Substance report

June 2022

Emerging Chemicals

HBM4EU science and policy for a healthy future

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Table of contents

Authors and Acknowledgements

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The EEA has since updated this document to reflect the work developed before the conclusion of HBM4EU, with the support of the CGL and other colleagues.

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Glossary

Abbreviations	
APCI	atmospheric pressure chemical ionisation
APPI	atmospheric pressure photo ionisation
CEC	Chemicals of Emerging Concern
C&L	Classification and Labelling
CLP	The ‘Classification, Labelling, Packaging’ Regulation Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures
CoRAP	Community Rolling Action Plan under REACH
EC	European Commission
ECHA	European Chemicals Agency
EDC	Endocrine disrupting chemicals
EFSA	European Food Safety Authority
EI	electron ionisation
ESI	electrospray ionisation
EU	European Union
GC-MS	Gas chromatography - mass spectrometry
GC-HRMS	Gas chromatography - high resolution mass spectrometry
HBM	Human Biomonitoring
HBM4EU	European Human Biomonitoring Initiative
HRMS	High resolution mass spectrometry
IARC	International Agency for Research on Cancer
InChi	International Chemical Identifier
LC-MS	Liquid chromatography - mass spectrometry

LC-HRMS	Liquid chromatography - high resolution mass spectrometry
MS/MS	Tandem mass spectrometry
NTS	Non-targeted screening
OEL	Occupational Exposure Limits
POPs	Persistent Organic Pollutants
REACH	The 'Registration, Evaluation, Authorisation and Restriction of Chemicals' Regulation Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
SMILES	Simplified molecular-input line-entry specification
SRM	Selected reaction monitoring
SVHC	Substance of very high concern
WP	Work package
WPL	Work package leader

1 Key messages

- An ambitious open access EU database of known substances to be addressed as CECs was elaborated, including more than 70,000 parent-compounds and more than 300,000 metabolites. This is a new resource for the scaled-up deployment and implementation of suspect screening analyses and associated support to policy capacity.
- A competent and harmonised EU network for suspect and non-targeted screening approaches dedicated to emerging chemicals in human fluids and tissues was established. This allows for more data generated in the environment-food-human nexus communities, in support of harmonized approaches and comparable data for improved support to policy.
- Several proofs of concept were conducted to illustrate the effective application of these new screening approaches and their potential for supporting policy through a contribution to early warning, mixture assessment, and prioritization. This led to the first candidates of exposure markers and real-life mixtures to be deeply considered in risk assessment.

2 Introduction

HBM4EU is a project funded under Horizon 2020 and runs from 2017 until June 2022. It generates knowledge to inform about the safe management of chemicals, and hence protect human health in Europe. HBM4EU uses human biomonitoring (HBM) to monitor the actual human exposure to chemicals and resulting health impacts to build upon existing evidence bases and improve chemical risk assessment. HBM4EU compares data from across Europe which allows an understanding of regional differences and can help to identify vulnerable groups to inform targeted measures to reduce exposure. The results of the HBM4EU project are aimed at supporting policy

development, by providing a key evidence base in the understanding of exposure and impacts to toxic chemicals.

If you would like to read more about the project itself, please visit the HBM4EU website.

2.1 How to use this document

This document provides a summary of the known and suspected adverse human health effects of emerging chemicals and describes the main exposure pathways for humans. It also indicates where HBM could be of value in the development of EU policy, along with the remaining challenges in determining human emerging chemicals exposure. This substance report is intended to inform scientists, relevant stakeholders and policy makers on the value of HBM to establish the EU population's exposure to emerging chemicals.

This substance report is based largely on the Scoping Document for Emerging Chemicals (2020), and HBM4EU Work Package 16 related deliverables.

The aim of this document is to summarise the work done by HBM4EU on emerging chemicals, the results so far, remaining challenges and where HBM data could be of value in the development of future EU policy.

Despite increasing societal, scientific and policy concerns, there is no consensus about the definition of nor the terminology of this "substance class". Following the definition proposed by Sauvé et al. (2014) that has been adopted in the context of HBM4EU, emerging chemicals - or preferably Chemicals of Emerging Concern (CECs) – have to be understood as either 1) novel chemicals (recently developed substitutes for substances currently subject to regulation or that have been banned) or 2) chemicals which have been present for a period of time in the environment-food-human continuum but for which a "new concern" has been identified (for instance, due to progress of analytical performances, newly identified sources, uses, and/or routes of exposure, particularly exposed sub-population, new toxicological evidence, etc).

Potential routes of exposure may be via direct usage of consumer products or uptake via food or the environment. At a regulatory and policy level, the main challenge associated to CECs is to develop early warning capability to rapidly handle these chemicals through biomonitoring program and further risk assessment process. At a scientific level, the main challenge associated to CECs is to develop new methodological strategies to rapidly document the reality of exposure and the related health impact for these chemicals, then to detect and prioritise these chemicals on the basis of relevant and well-integrated exposure and toxicological data.

2.2 Overview of emerging chemicals

3 Human exposure to emerging chemicals

Human exposure to Chemicals of Emerging Concern (CECs) cannot be easily documented or listed, as CECs do not refer to a specific substance group or have a grouping rationale based on a particular toxicological characteristic or common physico-chemical properties. Conversely, CEC refers to a very broad range of chemicals from various substances classes, each of them being associated with particular exposure sources, patterns, and levels.

