Suggested list of biomarkers, matrices and analytical methods for the 2nd prioritisation round of substances

In order to come up with a prioritised list of biomarkers, matrices and analytical methods for the 2nd prioritisation round of substances, partners in task 9.1 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 and experts in WP9 and WP16 were consulted and gave their input to the draft of the deliverable. The resulting list of suggested pairs of exposure biomarker/matrices as well as analytical methods is presented in Table 2. This, together with the suggested matrix, volume and LODs, are to be considered as advice on what a state-of-the art method should aim at, and will not be used as criteria for qualification of laboratories, which is taken care of through the carefully established QA-program in task 9.4. The substances have been categorised according to the scoping documents. 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 detail in the appendix. Information on the inventories will be available upon request. The inventories and evaluations will be revised and updated when new information is available in year four/five of HBM4EU.

In accordance with the annual work plan, the inventories and evaluations were revised and updated spring 2020, taking new information and input from the chemical group leaders into consideration. Table 2 is updated and details of the revision is presented in the chapter of the respective chemical group in the Appendix.

In accordance with the annual work plan, the inventories and evaluations were revised and updated spring 2021, taking the updated scoping documents (Dec 2020) for the individual substance groups into consideration. The progress made with respect to quality and harmonization/comparability of methods as a result of the HBM4EU QA/QC-program in task 9.4 is also elaborated. Table 2 is updated and details of the revision is presented in the chapter of the respective chemical group in the Appendix.

Additional relevant information generated by WP9 in HBM4EU regarding the human biomonitoring capacity in Europe can be found in the HBM4EU library under "Laboratories", e.g. qualified laboratories, tentative costs for chemical analyses, and ICI/EQUAS-reports.

https://www.hbm4eu.eu/online-library/

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

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL
Acrylamide				

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL
Category B				
Acrylamide	N-Acetyl-S-(2-carbamoyl-ethyl)cysteine (AAMA)*	Urine (0.1-4 mL)	LC-MS/MS	0.1-8.7 ng/mL
	N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine (GAMA)*	Urine (0.1-4 mL)	LC-MS/MS	0.3-1.7 ng/mL
	N-(2-Carbamoylethyl)valine (AAVal)	(Haemo)globin (20-100 mg)	GC-MS, LC-MS/MS	0.2-12 pmol/g
	N-(2-Carbamoyl-2-hydroxyethyl)valine (GAVal)	(Haemo)globin (20-100 mg)	GC-MS, LC-MS/MS	0.3-7.0 pmol/g
Aprotic solvents				
Category B				
N-methyl-2-pyrrolidone (NMP)	5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP)	Urine (0.6 mL / 2 µL)	GC-MS/MS, UPLC-MS/MS	0.8 ng/mL / ~0.3 ng/mL
	2-hydroxy-N-methylsuccinimide (2-HMSI)	Urine (0.6 mL)	GC-MS, GC-MS/MS	0.7-15 ng/mL
N,N-Dimethylformamide (DMF)	N-methylformamide (NMF)	Urine (0.5-10 mL)	GC-MS, GC-FPD, GC-NPD	0.2-0.5 µg/mL
	N-Acetyl-S-(N-methylcarbamoyl)cysteine (AMCC)	Urine (0.05-1 mL)	LC-MS/MS	0.67-5.5 ng/mL
Category C				
N,N-Dimethylacetamide (DMAC, DMA)	N-methylacetamide (NMAC, NMA)	Urine (0.1-2 mL)	GC-MS, GC-FPD, GC-NPD, LC-MS/MS	0.05-1.5 µg/mL
	S-(acetamidomethyl) mercapturic acid (AMMA)	Urine (0.1 / 0.5 mL)	LC-MS / LC-MS/MS	0.02 / 1.5 µg/mL
	N-hydroxymethyl-N-methylacetamide (DMAC-OH)	Urine (0.1 mL)	LC-MS/MS	0.02 µg/mL
Category D				
N-ethyl-2-pyrrolidone (NEP)	5-hydroxy-N-ethyl-2-pyrrolidone (5-HNEP)	Urine (0.6 mL)	GC-MS, GC-MS/MS	0.8-15 ng/mL

