EVENT REPORT

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EFSA International Workshop on RA of Combined Exposure to Multiple Chemicals

European Food Safety Authority

Jean-Lou CM Dorne, Christer Hogstrand, Bruno Dujardin, George EN Kass, AK Djien Liem, Jose Tarazona, Kyriaki Machera, Tobin Robinson, Paola Manini, Emilio Benfenati, Jan Dirk te Biesebeek, Josef Schlatter, Paul Price, Patience Browne, Alan R Boobis, Angelo Moretto, Anthony Smith, Emanuela Testai, Antonio Hernández-Jerez, Susanne Hougaard-Bennekou, Vittorio Fattori, Stephanie Bopp, Heather Wallace

Abstract

Human Risk Assessment (RA) of combined exposure to multiple chemicals follows the steps of RA namely problem formulation, exposure assessment, hazard assessment and risk characterisation. Over the last decade, scientific advisory bodies have published a range of harmonised guidance documents to provide practical approaches for risk assessors. These include the WHO, the US-EPA, the Joint Research Centre (JRC) of the European Commission, the OECD and the recent EFSA MIXTOX guidance document dealing with harmonised methodologies for the human health, animal health and ecological RA areas. On May 21st 2021, the Scientific Committee of EFSA published a draft guidance document on scientific criteria to group chemicals in assessment groups using hazard-driven criteria and prioritisation methods using exposure-driven and risk-based approaches. The latter guidance and future challenges were the topic of this international workshop which was held online in the afternoons of the 18th, 19th and 20st October 2021. First, a plenary session provided overviews of European and international activities in the field including WHO, OECD, US-EPA, JRC and EFSA. With a total of 118 participants including national and international scientific advisory bodies, academics and researchers, NGOs, industry, risk assessors, risk managers, four discussions groups were created for three break-out group sessions: 1) Hazard-driven criteria for grouping multiple chemicals into assessment groups; 2) Exposure-driven and risk-based criteria for the prioritisation of multiple chemicals 3) Data gaps and future challenges for human health and environmental RA of combined exposure to multiple chemicals to identify priority topics of international relevance and a way forward to address these topics. A final discussion and wrap-up session concluded the meeting. This event report provides a summary of the discussions together with conclusions and recommendations.

Key words: RA, combined exposure, multiple chemicals, grouping, scientific criteria, future challenges

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Correspondence: any enquiries related to this output should be addressed to MESE@efsa.europa.eu

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Summary

Human Risk Assessment (RA) of combined exposure to multiple chemicals "chemical mixtures" follows the standard steps of RA: problem formulation, exposure assessment, hazard assessment and risk characterisation. Over the last decade, scientific advisory bodies have published a range of harmonised guidance documents to provide practical approaches for risk assessors. These include the WHO, the US-EPA, the Joint Research Centre (JRC) of the European Commission, the OECD and the recent EFSA MIXTOX guidance document on "harmonised methodologies for the human health, animal health and ecological RA of combined exposure to multiple chemicals" to cite but a few.

First, the problem formulation considers the question to be addressed including the regulatory and scientific context and the approach taken for the RA in the light of data availability, time, and resources. Tiered approaches allow a risk manager to deliver fit for purpose RA and there are multiple tiers for the exposure assessment, hazard assessment and risk characterisation portions of a risk assessment. Low tiers often reflect prioritisation exercises, data poor situations, or urgent requests. Such tiers make use of conservative assumptions. In contrast, higher tiers are applied to situations where a full RA is required either under a regulatory framework or in a data rich situation and allow the use of more quantitative approaches (i.e. probabilistic approaches) and a better characterisation of uncertainties.

This principle implies that the RA can be finalised when the assessor determines that the exposed population has met the protection goals defined in the problem formulation. In contrast, when an assessor concludes that insufficient protection has occurred, a higher tier assessment, risk management, or risk mitigation measure may be needed.

Depending on the question defined in the problem formulation, the nature of the mixture and the characterisation of its individual components, two main approaches are applied: the whole mixture approach and the component-based approach (WMA and CBA). In principle, the WMA aims to assess the whole mixture as a single compound. On the other hand, the CBA, as the preferred approach, aims to group each component into assessment groups and then apply dose addition as the default model for assessing combined toxicity (unless evidence for interactions e.g., synergism, potentiation, antagonism) is available).

A critical step for the CBA is the application of scientific criteria to group chemicals in assessment groups and such grouping is performed in the problem formulation step. These criteria are very often hazard-driven using available hazard information (e.g. mode of action, adverse outcome pathways, target organ toxicity, specific toxicological effects) but in some instances, the approach can be refined using exposure-driven and risk-based criteria.

After the publication of the MIXTOX guidance, EFSA's Scientific Committee has been requested to address through a guidance document such scientific criteria for grouping chemicals into assessment groups while taking into account:

- Scientific principles from EFSA MIXTOX guidance as well as other relevant cross-cutting guidance documents (weight of evidence, biological relevance, uncertainty).
- The context of the RA (prioritisation, urgent RA, pre- and post-market RA), data availability, time and resources for the grouping of chemicals defined in the problem formulation.
- Tiering principles, fit for purpose scenarios considering available hazard, exposure information and additional considerations of relevance (e.g. adverse outcome pathways, TK and HBM.
- Relevant EFSA sectoral regulatory provisions and activities on cumulative assessment groups (CAG) for pesticides, relevant RA activities on contaminants, any other relevant panel and related European activities (European Commission, JRC, ECHA, EMA, EEA, Horizon 2020 projects).
- Relevant international activities (e.g. OECD) and the recent practical approach developed during the WHO/FAO consultation to be piloted by JMPR and JECFA.

This international workshop was held online from in the afternoons of 19th, 20th and 21st October 2021. First, a plenary session provided overviews of European and international activities including WHO, OECD, US-EPA, JRC and EFSA. The workshop then aimed to provide scientific discussions with international stakeholders (national and international scientific advisory bodies, academics and researchers, NGOs, industry, risk assessors, risk managers) and was split into three break-out group sessions followed by plenary sessions and a final wrap up plenary discussion. The three break-out group sessions discussed the following topics:

- Scientific criteria for grouping chemicals into assessment groups for human RA of combined exposure to multiple chemicals based on the EFSA draft guidance document published on 25 May 2021 for public consultation and other international documents and activities (FAO/WHO, OECD, JRC etc). This discussion allowed EFSA to further support harmonisation while integrating an international perspective in the final guidance document.
- Exposure-driven and risk-based criteria for the prioritisation of multiple chemicals
- A discussion on data gaps and future challenges in RA of combined exposure to multiple chemicals for the human health and the environmental areas to support EFSA and the international community while identifying priority risk topics of international relevance and a way forward to address these topics.

For each of the three break-out sessions, the four break-out groups were given the task to address the following questions:

Break-out session 1: Hazard-driven criteria for grouping chemicals into assessment groups

Question 1. The decision tree provided in the briefing notes for grouping chemicals into assessment groups uses toxicity information and mechanistic data as the gold standard. When such data are not available, phenomenological effects, target organ/system toxicity, common adverse outcome or in vitro, in silico bridging data' can be used for the grouping. Please comment on these proposals (including the example for the WoE approach in Appendix A), can you identify any challenges to implement such methodologies? It is proposed to focus on data rich, data poor chemicals and groups of chemicals with heterogenous data available (data rich and data poor).

Question 2. The use of toxicokinetic data and models for grouping chemicals into assessment group can support the grouping of chemicals. Please comment on these proposals, can you identify any challenges to implement such methodologies? It is proposed to focus on data rich, data poor chemicals and groups of chemicals with heterogenous data available (data rich and data poor).

Question 3. What are the current data gaps to apply hazard-driven criteria for grouping chemicals and how can they be overcome? Can NAMs (i.e. in vitro data, in silico models etc.) support filling such data gaps?

Break-out session 2: Exposure-driven and risk-based criteria for the prioritisation of multiple chemicals

Question 1. The workflow provided in the briefing notes describes the application of prioritisation methods for risk-based and exposure-driven approaches and supports the identification of low priority chemicals in different RA contexts. Please comment on the workflow proposal (as well as Appendices C, D and E). Can you identify any challenges to implement such methodologies? It is proposed to focus on data rich, data poor chemicals and groups of chemicals with heterogenous data available (data rich and data poor).

Question 2. Can you identify any challenges to apply threshold values for the implementation of prioritisation methods? It is proposed to focus on data rich, data poor chemicals and groups of chemicals with heterogenous data available (data rich and data poor).

Question 3. What are the current data gaps to apply such prioritisation methods and how can they be overcome?

Break-out session 3: Future Challenges for the RA of combined exposure to multiple chemicals

Question 1. What are the future challenges in the area of methodologies and guidance document development for the RA of combined exposure to multiple chemicals area? Please address both whole mixture and component-based approaches and steps of the RA process for human health, animal health and the environment. Identify key activities that would support solving such challenges?

Question 2. What are the future challenges in the area of data and computational tools for the RA of combined exposure to multiple chemicals area? Please address both whole mixture and component-based approaches and steps of the RA process for human health, animal health and the environment. Identify key activities that would support solving such challenges?

Question 3. What are the key challenges to implement the use of NAMs to RA of combined exposure to multiple chemicals area in the different areas including human health RA, animal health RA and ecological RA. Provide practical contexts and give examples.

This event report provides a summary of the discussions for each break-out sessions together with conclusions and recommendations for future activities.

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1. Introduction

Human RA of combined exposure to multiple chemicals "chemical mixtures" follows the standard steps of RA: problem formulation, exposure assessment, hazard assessment and risk characterisation. Over the last decade, scientific advisory bodies have published a range of harmonised guidance documents to provide practical approaches for risk assessors. These include the WHO, the US-EPA, the Joint Research Center (JRC) of the European Commission, the OECD and the recent EFSA MIXTOX guidance document on "harmonised methodologies for the human health, animal health and ecological RA of combined exposure to multiple chemicals" to cite but a few (WHO, 2010; Meek et al., 2011; OECD, 2018; EFSA SC, 2019, US-EPA, 2019).