At a scientific level, the main challenge associated to the detection of CECs and relevant chemical mixtures is to develop new methodological strategies to rapidly document the reality of exposure

and the related health impact for these chemicals, then to detect and prioritise these chemicals based on relevant and well-integrated exposure and toxicological data.

The same approaches are also helpful to elucidate the composition of complex mixtures by simultaneously generating exposure data for a wide range of markers from each individual sample, be it at environmental, occupational and/or consumer exposure level.

4 Health impacts of emerging chemicals

4.1 Overview of key health impacts from emerging chemicals

Chemicals of emerging concern are currently a growing issue for the scientific community, societal actors and public authorities. CECs do not refer to a specific substance group or have grouping rationale based for instance on a particular toxicological characteristic or a common physico-chemical property. Moreover, one part of those CECs refers to markers of exposure not yet fully characterised or are possibly not known. Therefore, documenting their impact on human health is not achievable in a same manner as other well-defined substances or substance groups. CECs also encompass a certain number of already known chemicals that belong to various substance classes, for example pesticides, UV-filters, drugs and novel flame retardants.

A significant proportion of these chemicals are known or suspected to be endocrine disrupting chemicals, with potential health impacts for instance on hormonal, reproductive, or metabolic functions. Some of these CECs may also be associated to persistent organic pollutants (POPs) properties that refer to other toxicological impacts, or potential carcinogenics. As such, CECs are not well addressed in existing HBM programmes, in part due to a lack of effective analytical methods to detect simultaneously a large number of such markers of exposure of various nature. The subsequent lack of exposure data results in uncertainty regarding the actual burden of those substances in the human population.

Increasing numbers of chemicals are being detected due to improvements of analytical techniques; However being detected does not necessarily mean that these compounds pose a risk. It depends on their concentration and their toxicity which is often not yet known.

4.2 Societal concerns

Concerning the use of Human Biomonitoring of toxic chemicals, 87 % of the respondents of the HBM4EU citizens' survey supported the use of HBM and said it should be used more, with 50 % saying it should be undertaken as regularly as food and water quality tests, with a stronger coordination at the European level, and near 60 % considered it should be included in the National Health Surveys.

Over 65 % of the respondents strongly supported the importance of HBM studies for the purposes of: evaluating chemical exposure of the population, study the health impacts of chemical exposure, the development of health policy that promote the safe use of chemicals, to support occupational health policies and the safe use of chemicals at work, to raise awareness/understanding the impact of chemical exposure amongst the population and to raise awareness/understanding of the impact of chemical exposure amongst health professionals and policy makers.

Overwhelmingly, citizens chose food, the environment and drinking water as priority areas of chemical exposure to be addressed by human biomonitoring studies (see Figure 1)

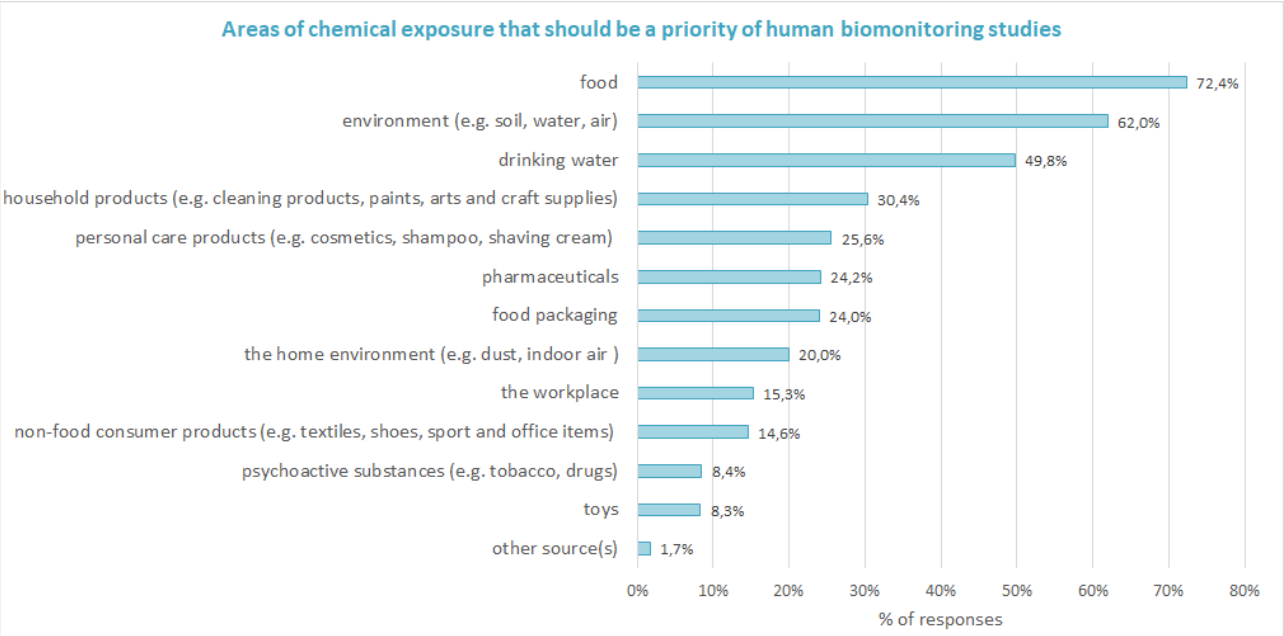

Figure 1 Priority areas in human biomonitoring

It is also noteworthy that, conversely, 13 % of the survey respondents supported the idea that HBM should not be done at all. A round of focus groups discussions in several countries that were also part of the survey provided additional insights (Matisāne et al., 2022; Uhl et al., 2021). Notably, the level of awareness about human biomonitoring was relatively low across countries and was not related with educational attainment. Moreover, beyond human biomonitoring, the awareness of the potential ill health effects from chemical exposures was also low, underscoring the importance of awareness raising and public education activities and policies.

