1 Prioritised substance group: Mixtures

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The phenomenon of mixtures (in the context of HBM) refers to the common occurrence of chemical xenobiotic substances in the body. 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. 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. The HBM4EU project addresses how HBM data can contribute to both the science and policy/regulation of dealing with the phenomenon of mixtures. Within the HBM4EU project, the focus for chemical mixtures will be on chemicals with exposure routes through the environment, food, occupation and/or consumer products.

The proposed activities on mixtures in HBM4EU were developed by a working group of experts from the member states. This comprised a first inventory in member states of available data, a preliminary inventory of policy needs in EC institutions combined with a preliminary inventory of specific policy needs in member states, a discussion at the Workshop HBM4EU Proposal Development (16-17th of November 2015, Utrecht), a EEA Workshop Activities on Mixtures under the European Human Biomonitoring Initiative (11th February 2016). In the latter, experts and policy makers jointly outlined the challenges that mixtures pose to science and policymaking. The proposed activities on mixtures in HBM4EU were further developed through e-mail exchanges, with periodic presentations to the HBM4EU Steering Group Meetings. The activities have been grouped into various tasks under WP15. Since the start of the HBM4EU project, these tasks have been refined and/or modified where deemed necessary, based on discussions in dedicated WP15 workshops (November 2017, Utrecht; May 2018, Lisbon; November 2018, Paris; July 2019, Berlin).

Hazardous properties

Since a wide range of chemical substances comprise the mixture of chemical substances in the body, and metabolites thereof, all classes of hazardous properties are potentially involved. This poses the challenge to identify where antagonism, addition or synergies in effects come into play, based on the mode(s) of action underlying adverse health effects.

Dealing with mixtures in research poses specific challenges e.g. (Kortenkamp 2007, Slama 2015). In toxicological research, the mode(s) of action of the different chemicals included in a mixture can be studied in more detail, but typically only a few permutations of possible mixtures can be assessed. This does not do justice to the wide array of substance to what populations are exposed to. On the other hand, observational studies in humans may capture these multiple substances, but often fall short in characterising the dynamics of exposure and ADME characteristics (absorption, distribution, metabolism, and excretion) and typically cannot document the modes of action involved and the causality between exposure and observed adverse health effects. Developments in modern techniques such as in sensor technologies, and in epigenomics, transcriptomics, metabolomics, as well as development in biostatistics now allow more in depth research of multiple exposures, body burdens and their effects in humans e.g. (Woodruff 2011, Lenters 2015, Agier 2016). To optimally benefit from these developments new forms of cooperation between traditionally separated research communities and projects need to be built. Based on discussions held in the WP15 workshop in May 2018, five case studies were developed to explore how existing methods can be best applied for human health risk assessment of chemical mixtures and how these methods may inform biomonitoring strategies. The case studies are as follows:

- Developmental neurotoxicity beyond polybrominated diphenylethers
- Heavy metals and nephrotoxicity
- Anti-androgenic chemicals and male reproductive health
- Chromium (VI), nickel and polycyclic aromatic hydrocarbons and lung cancer
- Differential exposure misclassification in HBM mixture data due to differences in half-lives between substance

Thus far, the case studies have resulted in an advanced decision tree and workflow scheme for the hazard assessment of chemical mixtures, including decision rules to define when analyses should be refined or discontinued, and adopting clear criteria for the grouping of chemicals to be subjected to mixture risk analyses. These grouping criteria are based on Adverse Outcome Pathway considerations. The lessons learned from the five case studies will comprise relevant insights into the possibilities and limitations of existing methods for human health risk assessment of chemical mixtures, and provide valuable input to recommendations for policy-making and further research.

Exposure characteristics

A central problem in the discussion on mixtures is the virtual absence of adequate exposure data. In many HBM projects, as well as in cohort studies and biobank studies, multiple (groups) of pollutants have been studied; yet the reporting is typically restricted to distributions and central tendency measures of single compounds or groups of compounds. The groups are often clustered on:

- chemical families, e.g. phthalates, bisphenols, dioxins, PCB's, PAH's, VOC's
- exposure routes, e.g. food, household dust
- type application such as plasticisers, flame retardants, pesticides
- supposed working mechanisms e.g. endocrine disruptors, carcinogens, neurotoxins.

In few cases, the distribution of a measure/indicator of cumulative body burdens in individuals is reported. If so, this only summarises body burdens within the clusters mentioned above and hardly ever overarching indicators are used and reported.

Thus, it is largely unknown whether specific profiles of high exposures exist, i.e. individuals high in PCB's are also in pesticides, flame retardants or poly fluorinates compounds or mycotoxins. Meaningful indicators to capture such profiles need to be developed for mixtures in the wider meaning of the word. With such aggregated mixture indicators exposure profiles of concern and potential hotspots or risk groups can then be identified in existing data and in new studies. Therefore, also existing data merit re-evaluation from a mixture perspective.

Policy relevance

Dealing with mixtures poses substantial regulatory challenges, with numerous pertinent EU and national regulations.
In the European Directive 396/2005 EFSA was appointed to be responsible for establishing the methodology for risk assessment of mixtures. It states among other things *"…It is also important to carry out further work to develop a methodology to take into account cumulative and synergistic effects. In view of human exposure to combinations of active substances and their cumulative and possible aggregate and synergistic effects on human health, MRLs should be set after consultation of the European Food Safety Authority….*". Since 2005 EFSA has published four Opinions and one Guidance on how to perform risk assessment for pesticide mixtures. The full methodology was discussed during an EFSA info session organised to discuss the methodology with the stakeholdersi. Also JRC has published several reports on assessment of mixtures, that advocate a new test strategy to define the relevant mixtures. EFSA takes pesticides as a concrete point of departure to develop strategies for dealing with mixtures. Such strategies, once developed, will then be generalised to other forms of mixtures. Central in this approach is the grouping of substances into so-called CAGs, i.e. cumulative assessment groups. Such CAGs are developed on the basis of similar target organs and/or mode of action (Rotter et al. 2019).

