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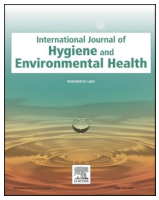

Mixture risk assessment and human biomonitoring: Lessons learnt from HBM4EU

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ABSTRACT

Unintentional chemical mixtures that are present in the environment are of societal concern as the (environmental) chemicals contained therein, either singly or in combination, may possess properties that are hazardous (toxic) for human health. The current regulatory practice, however, is still largely based on evaluating single chemical substances one-by-one. Over the years various research efforts have delivered tools and approaches for risk assessment of chemical mixtures, but many of these were not considered sufficiently mature for regulatory implementation. This is (partly) due to mixture risk assessment (MRA) being very complex because of the large number of chemicals present in the environment. A key element in risk assessment is information on actual exposures in the population of interest. To date, information on actual personal (internal) mixture exposures is largely absent, severely limiting MRA. The use of human biomonitoring data may improve this situation. Therefore, we investigated within the European Human Biomonitoring Initiative (HBM4EU) various approaches to assess combined exposures and MRA. Based on the insights and lessons learnt in the context of the HBM4EU project, conclusions as well as recommendations for policy development regarding chemical mixtures and for further research were drafted. These conclusions and recommendations relate to both exposure and adverse health effects in humans. The recommendations were discussed with stakeholders in a workshop held in October 2021. There was considerable support and agreement with the spirit, scope and intention of the draft recommendations. Here we describe the lessons learnt on mixture risk assessment through the HBM4EU project and present the final recommendations. Overall, 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. Also, 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. The 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. Moreover, 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.

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1. Introduction

Risk assessment of chemical mixtures is complex and poses a number of challenges for scientists, risk assessors and risk managers due to the large number of chemicals people are exposed to through the environment, food, occupation, lifestyle choices, cosmetics and consumer products. The issue of mixture risk assessment (MRA) is high on the agenda in Europe, in research as well as in the regulatory and policy arenas. Generic recommendations for future research and policy development have been drawn up earlier (Bopp et al., 2018; Kienzler et al., 2016; Kortenkamp and Faust, 2018; Rotter et al., 2018). More recently, a group of scientists published a Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment (Drakvik et al., 2020). Also, the EU's Chemicals Strategy for Sustainability (European Commission, 2020) 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 European 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 (MAF) in the chemical safety assessment of single substances (European Commission, 2020). The MAF is considered a pragmatic solution to a 'wicked problem', because it circumvents the need to assess many different combinations of substances. Thus far, most efforts have focused on the sizing of a MAF for ecological risk assessment, while efforts exploring the sizing of a MAF for human health risk assessment are limited (Socianu et al., 2022). EFSA, the European Food Safety Authority responsible for risk assessments of food and feed, rather prefers a different approach for MRA and recently published a guidance providing methodologies for applying scientific criteria and prioritization methods to group chemicals for human risk assessment of combined exposures to multiple chemicals (Scientific Committee et al., 2022).

The European Human Biomonitoring Initiative (HBM4EU; hbm4eu.eu), a joint project funded under the Horizon 2020 programme, addressed how Human Biomonitoring (HBM) data can contribute to both the science and the regulations dealing with the phenomenon of combined exposure to multiple chemical substances. Within HBM4EU, the focus for chemical mixtures was on chemicals with exposure routes through the environment, food, occupation and/or consumer products. We investigated various approaches for combined exposure and MRA, with the aim of exploring how HBM data could be best used to improve current procedures for MRA. The approaches studied included: *1*) estimating exposure to mixtures through correlation network analyses of existing HBM data, *i.e.* a graphical method that allows for the identification of groups of exposure biomarkers that are more densely related amongst each other than with other biomarkers; *2*) estimating exposure to pesticide mixtures using suspect screening analyses based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS); and *3*) establishing an advanced workflow for assessing mixture health effects. These activities were carried out by HBM4EU partners working closely together and in dialogue with European regulatory agencies and similar initiatives in the European Union.