5 EU policies on emerging chemicals

Substance evaluation under REACH (Regulation (EC) No 1907/2006) aims to determine whether the use(s) of a substance poses a risk to human health or the environment. The objective of this stage of the REACH process is to seek further information from registrants to inform as to a potential concern of the substance(s). Where substance evaluation deems the risks are sufficiently controlled, no further action needs to be taken. If the risks are not deemed to be sufficiently controlled then there may be a proposal for EU-wide risk management measures such as restriction, identification as a SVHC or, under CLP, establishment of a harmonised classification. Such substances are listed in the CoRAP.

Under Article 8 of Directive 2008/105/EC, the requirements for the establishment of a watch list of substances for which Union-wide monitoring data are gathered for the purpose of supporting prioritisation exercises in accordance with Article 16(2) of the Water Framework Directive. The substances selected for the watch list are those for which data indicate that they may pose a significant risk at the EU level to/or via the aquatic environment but for which the existing monitoring data is insufficient to confirm the nature of the actual risk. This may include substances of toxicological concern which are discharged in the environment-food-human continuum but are rarely or not measured. Through monitoring these substances, high-quality data should be generated which can then be used to support the risk assessments which underpin the identification of priority substances. This watch list is intended to be updated every two years. The

first watch list was produced in 2015 and contained 10 substances. The second watch list was outlined in Commission Implementing Decision 2018/840/EU.

The Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) released a position paper in 2018 on "Emerging Issues and the Role of the SCHEER". One of the responsibilities of the SCHEER is to draw attention to, and advise the European Commission on, emerging issues. Two complementary approaches were identified that enable the early identification of emerging issues:

- A proactive approach based on Committee brainstorming sessions (held to identify emerging issues of principal concern, including discussion of procedures to detect and characterise development); and
- A reactive approach based on their prior identification of change and monitoring to detect emerging issues.

The SCHEER highlight that identifying emerging issues early can raise awareness, thereby allowing the authorities to take appropriate and timely action to ensure public safety and/or environmental protection. However, they recognised that because such issues are necessarily "emerging" in nature, only limited data will be available to identify the issues and measure the impact. As such, the SCHEER aim to review any relevant new developments at each of plenary meeting and update the position paper accordingly. The SCHEER also wish to work closely with other EU scientific advisory committees which are also mandated to identify emerging issues. The working definition of an emerging issues is "one that has very recently been identified and for which the available database to conduct a risk assessment is very limited". The working definition for an emerging risk refers to "an effect resulting from a newly identified hazard to which an exposure may occur or from new or increased exposure and/or susceptibility to a known hazard" (SCHEER, 2018).

The Ministry of I&M/RWS (The Netherlands) and the OVAM (Flanders) have commissioned an inventory on the awareness and policy on emerging contaminants in Europe. This inventory aims to compile available knowledge and experience related to legislation, governance and policy. This project is focused on man-made emerging contaminants which are already present in environmental compartments (soil, sediment and groundwater). In order to gain an overview of available knowledge, a website was created and a questionnaire distributed to more than 500 experts in the fields of soil, groundwater and sediment in Europe. Only 12 questionnaires were completed but additional information was gathered at international meetings. It was found that scientific research was summarised and interpreted by a number of networks, such as the Norman Network and it was suggested that knowledge from these networks should be used to prioritise data collection and environmental modelling. It was determined that the lack of understanding of the effects and the lack of data from soil, sediment and groundwater samples were the main obstacles to developing a practical approach to emerging contaminants. It was concluded that the most effective approach in Europe would be Union-wide collaboration since this would allow for all stakeholders to maintain a similar level of knowledge. This project also concluded that determining concentrations in environmental compartments should be a priority to ascertain whether the theory that risks to human health are more acute nearer to contaminated hotspots (RWS Leefomgeving and OVAM, 2016).

At present CECs are thus mostly monitored in environmental matrices and compartments, especially water for which several structured initiatives, networks and organization exist (Norman network, 2019). Consideration of CECs in the chemical food safety area is also gaining in maturity. Conversely, CECs remain at this stage less investigated in the field of human biomonitoring, except for particular applications focussed on specific classes of compounds. This discrepancy

can be explained by (i) the lower chemical concentration levels and/or lower abundance of biological material available for analysis typically observed in human samples compared to environmental and food matrices (resulting in lower possible enrichment factors for the expected markers of exposure), (ii) that in many cases the relevant markers of exposure in humans are not the parent CECs, but rather their metabolites which may not have been identified yet and (iii) the level of collaboration and networking among expert laboratories in biomonitoring has not yet reached the same maturity as in other fields (e.g. water analysis). One current challenge is to make these different fields more interacting especially with regard to the development and implementation of relevant and necessary harmonization dispositions.

6 Policy questions for emerging chemicals

6.1 Introduction

For each of the HBM priority substances stakeholders were asked to identify policy related questions that HBM4EU should address in order to contribute to the strengthening of policy ambitions on emerging chemicals. Further background detail on emerging chemicals and how the policy questions were selected is available in the scoping document and the report on stakeholder consultation and mapping of needs.

The current situation for emerging chemicals' policy questions is summarised in the next section and they were based on a document (AD5.7 Reporting CGLs 2022) updated by the CGLs and work package leader (WPL).

