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First report on biobank activities

Deliverable Report

D8.2

WP8 - Targeted field work surveys and alignment at EU level

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Table of Contents

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2 Glossary

2-ethyl-5-hydroxyhexyl phthalate	5OH-MEPH
2-ethyl-5-oxohexyl phthalate	5oxo-MEHP
6-OH-Mono-propyl-heptyl phthalate	OH-MiDP
7-Carboxy-(mono-methylheptyl) phthalate	cx-MiNP
7-OH-(Mono-methyl-octyl) phthalate	OH-MiNP
bis(1,3-dichloro-2-propyl) phosphate	BDCIPP
bis(1-chloro-2-propyl) phosphate	BCIPP
Bis(2-chloroethyl) phosphate	BCEP
Bisphenol A, S, F	BPA, S, F
cyclohexane-1,2-dicarboxylate-mono-(7-carboxylate-4-methyl)heptyl ester	cx-MINCH
cyclohexane-1,2-dicarboxylate-mono-(7-hydroxy-4-methyl)octyl ester	OH-MINCH
cyclohexane-1,2-dicarboxylate-mono-(7-oxo-4-methyl)octyl ester	oxo-MINCH
Di(2-propylheptyl) phthalate	DPHP
Di-isononyl phthalate	DINP
Di-isononylcyclohexane 1,2-dicarboxylate	DINCH
Mono(2,7-methyl-7-carboxy-heptyl) phthalate	cx-MiDP
Mono(2-ethyl-5-carboxypentyl) phthalate	5cx-MEPP
monobenzyl phthalate	MBzP
Mono-cyclo-hexyl phthalate	MCHP
monoethyl phthalate	MEP
monoethylhexyl phthalate	MEHP
monoisobutyl phthalate	MiBP
mono-n-butyl phthalate	MnBP
Mono-n-octyl phthalate	MnOP
Mono-n-pentyl phthalate	MnPeP
Polyvinyl chloride	PVC
Tetrabromobisphenol A	TBBPA

3 Abstract/Summary

Task 8.2 investigates data sources and gaps for the purpose of evaluating time trends in chemical exposure across Europe. Exposure data covering three time points, two age groups and four geographical regions is aimed for. This first report focuses on chemicals that can be analysed in urine samples, and specifically on DINCH. Data on DINCH reported in scientific journals was reviewed and possible data sources including biobank urine samples reported in Task 7.1 questionnaire were investigated.

There is clearly a lack of exposure data for DINCHfor the first time point (2006-2010). DINCH was introduced in 2002, and national time trend studies have revealed increasing DINCH exposure levels from 2006 and forward. For the other chemicals on the first priority list that can be analysed in urine, i.e., BPA and certain phthalate metabolites, more information is available (work in progress).

We suggest relying on literature data for the first time point for DINCH. However, it may be possible to access biobank samples to gain more information on DINCH exposure from 2006 and onward, but the availability of samples (and their quality) and budget for new analysis need to be evaluated and decision taken in 2018.

We suggest using DEMOCOPHES samples, collected in 2011-2012, as a start for a European time trend analysis of several chemicals. DEMOCOPHES biobank samples are currently available from nine countries and include 1682 samples from mother-child pairs. These samples should preferably be analysed by laboratories approved through the HBM4EU ICI exercise. First priority is DINCH, but at the same time other substances can be analysed in the same samples, e.g., BPA, S and F, PAH metabolites, certain phthalate metabolites and certain flame retardants. Finally, for the last time point (2014-2019) we rely on the data collected within Task 8.1.

4 Introduction

The aim of Work Package 8 (WP8) is to collect biomonitoring data across Europe in order to determine exposure to the HBM4EU priority list of chemicals either in the general population or via an occupational setting. Three strategies were proposed. Firstly, to align current studies in partner countries and collect new samples (both for population and occupational exposures) in a harmonised way to achieve comparable exposure data, secondly, to access biobank samples for retrospective analysis of chemical exposure, and lastly to collate data which has already been collected and/or published. The Human Biomonitoring data collected will serve different purposes, among those, to determine status and trends of chemical exposure in Europe.

The aim of Task 8.2 is to evaluate time trends in chemical exposure across Europe. Three time-points will be assessed: 2006-2010, 2011-2013 and 2014-2019. The aim is to include one or two age-groups and four European geographical areas, provided data is available. Time-trends will initially be assessed for DINCH, followed by other chemicals on the first priority list. For DINCH and the first time point we suggest to use published exposure data, for the second time point we will use DEMOCOPHES samples from biobanks (new analyses), and for the third time point we will use new data collected in Task 8.1 (alignment of studies). This is the first progress report for Task 8.2.