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 of potential information needs from a governance perspective for mixture risk management, carried out in the framework of HBM4EU (Lebret, 2015; Lebret et al., 2020). 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. One of the main conclusions was that information needs from policy makers and experts were, at the time of the interviews, still rather diffuse and unarticulated, in line with the 'systemic risk' nature of mixtures. The term 'systemic risk' refers to the broad and deep embeddedness of risks to health and environment, rooted in societal, technological, economic, cultural, political and regulatory systems and developments (Klinke and Renn, 2006; OECD, 2003; Renn, 2004); governance of systemic risk goes beyond traditional risk management through technical and regulatory action. Thus, consequences for functionality of HBM mixture information could not easily be derived through the interviews. Also, views on responsibilities and criteria to guide risk reduction strategies varied considerably. Potential problems in cooperation between silos from different policy domains were seen as mainly stemming from differences in regulations and the absence of a common regulatory framework. The term 'silo' is used here to denote an established set of dedicated, explicit, legislation-based rules and protocols combined with implicit knowledge and procedures among practitioners within a specific policy domain, *e.g.* 'food safety', 'classification, labelling and packaging', or 'environmental policies'. Typically, silos develop over decades following state-of-the-art knowledge and societal demand, relatively independent of each other, leading to often different procedures to chemical risk assessment. In a broader sense, 'silos' can be seen as the 'cognitive distance' between different practitioners of chemical risk management (Lebret, 2015).

Based on the insights and lessons learnt from the HBM4EU activities on chemical mixtures, a team of experts involved in HBM4EU drafted recommendations for mixture risk assessment and for further research. The recommendations were discussed with HBM4EU and other experts and stakeholders in a workshop held in October 2021. Here we describe the lessons learnt on mixture risk assessment through the HBM4EU project and the outcomes of the workshop.

Abbreviations

ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
HBM	Human Biomonitoring
HBM4EU	European Human Biomonitoring Initiative
MAF	Mixture Assessment Factor
MRA	mixture risk assessment
SNMU	sparse non-negative matrix under-approximation
SPECIMEn	Survey on PEstiCIde Mixtures in Europe

2. HBM4EU workshop on mixture risk assessment

2.1. Scope and purpose

The HBM4EU workshop, entitled 'Chemical Mixtures: Lessons we are learning from HBM4EU', was held online on 14-15 October 2021. The scope of the workshop, defined by a preparatory group (primarily the authors of this publication), was to discuss the insights and lessons learnt within HBM4EU in relation to risk assessment of chemical mixtures. These were presented in detail in a set of pre-workshop webinars. More specifically, the goal of the workshop was to present a brief summary of the results obtained from the HBM4EU activities on chemical mixtures and to discuss recommendations for further research and for policy development regarding MRA. The workshop consisted of sessions where researchers presented the highlights of their findings from the respective activities on mixtures, intertwined with presentations from regulatory authorities, *i.e.* the European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA). The draft recommendations were presented in three sessions, followed by discussions on these recommendations in six breakout groups. Each of these breakout groups provided a concise summary of the discussions held in the plenary session; the main outcomes of the discussions are described below. In addition to the discussion sessions, an online poll (using Mentimeter; www.menti.

com) was held with the aim to get an impression about the extent to which participants of the workshop agree with the intention and scope of the recommendations drafted (Luijten et al., 2021). In total, almost 100 persons participated in the workshop representing a wide group of stakeholders, ranging from the European Commission to academia to NGOs.

2.2. Pre-workshop webinars

To allow workshop participants to familiarise themselves with complex (technical) material, methodologies and results that emerged from the HBM4EU activities on chemical mixtures, and to share the lessons learnt and to sketch the regulatory context, a set of four dedicated pre-workshop webinars was organised. These webinars focused on the key results of three different mixture activities: 1) Suspect screening of pesticide mixtures, results from the Survey on PEstiCIde Mixtures in Europe (SPECIMEn) study; 2) Patterns in real-life exposures to mixtures, results from HBM network analyses; and 3) Health risk from exposure to mixtures, results from the HBM4EU case studies. The fourth webinar was about the EC's Chemicals Strategy for Sustainability; this webinar was added to allow the scientists involved in the workshop to become familiar with the future policy and regulatory arena of EU's Chemical Strategy for Sustainability that will form the context for future mixture risk assessment and management within Europe.

