Substance report

June 2022

Chemical Mixtures

HBM4EU science and policy for a healthy future

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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	
ADME	abbreviation used in toxicology and pharmacology for "Absorption, Distribution, Metabolism and Excretion" which describes the distribution and fate of a chemical within an organism.
Adverse Outcome Pathways	A structured representation of biological events leading to adverse effects that are considered relevant for regulatory risk assessment.
Biomarker of exposure	The primary chemical or its metabolites that are used to estimate the extent of exposure of an organism.
Carcinogens	A chemical that induces or increases the risk of cancer
C&L	Classification and Labelling
CLP	The ‘Classification, Labelling, Packaging’ Regulation
Developmental effects	Effects in the developing offspring from chemical exposure of a parent before conception or during the period of embryonic or fetal development; such effects may include skeletal, soft tissue or functional changes in the offspring and may be observable prenatally, postnatally or at puberty.
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
Endocrine disruptor	A chemical that interferes with the normal functioning of the endocrine system which may result in adverse effects on physiological or neurological development, or on the functioning of the reproductive, immune and other body systems.
EU	European Union
GC	Gas Chromatography
Genotoxicantes (of which Mutagens are a subset)	Term used to define a chemical or other agent that causes permanent change in the amount or structure of the genetic material in a cell; such changes may affect the exposed organism or, if the cell exposed is a germ cell, any future generation(s) of offspring.
HBM	Human Biomonitoring
HBM4EU	European Human Biomonitoring Initiative

HRMS	High Resolution Mass Spectrometry
IARC	International Agency for Research on Cancer
LC-HRMS	LC-HRMS: Liquid Chromatography and High-Resolution Mass Spectrometry
Mode of Action combination of chemicals.	Change at the biochemical or cellular level that result from the exposure to a chemical or combination of chemicals.
MRA	Mixture Risk Assessment
Mutagens	Induce gene mutations but not changes in chromosome number
Neurotoxins	Term used to define a chemical or other agent that damage, destroy or impair the functioning of the cells of the nervous system.
OEL	Occupational Exposure Limits
PAHs	Polycyclic Aromatic Hydrocarbons.
PCBs	Polychlorinated biphenyls.
REACH	The 'Registration, Evaluation, Authorisation and Restriction of Chemicals' Regulation Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
Reproductive toxicant	Reproductive toxicant: Chemicals or other agents (e.g. radiation) that adversely impact on the sexual function, fecundity and/or fertility of a parent or on the development or viability of an offspring.
WP	Work package
WPL	Work package leader

1 Key messages

- Clusters of co-occurring substances can be identified through network analysis of existing human biomonitoring (HBM) databases. Several of the clusters identified spanned regulatory frameworks, stressing the need to strengthen mixture risk assessment across different sectors.
- Prioritization of clusters of co-occurring substances based on the level of toxicological concern is feasible in principle, but the current limited availability of HBM health-based guidance values hampers wider application.
- The SPECIMEn study revealed a total of 95 pesticide-related markers in urine samples from parent-child pairs in five European countries using suspect screening techniques. A subset of the markers was identified with a high level of confidence; these relate to 30

parent pesticides. Their detection frequency varied substantially between countries. However, consistent strong contributions from agricultural application to detection rates in hotspots or in spraying season were not observed.

- HBM4EU case studies focused on human health effects clearly underline that chemical mixtures are of public health concern, within and across sectors. The case studies demonstrate that assessment of health risks associated with combined exposures is generally possible by using the Hazard Index and/or the Point of Departure Index method. Furthermore, they show the usefulness of the identification of risk drivers that contribute most to the mixture risk.

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 in order 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 exposures and co-occurrence patterns of chemical mixtures and possible human health risks. It also indicates where HBM could be of value in the development of EU policy, along with the remaining challenges in determining human chemicals mixture exposures. 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 mixtures.

Humans are constantly exposed to multiple chemicals via multiple sources and routes. The focus of this document is specifically on the co-occurrence of chemicals as they circulate in the human body and can be assessed through HBM. Thus, they are a combination of sequential exposures of long-lived chemicals, as well as simultaneous combined exposures from single or multiple sources and pathways. Examples include exposure to mixtures through air pollution, chemicals in food, ingredients of consumer products and building materials cosmetics, etcetera.

This report is to illustrate the following:

- the potential use of human biomonitoring (HBM) in EU policy development;
- the actual mixture exposure levels in the European populations and co-occurrence patterns of chemicals
- the potential adverse health effects from exposure to unintentional chemical mixtures
- remaining challenges associated with use of HBM for mixtures; and
- the main findings to date from HBM4EU relating to mixtures.

This substance report is based largely on the scoping document for chemical mixtures produced in 2017 by the EEA, the position paper produced in the same year by Horizon 2020 funded studies

on chemical mixtures (including HBM4EU), and the deliverables produced to date for chemical mixtures.[1]

This substance report is intended to inform policy makers and other interested stakeholders on the potential value of HBM to establish the EU population's exposures to mixtures and to promote the uptake of HBM exposure data in the development and application of EU policy for mixtures.

2.2 Overview of mixtures

3 Human exposure to mixtures

Unintentional chemical mixtures present in the environment are of societal concern as the chemicals contained therein, either singly or in combination, may possess hazardous (toxic) properties for human health.

There is no broadly accepted operational definition of mixtures. In principle, every single substance, once it enters the body, will exhibit its health effects in interaction with a person's genetic makeup and acquired characteristics, and in concert with all other (xenobiotic) substances from previous and simultaneous exposures. These combined and/or simultaneous may come involuntarily or voluntary through different exposures routes from ambient environments, indoor and occupational environments, food, food additives, consumer products, medication, (medical or voluntary) implants, recreational drugs, performance enhancing drugs and food supplements, tattoo ink, etcetera (Figure 1). These mixtures thus form a challenge to (experimental and observational) science, to scientific assessment of risks and to regulation of substances and general risk management policies.

One particular concern is the potential impact of exposure to mixtures of pesticides arising from dietary and occupational exposure, proximity to areas of pesticide applications, domestic use or via the wider environment. However, in the absence of real-life mixture exposure data, documentation of actual health risks in the population to date is rather limited and the current regulatory practice is still largely based on considering single chemical substances.

Therefore, a group of scientists from several EU research projects dedicated to study mixture health risks (Bopp et al., 2018) published a Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment (Drakvik et al., 2020).

