SPE+acid silica and LLE+GPC followed by GC coupled either to LRMS(ECNI) or HRMS(EI). Among the serum methods, especial attention should be paid to one using only 0.5 mL. This method (LLE+GPC and GC-QqQ(ECNI)) determined 8 congeners giving MDL and recoveries in the range 0.02-5 pg/mL and 55-109%, respectively. Unfortunately, the rest of QA/QC parameters from the inventory were missing, but the method used 13C-IS. In hair samples, the lowest MDL ranged from 0.015-0.37 ng/g with recoveries between 60 and 120, and precision <20% for 38 different congeners. The method was based in MAE+GPC and GC-QqQ(EI). This method is more sensitive than the one developed for 10 congeners in nails (0.12-2.4 ng/g). Despite of being well-known substances, methods still strive to reduce the amount of sample or use alternative matrices obtaining satisfactory results.

Summary: Inventories were created with new methods from the last decade (approximately), and therefore for Category A substances only few and recent developed methods have been assessed. The inventory for PBDEs was performed considering all of them as a group. Due to the different physico-chemical properties of BDE-209, in the future, it would be highly advisable to assess it individually.

Most relevant matrices are serum and breast milk and they are analysed by GC-LRMS with a previous extraction (SPE or LLE) and clean-up (acid silica or GPC). The exception is HBCD, which is usually analysed by LC-MS (QqQ). The QA/QC measures are still not abundant for this class of substances: 50% did not report intra-precision, only 14% reported accuracies and 1 out of 23 methods reported inter-precision and only one used certified material.

Even though robust and reliable methods have been developed for non-invasive matrices (e.g., hair and nails), the lack of proper QA/QC measures (e.g., reference materials, interlaboratory comparisons, etc.) and the difficult interpretation of the results in terms of internal/external exposure, limits the use of these matrices for biomonitoring surveys. Furthermore, use of alternative matrices (e.g., urine, nails, faeces, etc.) might result in new challenges for Category A substances (e.g., correct biomarker, kinetics, risk assessment, etc.), which might imply a change in their categorisation (e.g., from Category A to B or C).

2.2.3.2 Category B substances

DBE-DBCH: 1 GC-HRMS method (breast milk 8-10 mL). Good MDL (0.008 ng/g lw) and QA/QC measures (recovery 67-105%, inter and intra precision <24%). Method applied to n=120 samples. Considering the lack of literature more research on other matrices like serum, plasma, etc. is needed to better assess the optimal pair biomarker/matrix.

BEH-TEBP: 7 GC methods in total. Determined in breast milk (5 mL), serum (2-4 g), hair (100 mg) and nails (0.03 g). The lowest MDL are for breast milk (GC-QqQ) and one serum method (GC-MS) (<0.5 ng/g lw). Both applied a clean-up using GPC, which is ideal for acid sensitive compounds. However, none of the methods provided satisfactory QA/QC measures.

BTBPE: 12 GC methods in total. Determined in breast milk (5-25 mL), serum (2-5 g), whole blood (7 g), hair (0.1-2 g) and nails (0.03 g). Methods developed for hair and nails provided good MDL and precision, but recoveries were above 139% and no 13C-ISs were used. Only two methods, GC-LRMS in serum and GC-HRMS in breast milk, reported inter and intra-precisions (<19 and <12%, respectively) with acceptable recoveries (67% spiking at 15 pg/mL and 110% at 1.78 ng/g lw, respectively) and MDL (2.3 pg/mL and 0.006 ng/g lw, respectively). Matrices such as hair and nails seem promising, but it is still unclear how to interpret the results. Methods must report QA/QC measures more clearly.

DBDPE: 8 GC methods in total. Determined in breast milk (5-25 mL), whole blood (7 g), serum (2-5 g) and hair (2 g). Only one method (serum) reported recovery, accuracy, inter and intra-precision. Recoveries are always lower than 50% (except one method (84-91%)) and labelled IS is seldom used. Need for increasing sensitivity to raise the detection frequencies. Hair method reaches satisfactory MLD (0.2 ng/g) although it does not report QA/QC results.

Dechlorane Plus: 22 GC methods in total. Determined in breast milk (2-10 mL), whole blood (5 g), serum (2-10 g), cord blood (10 mL), placenta (2 g dry weight (dw)), hair (0.1-2 g) and nails (0.03 g). Only two methods with full QA/QC parameters and there is a great variety of extractions, clean-up

procedures, and MS (LRMS and HRMS). Lowest MDL in serum (2 g) and milk (3 g) were 1.1-2.3 pg/mL (n=48) and 0.035-0.046 ng/g lw (n=44), respectively (both using GC-LRMS). Interesting MDL were obtained in hair samples (around 0.015 ng/g dw) and nails (0.2-0.6 ng/g, but n=5). The high number of developed methods in different matrices with satisfactory sensitivity plus the availability of 13C-IS indicate that, from an analytical point of view, this substance could be considered as Category A.