6.2 Early warning of presence of hazardous chemicals in EU population?

From the initial state-of-the-art at the early stage of the HBM4EU initiative, the work achieved within WP16 first permitted to build the basis of an EU network with harmonised competences in the field of suspect and non-targeted screening (SS/NTS) applied to human matrices, and secondly to develop and conduct several proof-of-concept studies illustrating the usefulness of these approaches. Overall, these efforts resulted in more than 3000 analysed samples and several hundreds of detected exposure markers associated to emerging chemicals. These generated data should be now a matter of deeper analysis for finally extracting useful information acting as a contribution to early warning system, based for instance on more frequently detected exposure markers not yet covered by HBM programs. This work also permitted to identify a number of limitations associated to these approaches, in particular the bottleneck associated to the identification of the exposure markers detected by the large-scale approaches, still impairing their real high throughput implementation.

6.3 Inform REACH process to identify substances of potential concern?

Within HBM4EU WP 16, we have successfully built the framework and capacities for generating comprehensive suspect lists and linked tandem mass spectral databases. Chemicals included in 51 publicly available databases related to CECs were collected and aggregated into a single and QA/QC consolidated database (CECScreen). In order to make the database applicable for suspect screening in HRMS data from human samples (so-called MS ready), chemical structures were standardised and required chemical properties were calculated. The database includes mainly parent compounds, however, most of these compounds are metabolised by the human body and,

as such, the parent compound might not be detected in human samples. Therefore, and as a first step, a number of phase I metabolites were simulated and included into a separate list which can also be used in suspect screening approaches. The number of compounds in the aggregated database is extensive (70,397) even more so in the simulated metabolites list (306,279). As a result, suspect screening efforts will yield a number of false positive annotations. In order to facilitate prioritisation for confirmation efforts and reduce the amount of chemicals to be considered further, metadata related to physicochemical properties, environmental fate and toxicity was included for the compounds in the CECs inventory. This new EU database finally act as a useful source of information for guiding prioritization of substances of potential concern.

6.4 Development of strategy for a non-toxic environment -> first step?

To identify new emerging substances in human samples, WP16 developed 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 collected, reviewed, acquired, and published so that they can be used and annotated to profiles that are generated when screening the HBM samples.

Reference spectra of 2600 compounds are now ready-to-use for compound annotation of non-targeted chemical profiles generated from human matrices for the identification of exposure markers. They were identified based on reviewing and benchmarking already existing spectral collection (MassBank and MassBank of North America).

New reference spectra for more than 1700 compounds were made available for new tandem mass spectral data acquisition which was achieved according to HBM4EU elaborated harmonisation guidelines. Furthermore, a workflow was developed for obtaining reference spectra of biotransformation products of CECs. This workflow included incubation of CECs with human liver S9 and subsequent acquisition of tandem mass spectra of putative biotransformation products for inclusion in a reference library. The usefulness of the developed workflow was exemplified for 22 pesticides.

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 highlights 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).

7 HBM4EU results

7.1 Categorisation

Substances under HBM4EU have been categorised depending on availability of HBM data. The categorisation indicates the information gaps allowing the development of targeted activities to fill the knowledge gaps. Substances will pass from Category E over D, C, B towards Category A as more information becomes available. Fully characterised substances should end up as category A substances.

Table 1.1 HBM4EU categorisation for emerging chemicals

Category		Priority substance(s)	Details
D	substances for which a toxicological concern exists but HBM data are not available. HBM4EU research may be focused on the development of suspect screening approaches permitting to generate a first level of data enabling to document the reality of human exposure and better justify further investment in a full quantitative and validated method development.	CECs	*a priori* already identified compounds but not yet measured in humans to be measured by suspect target screening
E	substances not yet identified as of toxicological concern and for which no HBM data are available. A bottom-up strategy will be applied, consisting to non-targeted screening approaches coupled to identification of unknowns capabilities for revealing, and further identifying, new (i.e. not yet known) markers of exposure related to chemicals of concern for HBM (parent compound or metabolite).	CECs	substances measured by non-target screening and (1) described in chemical databases or (2) not yet described (unknowns)

7.2 Key outputs

The main outputs from the HBM4EU to date include the following:

Work Package 16 (WP16) of HBM4EU is conducting a number of actions aiming to address this problem of CECs in human matrices, in particular through the development and implementation of large-scale suspect and non-targeted screening methods useful for HBM, environmental health studies and support to risk assessment purposes. The progress made so far is outlined below.

7.2.1 Creating an inventory of emerging chemicals relevant for HBM4EU and for which to date no biomonitoring data exists (Task 16.1)

The number of substances possibly considered as CECs is extremely high, encompassing compounds of diverse nature (substance group and use), properties (chemistry) and origin (sources of exposure). Various existing initiatives and/or sources of information are available that have proposed some lists of CECs in various contexts, for example regulation, environment, food and toxicology. These sources appear in some extend overlapping, and at the same time complementary. In that context, one goal of the HBM4EU WP16 was to provide an aggregated and consolidated picture about the existing lists or databases related to CECs at international level.

