Prioritised substance group: Emerging substances

Lead author	Greet Schoeters (VITO, BE)
Contributors	J-P Antignac, G. Koppen, J. Bessems, L. Debrauwer, A. Covaci

Short overview of results of the activities carried out within HBM4EU in 2020 to answer the policy questions with reference to corresponding deliverables.

Policy Question	Short Summary of Results
Early warning of presence of hazardous chemicals in EU population?	Early warning methods for chemicals of emerging concern in HBM4EU implies new measurements in human samples of chemicals that have been recently introduced in the environment, but for which few Human Biomonitoring data exist (WP9, WP16), or detection of early warning signals of toxicity of chemicals present in the human population (WP14, WP16). HBM4EU develops and applies new methods for targeted analysis of these emerging compounds (WP9, AD9.1) to obtain robust quantitative information on the corresponding human internal exposure levels. HBM4EU complements this information with strategies and implementation of suspect screening (qualitative determination of an extended number of a priori known markers as a support to further prioritisation) and non- targeted screening (detection of unknown compounds as a support to new marker discovery) (WP16).In addition early warning of the presence of hazardous chemicals in the population is addressed through the use of effect biomarkers (WP14) that may signal biological imprints of chemical exposures. Specific “emerging” substances are being measured for the first time in biobanked urine or blood samples of the general European population that have been collected after 2014. The strategy is described in D8.4 (WP8). HBM4EU focuses on substitutes and alternatives for hazardous substances. As such capacity has been inventarised and expanded in Europe for targeted analysis of new phthalates (MCHP, DnPeP, DiDP, DiNP) with between 5 and 19 qualified laboratories depending on the metabolite. For the DINCH metabolites 7 and 8 laboratories were qualified depending on the metabolite. For BPF and BPS, respectively 13 and 18 qualified laboratories were identified and for organophosphate flame retardants (OPFRs) 4 to 5 laboratories were qualified depending of the metabolite. WP9 has selected the most suitable biomarkers for the first set of prioritised substances (D9.2) and for the second set of priority chemicals (D9.5). The phthalates and substitutes are currently being analysed in samples from 2950 children (NO,DK, HU,SK,SL,PL,EL,IT,NL,FR,DE) and 2900 teenagers (NO,SE,PO,CZ,SK,SL,EL,ES,FR,BE,DE). Organophosphate flame retardants (OPFRs) are being analysed in 2950 samples of children, the bisphenols are being analysed in 3165 adult samples. Some of the newer perfluorinated compounds are being analysed in samples from teenagers (NO,SE,PO,CZ,SK,SL,EL,ES,FR,BE,DE).

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	Furthermore, new methods have been developed for detection of urinary metabolites of some reprotoxic classified and EU-regulated phthalates such as di(methoxyethyl) phthalate (DMEP), di-iso/npentyl phthalate (Di/nPeP), di-iso/n-hexyl phthalate (Di/nHexP) and di-C7-11 branched and linear alkyl ester phthalate (DHNUP). Smallscale ICIs are currently performed in order to expand the phthalate methodology for inclusion of these biomarkers for HBM investigations in the general population. Also, for analysis of CrVI in exhaled breath condensate (EBC) SOPs have been developed and a small sale ICI has been organised to allow implementation and generation of HBM data in the occupational studies. To identify new emerging substances, 5 European laboratories have joined forces and are applying their suspect screening capabilities to analyse 160 human urine/blood samples from various cohorts. They used a predefined list of suspect markers for which the expected detected signal characteristics have been inventoried within a MS reference laboratory. Based on this list, they identified dozens of markers from various substance groups including those for pesticides, plasticisers, UV-filters, and PFAS. Several were detected with high frequencies which demonstrates their widespread presence in the populations of various EU countries (D16.4). Another proof-of-concept illustrating the WP16 outputs is concerning the particular suspect screening of pesticides and their metabolites in human urine samples. The aim is to annotate a maximal number of exposure markers present in urine samples that are being collected in mother-child pairs of 5 different countries and in 2 different seasons (D15.6). A user-friendly software module- haloseeker – has been developed as an open access resource to identify markers of exposure to halogenated substances in human samples. It allows to handle large and complex datasets which are generated by LC-HRMS (https://www.ncbi.nlm.nih.gov/pubmed/30758179). Special emphasis is on human samples collected in the early life period, considered as the most vulnerable period of life and an important time window that should be protected from exposure to hazardous compounds. A first success story was then obtained on breast milk samples where 4-hydroxy-chlorothalonil was identified without any a priori (D16.3). The same approach is applied to meconium samples. 25 Spanish placenta samples have been also analysed using untargeted LC-HRMS profiling, metal, steroid hormones and PFAS profiling and an array of bioassays has been applied on the samples (AD14.4). The results of the suspect and non-targeted chemical analysis will be combined with the outcome of the bioassays to link the exposure profiles with biological activity.
Inform REACH process to identify substances of potential concern?	The substances that fall under the 1st and 2nd priority substance groups of HBM4EU have been categorised according to existing knowledge of internal exposure (WP4). Category C substances are substances for which HBM data are scarce or doesn't exists. Category D substances are substances for which a toxicological concern exists but HBM data are not available. Suspect screening is recommended to explore the presence of these chemicals in human samples and further prioritise the necessary investment in term of developing quantitative methods and the inclusion of certain markers in HBM programs. Category E substances are substances not yet identified as of toxicological concern and for which no HBM data are available. Non-targeted screening approaches are needed to identify yet unknown substances. The results of this categorisation for individual compounds of the prioritised substance groups in HBM4EU can be consulted in the scoping documents on the substance specific web pages at the HBM4EU web site. It is the ambition of HBM4EU to move substances gradually towards category A as more information in being collected. To make progress for the identification of less well-known substances or yet unknown substances, a strategic EU database for QA/QC consolidated, harmonised and sustainable annotation capability for markers of exposure has been produced under HBM4EU. Lists of known emerging chemicals have been combined from 51 existing international databases of emerging chemicals to orientate the selection of compounds to be further characterised (D16.1). The list contains more than 70 000 MS-ready structures with unique structural and stereochemistry properties and their exact masses. Further 306,279 unique transformation structures are added to the list after simulation of phase 1 metabolism.