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL
	2-hydroxy-N-ethylsuccinimide (2-HESI)	Urine (0.6 mL)	GC-MS, GC-MS/MS	0.7-5 ng/mL
Arsenic				
Category A				
Arsenic*	Arsenic (total)	Urine (0.2 mL)	ICP-MS, AAS	< 0.05 ng/mL
Category B				
Arsenic*	Arsenic (inorganic form)	Urine (0.2-1 mL)	HPLC-ICP-MS	< 0.05 ng/mL
	Arsenic (III)	Urine (0.1-0.5 mL)	HPLC-ICP-MS	< 0.05 ng/mL
	Arsenic (V)	Urine (0.1-0.5 mL)	HPLC-ICP-MS	< 0.05 ng/mL
	Arsenobetaine (AsB), Arsenocholine (AsC), Methylarsonic acid (MMA) and dimethylarsinic acid (DMA)	Urine (0.1 mL)	IC-ICP-MS	0.25 ng/mL
Diisocyanates				
Category A				
4,4‘-methylenediphenyl-diisocyanate (MDI)	4,4‘-methylenedianiline (MDA)	Urine 0.25-2 mL	GC-MS, LC-MS/MS	8.0-100 pg/mL
	MDI-Val	Erythrocytes, Globin 0.1 g	GC-HRMS	20 pg/g
2,4-Toluenediisocyanate (24TDI)	2,4-toluenediamine (24TDA)	Urine 0.25-2 mL	GC-MS, LC-MS/MS	2.0-100 pg/mL
2,6-Toluenediisocyanate (26TDI)	2,6-toluenediamine (26TDA	Urine 0.25-2 mL	GC-MS, LC-MS/MS	2-100 pg/mL
1,5-Naphthylene diisocyanate (NDI)	1,5-naphthylenediamine (NDA)	Urine 0.25-1 mL	GC-MS, LC-MS/MS	30-100 pg/mL
1,6-Hexamethylene diisocyanate (HDI)	1,6-hexamethylenediamine (HDA)	Urine 0.25-1 mL	GC-MS, LC-MS/MS	2.0-200 pg/mL
Category B				
MDI	MDI-lysine	Serum-Albumin 9 mg	LC-MS/MS	2.8 pg/mg
24TDI	4TDI2-lysine	Serum-Albumin 9 mg	LC-MS/MS	5.0 pg/mg

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL
	2TDI4-lysine			
26TDI	6TDI2-lysine	Serum-Albumin 9 mg	LC-MS/MS	5.0 pg/mg
Lead				
Category A				
Lead	Lead	Whole blood (0.2-1 mL)	ICP-MS or AAS	0.008 to 1 ng/mL
Mercury				
Category A				
Mercury	Mercury (total)	Urine/whole blood (0.2-1 mL)	ICP-MS or AAS, Direct Mercury Analyser	< 0.05 ng/mL
		Hair (1-50 mg hair shaft sample)	HPLC-ICP-MS, Direct Mercury Analyser	0.01 ng/mg
	Methyl mercury	Whole blood (0.2-1 mL)	HPLC-ICP-MS, Direct Mercury Analyser	< 0.05 ng/mL
		Hair (25-50 mg hair shaft sample)	HPLC-ICP-MS, Direct Mercury Analyser	0.01 ng/mg
Mycotoxins				
Category B				
Aflatoxin B1 (AFB)	AFB-lysine	Serum 0.15-0.25 mL	ELISA, LC-FLD, LC-MS/MS	0.2-9 pg/mg
	AFB	Urine 0.1-2 mL	LC-MS/MS, LC-HRMS	0.8-100 pg/mL
	Aflatoxin M1 (AFM1)	Urine 0.05-15 mL	ELISA, LC-FLD, LC-MS/MS, LC-HRMS	0.3-60 pg/mL
Aflatoxin B1 (AFB)	AFB	Plasma 0.1 mL	LC-MS/MS	40-50 pg/mL
	AFB	Dried blood spot, 0.1 mL	LC-MS/MS	12 pg/mL
	AFB-N7-guanine	Urine 10-20 mL	LC-MS/MS	0.04-3.0 pg/mL
	AFM1	Plasma 0.2 mL	LC-MS/MS	50-180 pg/mL