A European strategy needs to be set for the governance of combined exposure to multiple chemicals and mixtures. Without such a clear strategy, specific objectives and common priorities, research, and policies to address mixtures will likely remain scattered and insufficient.

The HBM4EU project addresses how HBM data can contribute to both the science and policy/regulation of dealing with the phenomenon of mixtures.

[1] The primary deliverables for mixtures are the Deliverables of work package 15 which specifically concerns chemical mixtures including the Survey on PestiCIde mixtures in Europe (SPECIMEn) and Deliverables 6.3 and 7.1

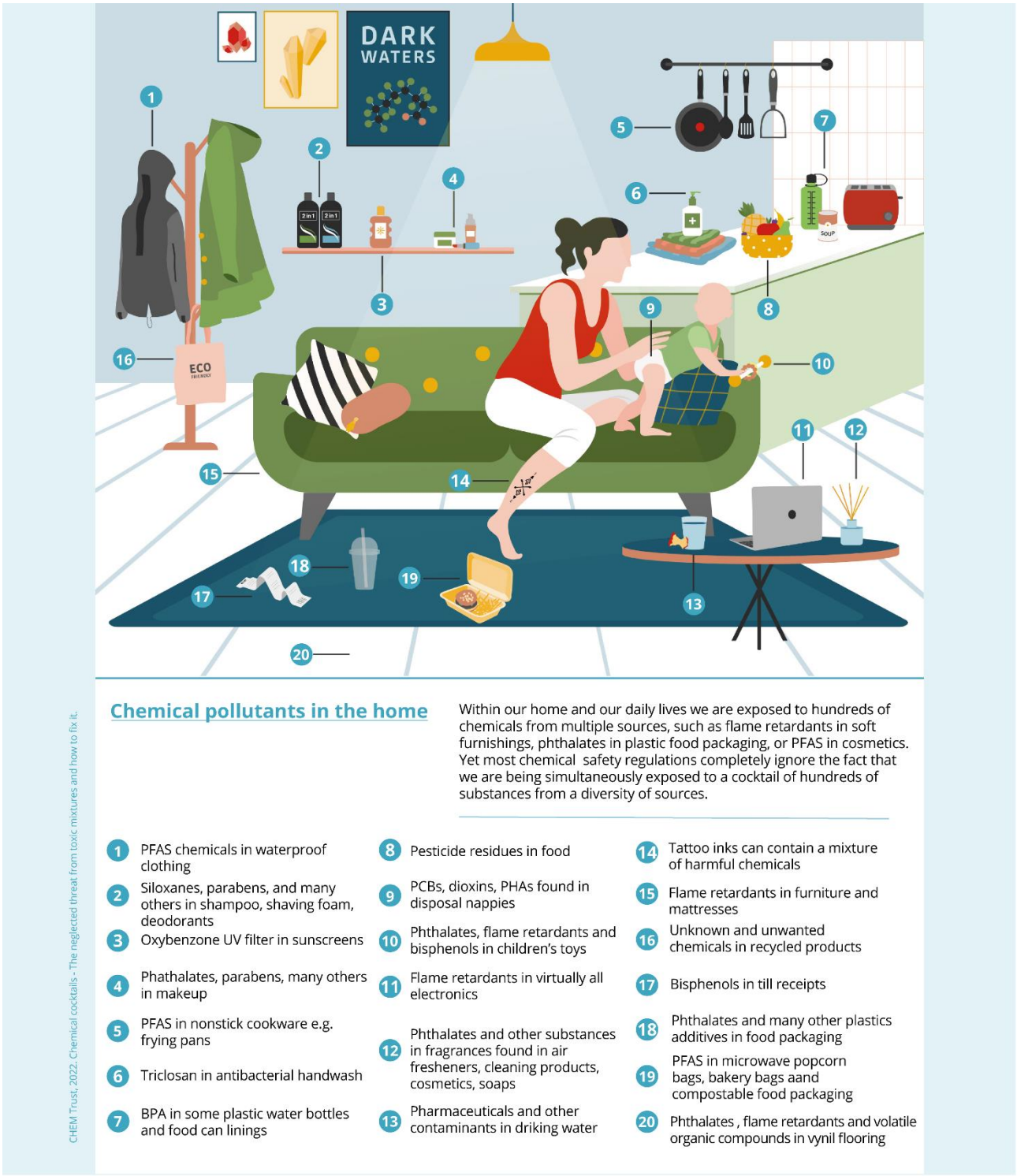

Figure 1 Overview of exposure routes for mixtures

3.1 Exposures

All exposures and exposure pathways described in HBM4EU Substance reports for priority chemicals also contribute to mixture exposure. Some chemicals can remain in the human body for a very long time, e.g. several years. This applies for instance for so-called 'legacy chemicals' such as lead which may deposit in bones and remain there for years to decades, or cadmium. Also,

PFAS have half-lives in humans of several years. Phthalates and modern pesticides are typically more short-lived and disappear from the body in a matter of hours or days. Thus, constituents from mixtures exposures may reflect past as well as very recent exposures, originating from different and common sources and pathways.

4 Health impacts of mixtures

4.1 Overview of key health impacts from mixtures

Chemical mixtures, which are present in the environment are of societal concern as the chemicals contained therein either singly or in combination may possess hazardous (toxic) properties (such as carcinogenic, mutagenic or reproductive effects) for human health. Previous HBM studies have mostly reported on the levels of individual chemicals, sometimes combined into chemical families, like PAHs or PCBs. Such studies do document the combined presence of many man-made chemicals in people (Knudsen et al, 2012 as cited in Position Paper, 2017), although few have documented or reported on co-occurrence of chemicals.

Generally, mixtures can be grouped in different ways, such as by chemical family (e.g. PAHs and phthalates), the exposure route (e.g. via food), the applications for which the chemicals are used (e.g. pesticides in agriculture, or cosmetics) or by their toxic mechanisms (e.g., mutagenicity).