First, the problem formulation considers the question to be addressed including the regulatory and scientific context and the approach taken for the RA in the light of data availability, time, resources. For this purpose, tiered approaches allow to deliver fit for purpose RA and are applied to exposure assessment, hazard assessment and risk characterisation. Low tiers often reflect prioritisation exercises, data poor situations or urgent requests and require conservative assumptions. In contrast, higher tiers are applied to situations under which a full RA is required either under a regulatory framework or in a data rich situation (e.g. well characterised contaminants). Such higher tier approaches allow more accurate and quantitative RA through a better characterisation of uncertainties through probabilistic approaches (WHO, 2010; Meek et al., 2011; OECD, 2018; EFSA SC, 2019, US-EPA, 2019). This principle implies that the RA can be finalised when sufficient protection for the exposed population is reached from the protection goals defined in the problem formulation. In contrast, when insufficient protection is concluded, a higher tier, risk management or risk mitigation measures may be needed.

Depending on the question defined in the problem formulation, the nature of the mixture and the characterisation of its individual components, two main approaches are applied: the whole mixture approach and the component-based approach (WMA and CBA). In principle, the WMA aims to assess the whole mixture as a single compound. On the other hand, the CBA, as the preferred approach, aims to group each component into assessment groups and apply dose addition as the default model for assessing combined toxicity unless evidence for interactions is available (i.e. synergism, potentiation, antagonism). A critical step for the CBA is the application of scientific criteria to group chemicals in assessment groups. These criteria are very often hazard-driven using available hazard information (e.g. mode of action (MoA), adverse outcome pathways (AOP), target organ toxicity, specific toxicological effects) but in some instances can be refined using exposure-driven and risk-based criteria.

After the publication of the MIXTOX guidance, EFSA's Scientific Committee has been requested to address through a guidance document, these scientific criteria for grouping chemicals into assessment groups while taking into account:

- Scientific principles from EFSA MIXTOX guidance as well as other relevant cross-cutting guidance documents (weight of evidence, biological relevance, uncertainty).
- The context of the RA (prioritisation, urgent RA, pre- and post-market RA), data availability, time and resources for the grouping of chemicals defined in the problem formulation.
- Tiering principles, fit for purpose scenarios considering available hazard, exposure information and additional considerations of relevance (e.g. AOPs, TK and HBM).
- Relevant EFSA sectoral regulatory provisions and activities on cumulative assessment groups (CAG) for pesticides, relevant RA activities on contaminants, any other relevant panel and related European activities (European Commission, JRC, ECHA, EMA, EEA, Horizon 2020 projects).
- Relevant international activities (e.g. OECD) and the recent practical approach developed during the WHO/FAO consultation to be piloted by JMPR and JECFA.

This international workshop aimed to provide scientific discussions with international stakeholders (national and international scientific advisory bodies, academics and researchers, NGOs, industry, risk assessors, risk managers) with regards to:

- Scientific criteria for grouping chemicals into assessment groups for human RA of combined exposure to multiple chemicals based on the EFSA draft guidance document published on 25 May 2021 for public consultation and other international documents and activities (FAO/WHO,

OECD, JRC etc). This discussion will allow EFSA to further support harmonisation while integrating an international perspective in the final guidance document.

- A discussion on data gaps and future challenges in the area of RA of combined exposure to multiple chemicals for the human health and the environmental areas to support EFSA and the international community to identify priority risk topics of international relevance and a way forward.

2. Break-out Sessions

The workshop has been split into a plenary session, 3 discussion groups associated with 3 plenary sessions and finally with a wrap-up plenary session:

- Break-out session 1: Hazard-driven criteria for grouping chemicals into assessment groups
- Break-out session 2: Exposure-driven and risk-based criteria for the prioritisation of multiple chemicals
- Break-out session 3: Future Challenges for the RA of combined exposure to multiple chemicals

2.1. Break-out session 1: Hazard-driven criteria for grouping chemicals into assessment groups

2.1.1. Briefing Notes

Human health assessment of combined exposure to multiple chemicals ("chemical mixtures") is a challenging topic for scientists, risk assessors and risk managers alike due to the complexity of the problem formulation, the large number of chemicals potentially involved, their toxicological profiles and human exposure patterns to these chemicals. In 2019, EFSA's Scientific Committee (SC) published the MIXTOX guidance document on "harmonised methodologies for human health, animal health and ecological RA of combined exposure to multiple chemicals". MIXTOX supports the harmonisation of methodologies for RA of combined exposure to multiple chemicals through whole mixture and component-based approaches. These methods can be implemented across EFSA's sectors in a fit for purpose manner depending on the question, regulatory context, data availability, time and resources available (EFSA SC, 2019).

In May 2021, EFSA published a draft guidance document "Scientific criteria for grouping chemicals into assessment groups for human RA of combined exposure to multiple chemicals". In this guidance, the use of scientific criteria for grouping of chemicals into assessment groups for human health is explored in the context of the component-based approach. The SC acknowledged that it is not feasible to start a RA from the whole universe of chemicals and, in practice, legal requirements or specific concerns often pre-define the chemicals to be assessed together and the assessment is restricted in the terms of reference (ToR) to specific groups of chemicals (e.g. plant protection products, contaminants) (EFSA SC, 2021). Regulatory criteria may provide a basis for a preliminary assessment group based on a common regulatory domain and are most often set by risk managers in the ToR, based on legislative requirements. In addition, prioritisation methods can also be considered using risk-based or exposure-driven methods and provide options to identify chemicals which are expected to contribute only marginally to the combined risk. Such 'low priority chemicals' may then be excluded from further grouping (see break-out session 2).

Hazard-driven criteria for grouping chemicals into assessment groups use similarity of toxicological properties for each individual chemical under consideration and the aim of this break out session is to discuss EFSA's proposal for application of such hazard-driven criteria. Overall, a decision tree has been proposed using mechanistic information on toxicity as the gold standard for grouping where available (i.e. common MoA or AOP). This top-down hierarchical process for grouping chemicals into assessment groups using hazard-driven criteria requires the application of a weight of evidence (WoE) approach to assemble, weigh, and integrate the available lines of evidence on toxicity through a structured approach and is described in figure 1 taken from the draft guidance on "scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals" and published on 25th May 2021 for public consultation (EFSA SC, 2017a,b; 2018, 2021) and an example is

provided in Appendix B of the Guidance (i.e. generic WoE Methodology for grouping multiple chemicals into assessment groups using hazard-driven criteria).

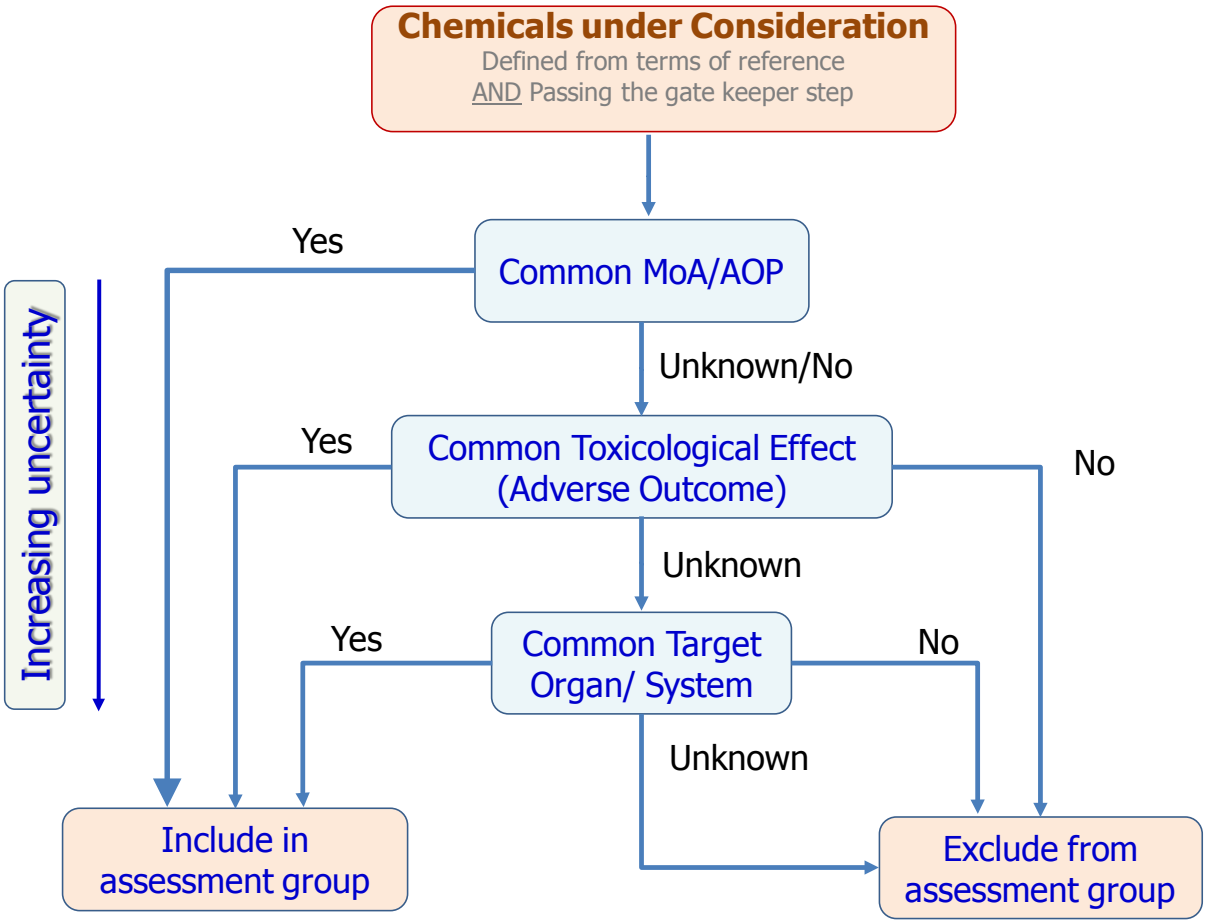


Figure 1: Top-down hierarchical process for grouping chemicals into Assessment Groups using hazard-driven criteria. The thickest arrow indicates the gold standard hazard-driven criteria ((MOA/Adverse Outcome Pathway) with the lowest uncertainty.