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	The curated data serves as a reference for HRMS screening. The database can be used to putatively identify measured chemical features in untargeted HRMS, facilitating the large-scale detection of chemicals of emerging concern in human samples in Exposome research. To further identify chemicals of potential concern, QSAR predictions related to physicochemical properties, environmental fate, toxicity and Absorption, Distribution, Metabolism, Excretion (ADME) processes could be performed for 64,684 structures(AD16.5 and publication in press). This database complements existing metabolomics databases focused on markers of effect / endogenous compounds.
Development of strategy for a non-toxic environment -> first step?	To identify new emerging substances in human samples, WP16 develops a data driven approach, a chemistry driven approach and a biology driven approach. Strategies for suspect and non- targeted screening are developed to identify yet unknown compounds of toxic concern in human biological matrices such as urine, blood, milk, meconium or placenta. A data driven approach: capacity on acquisition of high-resolution mass spectrometric data within the consortium is inventarised and brought together. The workflow for harmonisation and QAQC consolidation of the necessary reference MS data is laid down in AD16.4 “Annotation framework”. Reference mass spectrometric data are generated in a harmonised way so that they can be compared among laboratories and annotated to profiles that are generated when screening the samples. An inventory of screening techniques (AD16.1) and a first workflow for screening emerging chemicals (D16.2) has been published on the HBM4EU web site. A chemically driven approach: D16.2 highights crucial methodological questions of non-targeted analysis workflows including sample preparation, data acquisition, data mining and expert reviewing and proposes guidelines to implement NTA in Human Biomonitoring research. A set of QA/QC actions dedicated to sample collection, sample preparation and acquisition method specifically applied to the identification of chemicals of emerging concern in human matrices by non-target approaches is being developed and will be published. As a proof of concept non-targeted screening of halogenated emerging chemicals (incl. their metabolites) using gas/liquid chromatography coupled to high resolution mass spectrometry (GC/LC-HRMS) is developed and applied to various human matrices. A biology driven approach: combines suspect and non-targeted methodologies with effect directed analyses (EDA) (WP14). An overview of bioassays for analysing human samples and EDA approaches has been published (AD16.3) and a scientific publication is finalised. As proof of concept 25 placenta samples have been analysed with an array of bioassays including epigenetic markers (D14.4 and AD14.4). The results will be combined with the outcome of untargeted LC-HRMS profiling of the samples to link the exposure profiles with biological activity. In addition, effect markers are being selected and will be implemented in some of the HBM studies of WP8 as early warning signals for toxicity from exposure to multiple chemicals as occurs in real life. WP14 has defined effect markers as quantifiable changes in biochemical, physiologic or other parameters in the organism that occur as a result of exposure to chemicals. Criteria for selection of effect biomarkers (D14.1) and effect markers for the 1st set of priority chemicals (D14.2) have been identified. A distinction is made between novel effect markers, traditional effect markers with the novel markers relating more to early biological imprints of exposures, while the traditional markers are often clinical well validated markers that are reliable predictors of health risks but less specific for chemical exposures (D14.3).