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 2)

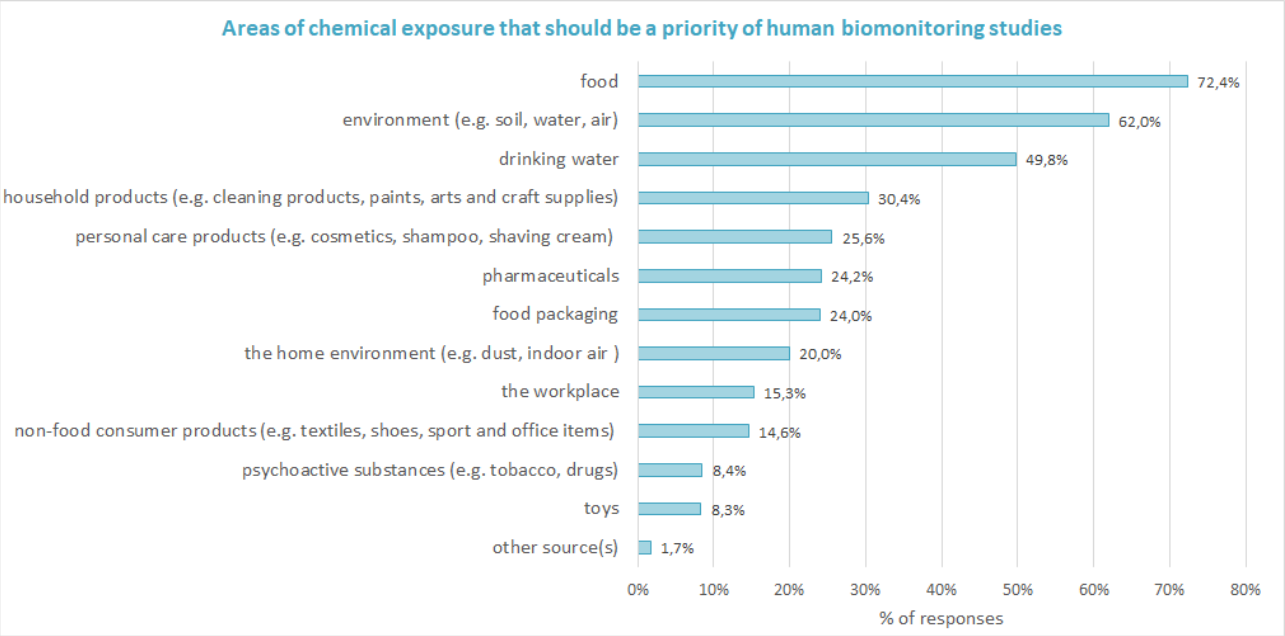

Figure 2 Priority areas in human biomonitoring

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 in several countries that were also part of the survey provided additional insights. 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.

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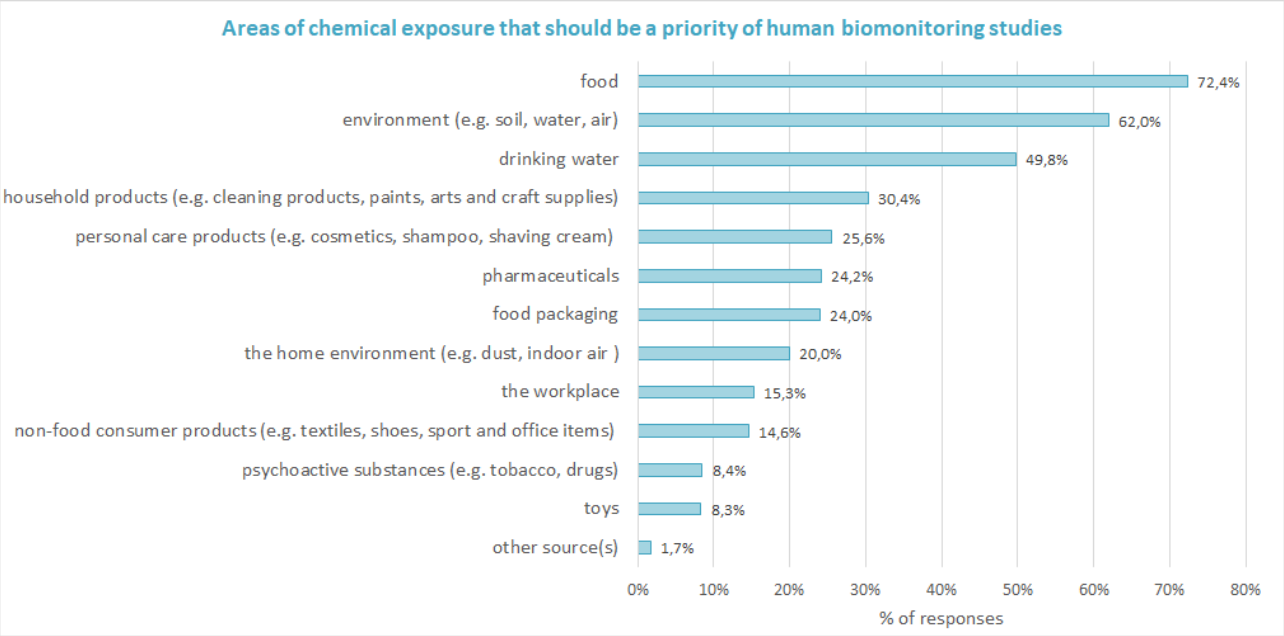

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5 EU policies on mixtures

Mixtures present substantial regulatory challenges due to the various exposure routes from both voluntary exposure and involuntary exposure. Generic recommendations for future research and policy development regarding mixture risk assessment have been drawn up earlier (Bopp et al., 2018; Kienzler et al., 2016; Kortenkamp & Faust, 2018; Rotter et al., 2018). Present regulation for chemical mixtures is limited in Europe (Rotter et al., 2018). However, EFSA recently published a guidance providing methodologies for applying scientific criteria and prioritisation methods to group chemicals for human risk assessment of combined exposures to multiple chemicals (EFSA et al., 2021).