In this context, grouping using such mechanistic information results in the lowest uncertainty. When such mechanistic data are not available, grouping may be performed using phenomenological effects or target organ/system toxicity, a common adverse outcome (AO) and the uncertainty associated with the resulting assessment group would be higher. 'In vitro or in silico bridging data' (e.g. in vitro toxicity assays, QSAR models and read-across techniques as part of New approach Methodologies (NAMs) can also be applied for the grouping of data poor chemicals, when no or scant toxicological information are available, along with data-rich members of an assessment group. However, the resulting uncertainty may be high. Structural similarity can also support grouping using software tools, such as the OECD QSAR Toolbox, molecular docking and machine learning tools but consideration of several features (i.e. chemical class, common functional groups, common precursor or breakdown products) should be used to increase the confidence in the assessment of similarity across the components. In this case, it is important to assess both similarities and dissimilarities between the chemicals with regards to the presence of specific chemical moieties or structural features, which may impact MoA or toxicity. Overall, it is essential to assess the applicability domain of each in silico model and integrate their prediction results for a given property using WoE methods (EFSA SC 2021).

Toxicokinetic (TK) data can also be useful for grouping chemicals particularly when common toxicologically relevant metabolites are shared among chemicals. While TK information should not be used in isolation for grouping, combining TK and toxicodynamic properties would provide a robust basis for this purpose. Such TK information for grouping includes: a) chemicals that are common substrates of transporters; b) chemicals producing the same metabolite(s) or are common substrates of enzyme isoforms (e.g. phase I or phase II xenobiotic metabolising enzymes). In addition, available TK data (e.g. body burden, clearance, half-life, elimination rate) or TK models in test species and humans (e.g physiologically-based kinetic models) can also be used to refine grouping, if needed, or can provide a basis to compare risk metrics based on internal dose (see prioritisation methods, break out session 2) (EFSA SC, 2021).

DISCUSSION POINTS

1. The decision tree presented above for grouping chemicals into assessment groups uses toxicity information and mechanistic data as the gold standard. When such data are not available, phenomenological effects, target organ/system toxicity, common adverse outcome or in vitro, in silico bridging data can be used for the grouping. Please comment on these proposals (including the example for the WoE approach in Appendix A), can you identify any challenges to implement such methodologies? It is proposed to focus on data rich, data poor chemicals and groups of chemicals with heterogenous data available (data rich and data poor).
2. The use of toxicokinetic data and models for grouping chemicals into assessment group can support the grouping of chemicals. Please comment on these proposals, can you identify any challenges to implement such methodologies? It is proposed to focus on data rich, data poor chemicals and groups of chemicals with heterogenous data available (data rich and data poor).
3. What are the current data gaps to apply hazard-driven criteria for grouping chemicals and how can they be overcome? Can NAMs (i.e. in vitro data, in silico models etc) support filling such data gaps?

2.1.2. Outcome of Break-out session 1

Discussion Point 1

The decision tree from figure 1 for grouping chemicals into assessment groups uses toxicity information and mechanistic data as the gold standard. Such data have however a limited availability for many chemicals. When such data are not available, phenomenological effects, target organ/system toxicity, common adverse outcome or in vitro, in silico bridging data can be used for the grouping.

The framework is using a top-down approach, which is the opposite used so far by other frameworks (i.e. bottom-up). Experts agreed that mechanistic information and AOPs are the gold standard for grouping with the least uncertainty and can also be used to capture potential interactions between chemicals with a WoE approach. It is foreseen that in the future, multiple chemicals will need to be assessed across regulatory domains. The break-out groups noted that the approach proposed in the framework is more easily/frequently applicable to intentional mixtures. For non-intentional mixtures, if the composition is known, it can also be applied, however, it is not the case very often. Further harmonisation of criteria for MixTox within different regulatory frameworks would be useful as well as the use of databases, exploiting their use with artificial intelligence (AI) applied on chemicals to identify relations between chemicals (groups of chemicals). On the other hand, different groups of chemicals are considered but the assessor should be reminded that particular chemicals can belong to several regulatory silos.

It is noted that the AOP does not consider the TK dimension whereas the MoA considers the TK dimension and this should be kept in mind. Even for data rich chemicals, relevant mechanistic data are not often available for grouping based on AOP.

However, the data gap issue on MoA/AOP information remains for many chemicals. *In silico* methods can support filling data gaps to feed AOPs and also provide an alternative means to group chemicals. Hence, the chemical structure can provide some information; however, the uncertainty must be considered. This is typically, the case for flavourings in food or feed for which the chemical structure can be a criterion for allocation of the substances in an assessment group.

In contrast, AOs are not always the most critical endpoints and may not be the best criteria for grouping. It has been noted that the pathway going to the AO box is the same independently on whether the MoA/AOP is known to be different (answer no) or is unknown. This may need some further consideration as the decision tree should be designed to stimulate generating or gathering further mechanistic information. NAMs and other mechanistic data can complement the apical AO from *in vivo* studies. This approach raises the question on connecting the *in vivo* outcomes with *in vitro* endpoints and how these are comparable for grouping. It is proposed to first assess the AO and then fill the AOP when the information is available even through the use of biomarkers of effect for AOPs when available.

As noted by the international community, one challenge is that heterogeneous information is available on chemicals within a group i.e. data rich vs data poor. Is it possible to group all of them? Probability for a substance to belong to an assessment group may differ. *In silico* data can refine such probabilities. It may be important to assess whether the issue of probabilities should be integrated in the guidance document.

Overall, the group concluded that the decision tree is mainly applicable to data rich compounds. It was also noted that for data rich scenarios, the organisation requesting a new authorisation may not be the data owner for substances leading to uncertain membership to an assessment group. In the cases of data poor compounds, a read across or in silico approach should be considered, taking into account both chemical determinants as well as biological determinants and the information from NAMs.

Further considerations and potential refinement of the framework were considered by the break-out groups:

- Criteria for inclusion and exclusion to be defined (wrapping also chemical similarity, TK, etc.) and adding a quantitative evaluation regarding the membership to AOP, AO, or overall effect
- Tools for grouping substances for common MoA: the same substance may be assigned to more than one MoA. In this case, the critical effect and its MoA may be identified and take precedence in the evaluation.
- Systemic exposure (depending on absorption) and bioactivation are 'prerequisite' and often part of the MoA (e.g. when the bioactivation is the key event in the MoA). Thus, TK is included into the MoA framework.
- The membership of an assessment group has a quantitative outcome, therefore, it would be important to introduce better definition moving away from categorical, binary classes.
- How to use the information about uncertainty (See the arrow in the flow chart) particularly when it cannot be quantified or when the levels of knowledge for different components in the mixture is highly variable? It was not clear whether the uncertainty in the grouping should be assessed as the last step of the grouping process (as part of problem formulation) or whether it should be assessed during the risk characterisation step (outside the grouping activity).
- The participants discussed that the use of an additional uncertainty factor for 'mixture' effect, which is not derived using data-based approaches, such as the Mixture assessment Factor (MAF) is not supported. Indeed, EFSA adopted some years ago an uncertainty and probability assessment document that can be used; furthermore the EUROMIX project also developed a similar document.

An important consideration is to better distinguish between data rich chemicals (for which *in vivo* data are available e.g. regulated products) and data poor chemicals (e.g. natural toxins), a new scheme using NAMs has been proposed during this break out session for data poor chemicals as presented below in figure 2. In the future, it is proposed to test and assess the scheme to conclude on its applicability and reliability in the context the use of NAMs for grouping chemicals in assessment groups.

Chemicals with different MIEs may trigger AOP networks that converge to a common AO. Such a network would indicate that the two chemicals had the same AOP and the same AO. Suggesting that they

belonged in the same assessment group. In addition, doses of the two chemicals may follow dose addition if both doses are sufficiently large as to trigger their respective MEIs. But at lower doses where one, or both, of the chemicals do not trigger their MEI's the response will not follow a dose addition. Such a relationship can be described as a threshold dose additivity (Price et al., 2020). For this reason, Figure 2 includes a test if the dose of the chemical is sufficient to result in dose additivity. It is noted that the dose that triggers a chemical's MEI may be lower than the chemical's NOAEL. Thus, two chemicals on a common AOP network but with different MEIs may still display a response when two subthreshold doses are administered (i.e., something from nothing).

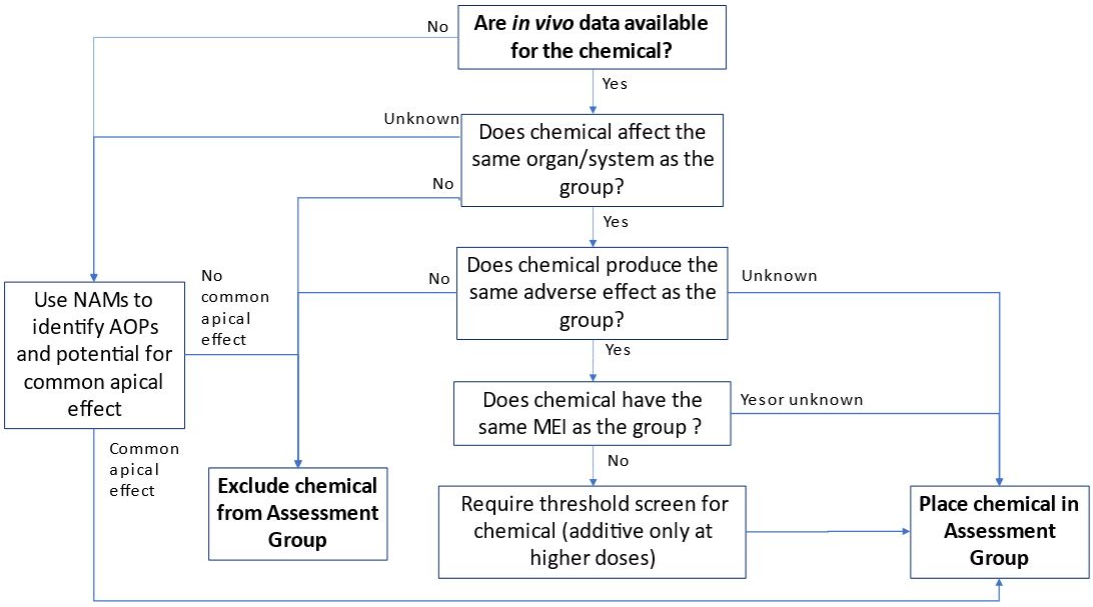


Figure 2: Integration of NAM-based approaches for the grouping of data-poor multiple chemicals using hazard-driven criteria

Discussion Point 2

The use of toxicokinetic data and models for grouping chemicals into assessment group can support the grouping of chemicals. Please comment on these proposals, can you identify any challenges to implement such methodologies? It is proposed to focus on data rich, data poor chemicals and groups of chemicals with heterogenous data available (data rich and data poor).