Data sources for elaborating this aggregated list include the databases incorporated in an exercise conducted by UBA, databases containing CECs suggested by HBM4EU partners and databases included in the US Environmental Protection Agency's Chemistry Dashboard. After combining these 51 databases, the assembled database contained 145,284 entries, 107,655 unique CAS numbers and 109,237 unique Simplified molecular-input line-entry specification (SMILES). After the exclusion of inorganic compounds and entries of which no SMILES could be generated, the aggregation work resulted in a list of 70,583 chemicals in terms of both structural and stereochemistry and 66,015 unique compounds in terms of structural chemistry only (Figure 1). Information retained in this list includes several compound identifiers, structural information and database origin. The compound identifiers included are ID number, CAS number and InChiKey. The structural information contains (canonical) SMILES, molecular formula and monoisotopic mass.

From this elaborated inventory, a new investment was made to (1) perform an additional level of curation and QA/QC consolidation (e.g. validate the unique identifier and exact mass associated to each compound on the list), and (2) to consider not only parent compounds as it is mainly the case in the current state but also the main known/expected/modelled biotransformation products susceptible to be present in human samples. This metabolite modelling is operated using high throughput approaches including the FAst MEtabolizer (FAME) and the OECD QSAR toolbox (Figure).

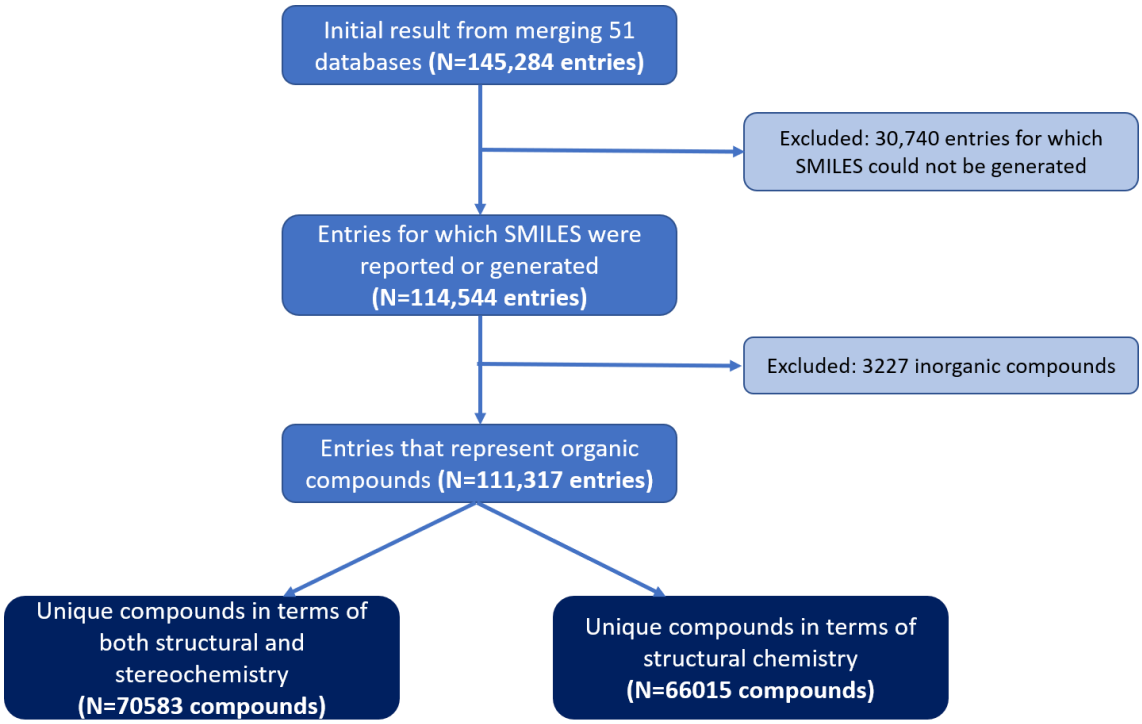

Figure 2 Flow diagram of the data aggregation and curation steps operated to elaborate a list of CECs for HRMS screening purpose

Beside the descriptive aspect of this work, this inventory is also of interest for guiding further developments foreseen within the HBM4EU WP16. Indeed, the data processing step associated to suspect and, in some extent, non-targeted screening approaches consists of using a reference library to compare and match each detected signal in the analysed sample to a list of already known and referenced compounds. Nowadays, the creation of such reference libraries is commonly driven by each laboratory's field of activity without much exchange, so that no global and integrated library is currently available, and a need of mutualisation and harmonisation clearly appears to reach more efficient use of these libraries. One goal of WP16 is to aggregate these existing capabilities for finally proposing an extended and qualitatively consolidated MS reference library to be used for suspect and non-targeted screenings of CECs. In that context, the present inventory of existing lists or databases related to CECs at international level represents a useful tool to orientate the selection of compounds to be characterised as reference standard and indexed in a global MS reference library (Figure 3).