In terms of human health legislation, CLP Regulation (EC) 1272/2008 has defined classification criteria for mixtures for human health. The pesticide regulation, under the plant protection products and data requirements (Regulation (EC) 1107/2009, Regulation (EU) 283/2013, Regulation (EU) 284/2013), considers mixtures for the constituents of the product but not for mixture assessment from different sources. Regarding biocidal products, Regulation (EU) 528/2012, mixtures are considered for the individual components of the product and if the biocidal product is intended to be authorized for use with other biocidal products. Maximum Residue Limits (MRL), Regulation (EC) 396/2005, cover pesticide residues from pesticide uses and other sources. Regarding dietary exposure regulations, the food law, Regulation (EC) 178/2002, considers cumulative toxic effects for food. Also the EU's Chemicals Strategy for Sustainability expresses the ambition to account for the cocktail effect of chemicals when assessing risks from chemicals, with the overall aim to work towards a zero pollution environment. Among others, the Commission aims to introduce or reinforce provisions to take account of the combination effects in relevant legislations, such as

legislation on water, food additives, toys, food contact materials, detergents and cosmetics. For REACH, it will be assessed how to best introduce a mixture assessment factor for the chemical safety assessment of substances.

5.1 H2020/FP7 projects on mixtures

There are five European Commission funded projects under H2020 and FP7 investigating chemical mixtures. These are: EDC-MixRisk which is focussing on the effects of endocrine disrupting chemicals; EuroMix which is investigating the delivery of a mixture test strategy and also test instruments using novel techniques for proving information for risk assessments concerning mixtures; EU-ToxRisk which is aiming to deliver testing strategies for ensuring there is reliable, animal free hazard and risk assessment of chemicals; SOLUTIONS which is looking at tools, methods and models for supporting decisions in environmental and water policies; and HBM4EU. The five research projects are collaborating on addressing the different aspects associated with mixtures and their effect on both human health and the environment.

5.1.1 Position paper

A number of key findings are discussed in the 2017 position paper (co-authored by all five research projects) for reducing risks to humans and the environment from hazardous chemical mixtures.

These include:

- The need for further research on the policy for using human biomonitoring data for exposure assessment;
- Improved source apportionment is required for the effective risk management of actual mixture exposures;
- Chemical monitoring is needed to enable an effect-based and holistic approach for mixture prioritisation for the prioritisation of mixtures using chemical and toxicity fingerprinting techniques; and
- There is the need for emission-based exposure and risk modelling to be performed alongside mixture monitoring approaches.

This has led to the publication of a Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment (Drakvik et al., 2020).

6 Policy questions for mixtures

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 mixtures. Further background detail on mixtures 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 mixtures' policy questions is summarised in the next section and they were based on a document updated by the CGL / work package leader (WPL) .

6.2 What is the information need of regulatory bodies and stakeholders?

In the absence of real-life mixture exposure data, documentation of actual health risks in the population to date is rather limited. The current regulatory practice is still largely based on considering single chemical substances. One of the main conclusions of a series of structured interviews with experts and policymakers was that information needs are still rather diffuse and unarticulated, in line with the 'systemic risk' nature of mixtures (Lebret et al. 2020). Thus, consequences for functionality of HBM mixture data cannot directly be derived. Moreover, views on responsibilities and criteria to guide risk reduction strategies varied considerably. Potential problems in cooperation between different policy domains (like DGs) were seen as mainly stemming from differences in regulations and the absence of a common regulatory framework. The statement published in 2018 and 2020 (Bopp et al., 2018, Drakvik et al., 2020) includes a call for action to ensure better management to protect public health and the environment from hazardous chemical mixtures. Such action should include initiatives that investigate the opportunities for including prospective mixture risk assessment in all relevant regulatory frameworks. Precautionary approaches and intermediate measures could already be applied for regulatory purposes, even though significant knowledge and data gaps remain. Furthermore, a European strategy needs to be defined for the governance of combined exposure to multiple chemicals and mixtures. The strategy would include research aimed at scientific advancement in mechanistic understanding and modelling techniques, as well as research to address regulatory and policy needs.

HBM4EU has provided first insights on actual mixture exposures by analysing the co-occurrence of multiple chemicals from HBM samples of individuals (Ottenbros et al, 2021; Luijten et al, 2022). New information on pesticide mixtures was generated in a so-called hotspot-control design in five countries (CZ, ES, HU, LV, NL) (Ottenbros et al, 2022) using advanced suspect screening techniques (Meijer et al., 2021; Oberacher et al., 2021) and an advanced workflow was established for the assessment of mixture health risks (Kortenkamp et al., 2021).

Results were shared among policy makers and experts through four webinars and lessons learnt discussed at a two-day online workshop in the fall of 2021. There, a series of recommendations for both research and mixture risk management policies were formulated in D15.8 (see below).

6.3 What are the common HBM mixture patterns in the European population?

The network analysis – an intuitive graphical analysis – and the more quantitative so-called Sparse Non-negative Matrix Underestimation (SNMU) analysis identified a number of mixture patterns in each dataset. Some were, as expected, chemical families such as PAH's, PCBs, and PFAS. Strongest co-occurrence patterns were found for metabolites of the same parent compound, e.g., among DINCH metabolites, or acrylamide. We also observed, however, examples of less expected combinations of substances in co-occurrence patterns. Notably selenium, chromium, antimony, and aprotic solvent HMSI. Also, the co-occurrence of parabens (MeP and EP) with Lysmeral (TBBA) was observed, which may point at the role of cosmetics. The analysis revealed co-occurrence patterns of chemicals that are regulated under different regulations, stemming from different regulatory domains. The main findings are listed below; for more details please refer to Luijten et al. (2021).

- Identification of co-occurrence of substances using HBM data is feasible through network and SNMU analyses, each with its own strengths and limitations. Combined application of these approaches to explore and quantify co-occurrence is recommended.
- Correlations amongst HBM levels were generally positive, with low to moderate values between parent compounds
- The SNMU analysis indicate that a substantial part of the variation in the HBM data (> 70 %) can be captured with a limited number of clusters of co-occurrence patterns. While this needs to be replicated in other datasets, there is no reason to believe that this will be very different in other HBM datasets.
- The role of HBM determinants can be assessed via the use of co-variates in the analysis, e.g. smoking, BMI, age, sex, education, diet, or other exposure information, in so-called Comparative Network Analysis (CNA).
- Existing databases of early HBM studies are useful for the first exploration of co-occurrence of substances in the human body. The number of individuals in which the full range of chemicals of interest is measured, however, is limited and needs to be expanded.
- The stability and consistency of identified networks and mixture communities deserves further study, particularly for high dimensional data when strata of covariates are being studied.
- Toxicity weighting of mixture communities/clusters is feasible, but severely limited by the shortage of available HBGVs or other indicators of toxic potency of the substances involved. More generic inroads need to be explored, the more so when suspect screening and untargeted screening is wider applied in HBM studies.
- So far, four existing datasets were subjected to network analysis techniques, in one dataset network analysis and SNMU were compared, and toxicity weighting was explored in one dataset. The datasets varied widely in study population, study design, matrixes under study and chemicals analysed. It is recommended to expand current analysis to a wider set of existing data.