TK includes absorption, including bioavailability, distribution, metabolism and excretion and can provide information on metabolites and metabolism, such as common metabolic pathway (e.g. the same CYP involved in metabolism). TK data are also critical to identify co-exposure as well as route(s) of excretion and excretion rate. Biomonitoring data provides information on TK. The Aggregate Exposure Pathway (AEP) framework defined in the EFSA MIXTOX guidance is used by the US-EPA to include TK information within the AOP framework. Some TK data are a 'prerequisite' which can support grouping: 1. No absorption results in no systemic exposure and the chemical can be out of a group 2. Part of a MoA analysis and linked to an upstream event i.e. bioactivation. Such information can be useful in the phase of the iterative process for both "grouping" and "ungrouping" for example if substance A and B have different TK, they may be classified as they belonging to 2 distinct assessment groups. However, TK data should not be used in isolation since such data can be relevant to any level in the decision tree. In addition, TK data and Physiologically-based-Kinetic models can provide information on potential interactions (Not expected to have a strong impact at the low exposure levels, e.g. pesticide residues). *In silico* methods are useful to predict TK properties (e.g. metabolism) but are associated with uncertainties that require assessment. For pesticides, there are still large data gaps for TK data, this

conclusion also applies to other chemicals i.e. food and feed additives, food contact materials, contaminants etc.

Discussion Point 3

What are the current data gaps to apply hazard-driven criteria for grouping chemicals and how can they be overcome? Can NAMs (i.e. *in vitro* data, *in silico* models etc) support filling such data gaps?

Many important data gaps have been identified for multiple chemicals and NAMs can support filling such data gaps. Knowledge of the AOP or MoA is often not available (or limited to few components in a mixture). In the broad context of mixtures, further experiments on toxicity of relevant whole mixtures are needed bearing in mind that it is not feasible to test all possible mixtures.

An important issue is to address a common level of granularity rand a definition of the effects (organ level, AO, or AOP, etc.). Participants concluded that data on interspecies differences are often missing, and this issue is relevant to human RA, but it is particularly important and relevant for environmental RA. Physiologically-based-kinetic-dynamic (PB-K-D) models B-KD models are needed. Consideration of toxic effects above the NOAEL can also be used for grouping using hazard-driven criteria including effects at LOAEL. Refinement of hazard-driven grouping can be achieved using exposure levels as well as multiple scenarios including different sources impacting on co-exposure. There is a need to develop a hierarchy of the effects through well-structured ontologies which will contribute to the development of network of effects and more relevant AOPs. Exploitation of Databases (US-EPA CompToxChemistry Dashboard, OECD, OFT, JRC…) and use of Artificial Intelligence will support filling data gaps and the integration of NAMs. AOPs should be fit for purpose and relevant, their development is not necessarily guided by regulatory needs and should have the quality that risk assessors would expect.

NAMs are endpoint driven so that the 'relevant/critical' end point might escape the NAM target. For those chemical groups which are well investigated, there are endpoints (any kind of outcome) for which reliable *in vitro* and *in silico* tools work well while for others they do not. In particular, the use of *in silico* tools or *in vitro* systems can sometimes lead to a lack quantitative information (i.e. what is the real concentration at the target: is it reached *in vivo*?). *In vivo* TK data are needed to estimate the 'internal dose', 'target dose'. Use of NAMs require adequate test systems, study design and QIVIVE models. NAMs can also be used for the identification of the AOP, but biomarkers of adversity should be known and available (TD information needed).

Different *in silico* tools can be applied for hazard-driven criteria and for grouping. Grouping can be supervised for a specific effect or generic effect (unsupervised) simply based on chemical similarity/structure. *In silico* methods can reduce the number of chemicals with data poor information (see Leonard et al., 2018; doi.org/10.1016/j.comtox.2017.10.003). Effects observed *in vitro* or modelled using *in silico* tools such as MIE for endocrine disruption do not always provide information regarding the ultimate apical effect. An appropriate battery of tests has to be applied.

With regards to the use of NAMs, identifying the relevant *in silico* or *in vitro* batteries to use can provide a sound basis for grouping using hazard-driven criteria. *In silico* tools and data require calibration. More specific models including PB-K instead of general models are needed. *In vitro* data can be useful to this end. NAMs can generate information in a faster manner, and at a lower societal cost, compared to *in vivo* testing and can be useful to take provisional decisions. NAMs can also contribute to fill data gaps particularly for the development of AOPs. However, these may not necessarily replace the AO which most of the time comes from *in vivo* testing.

However, there are a number of challenges in reaching consensus regarding the proper use of information generated by NAMs and increasing the confidence in using *in silico* tools requires mid- to long-term experience and case studies since experience is currently limited. This will depend on the scenario e.g. data rich scenarios with *in vivo* databases, *in silico* models may be needed less. In some cases, *in vitro* data may be more biologically-relevant compared to *in vivo* data, bearing in mind the reliability of such methods. Omics also provide a range of endpoints to derive reference points.

2.2. Break-out session 2: Exposure-driven and risk-based criteria for the prioritisation of multiple chemicals

2.2.1. Briefing notes

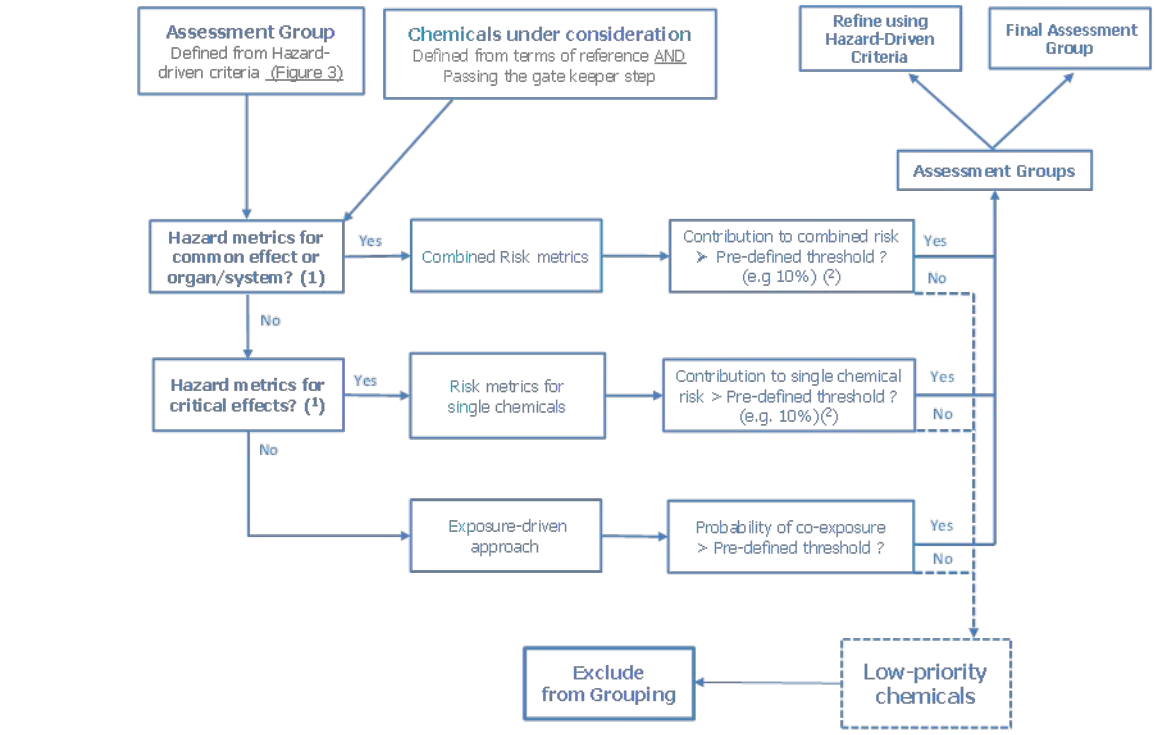
For a given RA of combined exposure to multiple chemicals, chemicals under consideration are pre-defined in the ToR and problem formulation mainly through regulatory or pragmatic criteria (EFSA SC, 2019; EFSA SC, 2021). When the number of chemicals under consideration is *a priori* vast and resources are limited, prioritisation methods can be applied to reduce the number of chemicals to be considered further within an already formed assessment group and these include (EFSA SC, 2021):

1) A combined risk-based approach generates risk metrics using hazard metrics for a common effect or target organ and exposure metrics for individual chemicals using dose addition as the default assumption. Chemicals with a marginal contribution to the combined risk can be identified and excluded from grouping. A threshold value can be used to quantify the marginal contribution to a combined risk for defining low priority chemicals. Such threshold values will depend on the context of the assessment, and the prioritisation method used should be documented. EFSA's SC had recommended threshold values of 10% i.e any chemical contributing more than 10% to the combined risk (threshold value) is retained for refinement of the assessment group using hazard-driven criteria. However, this threshold might not perform well under all circumstances, e.g., when a high number of chemicals have a contribution slightly below the threshold value and would need to be further assessed.

