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Guidance Document on Scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals

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Abstract

This guidance document provides harmonised and flexible methodologies to apply scientific criteria and prioritisation methods for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals. In the context of EFSA's risk assessments, the problem formulation step defines the chemicals to be assessed in the terms of reference usually through regulatory criteria often set by risk managers based on legislative requirements. Scientific criteria such as hazard-driven criteria can be used to group these chemicals into assessment groups. In this guidance document, a framework is proposed to apply hazard-driven criteria for grouping of chemicals into assessment groups using mechanistic information on toxicity as the gold standard where available (i.e. common mode of action or adverse outcome pathway) through a structured weight of evidence approach. However, when such mechanistic data are not available, grouping may be performed using a common adverse outcome. Toxicokinetic data can also be useful for grouping, particularly when metabolism information is available for a class of compounds and common toxicologically relevant metabolites are shared. In addition, prioritisation methods provide means to identify low-priority chemicals and reduce the number of chemicals in an assessment group. Prioritisation methods include combined risk-based approaches, risk-based approaches for single chemicals and exposure-driven approaches. Case studies have been provided to illustrate the practical application of hazard-driven criteria and the use of prioritisation methods for grouping of chemicals in assessment groups. Recommendations for future work are discussed.

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Keywords: harmonised methodologies, human risk assessment, combined exposure to multiple chemicals, scientific criteria, grouping, assessment groups, dose addition

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Summary

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 risk assessment of combined exposure to multiple chemicals'. MIXTOX supports the harmonisation of methodologies for risk assessment 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.

The present guidance document explores the use of scientific criteria for grouping of chemicals into assessment groups for human health in the context of the component-based approach. The Scientific Committee acknowledges that it is not feasible to start a risk assessment from the whole universe of chemicals. In practice, legal requirements or specific concerns often predefine 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). Thus, the group of chemicals or its components are identified and the grouping is often based on pragmatic considerations, regulatory criteria and scientific criteria. Then available hazard data are collected, and preliminary assessment groups can be formed. Regulatory criteria are most often set by risk managers in the ToR, based on legislative requirements and may provide a preliminary assessment group based on a common regulatory domain. Scientific criteria for grouping are hazard-driven and use similarity of toxicological and toxicokinetic properties for each individual chemical under consideration. Prioritisation methods also support grouping to filter the number of chemicals to be considered for grouping through pragmatic means, particularly when resources are limited. These methods are risk-based or exposure-driven and provide options to identify chemicals which contribute only marginally to the combined risk. Such chemicals are referred to as 'low-priority chemicals' and may be excluded from further grouping.

The application of hazard-driven criteria for grouping requires a weight of evidence (WoE) approach to assemble, weigh and integrate the available lines of evidence on toxicity. A framework is proposed to apply hazard-driven criteria for grouping chemicals into assessment groups using mechanistic information on toxicity as the gold standard while also considering toxic potency and toxicokinetic features (e.g. body burden). In practice, the lowest uncertainty in grouping can be achieved when knowledge on an adverse outcome pathway (AOP) is available, followed by knowledge on a mode of action (MoA) for the chemicals under evaluation. Grouping using phenomenological effects or target organ/system toxicity is linked to higher uncertainty. Data-poor chemicals (i.e. no or scant toxicological information) can be included in an assessment group along with data-rich members using 'in vitro or in silico bridging data' as part of the battery of new approach methodologies (NAMs). However, the resulting uncertainty is high. A generic structured WoE approach to group chemicals using MoA information is provided in Appendix B.

Structural similarity may also be used as criteria for grouping of chemicals into assessment groups but consideration of more than one feature (i.e. chemical class, common functional groups, common precursor or breakdown products) should be used to increase the confidence in the assessment of similarity of the components. There are also several software tools, such as the OECD QSAR Toolbox, available to support the identification of related substances. Many *in silico* methodologies can be used for this purpose, such as molecular docking and different machine learning tools. However, it is essential to assess the applicability domain of each model and integrate the results from multiple models for the prediction of the same property using WoE methods. It is also important to evaluate both similarities and dissimilarities between chemicals particularly for the presence of specific chemical moieties or structural features, which may impact on MoA or toxicity. Toxicokinetic data can also be useful for grouping, particularly when metabolism information is available for a class of compounds and common toxicologically relevant metabolites are shared.