6.4 Can we identify hotspots or risk groups with high mixture exposures?

In order to address this policy question, the HBM4EU-SPECIMEn (Survey on PEstiCIde Mixtures in Europe) study was performed (Ottenbros et al, 2022). This study was designed to assess concomitant/combined exposure to multiple pesticides in hotspot and control areas using human biomonitoring. The main aim of the SPECIMEn study is to generate new exposure data across Europe on a broad combination of pesticides and to assess possible local contributions (i.e. hotspot areas) and within-person variation. The approach used is a so-called ‘hotspot’ design, focusing on residential areas close to fields where pesticides are applied. The main research questions for this survey are:

1. Which combinations of pesticide-related compounds are most commonly detected?

2. Do patterns in pesticide-related compounds detected with suspect screening differ between age groups and study populations in different countries?

3. Do patterns in pesticide-related compounds detected with suspect screening differ between people living close to pesticide application sites and the general population?

4. Do patterns in pesticide-related compounds detected with suspect screening differ between seasons (spraying and non-spraying season)?

It is hypothesized to detect higher exposure levels within the population living close to the agricultural fields (hotspots), as well as within the spraying season (summer). Difference in detection frequencies between adults and children can be due to various reasons, such as differences in food consumption, product use, differences in activities like more hand-mouth contact (children) or occupational exposure (adults). Potential differences in the detected patterns will likely be influenced by, among others, food consumption and usage of pesticide containing products; these covariates will be covered by the application of a questionnaire.

In order to collect data geographically spread across Europe, countries from the regions as defined by HBMEU (North, South, East, West) were included: Hungary, Czech Republic, Spain, Latvia, and the Netherlands. Switzerland participated as well, with a slightly different study design. Data for this study started in winter 2019/2020 and finished in summer 2020. The corona pandemic necessitated some adaptions to the execution and planning of the work. Due to the delays, further and more in-depth analysis of the collected pesticide data is needed.

Through the novel suspect screening techniques, in total 95 pesticide-related markers were identified in the collected urine samples. Of these, 41 markers were identified with a high level of confidence. These related to 30 parent compounds that were identified with high confidence. Examples include acetamiprid, chlorpropham, boscalid, and clothianidin.

The detection frequencies for parent pesticides varied substantially between but also within countries. Across all countries, the pesticides acetamiprid, and chlorpropham were most frequently detected. Boscalid, fludioxonil, pirimiphos-methyl, and pyrimethanil were detected at frequencies above >10% in all countries except Hungary. Similarly, clothianidin, fluazifop, and propamocarb were detected at frequencies above >10% in all countries except Latvia. Finally, the markers for cyprodinil, flonicamid, and tebuconazole were detected at frequencies above 10 % in at least three countries. In Switzerland a comparable pattern of detection frequencies was detected compared to the SPECIMEn countries.

Many of the pesticides identified showed differences in detection rates when comparing hotspot areas versus control areas, samples collected in summer versus winter, and children versus adults; however, differences were in many cases not statistically significant. The statistically significant differences were not consistent across countries.

The co-occurrence patterns among the multiple compounds detected by the suspect screening, were described using a correlation network analysis. Eight different co-occurrence patterns were observed: The largest patterns were formed through co-occurrence of acetamiprid (insecticide), chlorpropham (herbicide and plant growth regulator), chlorpyrifos (insecticide), flonicamid (insecticide), pirimiphos-methyl (insecticide and acaricide), tebuconazole (fungicide), thiacloprid (insecticide) and triclosan (antifungal and algicide). Overall, however, the underlying correlations were weak.

In conclusion, using suspect screening analyses, HBM4EU generated valuable exposure data across Europe on a broad combination of pesticides. Consistent strong contributions from agricultural application to detection rates in hotspots or in spraying season were not observed.

Suspect screening is a valuable approach to get a broader overview and a semi quantitative evaluation of substance exposures across the EU. This allows for the prioritization of substances for targeted analysis and comparison of the suspect screening data with reported substance usage.

Mixture risk assessment would strongly benefit from a strategy for the measurement of multiple exposure and effect biomarkers in the same subject in HBM programmes, as demonstrated by e.g.

Vernet et al. 2019. This requires the development of an inclusive HBM/exposome infrastructure in Europe.

More in-depth analysis of the collected information is still ongoing.

6.5 Which sources and pathways contribute most to HBM mixture values?

Comparative Network Analysis (CNA) of HBM data greatly facilitate the identification of real-life mixtures, i.e. patterns of co-occurring substances. Late data availability and limited number of individuals in which the full range of HBM markers were measured in existing studies precluded adequate assessment of sources and pathways contributing most to HBM mixtures. Nonetheless, CNA was performed as a proof-of-concept, performing network analysis broken down by co-variates such as age, sex, BMI, level of education, smoking and dietary aspects. The analyses conducted (Luijten et al., 2022) revealed patterns of co-occurring substances in different studies, thereby nicely illustrating the potential of this method for policy making. However, it is too early to draw firm conclusions regarding sources and exposure pathways that led to the mixtures identified. Some co-occurrence patterns, like of parabens with Lysmeral hint to the role of cosmetics.

Also in the SPECIMEn study, time restraints resulting from the Covid-19 pandemic impeded the analysis of pesticide markers against co-variate information, e.g., obtained from questionnaires.

6.6 What are the impacts of chemical mixtures on human health?

The HBM4EU case studies (Kortenkamp et al., 2021) collectively highlight the need to take combined exposures into account in chemical risk assessment. It became evident that a disregard for combined exposures will lead to significant underestimations of the health risks associated with chemical exposures. This is true both for the general population and for more specific exposure scenarios, e.g., those relevant to occupational settings.