2) A risk-based approach for single chemicals aiming to derive individual risk metrics for each chemical under consideration when hazard metrics for the respective critical effect are available. Low priority chemicals can be identified and can be excluded from further assessment when individual risk metrics are below a pre-defined threshold. The FAO/WHO Expert Consultation on Dietary RA of combined exposure to multiple chemicals has proposed a pre-defined threshold value of 10% for a relevant health-based guidance value or 10-fold of the adequate MOE for each individual chemical (FAO/WHO, 2019). JECFA has recently explored the approach for the RA of multiple veterinary drug residues (diflubenzuron and halquinol) and concluded that for both compounds the estimated dietary exposure from veterinary use was 10% of the upper bound of the Acceptable Daily Intake in any population or subpopulation (FAO/WHO, 2020). EFSA's SC also recommended the use of such threshold values, when information on individual chemicals under consideration are limited while noting that this threshold value may be lowered on a case-by-case basis, depending on the context of the assessment and the experience gained. Threshold values need to be considered in relation to the protection goals defined by the risk managers and the rationale for deviating from the proposed threshold value should be documented.

3) Exposure-driven approaches allow to assess co-exposure to chemicals if hazard information is not readily accessible. Overall, chemicals which are unlikely to co-occur in humans can be considered of low priority for grouping. In essence, this method aims to determine the probability of combined exposure, identify and exclude low priority chemicals for which co-exposure probability is low. It can be applied when hazard metrics are not available to prioritise chemicals with risk-based methods or when large number of chemicals have to be evaluated in a short time frame; hazard metrics should then be collected or generated subsequently. So far, exposure-driven criteria have been applied to identify low priority chemicals from pre-defined chemicals present in the diet using probability of co-exposure patterns. Similar methods were also applied to biomonitoring data in body fluids (blood, breast milk) to provide correlations of internal exposure between multiple chemicals. The SC noted that exposure-driven approaches still have limited applications in the RA conducted by EFSA panels and recommended their use only when risk-based methods cannot be applied, and associated uncertainties should be assessed and documented.

The proposed workflow, which integrates the application of these prioritisation methods in different contexts, is provided below in figure 3.



(1) Hazard metrics may refer to either reference points, reference values or *in silico* predictions thereof.
(2) The definition of a threshold is relative and depends on the type of chemical and legal framework etc. This definition therefore needs to be carefully considered and validated for each assessment framework. Default threshold values of 10% contribution to combined risk and single risk metrics are proposed when no detailed information is available.

Figure 3: Workflow for risk-based and exposure-driven prioritisation methods applied to the grouping of chemicals into assessment groups

Overall, the application of such methods allows to identify low priority chemicals which can be excluded from grouping. For chemicals that have been prioritised, the assessor may conclude on a final assessment group or further refine the assessment group using hazard-driven criteria (see break-out session 1). Appendix C, D, and E of the guidance document (EFSA SC, 2021) provide: a) A concise account of statistical methods to study the probability of combined risk or combined exposure (Appendix C) B) Example of risk-based approach for single chemicals as a prioritisation method for grouping pesticides into assessment groups (Appendix D) and c) Exposure-driven approach as a prioritisation method for grouping multiple contaminants from breast milk and comparison with a risk-based approach for single chemicals (Appendix E).

To generate this guidance the TTC value for a chemical would be used with the exposure estimate to produce a conservative estimate of the HQ for the chemical in the CBA. If the value of the RQ is small (less than 0.1), then the chemical can be excluded from the assessment. However, if the sum of all chemicals with no toxicity data has a significant impact on the final value of HI, then the the approach could be reconsidered.

DISCUSSION POINTS

1. The workflow presented above describes the application of prioritisation methods for risk-based and exposure-driven approaches and supports the identification of low priority chemicals in different RA contexts. Please comment on the workflow proposal (As well as Appendices C, D and E). Can you identify any challenges to implement such methodologies? It is proposed to focus on data rich, data poor chemicals and groups of chemicals with heterogenous data available (data rich and data poor).

2. Can you identify any challenges to apply threshold values for the implementation of prioritisation methods? It is proposed to focus on data rich, data poor chemicals and groups of chemicals with heterogenous data available (data rich and data poor).
3. What are the current data gaps to apply such prioritisation methods and how can they be overcome?

2.2.2. Outcome of Break-out session 2

Discussion Point 1

The workflow presented above describes the application of prioritisation methods for risk-based and exposure-driven approaches and supports the identification of low priority chemicals in different RA contexts.

The workflow helps to capture the complexity of mixtures even if one does not have the same kind of information for all chemicals in the potential mixtures. The framework is useful in the case of absence of toxicology data and can be mostly used for prioritisation of chemicals. One general, key objective of mixture RA is to identify potential public health issues and identify the most relevant contributors for which measures should be taken to mitigate the risk and for such cases. Within the framework, the two entries and multiple use of the term 'assessment group' can be confusing. It should be clearly explained in the text, that the two flow charts are part of a circular, iterative process (changes in the workflow would make the picture too complicated). This iterative process depends on the time and resources available. The exposure driven cut-off value is expressed differently, i.e. as a probability for the chemicals to co-occur. A concrete figure for the probability as a (default) cut-off value was not proposed. Such a number would also be dependent on the protection goal.

The gatekeeper step can actually be read as: is there co-exposure? Does the gatekeeper step need additional clarification? It is not a simple consideration. It does not represent the whole universe of chemicals or the exposome as these are not manageable in practice. This goal may be achievable in the future. Whether several chemicals co-occur or not, i.e., likelihood of co-occurrence or co-exposure. However, defining co-exposure can be a challenge. If there is no co-exposure there is no reason for grouping. The hierarchy of steps in the scheme should be flexible. e.g. exposure might be very useful in the absence of toxicity data.

For method 1, it is not clear what are the advantages to decide on exclusion of chemicals when the RA for the whole group has been done already. In addition, it seems to be a circular process. For method 2, it is based on the individual compound risk level. Maybe it is more practical and possibly not necessarily a second choice after M1. Hazard driven criteria should have been considered before we start the flow chart. There is an option to start from exposure rather than from hazard and this should allow to set priority in specific cases. The workflow is not clear as for the contribution of exposure to the whole procedure.

The proposed cut-off value of 10% of total risk or 10% of the HBGV depends on the regulatory framework, protection goals and the number of substances present, and the prospective (e.g.: pre-marketing for pesticides) or retrospective (e.g: on monitoring data of pesticides) and has been shown to work well for pesticides. The participants also discussed an example on the evaluation of feed additives which reduces the numbers of compounds to be considered based on concentration levels in the considered mixture. It is not fully clear how the concentration threshold is set. This may possibly depend on exposure to the total "mixture" (e.g. based on occurrence and consumption data). Hence, the participants proposed to use the term 'cut-off value' instead of 'predefined threshold'.

How does the workflow perform in very complex situations? Experience should be gathered which will help in the future after implementation of the workflow for prioritisation which will provide a way forward. The consequences of different thresholds (cut-off values) should be further explored.

The participants also discussed the option to combine this screening approach with the TTC for data poor chemicals (when the chemicals do not belong to one of the TTC exclusion categories). The Cramer classes could be used to guide exposure thresholds. To generate this guidance the TTC value for a chemical would be used with the exposure estimate to produce a conservative estimate of the HQ for

the chemical in the CBA. If the value of the RQ is small (less than 0.1), then the chemical can be excluded from the assessment. However, if the sum of all chemicals with no toxicity data has a significant impact on the final value of HI, then the approach could be reconsidered.

A number of challenges have been identified:

- The prioritisation tool seems to work for pesticides, however, for contaminants participants were less confident. The RA of contaminants is not often based on MoA/AOP information since it is often lacking. For example, typically, PFAs are classified on immune suppression e.g. there is no information on MoA. Hence, contaminants are often grouped on chemical similarity. Another example includes, TCCD and even if human exposure is decreasing, this adds up to 50% of the risk. In this context, the assessment is always corrected for body burden because of the persistence of TCDD and prioritisation does not seem to be always logical.
- Biomonitoring of samples such as breastmilk supports the prediction of the exposure to multiple chemicals in infants. However, breastmilk samples will not always predict the body burden of the mother and/or other subpopulations. The ANSES breastmilk example provides a basis to predict which chemicals (e.g. POPs) are important in co-exposure and contributes to grouping. Hence, biomonitoring data contribute to assess chronic exposures even though such exposure data are not always available. A challenge was raised with regards to nutrients because of the big difference between nutrients and non-essential chemicals. RA of non-essential chemicals looks at parts of the population of the highly exposed, while for nutrients, assessors can be concerned with parts of the population with a 'too low intake'. Finally, a further complication is the fact some nutrients require a recommended intake, however, these are also considered toxic when exceeding recommended intakes e.g. copper, zinc or fluoride.

Discussion Point 2

Can you identify any challenges to apply threshold values for the implementation of prioritisation methods? It is proposed to focus on data rich, data poor chemicals and groups of chemicals with heterogenous data available (data rich and data poor).

Generally speaking, the 10% threshold/cut-off values need to be justified based on a range of different exercises and on actual data, under the different regulatory frameworks. It is difficult to generalise the default cut-off value. This should be implemented on a case-by-case basis, depending on the source of the chemicals and such value is independent from data rich or data poor chemicals. It was noted that lower thresholds may be applicable for chemicals which bioaccumulate and are persistent, compared to other chemicals, which may generate "only" acute effects.