5 Task 8.2 data collection strategy

5.1 Description of work

The overall aim of the Task is to derive time-trend data, in each of the four geographical regions described below. Three time-points will be assessed and if data is available, we will assess this for different age-groups. Time-trends will initially be assessed for DINCH, followed by BPA (and S and F if possible), PAHs, and certain flame retardants and phthalate metabolites, which can all be analysed in urine.

Task 8.2 builds on information collected by Task 7.1 questionnaires and the resulting database, literature reviews, and new HBM analyses. Based on the collected information and identified data gaps we suggest which data sets and/or biobank samples could be appropriate for the purpose of evaluating the first two time points. If available, we will approach appropriate biobanks that could be used to assess time-trends in exposure to the priority chemicals. KI and partners will request to access the samples and permission to perform new analyses on them according to the strategy developed in Task 7.4 for exchange of samples.

New chemical analyses will preferably be carried out at laboratories that have successfully passed the quality assurance program established by WP9 (end of 2018, beginning of 2019). The laboratories have to present Quality Control data, both internal and external analytical reference data.

For the last time point Task 8.2 builds on the information collected in Task 8.1. In collaboration with Task 8.1, we will identify appropriate studies that can provide data on DINCH (and the other prioritised chemicals that can be measured in urine) that can be used for time trend analysis.

Task 8.2 aims for two age categories to be covered by the time trend analyses: children age 6-11 years and women 20-45 years. This is in alignment with two of the age groups included in the strategy for the alignment of national studies (Task 8.1).

The number of data points needed for each time point in the trend analyses is based on statistical considerations and data availability. Time trends will be analysed in collaboration with WP10 (data management).

5.1.1 Strategy for exchange of samples

The strategy for exchange of samples was partly tested in M20-21 using the exchange of the first ICI Cr samples, organised by WP9, in collaboration with Task 7.4. All the samples arrived in good shape to the ten laboratories. A full test will be performed using the DEMOCOPHES samples as soon as we have the information on which laboratories that have been approved by WP9, and all the documents needed for new analyses are in place. After that the protocols (developed by Task 7.4) will be refined.

5.1.2 Time points for time trend analysis

Data for three time points will be investigated and collected

1. 2006-2010: Based on published data, aggregated or single data, may include new analyses of biobank samples to close data gaps
2. 2011-2013: Available information and new analysis of DEMOCOPHES-samples from biobank material
3. 2014-2019: Based on results obtained in Task 8.1

5.1.3 Age groups

For the first analysis of time trends we aim at two age groups. Other age groups (and gender) may be involved at a later stage of the project.

1. Children 6-11 years
2. Adult women 20-45 years

5.1.4 European geographical regions

Europe was divided into 4 geographical regions based on the report D 8.1: A strategy to collect EU wide HBM data.

1. North: Denmark, Estonia, Finland, Iceland, Ireland, Latvia, Lithuania, Norway, Sweden, UK
2. South: Croatia, Cyprus, Greece, Italy, Malta, Portugal, Slovenia, Spain
3. East: Bulgaria, Czech Republic, Hungary, Poland, Romania, Slovakia
4. West: Austria, Belgium, France, Germany, Luxembourg, The Netherlands, Switzerland

5.1.5 Prioritised substances

In the beginning of the project it was decided to start analysing time trends for DINCH. This work has been delayed because it is based on deliverables from other Tasks that have been delayed. Task 8.2 strategy is therefore to start collect information for those chemicals that were on the first list of prioritised chemicals, and that can be analysed in urine samples (since DEMOCOPHES biobank urine samples will be analysed for the second time point). Literature data is collected for a set of chemicals of the first prioritisation round. This work will be finalised in 2018. In the next step substances/biomarkers that are preferably measured in blood samples (e.g. the perfluorinated compounds) and possible data sources will be investigated.

Di-isononylcyclohexane 1,2-dicarboxylate (DiNCH) was introduced in 2002 to replace DEHP and other phthalates in PVC. A decrease in exposure to old and restricted phthalates and an increased exposure to new phthalates (e.g. DINP, DPHP) and phthalate substitutes (e.g. DINCH) is expected and has already been reported for some countries (Schütze et al 2014; Gyllenhammar et al 2017; Koch et al 2017; Shu et al 2018). The same trend can be expected for bisphenol A substitutes and brominated flame retardant substitutes (i.e. organophosphate flame retardants).