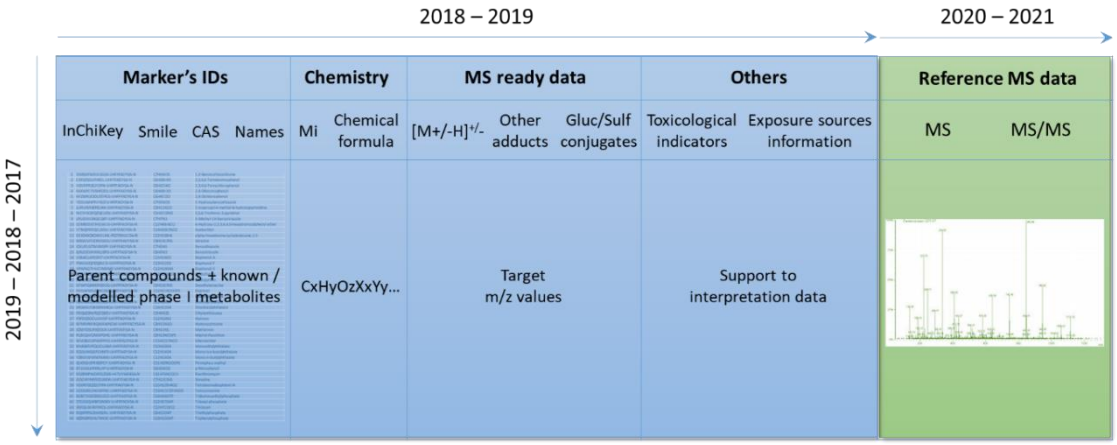

Figure 3 Structure of the annotation MS reference library (HBM4EU_EmergScreenDB) specifically developed within the HBM4EU initiative for markers of human internal exposure

The elaborated inventory of CECs and all associated files (i.e. CECscreen, CECscreen_Metabolites_DB, CECscreen_CompTox and CECscreen_OPERA_Predictions) are freely available on Zenodo17 via DOI: https://doi.org/10.5281/zenodo.3956586 and is hosted by the NORMAN Suspect List Exchange18.

Furthermore, the database is also included in PubChem via the NORMAN-SLE Classification Browser (https://pubchem.ncbi.nlm.nih.gov/classification/#hid=101) and in the CompTox Chemicals Dashboard12 (https://comptox.epa.gov/dashboard/chemical_lists/CECSCREEN).

Finally, the database is also incorporated into MetFrag19 (https://msbi.ipb-halle.de/MetFrag/).

Another approach to increase identification confidence and in due consequence reduce the number of false positive results involves the exploitation of tandem mass spectral data. A harmonized methodological guideline has been elaborated for generating the necessary reference spectrometric data to be used for annotating non-targeted chemical profiles generated from human matrices in the scope of identifying new exposure markers (AD16.4). We have then identified 2,624 compounds that are included in open repositories (MassBank and MoNA) with tandem mass spectral data of sufficient quality. Additionally, in a multi-partner effort, we have acquired reference data of another 1,258 compounds, which increased the coverage of CECscreen with tandem mass spectral data to 5.5 %.

7.2.2 Formatting of the inventory

The inventory created in Deliverable 16.1 also required formatting in a way which is more suitable and easily adaptable for integration into different suspect screening tools (AD 16.5). The aggregated data needed to be QA/QC consolidated to ensure that the data included were correct. This included the calculation of monoisotopic mass and the masses of several adduct species which are expected as MS descriptors for different markers. The list derived was (primarily) based on chemical compounds present in the external environment although many will undergo biotransformation reactions once present in the human body. As such, only the metabolites of the listed compounds may be detected in human samples and many of these biotransformation products are not covered by existing databases. These metabolites need to be identified (where known) or modelled (where unknown) and included in the list. Modelling of metabolites is to be carried out using the QSAR-ready SMILES and the BioTransformer software to predict properties that are related to exposure and toxicity.

7.2.3 Development and harmonization of suspect and non-targeted screening methods for CECs in HBM context (Task 16.2)

Depending on the level of pre-existing knowledge associated to the considered markers of exposure, three related methodological approaches can be used to stratify the human chemical exposome, namely i) targeted methods for known compounds, ii) suspect screening for known unknowns and iii) non-targeted screening for unknown unknowns (Figure 4).

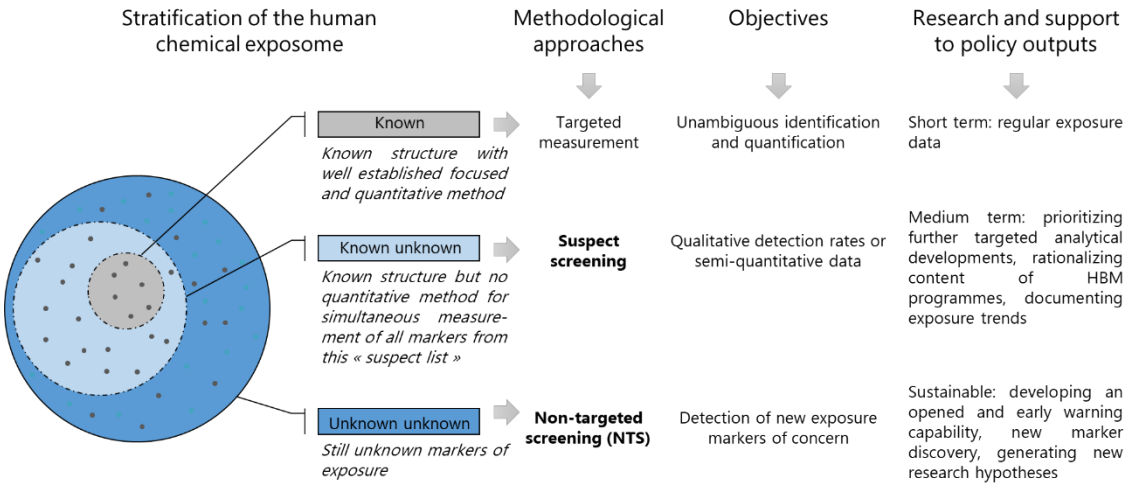

Figure 4 Conceptual view of the human chemical exposome, related methodological approaches, and associated objectives as considered within the HBM4EU project

Deliverable 16.2 requires the method development and harmonisation for such suspect screening of known emerging compounds and non-targeted screening of yet unknown compounds. So far, five EU laboratories have existing individual capabilities to test urine and blood samples. Suspect screening has been carried out on approximately 160 urine samples from various EU sources and cohorts. Each laboratory applied their own analytical workflow to capitalise on existing analytical capabilities based on LC-HRMS. The aim was to determine the presence of a predefined list of suspect markers for which the expected detected signal characteristics have been inventoried within a MS reference laboratory. A harmonized reporting template for suspect screening results has been first elaborated. Dozens of markers from various substance groups were then detected including those for pesticides, plasticisers and PFAS. Several were detected with high frequencies which demonstrates their widespread presence in the populations of various EU countries.