Based on the lessons learned in the case studies, an advanced decision tree and workflow scheme for assessing hazards from exposure to chemical mixtures were developed. In many cases, it was possible to identify drivers of mixture risks, i.e., chemicals that contribute more strongly to the estimated health risks than other chemicals in the mixtures. Therefore, it is recommended that methodologies for mixture risk assessment (MRA) by regulatory agencies and authorities should also include approaches for the identification of risk drivers that contribute most to the mixture risk, with the aim to focus and facilitate risk management. Furthermore, the case studies demonstrated that assessment of risks associated with combined exposures is generally possible by using the Hazard Index and/or the Point of Departure Index method. In the interpretation of results from the Hazard Index in a tiered approach, sufficient attention should be given to the underlying uncertainties in the applied assessment factors for the substance-specific Hazard Quotients used in the Hazard Index.

A bottleneck in the MRA is in the provision of exposure data. HBM markers measured in the same individuals may help solve this in the future. The co-occurrence patterns observed in the re-analysis of existing HBM mixture data may give guidance in this respect, but expansion to other suitable data sources is needed.

One of the case studies conducted underscored the need for more repeated HBM measurements for substances with a relatively short half-life in the human body, to assess the within-person variation against the between-persons variation. Single one-time measurements of such HBM markers may not adequately reflect longer-term exposure profiles and are therefore less suitable to

benchmark against health-based guidance values based on effects from longer-term exposures (Vernet et al, 2019).

6.7 What action perspectives are available to reduce mixture levels?

In policy terms, the risk management of 'mixture health risk problems' can be seen as a 'wicked problem', due to the nature of the problem (i.e., high uncertainty about risks, high complexity and diversity of interests with high ambiguity of values). Thus, a risk governance approach involving stakeholders is required to effectively and efficiently address the mixture health risk problem.

In line with the nature of 'wicked' problem of mixture risk governance, views on responsibilities and on criteria to guide risk reduction strategies varied considerably in the exploratory assessment of information needs (D15.1; Lebret et al, 2020). Concrete action perspectives, therefore, remain unarticulated.

Based on the insights and lessons learned thus far in the context of HBM4EU, conclusions as well as 14 recommendations for risk assessment of chemical mixtures and for further research have been drafted and were widely endorsed by policy makers, scientists and stakeholders. These are described in section 8.

7 HBM4EU results

7.1 Key outputs

Information needs of regulatory bodies and stakeholders

Chemical mixtures, that result from exposures from the ambient and indoor environment, occupation, consumer products and cosmetics, food, etcetera are of societal concern as chemicals in the human body may in combination possess hazardous (toxic) properties and may adversely affect human health. One particular concern is the potential impact of exposure to mixtures of pesticides arising from occupational exposure, proximity to areas of pesticide applications, dietary, domestic use or via the wider environment. In addition, new chemicals and new applications of existing chemicals are continuously introduced to the market. Thus, intentional and unintentional exposure to mixtures of chemicals possessing hazardous properties add to the total chemical burden to which the population is exposed.

While mixtures are high on the research, regulatory and policy agendas, opinions on how to deal with mixture risk problems vary considerably. This became apparent from a first exploratory assessment, carried out in the framework of HBM4EU WP15, of potential information needs from a governance perspective for mixture risk management. In this exercise, a set of questions was used in structured interviews with researchers and policy makers to delineate the current discourse on mixture risk governance. Information needs on mixtures was operationalised here as "How can we effectively and efficiently manage the health risks associated with chemical mixtures in the European population in such a way that residual mixture risks are considered acceptable from a public health and personal health point of view, while maintaining to the degree possible the societal and personal benefits of the products that lead to the mixture exposures".

The conclusions from the literature and interviews were:

- Mixture can be viewed as 'systemic risks' given the properties of uncertainty, complexity, and ambiguity and the general 'embeddedness' of chemicals in daily life; risk governance approaches for mixtures should therefore be targeted as such and the contextual aspects may require tailored approaches instead of generic regulation.

- The information needs from policy makers and experts are, at least at the time of the assessment, still rather diffuse and unarticulated.
- As can be expected from the literature on systemic risks, views on responsibilities and criteria to guide risk reduction strategies vary considerably; this warrants further exploration of views and mental models held by the stakeholders involved.
- A broader dialogue on information needs for mixture risk governance with stakeholders is needed.

Based on the literature and interviews, D15.1 also developed a long list of 'statements' (or positions in terms of argumentation analysis) for future use in exploration of information needs in policy makers and stakeholders. These can be used in further delineation of information needs.

In addition, HBM4EU contributed to initiatives from several EU research projects dedicated to study mixture health risks. They published a Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment (Bopp et al., 2018, Drakvik et al., 2020).

Exposure patterns in the European population

Initially, there was insufficient existing HBM mixture data available through the repository to directly address this question. Therefore, statistical scripts and approaches have been developed and tested on a simulated data set. These have been described in AD15.3 and D15.3. The scripts involve a combination of methods, both graphical and analytical, and combine alternative methods.

- In 2019 the scripts have been successfully applied to real HBM mixture data from the Flemish 'FLEHS' cohort under bilateral agreement. The results have been described in a scientific manuscript (Ottenbros et al. 2021).
- The developed and tested network analysis approaches were then applied more widely on four other data sets. Based on data availability, willingness to co-operate and availability of relevant expertise, similar analyses have been done for the Belgian '3xG' data, Czech data, the German 'GerES' V data, and Spanish 'Bioambient.es' data. For one of these datasets, i.e. GerES V, it was explored whether toxicity weighting could be used to prioritize specific mixtures. Also, an alternative approach, the SNMU (Sparse Non-negative Matrix Underestimation) was applied to the Flemish FLEHS data and compared to the network analysis results obtained for this dataset.

Exposure hotspots and risk groups

HBM4EU has run a survey of human internal exposure to mixtures of pesticides across five of the partner countries: Hungary, Czech Republic, Spain, Latvia and the Netherlands. Switzerland also collected urine samples, with a slightly different design. This survey, entitled 'SPECIMEn', explores exposure to pesticides and focusses on "hotspot" residential areas or close to agricultural fields where pesticides are applied, in comparison to control areas. The survey was designed to assess concomitant/combined exposure to multiple pesticides in hotspot and control areas using human biomonitoring. Details of the joint pesticide survey are described in AD15.7.