EH-TBB: 7 GC methods in total. Determined in breast milk (5-10 mL), serum (2-4 g), hair (0.1 g) and nails (0.03 g). The lowest MDLs are achieved using PLE and non-destructive lipid removal in breast milk followed by GC-HRMS (0.008 ng/g lw). This method provided good recovery (109%) and precision (<21%) The MDL using 2 g of serum and LLE+GPC with GC-QqQ was 0.38 ng/g lw with lower recovery (49%) and no precision reported. Lack of labelled IS in all matrices. There is a need to fully validate the methods for the specific compound/matrix.

HBB: 8 GC methods in total. Determined in breast milk (8-25 mL), serum (2-5 g), hair (0.1-3 g) and nails (0.03 g). One GC-LRMS method (serum) and one GC-HRMS (breast milk) method with full and satisfactory QA/QC parameters (MDL 0.3 pg/mL and 0.06 ng/g lw, respectively). Hair and nail samples showed good MDL (0.1-0.2 ng/g). For this compound, half of the methods were developed for non-invasive matrices.

HCDBCO: 2 GC methods in serum (2-3 g). The lowest MDL (0.21 ng/g lw) were obtained using LLE+GPC and GC-LRMS. This method was also applied to breast milk samples. More methods should be developed, although this substance might not be of relevance for the human exposure.

OBIND: 2 GC-QqQ methods. Lower MDLs were achieved using 5 g of breast milk (0.2 ng/g lw) than using 2 g of serum (1.5 ng/g lw). Recoveries were <50% and the rest of QA/QC parameters were missing. None used labelled IS. The methods are not reliable enough for this substance, although its relevance for human exposure might be rather low.

PBEB: 4 GC methods in total. Determined in serum (5 mL), breast milk (8-10 mL), hair (0.1 g) and nails (0.03 g). The methods applied to non-invasive samples presented good MDL (0.1-0.2 ng/g), but no QA/QC results and the sample size was very low (n=5). The method for serum followed SPE+acid silica and GC-LRMS without labelled IS achieving moderate MDL (2.3 pg/mL), good recovery (78-89%) and precision (<8%). The main drawbacks of the method was its applicability (n=12) and no labelled IS used. For the breast milk method (n=120) PLE+GPC and GC-HRMS was used giving a MDL of 0.003 ng/g lw, recovery of 121% and inter and intra-precision < 15%. More method development is required to achieve lower MDL in serum and apply it to a representative amount of samples.

PBT: 5 GC methods in total. Determined in serum (3-5 mL), hair (3 g) and breast breast milk (8-25 mL). Most sensitive methods were using 5 g of serum (1.6 pg/mL; SPE+strong acid and GC-LRMS) and 8-10 mL of breast milk (0.003 ng/g lw; PLE+GPC and GC-HRMS). Both provided full and satisfactory QA/QC parameters. Nonetheless, labelled IS was not used and the sample size was rather low in serum (n=12), but acceptable in breast milk (n=120). More research is needed to better assess an optimal method.

TBX: 2 GC methods (breast milk and hair). Breast milk method (8-10 mL) used GC-HRMS after PLE+GPC providing low MDL (0.001 ng/g lw), good recovery (80%) and inter- intra-precision (<15%). Hair method (3 g) used soxhlet extraction and acid silica as a clean-up, followed by GC-LRMS giving MDL between 19 and 727 pg/g dw (range for a group of compounds). No labelled IS used and no QA/QC results reported. The breast milk method seems robust enough. The hair

method is not reliable enough for this substance. More research is needed to better assess an optimal analytical method for the pair biomarker/matrix (e.g., serum).

TBBPA: 3 methods. Determined in serum (1 ml) and breast milk (15 mL). Lower MDL in serum (8 pg/mL by LC-QqQ or 4 pg/mL by GC-QqQ) compared to breast milk (60 pg/mL by LC-QqQ). Serum method provided good inter- and intra-precision (<18%). However, the serum method is more labour and time consuming because of the need of derivatisation. None of the methods used labelled IS. More research is needed to better assess an optimal method and use of labelled IS is highly recommended.