A non-targeted screening workflow has been also developed for halogenated markers of exposure using LC-HRMS. The first step was to develop user-friendly software which could handle large and complex datasets which are generated by LC-HRMS. The result of this is the **HaloSeeker** software which has been released as an open access resource available upon request at (contact.haloseeker@oniris-nantes.fr), under the GPLv3 license. The executable file is about 220. The second step was to develop sample preparation strategies which could isolate potential markers of exposure from human matrices. The focus was on biological compartments which are favourable to the bioaccumulation of halogenated compounds and POPs (e.g. adipose tissue, breast milk, meconium). Using this workflow, it was possible to identify 4-hydroxy-chlorothalonil in several breast milk samples. This method is now on-going for being applied at a larger scale in 2020-2021. The intention is to develop and apply a complementary GC-HRMS workflow to extend the exposome coverage.

7.3 Key data gaps and challenges

HBM4EU has helped to identify several specific data gaps that are needed to give policy makers relevant and strategic data to establish appropriate regulations and improve chemical risk management. However, some gaps and needs for action will remain after the end of HBM4EU which should be addressed in the future:

- The elaborated CECScreen database coupled to MS reference library are strategical pieces of SS/NTS workflow delivered as open access, that now require sustainable follow-up and resources for reinforced application range (Env-Food-HBM) and EU visibility
- Tandem mass spectral library search could significantly reduce time and effort spent for data processing and within HBM4EU, comparability of libraries produced in different laboratories and with different instrumental platforms was demonstrated. There is however a gap between the number of potential substances of interest for human biomonitoring applications (several hundreds of thousands) and available tandem mass spectral data (several tens of thousands).
- Within HBM4EU, comprehensive data collections, like CECScreen and MassBank-HBM4EU, were created for application in non-targeted LC-MS. The next step would represent the development of a computational framework for linking and exploiting the stored information. This data processing component of the SS/NTS approaches still represents a major bottleneck for reaching a higher throughput capacity.
- Adoption and implementation of the proposed harmonized QA/QC criteria for the identification of CECs in human matrices through SS/NTS can significantly improve the comparability of reported results. The methodological harmonization and QA/QC consolidation work conducted within HBM4EU represents a significant first stone but to be extended.
- The different proof-of-concept conducted led to significant amount of new data from which first results were extracted, but much more results should be expected from those now generated data, for which more resources are needed.
- The proof-of-concept studies demonstrated the vast potential of SS/NTS for application in human biomonitoring studies, but focused on certain types of biological material (e.g. blood, plasma, urine) only.

Based on the policy questions, ongoing work to address the knowledge gaps is summarised in the table below. If you would like to read more about the work packages (WP), please visit the HBM4EU website.

WP16 generated the following outputs:

- **1st collaborative application of existing suspect screening capabilities in EU**
    - Analysis of urine/blood samples by 5 different labs/countries using their own existing HRMS based suspect screening capabilities. The samples originated from EU cohorts.
    - Generation of new data (including detection frequencies and interindividual variability) as a support to further prioritization.
    - Harmonized reporting of the generated suspect screening results.
- **MS reference library for expanded suspect screening capabilities**
    - Strategical EU database for QA/QC consolidated, harmonized and sustainable annotation capability for markers of exposure. Innovative exposomic piece of

workflow complementary to existing metabolomics databases focused on markers of effect / endogenous compounds.

- **Proof-of-concept for large scale suspect screening of pesticides related markers**
    - Challenging multi-centric, harmonized HRMS based screening of > 1000 markers in 2000 human urine samples by 5 different labs/countries.
- **Proof-of-concept for non-targeted screening of halogenated related markers**
    - NTS of halogenated markers of exposure through a dedicated advanced software and appropriate sample preparation to data processing strategies.
- **Global framework for harmonized development/application of NTS in HBM**
    - EU network consolidation and guidelines for NTS adapted to the specific HBM field, linked but complementary to existing resources in the environmental or food areas.

7.3.1 Evidence base

So far, the evidence base available includes different international databases detailing emerging chemicals; these include the REACH Registration database; NORMAN Network; and the US EPA Chemistry Dashboard. As noted in Section 1.6.3, laboratories in four countries (France, Germany, Belgium and Austria) have undertaken suspect screening of blood and urine samples which originated from different cohorts. These include both the general population and those subject to occupational exposure.

When screening for substances the different possibly used approaches require different evidence bases. For suspect screening, a MS reference library can be used to identify the detected markers with various confidence level depending on the extent of available structural information. Some gradual scales have been proposed to rationalize and document this pivotal aspect that must be reinforced in terms of harmonization. A complementary biology-driven approach (effect directed analysis) can be employed which considers biological activity and toxicity of the chemicals, to reinforce this evidence base issue.