In the webinars the focus was on the introduction of the novel methodologies applied and on the results and conclusions obtained. At the webinars, no recommendations were discussed. Each of the webinars was recorded; the links to the recordings are available via the HBM4EU website (https://www.hbm4eu.eu/result/events/trainings/

2.3. Materials on which the conclusions and recommendations are based

The mixture activities under HBM4EU focussed on three elements.

1. The development and application of approaches to assess co-occurrence of chemicals by re-analysing HBM mixture data from previous HBM studies. Details about this body of work can be found in Ottenbros et al. (2021), in HBM4EU deliverables D15.3 and D15.7 (Luijten et al., 2022; Vlaanderen et al., 2019) and in Rodriguez Martin et al. (Rodriguez Martin et al., 2023). Correlation-network analysis is a method that allows for the identification of real-life exposure patterns to mixtures in the human body. It is a graphical method to represent the relationships between groups (so-called 'communities') in the data, allowing for the identification of groups of exposure biomarkers that are more densely related amongst each other than with other biomarkers. Similarly, sparse non-negative matrix under-approximation (SNMU (Gillis and Glineur, 2010; Gillis and Plemmons, 2013);) was applied to identify mixtures, followed by a hierarchical classification to cluster individuals regarding their co-exposure to the identified mixtures. In the context of HBM4EU, both approaches were further developed, tested on a Flemish data set and subsequently explored and used on existing HBM data from Belgium (3xG; https://studie3xg.be/nl), Czech Republic (CELSPAC - FIREexpo; https://www.recetox.muni.cz/en/services/celspac-population-studies/celspac-study), Germany (GerES V (Schwedler et al., 2020);) and Spain (BioAmbient.ES (Perez-Gomez et al., 2013);). Additionally, an approach to prioritize co-occurrence patterns resulting from the correlation network analysis according to toxicological concern was explored using human biomonitoring health-based guidance values (Loh et al., 2023; Luijten et al., 2022).
2. The preparation and execution of a pan-European study on pesticide mixture exposures, using pesticide suspect screening methods on collected urine samples. The suspect screening method, which is based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS), yields a list of tentative annotations of pesticides and pesticide metabolites present in a sample set (Huber et al., 2022). Hence, it provides insight into the exposure of humans to chemicals not covered in targeted monitoring programs. Within HBM4EU, this joint pesticide survey 'SPECIMEn' was executed in five partner countries: Czech Republic, Hungary, Latvia, Spain and the Netherlands. In addition, a pesticide suspect screening study with a different design was conducted in Switzerland. The main aim was 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 between pesticide spraying season and non-spraying season, for both adults and children. This so-called 'hotspot-control' design, focused on residential areas close (<250 m) to fields where pesticides are applied in comparison to control areas. Within each country, urine samples were collected from 50 parent-child pairs at each location, for both seasons. These samples (n = 2,088) were submitted to pesticide suspect screening in conjunction with HBM4EU work on emerging chemicals (Meijer et al., 2021; Oberacher et al., 2021; Ottenbros et al., 2022a; Ottenbros et al., 2022b).
3. Cases studies on mixture health risks to evaluate a proof-of-concept for the identification of mixture health effects. Selected case studies were directed to human health endpoints of concern: focusing on chemicals affecting neurodevelopment (*inter alia* PBDEs, organophosphate pesticides), chemicals with anti-androgenic properties (*inter alia* polychlorinated biphenyls, pesticides, phthalates), heavy metals causing nephrotoxicity (arsenic, cadmium, lead and mercury) and occupational carcinogens (chromium (VI), nickel and PAHs). As a generic issue, exposure misclassification was addressed resulting from the biomonitoring of multiple chemicals in single spot samples. To harmonise the case studies and for future mixture risk assessments, an advanced decision tree and workflow scheme were developed for the hazard assessment arm of mixture risk assessment (Kortenkamp et al., 2020, 2021).

Abstracts from the deliverables can be found in Appendix A (Supplementary data). Core conclusions and highlights of this work were presented at the pre-workshop webinars (https://www.hbm4eu.eu/result/events/trainings/) and form the basis of the conclusions and recommendations in the next section.