PFRs (e.g., TCEP, TCIPP, TPHP etc): 11 methods in total (LC and 1 GC). Metabolites were determined in urine (0.01-5 mL), breast milk (100 mL), hair (0.1 g) and nails (0.03 g). The preferred matrix and technique was urine and LC-QqQ, respectively, although the biomarkers for the same FR varied depending on the study. The lowest MDLs were 0.025-0.1 ng/mL for 10 metabolites, inter- and intra-precision were between 6 and 15%, and the deuterated ISs provided good accuracy (78-90%). The metabolites for monitoring are not always the most abundant. There is a need to assign the optimal metabolite to each PFR.

Summary: For the persistent FRs, GC-LRMS is the most suitable technique in all matrices. Extractions using SPE or LLE followed by destructive lipid removal are commonly used. However, GPC appears to be an interesting non-destructive lipid removal method, which enables the determination of compounds sensitive to acid in the same run. In terms of sensitivity, the use of HRMS(EI) improved the MDLs compared to LRMS(ECNI) for some compounds. Methods should report QA/QC measures more extensively and/or clearly and the applicability of the method is not widely proven. Hair or even nail samples seem to reach satisfactory MDLs for some FRs. The major concern to use such matrices is how to differentiate internal from external dose and the calculation of the body burden. These issues are unsolved yet.

For the non-persistent FRs (i.e., PFRs), LC-QqQ is the preferred technique using urine samples. There is a lack of 13C-IS, but deuterated ones seem to work fine. In some cases, the biomarker chosen to monitor certain FR is not specific or appears not to be the most abundant in *in-vivo* samples.

2.2.3.3 Category C substances

2,4-dibromophenol: 2 methods. Determined in breast milk (15 mL) and in serum (2-3 mL). The breast milk method (LC-QqQ) reported high MDL (200 pg/mL) with good recovery (89%) and precision (5%). However, the method in serum (GC-MS; need of derivatisation) does not provide units in its MQL (5), although precision is satisfactory (<10%). There is a need to use labelled IS and develop a more sensitive analytical methods for the determination of 2,4-dibromophenol.
2,4,6-tribromophenol: 4 methods in total. Determined in breast milk (15 mL) and serum (1 mL). Both MDLs are in the range 16-30 pg/mL using LC (breast milk) or GC (serum) coupled to QqQ. GC method is more tedious (need of derivatisation), but the method seems more robust because the QA/QC measures are more comprehensive. Nevertheless, the lack of optimal IS and the analysis of hundreds/thousands of samples make these methods not fully reliable.

Pentabromophenol: 2 methods in total. Determined in serum (1-3 mL) and breast milk (15 mL). The extraction of the analyte from the breast milk was carried out by shaking+clean up in C18. The extract was analised by LC-QqQ(ESI(-)) obtaining a MDL of 30 pg/mL, good recovery (82%) and

precision (4%), but no information about IS. The MQL in serum (0.5 without reported units) was achieved using SPE+acid silica and derivatisation followed by GC-LRMS. Lack of labelled IS, but good recoveries (104-131%) and precision (<14%). Both methods were applied to a satisfactory number of samples (n>50). Both seem to work fine, but still too few methods to decide whether these methods are optimal for this compound.

Dechlorane 602 and Dechlorane 603: 4 GC methods in total. Determined only in serum (2-10 mL). The most sensitive methods (2-3 mL) presented MLD in the range 0.02-0.14 ng/g lw using GC low and high resolution (LR and HR, respectively). Only one method (GC-LRMS) provided complete and satisfactory data for the QA/QC measures, but for Dechlorane 602, this method reported approximately 10 x MLD than GC-HRMS. Labelled IS not used during method development.

Dechlorane 604: 2 GC methods (serum). Only one provided MDL (3.6 ng/g lw) and recovery (84%). Lack of any assurance of the results. Extraction of the analyte by LLE and clean-up by GPC. More research is needed to better assess an optimal method.

BPA-BDPP and RBDPP: 1 LC method in urine (5 mL). The biomarker of exposure is diphenyl phosphate (CAS-No 838-85-7), which is a common metabolite for many FRs (e.g., TPHP (CAS-No 115-86-6), EHDPP (CAS-No 1241-94-7), etc). Extraction was carried out using SPE and the metabolite determined by LC-QqQ. The MDL was 0.2 ng/mL and recovery 95%. No more QA/QC data was reported in a small cohort (n=24). The biomarker and matrix do not seem the optimal ones. Therefore, there is a need to find out what are the best biomarkers and matrix for these two FRs.

Summary: For persistent FRs, GC-MS is the most common technique for serum and breast milk samples. The use of LRMS (QqQ) or HRMS is highly recommended for this group of substances, although some of the FRs might not be relevant for the human exposure.There is a need to have commercially available labelled IS, and the applicability of the methods has generally been tested in small cohorts.