7.3.2 Challenges

One of the key challenges when determining exposure to CECs is that current targeted methods do not reflect the simultaneous exposure to the wide variety of existing chemicals (i.e. combined exposure to multiple chemicals). There is also concern that a targeted approach does not allow the capture of as yet unidentified markers of exposure. Therefore, suspect and non-targeted screening approaches are being employed. Use of these approaches are in their infancy and so they have not yet been applied on a large scale. In order for these new screening methods to fit with the high level of expectations that they arouse, significant medium to long-term analytical and network development work is needed (Figure 5). Indeed, the emerging NTS area is characterised by both a contextual scientific background of high complexity and an underlying necessary methodological framework of high technicity. The analysis of human samples also requires specific methodologies and processes compared to other fields of application, such as environment or food. On one hand, rigorous harmonisation measures are required to achieve better consolidation and comparability of data generated from various studies, especially regarding their further use in a regulatory and support to policy context. On the other hand, this evolving field requires considerable flexibility to maintain its capacity in discovery and exploratory research.

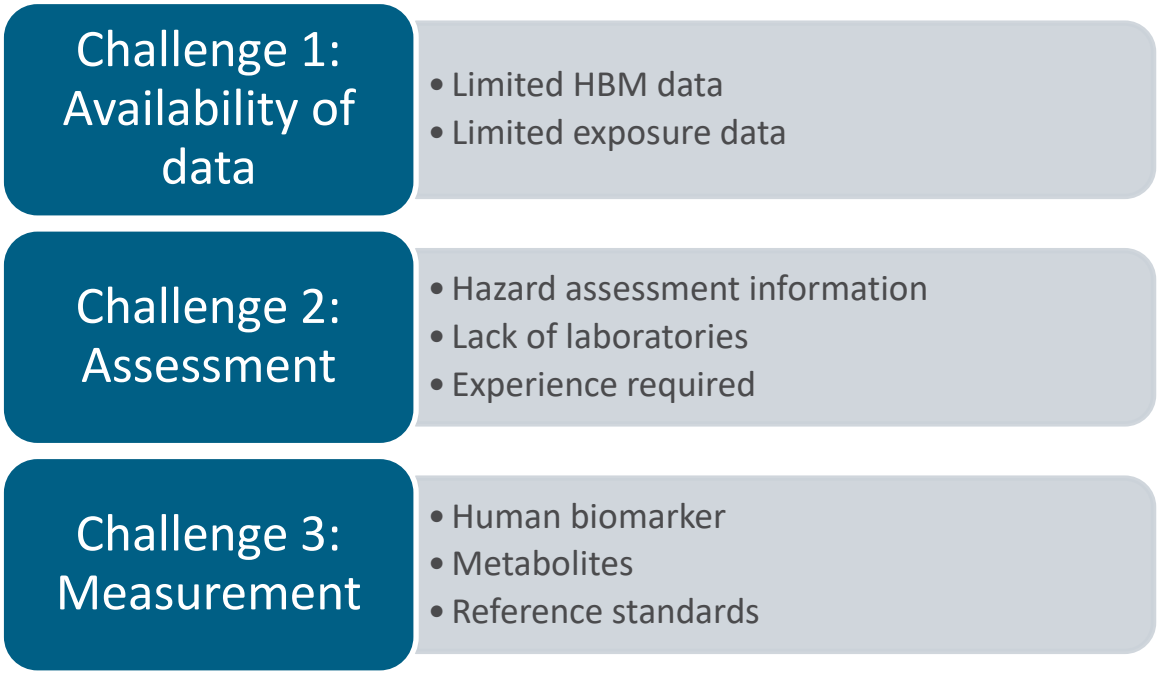

Figure 5 Challenges

7.3.3 Opportunities for policy uptake

Through the screening of available information drawn from REACH Registration dossiers and external data, ECHA and Member States identify substances of potential concern and potential substances of very high concern (SVHC). Currently, there are over 20 thousand substances registered under REACH. While some information on use and exposure is available in REACH registration dossiers, it is often limited and real exposure potential difficult to predict. The data gathered under WP16 on emerging chemicals could be utilised when prioritising substances for further data generation (under e.g. Substance Evaluation) and regulatory actions such as inclusion in the Candidate List under REACH.

8 Future recommendations

- Conduct a strategic reflection on the current and future (differentiating) positioning of the elaborated CECScreen database in view of other existing initiatives and tools worldwide, and include of this component in PARC to ensure medium term sustainability.
- Build a strong and dynamic consortium within scientific communities (e.g. within PARC or others) that will significantly increase the number of available reference spectra by sharing the associated workload. Develop of a computational framework for exploiting the information stored in tandem mass spectral libraries.
- Develop a more integrated and common computational tool for annotation serving suspect and non-targeted screening implementation, e.g. inspired from the Workflow for Metabolomics W4M/Galaxy user friendly environment. Include this component in PARC to conduct the necessary further development.
- Foster further collaborative trials at the European and/or international level to proof the added value of harmonized QA/QC measures and introduce minimum requirements for performance characteristics. Include this component in PARC to conduct the necessary further development, in particular with strong integration of environment-food safety-HBM backgrounds and views.

- Consolidation of high throughput effect-directed analysis (EDA) work and expansion of the application to include other endpoints/compound classes and different matrix types, within PARC, but also through other research funding.
- Development of a prioritisation strategy for managing CEC
- Develop strategies for linking the exposure data of CEC with hazard information using in silico information and read across strategies
- Develop strategies to link CEC detected in human matrices to potential exposure pathways and sources

9 References

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