The guidance document includes prioritisation methods to be applied when the number of chemicals to be assessed is *a priori* vast and resources are limited. These provide means to reduce the number of chemicals to be considered for grouping or within an already formed assessment group. Therefore, chemicals which are unlikely to co-occur in humans or otherwise would contribute only marginally to a combined risk can be considered of low-priority for grouping. Cut-off values applied for defining such low-priority chemicals will depend on the context of the assessment, the prioritisation

method used and should be documented and justified. These methods include combined risk- and single risk-based approaches, and exposure-driven approaches. An account of related statistical methods as well as practical examples are provided in Appendices C, D and E.

Recommendations for future work to test the applicability and implementation of the proposed scientific criteria for grouping chemicals into assessment groups are made. A testing phase in relevant EFSA Panels using specific case studies is proposed. In addition, inter-agency, Member States and international cooperation in this area are needed to facilitate data exchange and harmonisation of methods and tools. To support the implementation of the hazard-driven criteria, a further update of the OpenFoodTox database and the use of OECD international harmonised standards to structure data on chemical properties is proposed. In addition, harmonised WoE approaches should be applied to avoid divergence across EFSA Panels in the process of grouping chemicals into assessment groups. Finally, development and implementation of generic *in silico* approaches (e.g. Quantitative Structural Activity Relationship (QSARs), physiologically based kinetic (PB-K) models) to support grouping of chemicals are also recommended.

With regard to prioritisation methods, the Scientific Committee recommends identifying and testing the appropriateness of cut-off values for risk metrics in the context of regulatory requirements, data availability and number of chemicals under consideration. As a starting point, a default value of $\geq 10\%$ contribution of a single chemical to the combined risk is proposed.

Table of contents

Abstract.....	1
Summary.....	3
1. Introduction.....	6
1.1. Background and Terms of Reference as provided by the requestor.....	6
1.1.1. Background.....	6
1.1.2. Terms of Reference.....	6
1.2. Interpretation of the Terms of Reference.....	7
1.3. Audience and degree of obligation.....	7
2. General principles: Problem formulation and grouping.....	7
3. Hazard-driven criteria.....	8
3.1. Grouping using toxicity information.....	8
3.2. Grouping using Toxicokinetic information.....	12
4. Prioritisation methods for grouping chemicals into assessment groups.....	13
4.1. Introduction.....	13
4.2. Workflow for the prioritisation of multiple chemicals using risk-based and exposure-driven approaches.....	14
5. Recommendations.....	16
References.....	17
Abbreviations.....	20
Appendix A – Glossary.....	23
Appendix B – Generic Weight of Evidence Methodology for grouping multiple chemicals into assessment groups using hazard-driven criteria.....	26
Appendix C – Statistical Methods to study the probability of combined risk or combined exposure.....	29
Appendix D – Risk-based approach for single chemicals as a prioritisation method for grouping pesticides into assessment groups.....	31
Appendix E – Exposure-driven approach as a prioritisation method for grouping multiple contaminants from breast milk and comparison with a risk-based approach for single chemicals.....	35

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

Human health assessment of combined exposure to multiple chemicals (“chemical mixtures”) is a challenging topic for scientists, risk assessors and risk managers. This is 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 March 2019, the Scientific Committee of EFSA published the “guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals” (EFSA Scientific Committee, 2019). This document supports the harmonisation of methodologies for risk assessment of combined exposure to multiple chemicals including the setting of assessment groups for component-based approaches. The methods described in the guidance 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.

A number of relevant EFSA Panel activities in this field include:

- PPR Panel and Pesticide Units: grouping of pesticide active substances into “Cumulative Assessment Groups” (CAGs) based on specific toxicological effects and consideration of mode of action (MoA) as far as possible (EFSA PPR Panel, 2013a,b). In September 2019, the Pesticides Unit published Scientific Reports, which were subject to public consultation, on the establishment of CAGs of pesticides for their effects on the nervous system and the thyroid (EFSA, 2020a,b).
- Panel on Contaminants in the Food Chain (CONTAM): publication of a number of opinions involving case-by-case approaches to risk assessment of multiple contaminants. Component-based approaches have included Toxic Equivalency Factors (TEF) approaches for non-ortho polybrominated biphenyls and several groups of marine biotoxins (EFSA CONTAM Panel, 2009, 2010).
- Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF): risk assessment of combined exposure to rum ether [Flavouring Group Evaluation 500 (FGE.500)] and grouping of 84 reported constituents for 12 congeneric groups allocated based on structural and metabolic similarity (EFSA CEF Panel, 2017).
- Panel on Additives and Products or Substances used in Animal Feed (FEEDAP): A component-based approach was applied to assess the safety of an essential oil from the seeds of *Elettaria cardamomum* (L.) Maton when used as a sensory additive for all animal species as a mixture (EFSA FEEDAP Panel, 2019).