5.1.6 Number of data and time points

A certain number of time points and data is needed for statistical time trend analysis (D10.2). At least three time points of exposure data within each specific area are needed to assess a time trend. A number of approximately 150 subjects per gender and geographical region have been proposed to be sufficient. Depending on the variability in exposure data for a particular chemical, this number may have to be expanded. The assessment of exposure levels should be done in a selected, as far as possible homogeneous age group (D8.1). With a limited number of data points, comparisons of exposure levels before and after regulation of particular chemicals and introduction of substitute chemicals can be carried out to test for statistical differences over time and between geographical regions. For DINCH and the first round of chemicals, we aim to include 2 age groups (children 6-11 yrs, and women 20-45 yrs) and as far as possible 4 geographical regions, which adds up to 600 samples per time point for children (if there is no stratification by sex) and 600 samples for women.

5.2 Data sources

A number of deliverables and reports were used as a basis for this report.

1. Deliverable 7.1 Report on ongoing activities and existing data and data gaps for the 1st prioritised substances including a list of metadata that can be uploaded in IPCheM (M12)
2. HBM4EU 7.1 questionnaire database and literature search
3. Deliverable 7.2 Strategy and SOPs for human sample exchange, including ethical demands (M12)
4. Deliverable 8.1 Description of the national programs. A strategy to collect EU wide HBM data (M14)
5. Deliverable 8.4 Initial report on strategies adopted to align studies across Europe and preliminary results (M20)
6. Deliverable 9.2 Prioritised list of biomarkers, matrices and analytical methods for the 1st prioritisation round of substances (M9)
7. Deliverable 10.2 Statistical Analysis Plan (M12)
8. The HBM4EU Scoping documents on HBM4EU priority substances
9. New literature search Pub Med 2018-09-10

5.3 Results

Information on published chemical exposure data and biobanks that possibly have samples that can be used for new analysis is listed in table 1 and 2 (focusing on DINCH and work in progress for other compounds). The information obtained is categorised by time point, region, age group and chemical substance.

5.3.1 Literature search and Task 7.1 database inventory

We found no published information on DINCH exposure in children for the first time point (2006-2010), and little information for adults. Thus, there is clearly a lack of exposure data for DINCH, and less so for BPA and certain phthalate metabolites measured in urine, for the first time point. DINCH was introduced in 2002, and national time trend studies have revealed increasing DINCH exposure levels from 2006 and forward (Schütze et al 2014; Gyllenhammar et al, 2017; Shu et al, 2018).

We suggest relying on literature data for the first time point for DINCH. Data evaluation should be performed in collaboration with WP10. It may be possible to access biobank samples to gain more information on DINCH exposure from 2006 and onward, but the availability of samples (and their quality) and budget for new analysis need to be evaluated (see below).

Table 1: Literature data for the first time point 2006-2010

Region	Age group	Study (reference)	Age group	Country	Year of data collection	Chemicals analysed
North	Children	Urinary phthalate excretion in 555 healthy Danish boys with and without pubertal gynaecomastia (Mieritz et al 2012)	Boys 6-19 yrs	Denmark	2006-2008	Phthalates, BPA (not DINCH)
		High urinary phthalate concentration associated with	Girls, 6-19 yrs	Denmark	2006-2008	Phthalates (not DINCH)

Region	Age group	Study (reference)	Age group	Country	Year of data collection	Chemicals analysed
		delayed pubarche in girls (Frederiksen et al 2012)				
		Urinary phthalates from 168 girls and boys measured twice a year during a 5-year period: associations with adrenal androgen levels and puberty (Mouritsen et al, 2013)	Girls and boys,	Denmark	2006-2010, every six month	Phthalates (not DINCH)
	Adults	Temporal trends of phthalate exposures during 2007-2010 in Swedish pregnant women (Shu et al 2018)	Pregnant women	Sweden	2007-2010 (trend)	DINCH, Phthalates
		Diverging temporal trends of human exposure to bisphenols and plastizisers, such as phthalates, caused by substitution of legacy EDCs? (Gyllenhammar et al 2017)	First-time mothers	Sweden	2009-2015 (trend)	DINCH, Phthalates, BPA, BPF
West	Children	Determination of bisphenol a in urine from mother-child pairs-results from the Duisburg birth cohort study (Kasper-Sonnenberg et al 2012)	Mother-child pairs (children 6-8 yrs; mothers 29-49 yrs)	Germany	2007-2009	BPA
	Adults	Entering markets and bodies: increasing levels of the novel plasticizer Hexamoll® DINCH® in 24 h urine samples from the German Environmental Specimen Bank (Schütze et al 2014)	students 20-30 yrs	Germany	1999-2012 (trend)	DINCH

5.3.2 Biobank inventory

Biobank samples can be analysed for the purpose of achieving retrospective information on exposure levels. Investigation of possible biobank material for the first two time points (2006-2010, 2011-2012), and for analyses of prioritised chemicals in urine was performed.