3. Insights and lessons on chemical mixtures learnt from HBM4EU

The preparatory group drafted a set of 13 conclusions and 16 initial recommendations based on the results obtained until Summer 2021 in HBM4EU; these were subsequently discussed with the HBM4EU Management Board and partners involved in the work on chemical mixtures. Improved versions of the drafted conclusions and recommendations were shared with all workshop participants prior to the workshop. It should be noted that the focus was on conclusions and recommendations that emerge from the HBM4EU project and not on more generic recommendations regarding MRA. Based on the discussions held in the workshop and on additional input from the HBM4EU Management Board, the wording of the conclusions and recommendations was further improved. Based on these discussions, some recommendations were combined, leading to a set of 14 recommendations. The final versions of the conclusions (C1–C13) and recommendations (R1-R14) are presented below, accompanied by key points of the workshop discussions on the recommendations.

3.1. Conclusions from HBM4EU

In total, 13 conclusions for risk assessment of chemical mixtures were defined; these were clustered into three groups: generic conclusions, conclusions specifically related to exposure, and those specifically related to health risks.

3.1.1. Generic

C1. Mixtures matter: the different activities conducted in HBM4EU clearly indicate that chemical mixtures pose a concern regarding human health.
C2. HBM4EU results show that human biomonitoring data are a suitable instrument to obtain insight into the real-life mixtures the human population is exposed to.
C3. While HBM4EU activities did not specifically address the Mixture Assessment Factor (MAF), the results from the HBM4EU activities on chemical mixtures as well as the data generated in the HBM4EU Aligned Studies can contribute substantially to a scientific underpinning of the MAF; however, this requires further work.

3.1.2. Exposure

C4. Network analysis of existing human biomonitoring studies reveal that combined exposures to multiple chemicals are common and occur in all population groups.
C5. Network analysis of existing human biomonitoring studies shows clusters of co-occurring chemicals; chemicals in these mixture clusters are regulated independently under different legislative frameworks.
C6. Analysis of existing human biomonitoring data demonstrates clusters of co-occurring chemicals as body burdens of mixtures. This shows that the unspoken assumption in single-chemical risk assessment, of exposure to a chemical in an otherwise chemically pristine environment, is contradicted by the available evidence.
C7. The number of individuals with human biomonitoring data for a wide array of chemicals is relatively scarce (compared to the total number of participants) due to logistic and financial reasons. Most chemicals are measured in subpopulations and (due to the physico-chemical properties) in different media, most in urine, fewer in blood. Thus, the number of individuals with a 'complete human biomonitoring data set' is relatively small in comparison with the number of chemicals that could be measured. This may limit the stability and/or consistency of the network communities identified through network analysis, and thus our ability to identify mixture exposure patterns of concern.
C8. Financial and logistic limitations have prevented HBM4EU new data collections to cover the full range of priority substances to be measured in the same individuals. Different chemical families were measured in different subpopulations, thus none of the individuals studied in the context of the HBM4EU Aligned Studies have data on the full range of priority substances.
C9. National information on active ingredients of pesticide use per crop type is insufficiently available for a multi-country comparison on pesticide exposures.

3.1.3. Health risks

C10. HBM4EU case studies of human health effects clearly show that chemical mixtures are of public health concern (Kortenkamp et al., 2022; Tavares et al., 2022). In the majority of the cases, it was possible to identify risk drivers,[1] *i.e.* chemicals that contribute more strongly than others to the health risk (Kortenkamp et al., 2021).
C11. HBM4EU case studies of health effects identified several legacy compounds as important drivers of risks (Kortenkamp et al., 2021). Network analyses showed that several of such legacy compounds cluster with other newer chemicals (Rodriguez Martin et al., 2023).
C12. HBM4EU case studies showed that when assessment factors of chemicals combined in a Hazard Index[2] vary widely, the application of a Hazard Index becomes problematic; in such cases the use of the Point of Departure Index[3] is more appropriate.
C13. HBM4EU case studies revealed that exposure to some chemicals (primarily legacy chemicals) already exceed their single acceptable levels.

3.2. Recommendations

The recommendations for further research and for policy development regarding chemical mixtures were drawn up with the aim of contributing to and enriching existing recommendations through science-based recommendations derived directly from HBM4EU, rather than to reiterate earlier generic recommendations. As the conclusions, the recommendations were grouped into the categories 'generic', 'exposure' and 'health risks'. The recommendations and the main outcomes of the discussions held in the workshop are as follows.