The field work for this survey covered two seasons started in the fall of 2019 (non-spraying season) and was completed in the summer of 2020 (spraying season). Urine samples have been collected in 50 parent-child pairs in hotspots (residences within 250 m of agricultural application of pesticides) and 50 parent-child pairs in control areas. Samples and questionnaires were collected in a non-spraying and a spraying season. Samples were analysed through pesticide suspect screening in five different laboratories, using harmonised approaches for sample preparation, data acquisition, data processing, incl. annotation of pesticides and metabolites. This work was done in conjunction to CGL Emerging Chemicals (WP16). All samples from each single country were analysed in one laboratory. These suspect screening approaches are built on non-selective

analytical workflow and allow the qualitative monitoring of several hundred (up to several thousands) of exposure markers, including various pesticide classes under their parent or metabolite form. This approach should gain insight into the occurrence of extended exposure patterns of pesticide-biomarkers, differences across the countries participating in SPECIMEn, differences between two seasons (spraying season with active application, and non-spraying season with no active application) and/or location (living close to agricultural areas or not).

The results obtained contribute to the prioritisation of certain substances in terms of further exposure and risk assessment, and to possibly generate early warning information.

Exposure sources and pathways

Limitations in data availability, and delays due to the corona pandemic restricted possibilities to perform 'Comparative Network Analysis' (CNA) to study the role of co-variates at this time (task 15.1). Moreover, due to the pandemic it was not possible to collect house dust samples during the spraying season. Again, time constraints limited the ability to analyse observed pesticides against questionnaire information to date (task 15.2).

Exposure impacts

Within Task 15.3, seven case studies have been conducted to identify methods for the prediction of mixture effects that can be used consistently for human health risk assessments and can inform biomonitoring strategies.

- Case study 1: A mixture risk assessment for male reproductive health with a focus on semen quality
- Case study 2: Mixture risk assessment of antiandrogenic chemicals based on human-derived hazard and exposure data
- Case study 3: A mixture risk assessment for developmental neurotoxicity with a focus on declines in IQ
- Case study 4: Occupational exposure to hexavalent chromium, nickel and PAHs and lung cancer
- Case study 5: Hazard index assessment of the combined chronic dietary exposure to heavy metals in the general population
- Case study 6: Risk assessment of the occupational exposure to a mixture of four toxic metals
- Case study 7: Relying on repeated biospecimens to reduce the effects of classical-type exposure measurement error in studies linking the exposome to health.

The case studies produced a framework and an advanced workflow for the conduct of such mixture risk assessments.

Risk management of 'mixture health risk problems

The EU's Chemicals Strategy for Sustainability (European Commission, 2020a) expresses the ambition to account for the cocktail effect of chemicals when assessing risks from chemicals, with the overall aim to work towards a toxic-free environment. Among others, the Commission aims to introduce or reinforce provisions to take account of the combination effects in relevant legislations, such as legislation on water, food additives, toys, food contact material, detergents and cosmetics.

For REACH, it will be assessed how to best introduce (a) mixture assessment factor(s) for the chemical safety assessment of substances. More details can be found in the accompanying Staff Working Document (European Commission, 2020b), which describes the progress made since 2012 on the assessment of (un)intentional mixtures and to provide background information and evidence base to actions announced in EU's Chemicals Strategy for Sustainability. One of HBM4EU's recommendations is to apply HBM4EU (mixture) data and experience to support the

science-based derivation of an appropriate Mixture Assessment Factor (MAF). Simulation studies and sensitivity analyses, using HBM4EU (mixture) data, cases studies and overall experience would allow to assess consequences of a MAF on ensuing mixture exposures and HBM mixture levels, as well as gauge the impact of a MAF on the resulting mixture risk reduction.

While a range of positions have been brought forward in assessment of information needs and in discussions on action perspectives with respect to mixture risk governance, the overall picture as of yet is anecdotal. It is unclear to what degree the various options have support in a wider constituency of experts, policy makers or the general public and stakeholders. A more systematic and broader consultation will be needed, e.g., in the framework of PARC to gauge support for the (sometimes incompatible) alternative action perspectives to reduce mixture risks in the population.

In the final year of the project, results and lessons learnt were discussed in a two-day online workshop together with policy makers and stakeholders. This yielded a set of recommendations.

7.2 Key data gaps & challenges

HBM4EU has helped to identify a number of specific data gaps and has substantially added new information and insights into the actual mixture exposures in the European population and has produced an advanced workflow and cases studies for the assessment of mixture health risks. These are needed to give policy makers relevant and strategic data to establish appropriate regulations and improve chemical risk management:

- HBM4EU results demonstrated the potential of human biomonitoring as an instrument to obtain insight into the real-life mixtures the human population is exposed to.
- HBM4EU results demonstrated that chemical mixtures are of public health concern. In the majority of the cases, it was possible to identify risk drivers, i.e. chemicals that contribute more strongly than others to the health risk.
- HBM4EU novel approaches to identify co-occurrence patterns demonstrated clusters of co-occurring chemicals; chemicals in these mixture clusters are regulated independently under different legislative frameworks.
- HBM4EU data and expertise can support a science-based derivation of a Mixture Assessment Factor and gauge potential impacts on the population’s exposure to chemicals
- While further expansion is needed on various aspects of the mixture activities carried out in the context of HBM4EU, application of available methodologies for mixture risk assessment should already be implemented to the degree possible.

However, some gaps and needs for action will remain after the end of HBM4EU which should be addressed in the future. There is a clear need for MRA approaches across regulatory sectors, which is fully in line with the ‘one chemical, one assessment approach’ for chemical safety assessments proposed by the European Commission. Broader implementation may be hampered by insufficient data availability, in particular regarding observations at the level of the individual. This aspect should be addressed in a strategy for an inclusive European HBM/exposome programme, including the required infrastructure. In this context, the term ‘infrastructure’ not only relates to the collection and analysis of human biomonitoring samples (and thus the necessary network of laboratories), but also to data interpretation and making data FAIR (findability, accessibility, interoperability, and reusability), all in a harmonized fashion. EIRENE, the European research infrastructure on human exposome developed under ESFRI (European Strategy Forum on Research Infrastructures) could be considered as a step in the right direction (https://www.eirene-ri.eu/). Such a strategy should also cover collection of auxiliary information on

exposure routes, e.g. from questionnaires or indoor measurements (like indoor air, house dust, carpeting, etc).