A remark was made on the prioritisation default thresholds of 10% (or HQ is 0.1) used to exclude the chemical from the assessment group. It is not fully following the definition set in the 2019 WHO meeting i.e. if the estimated dietary exposure for a single chemical under evaluation is more than 10 percent of the relevant health based guidance value (HBGV), the need to include the chemical in a RA of combined exposure to multiple chemicals should be considered. The WG used 10% as practical cut-off value for chemicals with low priority for exclusion when below this cut-off. Based on its MoA/AOP, the chemical could belong to the assessment group, but its exposure pattern shows it to be less relevant to be considered for the higher tier assessment of combined exposure. It is proposed to use the cut-off value, in combination with the applicable exposure percentiles of risk.

The second footnote (Figure 3) provides very important information and should be made more visible. In addition, the second sentence should be made clearer "default values of 10% contribution to the combined risk and single risk metrics are proposed when no detailed information is available".

The creation of open access databases will support the further testing of this framework with 'real world' data on exposure (including HBM data). Potential co-exposure obtained from such databases could trigger the request to an applicant to produce toxicity data, particularly for regulated products. For other products, public institutions could take care of producing data.

A number of challenges have been identified:

- To what extent is the 10% threshold arbitrary? Is it the result of "harmonisation" across regulatory bodies (WHO, US-EPA, EFSA)? A "validation" step was taken for pesticides and the 10% cut-off values worked well (empirical approach following a kind of sensitivity analysis). Case studies or greater experience for validation and to see how these cut-off values are required.
- Defining criteria for selecting threshold values in the future or to refine those values is required.
- The level of the threshold applies to different levels of protection (e.g., pesticides 99.9th percentile exposure or 95th percentiles for contaminants)
- For data poor chemicals, uncertainties can be higher and a more conservative approach should be used to test if such a cut-off value is still valid. However, these may lead to un-realistically high conservatism. Exploring big data on exposure will allow an initial screening based on actual exposure ranges (possibility/plausibility of co-occurrence and at which level). This can be particularly useful for data poor compounds for which toxicity data are not available or challenging to generate.
- TK and TD interactions should be considered for applying such cut-off values when relevant

Discussion Point 3:

What are the current data gaps to apply such prioritisation methods and how can they be overcome?

Structured databases for HBGV need improvement, including more mechanistic information as well as non-critical endpoints, since such databases will facilitate the implementation of risk-based methods (e.g. EFSA's OFT). In some assessment contexts, this will support risk assessors to move straight away to the grouping exercise without the need for prioritisation.

More information on TK is needed as several chemicals might occur in the body and further development of methods to quantify the probability of combined exposure to chemicals should be developed and tested. Hence, probabilistic exposure assessment methods are needed particularly because very heterogeneous exposure patterns are known in human populations. This concept also applies to non-dietary exposure assessment. HBM data will also help to improve exposure assessment and grouping.

Participants commented that there are "experience gaps" rather than "data gaps" and require more validation studies.

HBGV are mainly based on animal data. Would it be possible to derive them from NAMs?

The Risk-based criteria: 10% as predefined (default) cut-off value for the risk metric boxes has been concluded as a pragmatic and reasonable starting point. However, its validity of the 10% cut-off value has been discussed and two questions were raised:1. What are the consequences of using the threshold of 10% in method 1 and 2? Are there any available methods to test its validity in a range of RA contexts? The combined overall risk is usually driven by few chemicals in a mixture (see EUROMIX data set analysis, other examples include the recent total diet study carried out in African countries). Identified main risk drivers contribute rarely <25% for "real-world" mixtures (e.g. indoor air, waste sites, water quality, environmental contaminants, HBM data). The situation may be different for 'artificial' mixtures created in the laboratory, when compared to the real-world situation since for the composition may be known and the number of multiple chemicals may be more limited. In the case of multiple drug treatments, the 10% value may be too high since doses are the same over time and comparatively high (i.e designed to produce a pharmacological effect), compared with food chemicals where exposure is highly variable and usually lower (i.e. below the HBGV). It was noted that if a compound out of 10 in a mixture is contributing to 10% of the combined risk, the same chemical will contribute less when the number of chemicals in the mixture is high (i.e. 100). However, the experience gained so far shows that often only a few compiunds contribute to driving risk. In addition, there would be value in the testing of the use of the TTC in the implementation of the RA of combined exposure to multiple chemicals. Hence, the cut-off value should be reconsidered on the basis of chemical-specific data and in the light of the assessment context and protection goals (e.g. in specific legislations).

A proposed pragmatic approach would be to select the top 50 chemicals as contaminants, additives, contact materials, etc. and to group them based on hazard-driven criteria including TK properties,

exposure-based methods and assumptions in order to test what are the risk conclusions. Since grouping is an iterative process, assessors gather information and then group. Based on additional information the assessment can be further modified. For examples, dioxins include a large number of congeners (n=201) and the use of TK showed that 17 congeners can be filtered out and in this case the risk is a function of hazard, external exposure + body burden (TK information).

In silico tools provide useful tools to predict migration of chemicals such as those from packaging materials and allow to process high number of chemicals. Such in silico tools thus help in prioritisation and reducing the need to select few chemicals using cut-off values.

For many years, WHO has been collecting breastmilk samples for POPs and panels of experts and risk managers weight the risk-benefit for the individual POPs. Two questions were raised: 1. How to deal with mixtures of POPs in breastmilk or foods? 2. How to compare risk-benefits of breastmilk containing mixtures of POPs, e.g. still present after so many years? *In silico* models have also been proposed as useful tools to address multiple targets simultaneously and to integrate risk and benefit assessments.

Finally, the participants raised the need to broaden the knowledge base through combining expert knowledge from academia, scientific advisory bodies (BfR, ANSES, RIVM, KEMi, etc.), EFSA/ECHA/JRC/US EPA) and active participation in relevant research projects (Athlete, Euromix follow-up, PARC, HBM4EU, etc..).

2.3. Future Challenges for the RA of combined exposure to multiple chemicals

2.3.1. Briefing Notes

RA of combined exposure to multiple chemicals is a multidisciplinary field which often requires dedicated development within various disciplines. Hence, available sources of information and data on combined exposure, hazard and risk are scattered but are also diverse in nature and definitions, models and metrics used in each field. However, such apparent divergences also mask an underlying high degree of similarity which provides an opportunity for harmonisation of methods, data collection and the development of computation tools as well as to identify remaining gaps (EFSA SC, 2019). With regards to future challenges, national and international guidance documents and research projects have identified data gaps and provided a number of recommendations to further support RA of combined exposure of multiple chemicals (e.g. US-EPA, WHO, OECD, JRC, ECHA, EFSA etc.). In this respect, the recent EFSA and FAO/WHO provided a number of recommendations for future work to support respectively harmonised RA of combined exposure to multiple chemicals (EFSA SC, 2019; FAO/WHO, 2019) and grouping of chemicals in assessment groups for human health (EFSA SC, 2021):

Recommendations from the EFSA guidance documents (EFSA SC, 2019, EFSA SC, 2021) include key aspects for the implementation of methodologies, data collection and open-source tools for exposure assessment, hazard assessment and risk characterisation of multiple chemicals and international scientific cooperation:

- Applicability of the guidance documents tested through a testing phase and the development of specific case studies. This including more complex scenarios on single and multiple species, relevant to the different EFSA panels.
- Probabilistic exposure assessment methodologies and guidance for aggregate exposure assessment methodologies for single and multiple chemicals
- Non-target chemical analysis (broad scope chemical screening) for the characterisation of chemical mixtures.
- Landscape modelling in ecological RA of multiple chemicals to integrate taxa-specific hazard information, exposure information, eco-epidemiological information in a spatial explicit fashion for different habitats and ecosystems.
- Methodologies for RA of exposure to multiple chemicals combined with other stressors (e.g. biological hazards, physical agents).
- Open source curated tools and databases for exposure and hazard assessment of multiple chemicals including production, use, occurrence (e.g. IPCHEM), consumption data and toxicity

including update of EFSA's OpenFoodTox database with systematic data collection for individual chemicals reporting hazard metrics (e.g. specific effects, target organs, MoA, AOPs, Kes, and related properties) and the development and implementation of generic in silico approaches to support grouping of chemicals for combined toxicity (i.e. QSARs) and TK properties (i.e. biologically-based and TK models). This will support the development of NAMs for grouping multiple chemicals based on a) predictions of the interaction between chemicals and their molecular targets, b) predictions of toxicological endpoints (i.e. phenomenological effects).

- Methodologies to investigate interactions using TK and TK-Toxicodynamic (TD) models including dose dependency of specific TK/TD interactions (inhibition, induction of phase I, II enzymes and/or transporters, inhibition of repair mechanisms), use of binary interaction data to predict effects of mixtures with more components, use of extra uncertainty factors for interactions when justified, particularly when components are present below their individual reference points.
- Using OECD harmonised templates (OHT) to structure data on chemical properties to:
  - Develop structured means for WoE approaches and avoid divergence for grouping chemicals into assessment groups across EFSA Panels in different assessments.
  - Support integration of NAMs (in vitro, OMICs, in silico models and generic physiologically-based TK and TK-TD models etc.) to support component-based approaches as currently investigated world-wide (OECD, US EPA, EFSA) and Horizon 2020 and Horizon Europe programmes (EuroMix, EUTOXRISK, HBM4EU, PARC etc.). NAM Implementation can be supported through the use of the OHT 201 template for intermediate effects which can improve the mechanistic basis for setting assessment groups using data on MoA, KEs and AOPs for multiple chemicals.
- For prioritisation methods a) appropriateness of threshold values for risk metrics needs to be considered depending on the regulatory context of the assessment (i.e., protection goals), data availability and number of chemicals under consideration particularly for the default threshold values of 10% (combined risk or to single risk metrics). b) Develop and implement user-friendly open-source tools to implement risk-based and exposure-driven approaches including simple deterministic and probabilistic methods for which further implementation.
- Improve inter-agency, Member states, and international cooperation to facilitate data exchange and harmonisation of methods and tools.

The FAO/WHO Expert Consultation on Dietary RA of combined exposure to multiple chemicals proposed key recommendations for the human health area including: a) database development, b) reporting of RA outcomes and future work for JECFA and JMPR experts. A particular recommendation was provided with regards to access to a suitable tool and any associated computational facilities for probabilistic modelling of combined exposures to multiple chemicals should be made available to JECFA/JMPR experts together with associated training (FAO/WHO, 2019).