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL
	AFM1	Human milk 10 mL	ELISA, LC-HRMS	4.3-1250 pg/mL
Category C				
Deoxynivalenol (DON) 3-acetyl-DON 15-acetyl-DON DON-3-glucoside	Deoxynivalenol* (total deoxynivalenol after deconjugation = sum free deoxynivalenol + deoxynivalenol-15-glucuronide + deoxynivalenol-3-glucuronide)*	Urine 0.5-2 mL	LC-MS/MS	≤ 0.3 ng/mL
Fumonisin B1 (FB1)	fumonisin B1 (FB1)	Urine (0.1-2 mL)	LC-MS/MS	0.01-0.05 ng/mL
Pesticides, including pyrethroids				
Category B				
Chlorpyrifos	3,5,6-trichloro-2-pyridinol (TCPy)*	Urine (0,5-5 mL)	LC-MS/MS	0.05-0.9 ng/mL
	Diethyl phosphate (DEP)[2]	Urine (0.25-1 mL)	LC-MS/MS	0.04-3.3 ng/mL
	Diethyl thiophosphate (DETP)[2]	Urine (0.25-1 mL)	LC-MS/MS	0.02-1.1 ng/mL
	Diethyl dithiophosphate (DEDTP)[2]	Urine (0.25-1 mL)	LC-MS/MS	0.01-1.6 ng/mL
Glyphosate	Glyphosate*	Urine (0.05- 0.5 mL)	LC-MS/MS / micro-LC-MS/MS/GC-MS/MS	0.02-0.5 ng/mL
	Aminomethylphosphonic acid (AMPA)*	Urine (0.05– 0.5 mL)	LC-MS/MS / micro-LC-MS/MS/GC-MS-MS	0.02-1.0 ng/mL
Bifenthrin, (lambda)cyhalothrin Tefluthrin	cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid (ClF3CA)	Urine 5 mL	GC-MS/MS	0.01 ng/mL
Allethrin, phenothrin, pyrethrum, resmethrin, tetramethrin	trans-Chrysanthemumdicarboxylic acid (trans-CDCA)	Urine 2 mL	GC-HRMS	0.05 ng/mL

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL
Acrinathrin, permethrin, deltamethrin, etofenprox, cypermethrin, phenothrin, (es)fenvalerate, fluvalinate, cyhalothrin, fenpropathrin, tralomethrin, cyphenothrin, flucythrinate, phenothrin	3-phenoxybenzoic acid (3PBA)	Urine (1-5 mL)	LC-MS/MS, GC-MS, GC-MS/MS, GC-HRMS	0.01-0.1 ng/mL
Flumethrin, cyfluthrin	4-fluoro-3-phenoxybenzoic acid (4F3PBA)	Urine (1-5 mL)	LC-MS/MS, GC-MS, GC-MS/MS, GC-HRMS	0.01-0.2 ng/mL
Cyfluthrin, cypermethrin, permethrin, transfluthrin	cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (cis-DCCA)	Urine (1-5 mL)	LC-MS/MS, GC-MS, GC-MS/MS, GC-HRMS	0.01-0.4 ng/mL
Cyfluthrin, cypermethrin, permethrin, transfluthrin	trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (trans-DCCA)	Urine (1-5 mL)	LC-MS/MS, GC-MS, GC-MS/MS, GC-HRMS	0.01-0.4 ng/mL
Deltamethrin	cis-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DBCA	Urine (1-5 mL)	LC-MS/MS	0.01-0.4 ng/mL
Category C				
Dimethoate	Dimethyl phosphate (DMP)	Urine (0.25-1 mL)	LC-MS/MS	0.1-1.2 ng/mL
	Dimethyl thiophosphate (DMTP)	Urine (0.25-1 mL)	LC-MS/MS	0.02-0.4 ng/mL
	Dimethyl dithiophosphate (DMDTP)	Urine (0.25-1 mL)	LC-MS/MS	0.01-0.6 ng/mL
	Diethyl phosphate (DEP)[3]	Urine (0.25-1 mL)	LC-MS/MS	0.04-3.3 ng/mL
	Diethyl thiophosphate (DETP)[3]	Urine (0.25-1 mL)	LC-MS/MS	0.02-1.1 ng/mL
	Diethyl dithiophosphate (DEDTP)[3]	Urine (0.25-1 mL)	LC-MS/MS	0.01-1.6 ng/mL
Fipronil	Fipronil sulfone	Serum/plasma (0.2-0.25 mL)	LC-MS/MS	0.01-0.02 ng/mL
UV filters- benzophenones				