Several Member States (MS) also have issues reports and opinions on dealing with mixtures. For instance in the Netherlands, avoidance of cumulative exposures (of all environmental agents, not just substances) is one of the corner stones of modern environmental policy[1]. In France, the new health law (currently under consideration) indicates that the identification of risks health should be done relying on the Exposome concept, integrating the effects of exposures to all non-genetic factors.

While there is a clear information need articulated from the side of policy makers, there is less insight in the possible action perspectives for policy makers and stakeholders in dealing with mixtures. Moreover, it is difficult to assess "value of information" for HBM data on mixtures: at what point would additional information on HBM and exposure to mixtures (based on HBM data, or the combined knowledge base) lead to other decisions and other/further policy actions? Should exposure to all substances in the mixtures be reduced, or the one with the highest impact on adverse health outcomes, the one with easy and safe alternatives/replacements, or the ones with the least costs to reduce, or should the cost-benefit ratio of each source/exposure route be taken into account. One can imagine that the cost-benefit ratio to reduce BPA exposure for babies, children, shop personnel, or in medical (emergency) equipment, may vary substantially.

Moreover, when mode of action (MoA) and adverse outcome pathways (AOP) are taken as point of departure to assess acceptability of the combined health impacts of exposure to mixtures, there may well be a need to compare across substances emerging from different types of applications, e.g. flame retardants, pesticides, plasticizers, and food additives/contaminants.

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[1] Ministry of Infrastructure and the Environment (2014). Explicitly dealing with safety' (in Dutch) Bewust Omgaan met Veiligheid, Rode Draden; Een proeve van een IenM-breed afwegingskader veiligheid. 's Gravenhagen, Ministry of Infrastructure and the Environment.

For HBM data on mixtures to be meaningful for policy development, it is necessary get further insight in and articulation of the expectations and primary policy needs already in the design phase of the research.

Based on, among other things, the above, the challenge from the Policy Board laid out for HBM4EU activities reads “We encourage the consortium to start addressing identification of chemical mixtures to which humans are exposed and develop concrete activities, across all three pillars, which would be carried out in the second half of the project. The pre-defined mixtures of substances having common mode of action could frame the initial perspective on this topic”.

1.1 Categorisation of Substances

Mixtures as a group fall into category C (Very little or no Human Biomonitoring data and/or information on toxicological/health effects or external exposure is available). While single chemicals, or even chemical family groups such as PCB’s may warrant a category A or B classification, the essence of the mixture issue is the many unknowns about joint and cumulative exposure, combined mode of actions and overall adverse outcomes and health risks and impacts. Data coming available under category D and E would ultimately also fall under the Mixture umbrella.

1.2 Objectives / Policy-related questions

The overarching objective of the mixture activities in HBM4EU is to improve the efficacy of HBM to inform science, policy/regulatory actions and societal debate with respect to dealing with mixtures.

Some underlying questions include:

- What is the information need of regulatory bodies and stakeholders?
- What are common HBM mixture patterns in the European population and how do these unintentional mixtures vary across countries?
- Can we identify hotspots or risk groups with high mixture exposures?
- Which sources & pathways contribute most to HBM mixture values?
- What are the impacts of chemical mixtures on human health, and how can this inform risk assessment for mixtures, including EFSA‘s work on pesticides?
- What action perspectives are available to reduce mixture levels?

The more specific objectives are:

- Develop summary indicators to describe the exposure and body burdens of mixtures with an emphasis on defining priority mixtures and drivers of mixture toxicity
- Re-evaluate existing HBM mixture data to identify real-life exposure patterns to mixtures
- Collect new HBM mixture data in selected European countries
- Further develop and apply practical approaches to assess the potential health risks and impacts of mixtures by conducting case studies
- Inform policy makers, stakeholders and the public at large about mixture exposures, possible health risks and action perspectives

1.3 Research Activities to be undertaken

Table 1: Listing of research activities to be carried out to answer the policy questions

Substance	Available knowledge related to policy question	Knowledge gaps / Activities needed to answer policy question
All	What is the information need of regulatory bodies and stakeholders?	In 2017 preparations started for develop exchange of information and establish cooperation amongst Horizon2020 funded projects on mixtures. To this end, two workshops have been organised, in which HBM4EU participated. This resulted in two publications (Bopp et al, 2018; Drakvik et al, 2020)
All	What are common HBM mixture patterns in the European population and how do these unintentional mixtures vary across countries?	In WP15, task 15.1 summary indicators are being developed to describe the exposure and body burdens of mixtures with an emphasis on defining priority mixtures and drivers of mixture toxicity. With these indicators we will re-evaluate existing HBM mixture data to identify real-life exposure patterns to mixtures and to obtain insight into the commonalities and differences across Europe.
All	Can we identify hotspots or risk groups with high mixture exposures?	In WP15, task 15.2, the SPECIMEn study is being conducted in five countries, with the aim to identify possible hotspots and risk groups.
All	Which sources & pathways contribute most to HBM mixture values?	In WP15, in concert with WP12, we are addressing source attribution to observed HBM mixture data
All	What are the impacts of chemical mixtures on human health, and how can this inform risk assessment for mixtures, including EFSA‘s work on pesticides?	In WP15, task 15.3, five case studies are being performed to obtain insight into the possibilities and limitations of existing methods for human health risk assessment of chemical mixtures
All	What action perspectives are available to reduce mixture levels?	In WP15, together with WP5, we will evaluate possible action perspectives and develop concrete recommendations for policy and further research

1.4 References

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