For the non-persistent PFRs (i.e., BPA-BDPP and RBDPP), there are no reliable methods for biomonitoring Category C substances according to the inventory provided.

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2.2.4 Bisphenols

2.2.4.1 Bisphenol A (Category A)

In summary, there are 16 entries in the inventory (contribution from ULPGC, Canary Islands, Spain). Eight entries concerning BPA, one entry is about Clx-BPA, one entry is about BPS but 6 entries do not allow to distinguish between BPA, BPS, BPF (category B) and other bisphenols (Category C). These 6 entries should be moved to the inventory from the Antwerpen University – UA and evaluated separately, and therefore, here we evaluated only the entries for BPA. We added one additional row for BPA measures in urine, extracted from entry no. 26, resulting in 10 entries for BPA.
Out of these, 4 entries describe the methods for determination of BPA in urine (1 entry only Clx-BPA), three for blood (serum/plasma), two for hair and one for saliva. BPA biomarker and labelled

standards are commercially available. Blank contamination was only reported for Clx-BPA, and matrix effects were between 41-95 %.

Methods: The techniques described are LC-MS/MS (one GC-MS/MS) with different sample preparation and clean-up procedures (SPE, LLE, L-L, ultrasound, shaking, centrifugation, and derivatisation). Limits of detection are usually between 0.003 and 0.15 ng/mL except of a method with hair (LOD=1.8 ng/mL). One study describes the use of a specific column "Glass AFFINIMIP® SPE Bisphenol A cartridge", developed to selectively retain BPA, and the LOD (0.03 ng/mL) is comparable with the other methods. Sample intake was 0.3-5 ml (urine), 0.3-2.5 mL (blood), 0.15-1.0 mL (hair), and 0.7 mL (saliva). Urine should be preferred as the matrix of choice for exposure assessment in the general population. Other matrices are more of interest for gaining insights into mechanisms of toxicity. In the general population urinary concentrations of BPA are within the ng/mL-range (generally a median concentration of 1 to 3 ng/ml). Therefore LOQ's around 0.1 ng/mL (LOD's around 0.03 ng/mL) are required for population based studies. The risk of contamination of the samples during the sampling/preparation procedure should be monitored by field and laboratory blank controls.

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2.2.4.2 Bisphenol S/F (Category B); other BP's (Category C)

There are 11 entries for BPS, 7 for BPF and 4 for other BP's in the inventory (contribution from the University of Antwerp - UA).

BPS: 4 entries describe the methods for determination of BPS in urine, 3 for serum, 1 for breast milk and 3 for other matrices (food, paper products).

BPF: 2 entries are for urine, 1 for breast milk and 4 are for other matrices (food, paper products, and aqueous environmental samples.

Other bisphenols: There are 4 entries but no measures in human matrices. Matrices are food samples (1) and paper products (2).

Methods: The described technics are predominantly LC-MS/MS (3 entries for GC-MS/MS) with different sample preparation and clean-up procedures (SPE, LLE, ultrasound, shaking, centrifugation, reflux extraction and derivatisation) for different matrices (urine, serum, breast milk, food samples…).

For **BPS** LOD's are between 0.01 and 0.03 ng/mL (urine) and 0.002-0.03 ng/mL (serum). For urine, LOQs are between 0.02-0.04 ng/mL. The LOQ for breast milk is 0.003 ng/ml. In human samples the reported concentrations are: < LOD - 212 ng/mL, < LOQ – 21 ng/mL, <LOD – 11.04 µg/g creatinine (urine – 3 entries); <LOD – 0.12 (serum – 2 entries) and, for breast milk, at 0.23 µg/kg (1 sample). Labelled BPS-standards are used in the US study from Thayer et al. 2016 (urine, serum). The others used deuterated- or 13C12-BPA as internal standards.For **BPF** in urine one LOD is at 0.06 ng/mL and one LOQ is at 0.5 ng/mL.The LOQ for breast milk is 0.018 ng/mL. The measured BPF concentration in urine is < LOD - 12.3 ng/mL (1 entry). In the study from Belgium a deuterated BPF-standard is reported. For the other entries only labelled BPA-standards are mentioned.

For **other bisphenols** no method for human matrices is reported. Isotope-labelled standard is deuterated-BPA.

Commercial available internal standards and biomarkers for BPS, BPF and other BP's are not well established. Urine should be preferred as the matrix of choice for exposure assessment in the general population. LOD's < 0.01 ng/mL for urine are required for population based studies but such low LOD is only reported in the study from Liao et al. (2012). Other matrices such as serum or breast milk are more of interest for gaining insights into mechanisms of toxicity. The risk of contamination of the samples during the sampling/preparation procedure should be monitored by field and laboratory blank controls.