3.2.1. Generic

R1. 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.
R2. 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.
R3. 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.
R4. 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.

The discussions at the workshop demonstrated that the majority of the participants was in strong support of, where possible, the implementation of available methodologies and approaches for MRA in processes for regulatory decision-making. Also, the need for MRA approaches across regulatory sectors (*e.g.* cosmetics, industrial

[1] The term 'risk driver' is used here within the context of the case studies in HBM4EU, in which the hazard index (HI) is used. Thus, risk drivers are those substances with hazard quotients (HQs) that substantially contribute to the HI and drive the HI above one. One should note that unmeasured substances or substances for which a health-based guidance value is not (yet) available and for which no HQ can be calculated (known unknowns), can also contribute to the (unobserved) generic mixture risks.

[2] The Hazard Index (HI) approach is the most common approach for applying dose addition for assessing risks from combined exposure to chemical components with the same adverse outcome (Rotter et al., 2018). The HI of a chemical mixture is calculated by summing the Hazard Quotients (HQ) of each mixture component. The HQ value per chemical component is derived by scaling its estimated exposure or dose in the population by a level of exposure considered safe or acceptable. An HI value > 1 indicates that exposure to the mixture is greater than a threshold level of concern and warrants further investigation.

[3] The Point of Departure Index (PODI) approach is similar to the Hazard Index (HI) approach. In the PODI approach, a point of departure (POD) such as a NOAEL (No Observed Adverse Effect Level) instead of a health-based guidance value is used. Thus, in the PODI approach no assessment factor is applied.

chemicals, chemicals used in food) was stressed, which is 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) was seen 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).

Regarding the MAF, there was general agreement that the value of a MAF should be science-based and data driven. Although HBM4EU has not directly looked at a MAF, building on the HBM4EU (mixture) data and experience would add value to the derivation of an appropriate MAF. In particular, through simulation studies and sensitivity analyses, the consequences of a MAF on ensuing mixture exposures and HBM mixture levels could be assessed through data from *e.g.* the HBM4EU network analysis on existing data (Ottenbros et al., 2021). Moreover, the impact of a MAF on mixture risk reduction could be gauged in this way.

3.2.2. Exposure

R5. 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.
R6. 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.
R7. 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.
R8. 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.

In the discussions at the workshop it was observed that data from individuals with a wide set of measured exposure biomarkers is still scarce. Therefore, there was general support 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. Several concerns were expressed about the feasibility and logistic and financial consequences; also the need for further standardisation and validation of suspect screening was mentioned. 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, it was noted that incorporation of 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.

3.2.3. Health risks

R9. Compliance or non-compliance of some chemicals with their single regulatory values should not distract from their possible contribution to mixture problems/risks.
R10. 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.
R11. 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.
R12. 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.
R13. 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.
R14. 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.

Overall, the discussions at the workshop showed general support and agreement with the recommendations drawn from HBM4EU's work on health risks. Apart from some semantic discussions regarding the wording, there was some variation in opinions between breakout groups. For instance, while in general there was support for the first recommendations, some thought it was only stating the obvious. Several feasibility and practical issues were brought forward, often relating more to mixture risk *management* than to the mixture risk *assessment* per se. Indeed, some noted that not the mixture risk assessment, but the mixture risk management is the issue here. On the more technical side, data availability was considered a limiting factor. Information on the toxicokinetics as a prerequisite for specific and sensitive exposure marker was also considered relevant here. A focus on the drivers of risk, instead of addressing the whole mixture, found general support in the discussions, though it was also noted that these drivers would only be known when the whole mixture is known first. Also, 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.

On the technical side again, it was stressed that 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 Hazard Quotients. 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. It was also suggested to use distributions of assessment factors to capture the underlying uncertainty and to initiate further research on development of scientific criteria for selecting assessment factors.

It was noted that existing exposures and body burdens may originate from different regulatory silos, which brings about further challenges to the risk management. In this context, it was argued that 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. The issue was raised whether 'old' existing chemicals on the market should be treated more lenient than new chemicals that are entering the market, because previous risk assessments did not consider co-exposures and body burdens from other chemicals. Another issue was what the implication would be in the situation of a new chemical authorisation, where body burdens of metals like lead, or cadmium, or persistent organic pollutants would already have a Hazard Index (HI) above or close to 1. Would new chemicals with similar modes of action be restricted because this would further increase the HI above 1.