It should be noted that EFSA is currently planning several activities that are intended to address part of the recommendations listed above. This includes:

- Transition of the MCRA platform, developed by Wageningen University & Research (WUR, Biometris) for the Dutch Institute for Public Health and the environment (RIVM), towards an Open MCRA platform. This web-based platform brings together relevant tools for RA of combined exposure to multiple chemicals, and will be improved in terms of transparency, interoperability, accessibility and harmonisation (across regulatory domains).
- Update of OpenFoodTox through data collection of hazard metrics (e.g. specific effects, target organs, MoA, AOPs and related properties) (OpenFoodTox 3.0).
- Development of biologically-based models for humans, animals and species of ecological relevance for single chemicals, multiple chemicals and multiple stressors through procurements and implementation in EFSA's TKplate: article 36 grant "Modelling human variability in toxicokinetic and toxicodynamic processes using Bayesian meta-analysis, physiologically-based modelling and in vitro" (GA/EFSA/SCER/2015/01) (Testai et al., 2021); procurement "Data

collection, update and further development of biologically-based models for humans and animal species to support transparency in food and feed safety" (OC/EFSA/SCER/2020/03) and procurement call "TKplate 2.0: An Open source platform integrating physiologically-based kinetic and physiologically-based kinetic-dynamic models and machine learning models for RA of single and multiple chemicals and biological stressors in animal species" (OC/EFSA/SCER/2021/07) (Bossier et al., 2020).

DISCUSSION POINTS

1) What are the future challenges in the area of methodologies and guidance document development for the RA of combined exposure to multiple chemicals area? Please address both whole mixture and component-based approaches and steps of the RA process for human health, animal health and the environment. Identify key activities that would support solving such challenges?
2) What are the future challenges in the area of data and computational tools for the RA of combined exposure to multiple chemicals area? Please address both whole mixture and component-based approaches and steps of the RA process for human health, animal health and the environment. Identify key activities that would support solving such challenges?
3) What are the key challenges to implement the use of NAMs to RA of combined exposure to multiple chemicals area in the different areas including human health RA, animal health RA and ecological RA. Provide practical contexts and give examples.

2.3.2. Outcome of Break-out session 3

Discussion Point 1

What are the future challenges in the area of methodologies and guidance document development for the RA of combined exposure to multiple chemicals area?

The participants highlighted that the first future challenge for the RA of combined exposure to multiple chemicals is methodological since it is not possible to find a single methodology that would fit the human health, animal health and ecological RA areas even though EFSA has made efforts to harmonise the methods across these areas. A point of discussion was the need for further harmonisation between OECD and EFSA guidance documents. There is a need for a roadmap in this field to be discussed with risk managers since current regulations are not fully fit-for-purpose. In this context, EFSA is now planning to develop roadmaps and to contract (externalise) to do so and to move towards open-source platforms, data consolidation (e.g., pesticides), food additives and food contact materials are also being considered now. In addition, model development for the use of NAMs including OMICs data to improve grouping and aggregate exposure to integrate non-dietary exposure. More concrete areas to be considered for mixture RA including ecological RA for which EFSA does not have a dedicated guidance document. For the environmental RA, more interaction with EEA would be required, particularly in the field of feed additives and consideration of a system approach as the one included in the Must-B opinion is an option.

Whole mixtures are complex with many compounds for which analysis from the chemistry point of view is a challenge and toxicological data are missing. Further work in this area is needed.

NAMs could be very useful in this area although the terms is sometimes too generic and specific NAMs should be identified to answer specific questions, e.g. species differences or identification of apical effects. NAMs should be evaluated for their applicability to the RA of whole mixtures. A level of complexity to tackle is the fact that these whole mixtures can be very dynamic and diverse in time and therefore may not show homogenous structural similarity and further guidance on how to tackle such complexity is recommended. While using the whole mixture approach, it is practically impossible to have an accurate characterisation of mixtures of natural origin.

Since RA methodologies have been developed, risk assessors are now in a position to perform RA for mixtures and further refinement of methods and models is needed including methods for assessing and defining co-exposure.

The participants proposed a range of future activities:

- International harmonisation on hazard identification and characterisation.
- Develop harmonised approaches for monitoring of chemicals of concern.
- Need for global data repository on hazard (HBGVs, target tissue/organ) and exposure data.
- Develop ERA and whole mixture specific guidance documents in this area
- Bringing access to HBM data and use the produced data from EU funded and national funded projects in the human health area
- Horizon scanning and harmonised methods are needed for the preparedness in cases of pollution crisis at local or regional level to perform (e.g. PFAS, dioxins).
- Results from toxicity studies may differ from what expected from chemical composition due to presence of e.g., compounds with genotoxic alerts.

Discussion Point 2

What are the future challenges in the area of data and computational tools for the RA of combined exposure to multiple chemicals area?

Some general considerations were first discussed. These include the need to improve and increase the number of risk assessment that are performed for single chemicals across all sectors (food, feed, industrial chemicals, pharmaceuticals etc), the need to perform RA across regulatory silos and the question as to whether we are investigating the right stressors?

Future challenges in the area of data for the RA of combined exposure to multiple chemicals area include:

- Need to develop databases on human exposure, both dietary, occupational and information from biomonitoring from different European population groups are needed.
- Although there is quite some qualitative information on chemicals, quantitative data are still a challenge
- In the area of exposure assessment, non-target analyses provide information in various media – data are qualitative but again quantitative data and the identification of biomarkers is a challenge. Data to validate the approaches are still not available.
- In the future, can the grouping be also based on epidemiological data using e.g. QUALYs and DALYs? There is currently a lack of available methodologies to do so. The HBM4EU project has proposed to derive HBM Guidance Values for chemicals amongst others using HBGVs based on epidemiological data.
- For ERA, more information on chemicals occurring in drinking water and potential interactions in species of ecological relevance.
- Limitation of some HBM data, mainly focussing on POPs, chemicals with a short half-life are not captured most of the time.
- RA of combined exposure to multiple chemicals is a good opportunity for NAMs and in silico to be developed and gain acceptance but further work is needed in this area
- Need more data to support TK grouping – determining common metabolites, providing means to identify metabolite mixture effects from enzymes and common target transporters (e.g. OpenFoodTox).
- Classification of substances using the MoA/AOP framework, structuring data for same MoA/AOP in databases (e.g OpenFoodTox, US-EPA CompTox chemistry dashboard)

Future challenges in the area of computational tools for the RA of combined exposure to multiple chemicals area:

- Move away from animal data and use NAMs particularly *in silico* tools
- Computational capacity is very demanding to run RA of combined exposure to multiple chemicals
- Computational tools would be needed to determine when the whole mixtures are similar or not (physico-chemical similarity or toxicological similarity to be comparable)
- Quantifying the uncertainty for membership to an assessment group in a software tool would be useful
- Use the computational tools available for pesticide RA in other areas and regulatory frameworks
- Development of a workflow and computational tools to identify the possibility of interactions (e.g., synergism/antagonism) as performed in the pharmacology area.
- Equipotency is applied using dose addition but more *in silico* models need to be developed to apply this method and other methods (e.g. response addition, interactions).
- Determine concentrations for chemicals with no- or low-concern versus chemicals of concern. Relate them to mixture effects in human health, animal health and ecological RA
- Take into account the importance of body burden to move towards a more realistic situation and develop in silico tools
- Develop guidance on battery of NAMs as the minimum requirements and evidence to conclude on specific potency

Discussion Point 3

What are the key challenges to implement the use of NAMs to RA of combined exposure to multiple chemicals area in the different areas including human health RA, animal health RA and ecological RA? Provide practical contexts and give examples.

NAMs, depending on data availability, can be used for several applications in the context of RA of combined exposure to multiple chemicals area within human health RA, animal health RA and ecological RA. These are particularly useful to assess properties for single chemicals which can be then integrated for the RA of multiple chemicals. However, participants concluded that it is important to select the NAMs that fit the purpose of the assessment and have discussed some practical applications of NAMs which include:

- Use of Omics useful for the grouping of chemicals and option to compare the exercise with current approach used for pesticides and other chemicals to assess their performance.
- Preliminary screening for common targets using QSARs for a range of species
- Identifying the main drivers of exposure using probabilistic exposure assessment methodologies (including aggregate exposure)
- New computational tools allow to address both hazard and exposure (e.g. VERMEER: www.vegahub.eu)
- Assessment of TK using generic TK models are available for humans, animals and species of ecological relevance
- Assessment of species sensitivity using species sensitivity distributions or models available for single components for aquatic and terrestrial species (less developed for terrestrial animals)
- NAMs can be of importance for whole mixtures.

Challenges and recommendations have been discussed and include:

- Identifying the most urgent need to develop NAMs for mixture RA
- NAMs approach depends on the availability of data in databases. NAMs data are in a different format than the currently used for RA. There is a need to organise datasets in an appropriate manner.
- Multidisciplinary approach is required to make a full use of NAMs and more expertise is needed.
- Who will prioritise NAMs and who will accept them? Potential conflict between scientists, risk assessors and risk managers since they may have different needs and framework
- Acceptance is a barrier, in particular regarding the potency evaluation using NAMs. The acceptance of NAMs results may require years of validation unless there is a consensus on their mechanistic value for RA. Transparency in method development will contribute to an easier validation. A structured approach will accelerate the verification by regulators
- Harmonisation of regulation and an overall common framework. Learn from related directives. e.g. Pharmaceuticals regulation addresses interactions and requires information if a drug is substrate or inhibits a cytochrome P-450 and this can be performed using *in vitro* and *in silico* tools.
- Investigation on how exposome data can be compared with *in silico* predictions is needed
- Assessment of the applicability domain of in silico methods for food bioactive compounds in mixtures requires further work
- NAMs are mainly validated for human RA and not yet applicable for animal RA (except for test species) because of lack of available data, in vitro methods and in silico models. Further work required in this field for farm animals and pets.
- For ERA, RA methodology has been developed for single substances and partially for multiple chemicals but still needs more development. Spatial and temporal variations in combined exposures need to be better addressed. Computational tools: In addition, more in silico tools needed for ERA including grouping tools.
- Importance of dissemination and communication: access of the tools to third countries, and in general world-wide. Some tools have high dissemination potential: in silico tools, while other tools (omics) require high level of technology and training.