For BPS, BPF and other BP's there is a general lack of peer-reviewed, published methods, especially in urine, and experience in HBM studies is not well established. Up to now quantifiable measures in urine with quality assured (labelled) internal standards for BPS and BPF are not available in Europe.

Summary: For **BPA, BPS and BPF** urine should be preferred as the matrix of choice for exposure assessment in the general population. Other matrices such as serum or breast milk are more of interest for gaining insights into mechanisms of toxicity. The risk of contamination of the samples during the sampling/preparation procedure should be monitored by field and laboratory blank controls.

In the general population urinary concentrations of **BPA** are within the ng/mL-range (generally a median concentration of 1 to 3 ng/ml). Therefore, LOQ's around 0.1 ng/mL (LOD's around 0.03 ng/mL) are required for population based studies.

Commercial available internal standards and biomarkers for **BPS, BPF and other BP's** are not well established. LOD's < 0.01 ng/mL for urine are required for population based studies but such low LOD is only reported in one study (Liao et al. 2012). For BPS, BPF and other BP's there is a general lack of peer-reviewed, published methods, especially in urine, and experience in HBM studies is not well established. Up to now quantifiable measures in urine with quality assured (labelled) internal standards for BPS and BPF are not available in Europe.

While BPA (determination as biomarker of exposure in urine) can clearly be regarded as a Category A substance as categorised in the scoping documents. However, the current method and biomarker/matrix inventory does not fully support the Category B categorisation of BPS and BPF from the scoping documents. Currently, due to the lack of peer-review, internationally published and cross validated methods a categorisation of BPS and BPF as Category C substances seems more appropriate.

Additional questions in regard to natural occurances of BPF in mustard (Zoller O et al. 2016) need to be answered in order to ensure the specificity of this biomarker to represent non-natural exposures to BPF. A more detailed investigation of the isoforms of BPF (4,4'-BPF,2,2'-BPF and 2,4'-BPF) in terms of exposure biomarker validity might be warranted.

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2.2.5 Polyaromatic hydrocarbons (Category B)

There are 18 entries for PAHs in the excel sheet inventory from Las Palmas University, only one of them was removed for not showing the analytical requirements to be assessed properly (methods without information in sample intake, MDL, QA/QC and reference materials, in-house controls or interlaboratory comparison will be disregarded). Each entry shows an analytical method where several PAHs, from 8 to 24, are determinated in biological matrices such as urine, whole blood, hair, breast milk and cord blood.

Methods: Most methods are based on the analysis of several PAH's using liquid chromatography (LC) with Electrospray (ESI) or atmospheric pressure chemical ionisation (APCI) as ionisation sources. Also, gas chromatography (GC) is employed with electron impact (EI) or chemical ionisation (CI) as ionisation sources. All of them are coupled to mass spectrometers as single quadrupole (Q), triple quadrupole (QqQ) and also there is one entry which used a high-resolution mass spectrometer (HRMS). Only two entries show a LC coupled to fluorescence detector (FLD) and one entry a GC coupled to Flame ionisation detector (FID), but these latter methodologies may be considered somewhat obsolete. However, most updated methods employ LC-MS-MS for PAH biomarkers and GC-MS-MS or LC-MS-MS for parental compounds.

Most common sample preparation is liquid-liquid extraction (LLE), solid phase extraction (SPE), Florisil or silica and also deconjugation steps are included in the excel sheet. Sample intake depend on the matrix selected being 2 mL for whole blood, 0.1-0.2 g for hair, between 10 to 50 mL for breast milk, 0.03 mL for cord blood and finally from 1 to 36 mL for urine.

LOQ's for urine are between 0.02 and 0.18 ng/mL (being similar when MS, FLD or FID are employed as detector). LOD´s between 0.001-0.1 ng/mL, 0.012-0.30 ng/mL, 0.012-0.019 ng/mL and 20 ng/mL are reported for hair, breast milk, cord blood and whole blood respectively. The accuracies at spiking level around 0.050 ng/mL for urine, hair, breast milk, cord blood and whole blood were 66-110%, 70-145%, 68-133%, 81-126% and 80-120% respectively. Finally, the reported precision were lower than 30% in all cases.

All methodologies used internal standards as deuterated or 13-C labelled PAHs compounds, internal in house controls and the chromatography time was from 12 to 40 minutes independently wether LC o GC was selected.

Remarks: According to the inventory for PAHs, urine seems to be the preferred matrix to monitor this kind of compounds. The analytical features as LOD, accuracy and precision reported are similar for urine and other matrices. However, for human biomonitoring urine is the best matrix due to the following features: it is the easiest to sample, the volume required could be easily collected, the sample preparation is based on LLE or SPE in most cases and until 24 compounds were determined in this matrix.