3.3. Level of support to individual recommendations

The level of support to the individual recommendations was assessed through an online Mentimeter poll among workshop participants. Participants scored each recommendation on a 5-point Likert scale ranging from 'Strongly disagree (−2)', 'Disagree (−1), 'Neutral (0); 'Agree (+1)', to 'Strongly agree (+2)'. The average was derived by calculating the product of number of participants that scored a scoring category and the value of that category, and summing across the five categories and then dividing by the total number of scoring participants. A total of 43 participants responded to the online poll and scored the 16 initial recommendations at the end of the workshop, having heard the earlier discussions.

Overall, there was generally good agreement with the presented recommendations, with only a few participants (strongly) disagreeing with a specific recommendation. A maximum of two participants strongly disagreed on the same question (Supplementary Material B). All recommendations had positive average scores, most above 1. Only one recommendation (*i.e.* regarding re-analysis of samples from the HBM4EU Aligned Studies by suspect screening and non-targeted screening) had two 'Strongly disagree' votes; this was primarily based on the initial wording that focused on these particular HBM4EU Aligned studies, while other available HBM studies may be suited just as well. Still, the average score was positive for this recommendation with average 0.4. Thus, there was general consensus among participants from science as well as policy makers and other stakeholders that participated in the workshop and the Mentimeter poll.

4. Conclusions and discussion

While the focus during the workshop was on lessons learnt from HBM4EU and conclusions and recommendations are based on HBM4EU results, several of the recommendations corroborate earlier recommendations, *e.g.* from Bopp et al. (2018), Rotter et al. (2018), Kortenkamp and Faust (2018) and Drakvik et al. (2020). They underscore the importance of mixture risk assessment and mixture risk management. In summary, the lessons learnt form HBM4EU are as follows.

1. 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.
2. 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.
3. 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.
4. 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
5. 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.

HBM4EU has contributed substantially to our understanding of the actual mixture exposures and potential health risks in the European population from man-made chemicals. Yet, much work remains to be done on virtually all aspects. The database of HBM levels measured in the same individuals need to be enlarged, both in number of chemicals and in number of individuals in which they are measured. For suspect screening and non-targeted analysis, harmonisation and QA/QC procedures across laboratories needs to be strengthened and further validated; also a wider application of these techniques in HBM is worth striving for. Approaches to address the co-occurrence of chemicals need to be expanded, methodologically and in broader application to other datasets. Toxicity weighting of co-occurrence patterns needs to be brought forward to focus attention on mixtures of concern, for research and for mixture risk management. Drivers of risk, as well as drivers of body burdens, *i.e.* the exposure sources and routes need further attention. Parts of this needed work can be carried forward in Horizon Europe Partnership for the Assessment of Risk from Chemicals (PARC) and in other projects, *e.g.* in exposome research projects.

The discussions during the workshop further illustrated the earlier findings in the initial HBM4EU assessment of potential information needs on mixtures. There, it was observed that opinions on how to deal with mixture risk problems vary considerably between experts and between policy makers. Information needs from policy makers and experts were, at the time of the interviews, still rather diffuse and unarticulated, in line with the 'systemic risk' nature of mixtures. The workshops discussions, particularly the discussions on health risks of mixtures, often addressed issues with the risk *management* practices, more than the technical risk assessment itself. These discussions also demonstrated the diversity of opinions and preferences regarding risk management avenues. Some favor a uniform MAF, other see potential controversy in treatment of 'old' legacy chemicals versus authorization of new chemical. Also, the risk acceptability of different drivers of mixture risk may vary, both in regulatory context as in public perception, based on, for instance, (in)voluntariness of exposure and risk/benefit and 'essential use' considerations.

While there is general agreement with the need for a cross-silo legal framework for mixture risk management, the mixture risk problem is also seen as a 'systemic risk' and a 'wicked problem' from a governance perspective. This implies that (technical) regulations alone cannot bring resolution to the wicked problem. Broader stakeholder involvement and consultation is needed in that respect, in addition to improved mixture risk assessment procedures and practice.

Declaration of competing interest

None.

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Appendix A. Supplementary data

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