3. Conclusions and Recommendations

The aim of this workshop was to bring together diverse scientific communities to discuss the challenges facing RA in term of identification of drivers for grouping chemicals into assessment groups and prioritisation methods of the combined exposure to multiple chemicals (chemical mixtures) as well as consider the future challenges for RA in this area. The workshop was structured into 3 break-out sessions for each of the four break-out groups.

Break out session 1 focused on Hazard-driven methods and the outcome of the workshop highlighted:

- The gold standard for grouping chemicals into assessment groups are mechanistic data namely MoA/ AOP data
- Limited availability of such data for many chemicals highlights that such an approach is currently mostly suitable for data rich chemicals
- A decision tree was designed during the workshop to stimulate the generation /gathering of mechanistic data
- In the proposed scheme of the draft guidance on grouping, "Unknown/no" leading to same decision between MoA and AO, this should be reviewed.

- Other type of information can be used to group chemicals into assessment groups including structural information, TK (common metabolites/ metabolic pathways)
- NAMs provide relevant mechanistic information to be combined with information on adversity/apical effect
- NAM results often lack comparative /quantitative information and need to be combined with toxicokinetic information and models such as Quantitative In Vivo in vitro extrapolation models

Break-out sessions 2 focused on prioritisation methods

- Consider "cut-off" instead of "threshold", driven by public health concerns in the prioritisation scheme proposed in the draft guidance on grouping, Step 1 is rather demanding, while Step 2 seems more practically feasible
- The gate keeper step (entry point) might have addressed already co-exposure
- Hierarchy of steps should be flexible; exposure might be very useful in absence of toxicity data
- Should the TTC be considered for use in the assessment of the contributions from data poor chemicals?
- Thresholds will depend on regulatory framework and protection goals etc
- The 10% threshold needs further consideration; the 10% for step 1 and step 2 are rather different. Such a threshold has been proven suitable for pesticides but more real case examples needed are needed for other areas. In addition, consequences of different thresholds should be explored.
- Additional TK data to address co-exposure are needed
- More probabilistic exposure assessment to address population heterogeneity
- HBM data to inform exposure assessment
- Improved structured databases to help prioritisation
- Co-exposure evidence from databases could trigger toxicity data generation

Break-out session 3 on future challenges:

- Probabilistic exposure assessment (including aggregate exposure) is crucial for RA of combined exposure to multiple chemicals.
- For ERA, more data needed in area of exposure, considering spatial/temporal variability and specific guidance document is needed.
- Big challenges for very complex mixtures, with limited possibilities to identify chemicals and lack of toxicity data
- We have many methods and tools at hand. Need training to use them.
- NAMs very useful here and the mixture context provides an opportunity for NAMs to gain acceptance. Such NAMs should be used in combination with TK data. There is a need to prioritise NAM development needed in regulatory context, fit for purpose. So far limited acceptance by many regulators e.g. for NAM-based potency factors. Guidance needed on NAM use (for whole and component-based mixture approaches). Guidance on the use of TTC in this context.
- In the future the grouping can be based on epidemiological data? Can we make use of concepts from Life Cycle Analysis such as the Qualy or Daly? HBM useful but still has limitations, retrospective, limited number of compounds analysed, non-persistent compounds.
- Overcome regulatory silos

Finally, a number of general topics were mentioned repeatedly during the workshop:

- Differences between data rich and data poor chemicals, workflows working well for data rich chemicals
- Take advantage/learn from the experience already gained across areas, e.g. pesticides, contaminants, feed additives
- Different EFSA Panels might have different needs and different processes
- Need for further case studies to test the proposed schemes with real examples
- Improve databases to facilitate access to relevant data (mechanistic, exposure)
- Need for access to data, tools and training on the methods and tools
- For the suggested workflows in the guidance on grouping, make clear that they are used in an iterative process. Some places of the guidance need further clarification/explanation.

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Abbreviations

ADI	Acceptable daily intake
AG	Assessment Group
AOP	Adverse Outcome Pathways
CAG	Cumulative Assessment Group
CONTAM	EFSA Scientific Panel on Contaminants in the Food Chain
EC	European Commission
ECHA	European Chemicals Agency
EEA	European Environment Agency
ERA	Ecological/Environmental RA
HBGV	Health-based guidance value
HI	Hazard Index
HQ	Hazard Quotient
JRC	Joint Research Centre of the European Commission
KEs	Key events
MOE	Margin of Exposure
NOAEL	No observed adverse effect level
PB-K	Physiologically-based kinetic models
PB-K-D	Physiologically-based kinetic-dynamic models
POD	Point of departure
PPR	EFSA Scientific Panel on Plant Protection Products and their Residues
QSAR	Quantitative Structural Activity Relationship
RA	Risk Assessment
RP	Reference point
RVs	Reference values
SCER	EFSA's Scientific Committee and Emerging Risks Unit
SCHER	Scientific Committee on Health and Environmental Risks
TTC	Threshold of Toxicological Concern
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
WOE	Weight of Evidence

Annex A – Programme of the workshop

International Workshop on RA of Combined Exposure to Multiple Chemicals| Online,18-19-20 October 2021

DAY 1 – 18 October 2021

OPENING SESSION: International activities on RA of combined exposure to multiple chemicals: Towards harmonisation of methodologies **Chair: Heather Wallace, University of Aberdeen (UK)** **Rapporteur: Stephanie Bopp, Joint Research Center, Ispra (Italy)**		
13:30-13:45	**Welcome**	Juliane Kleiner, EFSA
13:45-14:00	**Overall Objective of the Workshop**	Heather Wallace, University of Aberdeen (UK)
14:00-14:25	**EFSA MIXTOX Guidance Documents** Questions	Christer Hogstrand, Kings College London (UK)
14:25-14:55	**US-EPA activities on Combined Exposure to Multiple Chemicals: Latest Developments** Questions	Paul Price, US-EPA, Durham (US)
14:55-15:20	**FAO/WHO Consultation on Combined Exposure to Multiple Chemicals for JECFA and JMPR** Questions	Alan Boobis, Imperial College London (UK)
15:20-15:40	**Coffee/Tea break**	
15:40-16:05	**OECD Activities on Combined Exposure to Multiple Chemicals** Questions	Patience Browne, OECD (FR)
16:05-16:30	**JRC EURL-ECVAM Activities on Combined Exposure to Multiple Chemicals** Questions	Stephanie Bopp, EC-JRC
16:30-16:40	**Introduction to Break-out Sessions and Break-out Groups**	Jean Lou Dorne, EFSA
BREAK-OUT SESSION 1: Hazard-driven Criteria for grouping chemical into assessment groups : from systemic toxicity to mechanistic understanding		
16:40-18:40	**Break-out Session 1: Hazard-driven Criteria**	Chair and Rapporteur per Break-out group

DAY 2– 19 October 2021

PLENARY SESSION: Reporting from Break-out session 1 **Chair: Heather Wallace, University of Aberdeen (UK)** **Rapporteur: Stephanie Bopp, Joint Research Center, Ispra (Italy)**		
14:00-14:40	**Reporting from Break-out session 1: Hazard-driven criteria** Reports from each Breakout group (10 min each)	Rapporteur from all 4 Break-out groups (10' each)
14:40-15.40	**Plenary Discussion and Take-home messages** Questions, answers and discussion	Chair/Rapporteur
15:40-16:00	**Coffee/Tea break**	
BREAK-OUT SESSION 2: Exposure-driven and Risk-based Criteria: Priority settings and tools		
16:00-18:00	**Break-out session 2: Exposure-driven and Risk-based Criteria**	Chair and Rapporteur per Breakout group

DAY 3 – 20 October 2021

PLENARY SESSION 2 : Reporting from Break-out session 2 **Chair: Heather Wallace, University of Aberdeen (UK)** **Rapporteur: Stephanie Bopp, Joint Research Center, Ispra (Italy)**		
13:30-14:10	**Reporting from break-out session 2:** **Exposure-driven and Risk-based criteria** Reports from each break-out group (10 min each)	Rapporteur from all 4 Break-out groups (10' each)
14:10-15:10	**Plenary discussion and take-home messages** Questions, answers and discussion	Chair/Rapporteur
BREAK-OUT SESSION 3: Future challenges in RA of combined exposure to multiple chemicals		
15:10-16:40	**Breakout session 3: Future challenges**	Chair and Rapporteur per Break-out group
16:40-17:00	**Coffee/Tea break**	
PLENARY SESSION 3 : Reporting from Break-out session 3 **Chair: Heather Wallace, University of Aberdeen (UK)** **Rapporteur: Stephanie Bopp, Joint Research Center, Ispra (Italy)**		
17:00-17:40	**Reporting from Break-out session 3:** **Future challenges in RA of combined exposure to multiple chemicals** Reports from each break-out group (10 min each)	Rapporteur from all 4 Break-out groups (10' each)
17:40-18:00	**Plenary discussion and Take-Home messages** Questions, Answers and Discussion	Chair/Rapporteur
FINAL SESSION: Final take home messages, way forward and closing remarks **Chair: Heather Wallace, University of Aberdeen (UK)** **Rapporteur: Stephanie Bopp, Joint Research Center, Ispra (Italy)**		
18:00-18:15	**Wrap up and Final Take-Home messages**	Chair/Rapporteur
18:15-18:20	**Closing remarks**	Juliane Kleiner, EFSA

Annex B – Presentations

All the presentations of the International Workshop on Human RA of Combined Exposure to Multiple Chemicals can be found in the online version of this output ('Supporting information'section)

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