Recently, two reference materials for hydroxylated PAHs in urine have been developed, so they could be used as an external control for the analysis of PAHs. Also, there is a reference material with 16 PAHs in acetonitrile.

- 11 OH-PAHs: SRM 3673 - Organic Contaminants in Non-Smokers' Urine (Frozen).
- 11 OH-PAHs: SRM 3672 - Organic Contaminants in Smokers' Urine (Frozen).
- 16PAHs: SRM 1647f - Priority Pollutant Polycyclic Aromatic Hydrocarbons in Acetonitrile.

Summary: Untransformed PAHs (parent compounds) can be used in human biomonitoring studies. In this case the preferred matrix is the serum or plasma and they may be determined interchangeably by liquid or gas chromatography, preferably coupled to triple quadrupole mass spectrometry (although they could also be analysed in ion trap mass spectrometry, TOF or by single quadrupole operated in the selected ions monitoring mode). Human exposure to PAHs can also be monitored through biomarkers (metabolites) in urine, and in this case the technique of choice would be LC-MS-MS. LOQs obtained with these methods are very low (well below 1 ng/mL). Accuracies and precision are good enough for all parent compounds and metabolites, and therefore can be considered suitable for human biomonitoring studies.

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2.2.6 Anilines and MOCA (Category A, B and C)

This is a very complex chemical group in which not only aniline, but also many aromatic amines (aniline-like compounds) may be included. However, many of these related compounds may appear in urine or other biological samples as a result of exposure to a wide variety of substances, including tobacco, drugs, or industrial chemicals different from aniline. Therefore, the first decision made by the responsible of the inventory was to exclude all the chemicals and biomarkers that are not directly related to the human exposure to aniline or aniline-like compounds, such as isocyanate exposure.

These most frequently studied aniline-related compounds (MOCA, 4,4'-MDA, and o-toluidine) have been included as Category A/B substances. However, we have also included a selection of other aniline-related aromatic amines as Category B/C substances, as many of these may be used as interesting biomarkers of the exposure to anilines and other related industrial chemicals.

All selected methods used as separation techniques either liquid chromatography (LC, and mostly UHPLC) or gas chromatography (GC), and as detector: mass spectrometry (MS, single quadrupole (Q), triple quadrupole (QqQ)), or quadrupole time-of-flight.

Sample preparations described in the methods are based on:

- Liquid-liquid extraction (LLE).
- Solid phase extraction (SPE).
- Also derivatisation steps are included for LC and GC in the inventory.

Sample intake depends on the matrix selected being:

- 4.5mL for plasma.
- 2.5mL for erythrocytes.
- 6 mL for whole blood.
- From 0.9 to 25 mL for urine (which is mainly used as preferred matrix for biomonitoring studies).

Limits of detection:

- For MOCA (4,4-methylenebis(2-chloroaniline)) and MDA (4,4-methylenedianiline) (both Category A) are from 1 to 3 ng/mL, independently whether UVD, ECD or MS are selected.
- For aniline and o´-toluidine (both Category B) are between 0.05 and 1 ng/mL (being twenty times lower when MS is employed compared to ECD).
- Other aromatic amines (Category C) ranged from 0.01 to 1.6 ng/mL

Accuracies (at a spiking level around 50 ng/mL):

- Category A: MOCA: 96-101% and MDA: 68-124%
- Category B: Aniline: 80-117% and o´toluidine: 85-112%
- Category B: Aromatic amines: 87-109%

The precisions for the anilines, and related compounds, were lower than 20%.

Internal Standards: The internal standard has not been specified in the inventory for each compound, only some entries include surrogate as internal standard.

Chromatography time: from 20 to 67 minutes.

QA/QC: All methodologies were checked using internal house controls.

Remarks: The inventory for anilines compounds in Category A/B has resulted, from our point of view, a little bit scarce, as it included only 4 references for aniline, 2 references for MOCA, 5 references for MDA and 4 references for o´-toluidine, which could finally be taken into account in this evaluation.

For the inventory of substances in Category C we have selected some aromatic amines from a fully validated, but not published method employed in the Institute of Biomonitoring, Currenta GmbH & Co, Leverkusen, Germany by Prof. Dr. Gabriele Lang et al. For other substances within this list published methodologies exist, but many do not provide data on full validation experiments. Aniline, arylamines (AAs) and heterocyclic aromatic amines (HAAs) are known for their ability of forming adducts with DNA, hemoglobin and other proteins. It is possible to biomonitor the presence of certain of these adducts. However, this is a field under research, because not all the possible adducts are known and these are highly dependent on the exposure to a particular chemical. Therefore, not a common or standardised methodology is available, and there are few (if any) laboratories which employ these biomarkers in routine studies for biomonitoring exposure to aniline and related industrial chemicals. Subsequently these biomarkers have not considered in the context of a project such as HBM4EU.