For the first time point (2006-2010) we found eight institutes reporting biobank material in the 7.1 database that fit the purpose of gaining exposure data for the selected time frame and two age

groups (6-11 and women 20-45 yrs; table 2). In some of these studies DINCH and other prioritised chemicals have already been analysed and data has been published. Access to these biobank samples and/or data is possible according to the 7.1 database. There may be other biobanks that keep appropriate urine samples, and that are not part of the HBM4EU project or not yet reported to the 7.1 questionnaire.

Table 2: Biobank material (urine samples)

Region	Country, study (institute), year of sampling)	Target group	Chemicals analysed
North	Denmark, Copenhagen puberty study, CPHPUB (Rigshospitalet), 2006-2017	5.6-20 yrs	Phthalates, bisphenols
	Norway, HBM Within the Norwegian Mother and Child Cohort Study, MoBa (NIPH), 2009-ongoing	15-55 yrs	phthalates
South	Slovenia, National HBM Survey, SLO_HBM (JSI), 2007-2015	20-45 yrs	Cd, other metals
	Spain, BIOAMBIENT.ES survey (CNSA-ISCIII), 2009-2010	16-65 yrs, workers	Phthalates, flame retardants, Cd, other metals
West	Germany, German Environmental specimen bank, ESB (UBA), 1985-2015	Adults 20-29 yrs	DINCH, phthalates, BPA, flame retardants
	France, PELAGIE Mother-Child Cohort (INSERM), 2002	Children 0-6 yrs, women 18-44 yrs	Phthalates, flame retardants
	France, French National Nutrition and Health Survey, ENNS (ANSP), 2006-2007	18-74 yrs	Metals, pesticides
East	Czech republic, (Central) European Longitudinal Study on Parents and Children, (C)ELSPAC (MU), 1991-2030	0-11 yrs, mothers from 25 yrs	Phthalates, bisphenols, flame retardants

For the second time point we suggest to analyse DEMOCOPHES samples collected in 2011-2012. Several countries that participated in the DEMOCOPHES study have stored urine samples (table 3). In response to a questionnaire sent in Sep 2017, and further contacts, nine DEMOCOPHES partners have replied positive to perform new analyses in their biobank urine samples, and also to co-finance the analyses with 50%. Data on seven phthalate metabolites and BPA exposure levels in children (6-11 yrs) and their mothers has previously been analysed and published (Den Hond et

al 2015; Covaci et al 2015). The management board has approved analysis of DINCH in these samples. We suggest analysing also other metabolites of interest in the same urine samples as long as analytical quality can be evaluated and it fits the budget allocated. A new contact will be taken with the DEMOCOPHES partners in M22, for update of the information previously reported.

Table 3: DEMOCOPHES biobanks

Region	DEMOCOPHES biobanks	Number of samples
North	Denmark/RegionH, Sweden/SEPA/KI, UK/DH	482 mother-child pair
South	Cyprus/ MOH-CY, Slovenia/JSI, Spain/ISCIII	600 mother-child pair
East		
West	Belgium/VITO, Germany/UBA, Luxembourg/LNS	600 mother-child pair

Possible groups of chemicals of the first prioritisation round that can be analysed in the DEMOCOPHES urine samples, and that are covered by WP9 QA/QC programme, are listed below (table 4). Ethical approvals of the studies as well as the permission to transfer the single measurement data for statistical analysis at EU level should be made available to the project coordinator before study start. Concerning the second prioritisation round, chemicals that can be analysed in urine include PAH, antibacterial agents, pesticides, UV-filters, and nicotine/cotinine.

Table 4: Examples of chemicals on the first chemical priority list that can be analysed in urine samples

Chemical group	Metabolites	Reported in DEMOCOPHES study
DINCH	OH-MINCH, cx-MINCH, (*oxo-MINCH)*	
Phthalates	MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP, MEP, MBzP, MiBP, MnBP, MCHP, MnPeP, MnOP, OH-MiNP, cx-MiNP, OH-MiDP, cx-MiDP	MEHP, 5OH-MEPH, 5oxo-MEHP, MEP, MBzP, MnBP, MiBP
Bisphenols	BPA, BPF, BPS	BPA
Organophosphate flame retardants	DPHP, BDCIPP, BCEP, BCIPP, *TBBPA*	

5.3.3 Alignment study data

Data for the third time point (2014–2019) in the time series can be obtained from Task.8.1 Alignment study. It includes all four geographical regions and the two age groups children 6-11 yrs and women 20-39 yrs, in total 33 studies from 21 different European countries. Chemicals that will be analysed in urine samples in those age groups are for children: phthalates, DINCH and flame retardants in urine. In adults; bisphenols, PAH metabolites and Cd will be analysed. As for Cd, data is already available from the DEMOCPHES database.

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