HBM4EU has clearly demonstrated the added value of HBM for addressing the challenges associated with MRA. However, data from individuals with a wide set of measured exposure biomarkers is still scarce. Therefore, it is recommended to 1) explore the possibilities to expand the knowledge base by applying suspect screening of existing samples from earlier studies; 2) expand in future studies the number of individuals in which the full range of targeted substances is measured. Not all HBM studies need to aim at fulfilling all recommendations. Also, in suspect screening, careful selection of relevant suspects is needed. Relevant consideration may be to include chemicals across regulatory domains, applications to hotspots to prioritize targeted analysis and ability to address time trends, i.e. replacement and emerging chemicals.

With respect to approaches to identify co-occurrence patterns in HBM mixture data, toxicological potency information, drivers of toxicity and grouping chemicals according to their mode of action should be included. Also, for risk management purposes, there is a clear need of knowledge not only about the HBM biomarker levels, but also about the preceding exposures, exposure routes and frequencies and source contributions. This is essential to allow effective interventions and exposure reduction strategies.

Regarding health risks due to exposure to chemical mixtures, the focus should be on risk drivers. These drivers of risk may change over time when the composition of the mixture changes, e.g., due to replacement of chemicals. On the other hand, diffuse exposures to persistent legacy chemicals may act as risk drivers, but may be difficult to further manage. It should be noted that information on toxicokinetics is a prerequisite for specific and sensitive exposure markers. The Hazard Index (HI) approach is a simple low tier approach where conservative assessment factors are being applied. Interpretation of HI should always be done with great care, taking into account uncertainty and the origin and precise nature of the applied assessment factors used to derive individual substance HQs. A Point of Departure Index (PODI) approach would be more robust in that sense. Nevertheless, in both cases (HI and PODI) the origin and precise nature of the common effect (used in the reference doses or toxicological reference values) deserves attention.

Existing exposures and body burdens may originate from different regulatory silos, which brings about further challenges to the risk management. In this context, the practical feasibility of mixture risk management is a concern, given the current absence of a legal framework to do so. Both the delineation as well as the practice of risk assessment varies across regulatory silos and for medication risk/benefit considerations may play a different role, as do the (in)voluntariness of the exposures.

8 Future recommendations

Based on the insights and lessons learned thus far in the context of HBM4EU, conclusions as well as 14 recommendations for risk assessment of chemical mixtures and for further research have been drafted and were widely endorsed by policy makers, scientists and stakeholders (Luijten et al., 2021). These are as follows:

1. Implementation of available methodologies for mixture risk assessment by (national and international) regulatory agencies should be accelerated to the degree possible, mainly based on the evidence HBM4EU has generated on mixture exposures and health risks.

2. HBM data of appropriate quality and granularity, particularly data on the common occurrence of chemicals, need to be more widely utilized, both in the design of toxicological mixture studies, epidemiological studies and in risk assessment as input to mixture risk management.

3. An HBM strategy for the measurement of biomarkers of multiple exposures and effects in the same subject needs to be developed, building on the HBM4EU experience. This requires the development of an inclusive HBM/exposome research infrastructure in Europe.

4. HBM4EU (mixture) data and experience should be applied to support the science-based derivation of an appropriate Mixture Assessment Factor (MAF). Simulation studies and sensitivity analyses, using HBM4EU (mixture) data, cases studies and overall experience would allow to assess consequences of a MAF on ensuing mixture exposures and HBM mixture levels, as well as gauge the impact of a MAF on the resulting mixture risk reduction

5. Future HBM studies should aim to collect data on the full range of chemicals of interest by targeted analysis in sufficiently large study populations measured in the same individuals, to assess the actual mixture exposures in the population and co-occurrence in the body.

6. When data on substance use and exposures is limited, it is recommended to apply suspect screening analysis in human samples to get a broader overview and a semi-quantitative evaluation of substance exposures across the EU. This will support prioritization of substances for targeted analysis and for comparison of the suspect screening data with reported substance usage.

7. Further research should focus on broadening and refinement of a combination of approaches (like network analysis and SNMU (sparse non-negative matrix under-approximation) method and toxicity weighting) to identify real-life chemical mixtures of concern to which the population is exposed. This will allow prioritization of mixtures of concern and support policy decisions. This involves data-driven approaches and methodologies to incorporate toxicological potency information and to group substances with common modes of action.

8. Existing samples collected within the HBM4EU WP8 framework and earlier relevant HBM studies should be screened on feasibility aspects for re-analysis through suspect screening and untargeted analysis. This will allow to expand the assessment of actual mixture exposures in the population and to assess time trends.

9. Compliance or non-compliance of some chemicals with their single regulatory values should not distract from their possible contribution to mixture problems/risks.

10. In the risk assessment for the authorization of a new chemical, existing mixture exposures and body burdens of substances with similar adverse outcomes, need to be taken into account to the degree possible.

11. In the risk assessment and management of mixtures, chemicals from other sources, e.g. medication or recreational drugs, that produce similar adverse outcomes, should also be taken into account to the degree possible. A legal basis to do so needs to be further developed.

12. Methodologies for mixture risk assessment by regulatory agencies and authorities should also include approaches for the identification of risk drivers that contribute most to the mixture risk, with the aim to focus and facilitate risk management.

13. In the interpretation of results from the Hazard Index in a tiered approach, sufficient attention should be given to the underlying uncertainties in the applied assessment factors for the substance-specific Hazard Quotients used in the Hazard Index.

14. The identification of groups of co-occurring substances, regulated in different domains and sectors, and of toxicological concern, as through network analysis and mixture risk assessment, underscores the need to strengthen mixture risk assessment across regulatory domains and sectors.

8.1 Opportunities for policy uptake

To ensure risk assessment of mixtures utilise HBM data, the requirement for such information should be included in relevant legislations to drive the collection of such data. The need for this is emphasised by the current general lack of such information in existing human health and ecosystem risk assessments. It is emphasised that to be of value, such data must be collected using transparent and systemic approaches and that there should be a uniformity in requirements across different regulations (Drakvik et al., 2020).

Effect-based regulatory values for mixtures that have similar adverse effects need to be developed; this could be informed by HBM. The nature and role of the various routes (e.g. occupational exposure, intentional exposure and unintentional exposure) of exposure for mixtures also needs consideration.

Looking to the future, grouping of chemicals into "cumulative assessment groups" may be a possibility, together with development of a multi-causal framework. Such a framework would allow the integration of exposure data and inform the development of strategies to prevent or reduce such mixture exposures (Drakvik et al, 2020).

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