Summary: Urine is the preferred matrix for biomonitoring anilines, and LC-MS-MS the most employed technique, although GC-MS performs also satisfactory after sample derivatisation. The sensitivity achieved in the LC methods is good across the three categorisation groups (A, B and C). However, there is a lack of QA/QC measures, which guarantee the validity of the selected biomarker for compounds in Category B and C.

Determination of native anilines or hemoglobin adducts in blood is also possible, but this combination biomarker/matrix has not been explored thoroughly.

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2.2.7 Cadmium (Category A)

There were a total of 44 entries for Cd in the inventories sent from Department of Environmental Sciences, Jozef Stefan Institute, Slovenia; Faculty of Medicine, University of Lisbon; and State Education Development Agency, Republic of Latvia.

In general terms the information in these inventories was incomplete. Most of the required fields have not been completed. Thus, only 11 methodologies fulfilled the analytical requirements to be assesed properly, and these were those employed in the present evaluation. In addition, one highly relevant article based on the elimination of molybdenum oxide interference in the Cd determination has been considered (Jarrett et al. Journal of Analytical Atomic Spectrometry Volume 23, 2008, Pages 962-967; Cañas A et al. Referencia: ICHMET 2012 Conference proceedings. 2013. doi: 10.1051/e3sconf21003.).

All selected methods are based on the ICP-MS technique. Cd is analysed in urine, serum, whole blood, breast milk and many other matrices of minor interest in the context of HBM4EU (such as teeth dentine, brain, liver, bone, kidney and lung). Cadmium exposure reflecting the internal dose is best monitored either in urine or blood. As cadmium is accumulated in the kidney and has a long half-life, the urinary excretion corresponds approximately to the daily intake at steady state, which is obtained after long-term exposure. Blood cadmium reflects short-term exposure over the past three to four months, and is usually assessed in whole blood. It has been shown that the determination of Cd is affected by the interference of tin (^{114}Sn) and molybdenum oxide. These interferences contribute to overestimate the concentrations.

Methods: All described methods use Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Sample preparations described in the methods are based on:

- Dilution up to ten times using triton, EDTA and ammonia for whole blood.
- Microwave digestion with HNO_3 and H_2O_2 for whole blood and serum.
- Acid dilution with HNO_3 for urine.

Limits of detection:

- 0.05 – 0.2 ng/mL for whole blood (range for the 6 methods finally assessed).
- 0.03 – 0.09 ng/mL for urine (range for the 4 methods finally assessed).
- 0.07 ng/mL for serum (only one method has been reported and considered).

Internal Standards: The internal standards (IS) employed were a mixture of Ga, Gd, Y and Sc.

Also radioactive metals have been described to be employed as internal standard, but those have not been considered in this evaluation (see remark nº 3).

QA/QC: All 11 methods that were finally evaluated were assessed using external quality control and also reference materials.

Remarks: Cadmium is a metal that has been extensively analysed in all kinds of biological samples and there are numerous studies of human biomonitoring that include its measurement. There is therefore a long accumulated experience for its analytical determination. However, most of the modern methods are based on the inductively coupled plasma mass spectrometry (ICP-MS) since it allows reaching the required sensitivity in this type of studies.

However in doing this evaluation we have encountered some difficulties as consequence of the incomplete inventory of this substance. Following there are some remarks:

1. First, we found that very few of the methods included in the inventories submitted met the minimum requirements in order to be properly evaluated. That is, the information required is insufficient in most cases (there is no information about the preparation of the sample, the internal standard used, precision, accuracy, or matrix effect, limits of detection, etc…).
2. No analytical chemistry journal has been consulted (or at least none has been reported in any of the inventories). All inventoried methods have been obtained from epidemiological studies, in which the methods have been referenced and have only been briefly described in the material and methods. The original sources were not consulted in order to obtain the required information.
3. Some of the methods selected use radioactive metals as internal standards. We consider that these methods were not appropriate for their inclusion in the evaluation because they are obsolete (there are many alternatives to avoid such highly contaminant and dangerous compounds).
4. No references describing the methodology for analysis of Cd by isotopic dilution have been included in the inventories provided. This is a well-known and used methodology, which can be considered the **reference method**, and which is based on the measurement of isotope ratios in samples where the isotopic composition has been altered by the addition of a known amount of an isotopically enriched element (^{114}Cd). The quantification is based on the dilution of the sample, addition of the spike and the analysis using ICP-MS. The determination is performed without any calibration curve and the analytical features could be correctly described. Although it was not included in the inventory, given the importance of this methodology, we have considered it important to note that this methodology should be recommended. Regarding this, there are at least three references that should be taken into account (see inventory above).