Substance	Biomarker	Matrix (amount)	Analytical Method	MDL
Benzophenone-3 (BP3)	BP3	Urine (0.1-5 mL) Serum (0.1-1 mL)	LC-MS/MS, GC-MS, GC-MS/MS LC-MS/MS	0.06-0.5 ng/mL 0.1-2ng/mL
Benzophenone (BP)	BP	Urine (0.1 mL) Urine (1 mL)	LC-MS/MS GC-MS	2.4 ng/mL 0.02 ng/mL
Benzophenone-1 (BP1) (also metabolite of Benzophenone-3 (BP3)	BP1	Urine (0.1-5 mL) Serum (0.1-1 mL)	LC-MS/MS, GC-MS, GC-MS/MS LC-MS/MS	0.03-0.5 ng/mL 0.1-0.2 ng/mL
Benzophenone-2 (BP2)	BP2	Urine (0.1-5 mL) Serum (0.1-1 mL)	LC-MS/MS LC-MS/MS	0.02-0.4 ng/mL 0.06-0.2 ng/mL
Benzophenone-6 (BP6)	BP6	Urine (5 mL) Serum (1 mL)	GC-MS/MS LC-MS/MS	0.06 ng/mL 0.3 ng/mL
Benzophenone-7 (BP7)	BP7	Urine (0.1 mL) Serum (0.1 mL)	LC-MS/MS LC-MS/MS	0.4 ng/mL 0.2 ng/mL
Benzophenone-8 (BP8)	BP8	Urine (0.3-5 mL) Serum (1 mL)	LC-MS/MS LC-MS/MS	0.06-0.5 ng/mL 0.1 ng/mL
4-Hydroxybenzophenone (4HBP)	4HBP	Urine (0.1-5 mL) Serum (0.1-1 mL)	LC-MS/MS LC-MS/MS	0.02-0.2 ng/mL 0.2 ng/mL
4-Methylbenzophenone (4MP)	4MP	Urine (0.1 mL) Serum (0.1 mL)	LC-MS/MS LC-MS/MS	0.5 ng/mL 0.3 ng/mL

[1]Sample intake, technique and MDL were extracted from the inventory.
Green row: information for this substance has been updated.
Blue row: new substance.
*Substance was included in the HBM4EU QA/QC program.

[2]DEP and DETP are unspecific biomarkers of chlorpyrifos, and DEDTP is an unspecific biomarker of other organophosphorus pesticides. These are not the suggested biomarkers for chlopyrifos, but are included here since they are usually determined by the same analysis method as the unspecific biomarkers of the pesticide dimethoate (DMP, DMTP and DMDTP) without increasing the analysis cost. Measuring these unspecific biomarkers also enables comparison with earlier studies when the TCPy were not assessed.

[3]Not biomarkers of dimethoate but included here as these three are usually determined in the same analysis method as DMP, DMTP and DMDTP without increasing the analysis cost. See also previous footnote.