Summary: Urine is a preferable matrix due to non-invasive sampling. In HBM surveys, dealing mostly with low exposure levels, blood cadmium is an alternative exposure biomarker. The most sensitive technique for the determination of cadmium in both matrices is ICP-MS. However, for the urinary levels of cadmium, correction measures must be taken for the interferences of tin and molybdenum oxide during the analysis, especially at low levels. Therefore, the most suitable technique would be inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS), which reduces drastically the molybdenum-based polyatomic interferences.

Alternative analytical methodologies able to overcome the inherent problems of the interferences should be explored.

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2.2.8 Chromium (Category C)

Matrices: Urine, whole blood, exhaled breath condensate (EBC), red blood cells (RBC or erythrocytes) and other non-useful biological matrices of minor interest in the context of HBM4EU such as water, soil and tooth.

Evaluation: There were a total of 16 entries for Cr in the inventory, but only 8 were taken into account for the assessement. In general, the inventory was found to be incomplete, with relevant information lacking for most methods. Thus, this evaluation was made based only in the 8 methodologies that fulfilled the analytical requirements to be assesed properly.

The selected methods are based on four different techniques:

- ICP-MS (Inductively coupled plasma mass spectrometry).
- GF-AAS (Graphite Furnace Atomic Absorption Spectrometry).
- EAAS (electrothermal atomic absorption).
- AAS (Atomic Absorption Spectrometry) techniques.

Cr is analysed in urine, whole blood, EBC and RBC. Other potentially relevant matrices in human biomonitoring studies were not included in the inventory.

Methods: All described methods use ICP-MS, GF-AAS, EAAS and AAS, and the most frequent sample preparations are: liquid liquid extraction, centrifugation and clean up using strong acid.

Limits of detection and quantification:

- ICP-MS: 0.002-0.04 ng/mL for RBC, EBC and whole blood (range for the 3 methods finally assessed).
- GF-AAS: 1.0-1.5 ng/mL (LOQ) for urine and RBC (range for the 2 methods finally assessed).
- AAS: 0.06 ng/mL for RBC (only one method has been reported and considered).
- EAAS: 0.2 ng/mL for RBC and Whole blood (one method has been taken into account because for whole blood the LOD was not showed).

Internal Standards: There is no information about the internal standard used.

QA/QC: Only 4 methods contained information about the external controls employed as reference materials, internal house controls, etc.

Remarks: Cr (III) is a beneficial micronutrient although can be toxic at living organisms at high concentrations, while Cr(VI) is known to be highly toxic and carcinogenic. The methodology selected to determinate this element shoud control the conversion from Cr (VI) to Cr (III) after the take up. Nowadays, there are a lot of methodologies focus on the determination of the element using ICP-MS. This technique may be used without separation technique, analysing the total concentration of Cr, but also, it could be coupled to liquid chromatography to carry out speciation analysis. The low detection limits that ICP-MS offer in biological matrices makes this technique an alternative to measure Cr with high analytical quality.

However in doing this evaluation we have encountered some difficulties as consequence of the incomplete inventory of this substance. Following there are some remarks:

1. First, we found that very few of the methods included in the inventories submitted met the minimum requirements in order to be properly evaluated. That is, the information required is insufficient in most cases (there is no information about the preparation of the sample, the internal standard used, precision, accuracy, or matrix effect, limits of detection, etc…).
2. No analytical chemistry journal has been consulted (or at least none has been reported in any of the inventories). All inventoried methods have been obtained from epidemiological studies, in which the methods have been referenced and have only been briefly described in the material and methods. The original sources were not consulted in order to obtain the required information.
3. Several of the methods provided in the inventories have been developed for matrices of little utility in human biomonitoring studies (water, soil and tooth) and therefore have not been taken into account in the present evaluation. Only one method has been evaluated for each matrix which is probably too little to performing a proper assessment.

Summary: The preferred technique for chromium determination is ICP-MS. Currently the chosen matrix for the determination of Cr (VI) is the analysis of erythrocytes because only Cr (VI) can enter into them. Chromium speciation in biological fluids is questionable since Cr (VI) is rapidly converted into Cr (III).

An alternative to invasive matrices is the determination of Cr (VI) and Cr (III) in exhaled breath condensate, although so far only atomic absorption spectrometry has been used.

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