1.1.2. Terms of Reference

EFSA requests the Scientific Committee to develop a guidance document addressing scientific criteria for the grouping of chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals, taking into account:

- The scientific principles laid down in the recent Scientific Committee guidance on ‘harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals’ as well as other relevant cross-cutting guidance documents (i.e. weight of evidence, biological relevance, uncertainty).
- The need for prioritisation methodologies to accommodate risk assessments within the context of data availability, time, and resources for the grouping of chemicals defined in the problem formulation.
- The context of the risk assessment (pre- and post-market).
- Tiering principles and a range of fit for purpose scenarios should be developed, considering available hazard information (e.g. reference points, specific toxicological effects in target organs, mode of action) and exposure information. Additional considerations may be of relevance including adverse outcome pathways (AOP), toxicokinetics and human biomonitoring.
- Relevant EFSA sectoral regulatory provisions and activities including the work on CAGs for pesticides by the Pesticide units, relevant risk assessment activities on contaminants, any other relevant panel (FEEDAP, FAF, CEP, NDA) and other related European activities (European Commission, JRC, ECHA, EMA, EDC-MixRisk, EuroMix and HBM4EU Horizon 2020 projects).

- Relevant international activities including the recent guidance documents of the OECD and the practical approach developed during the WHO/FAO consultation to be piloted by JMPR and JECFA in 2019. This will ensure consistency and harmonisation, provide an international dimension to the statement, and avoid duplication of the work.

In line with EFSA's policy on openness and transparency (EFSA, 2020c), EFSA will publish a draft version of the scientific opinion for public consultation. Following the public consultation, the finalised opinion will be published after adoption by the Scientific Committee together with the technical report of the public consultation.

This activity should be delivered to the Scientific Committee by the autumn 2021.

1.2. Interpretation of the Terms of Reference

The MIXTOX guidance document (EFSA Scientific Committee, 2019) provides general principles for 'harmonisation of methodologies for human health, animal health and ecological risk assessments of combined exposure to multiple chemicals'. The present guidance document provides the scientific criteria for grouping chemicals into assessment groups for human health in the context of the component-based approach and applies to dietary exposure. The Scientific Committee recognises that it is not feasible to start a risk assessment of combined exposure to multiple chemicals from the whole universe of chemicals. The Scientific Committee notes that in practice, legal requirements or specific concerns often predefine 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 or chemicals in human breast milk). Thus, the group of chemicals to be considered in an assessment by EFSA is defined and frequently based on regulatory criteria or pragmatic considerations. The scientific criteria for grouping chemicals into assessment groups for human health as proposed in this document therefore relate to the predefined group of chemicals in the ToR or in problem formulation.

1.3. Audience and degree of obligation

This guidance document provides scientific criteria for grouping chemicals into assessment groups using harmonised and flexible stepwise procedures. These criteria will allow EFSA to conduct human risk assessments of combined exposure to multiple chemicals using component-based approaches. This guidance document is unconditional (i.e. required, see EFSA Scientific Committee, 2015) for the EFSA panels and EFSA units performing combined exposure risk assessments in the food safety area. Acknowledging the different types of questions in the problem formulation and data availability, this document provides recommendations on the most appropriate and fit-for-purpose scientific criteria for grouping chemicals (from a predefined group of chemicals in the ToR) into assessment groups. Readers and users of this guidance document are assumed to be experienced in human risk assessment of single chemicals, and emphasis is on the specific aspects to deal with grouping multiple chemicals for combined exposure risk assessment.

2. General principles: problem formulation and grouping

In the problem formulation, it is decided whether a risk assessment of combined exposure to multiple chemicals is required ('gatekeeper step') and, if so, a component-based or a whole-mixture based approach should be followed. If the decision is to embark on a component-based approach, it will be necessary to discuss which chemicals should be considered together in an assessment group. In the context of EFSA's remit, the 'gatekeeper step' is often outlined in the ToR, which is most often developed by the European Commission in consultation with experts from Member States, before a request for a risk assessment is sent to EFSA (EFSA, 2015; EFSA Scientific Committee, 2019). The question to be addressed is then described within EFSA outputs in the '[Interpretation of the Terms of Reference](#)' section.

Component-based approaches for multiple chemicals are relevant to both regulated products (e.g. plant protection products; feed additives; food contact materials) and contaminants in the food chain (e.g. environmental contaminants, natural toxins, food and/or feed processing contaminants).

The general principles for the grouping of chemicals into assessment groups have been described previously by EFSA (EFSA PPR Panel, 2013a,b; EFSA Scientific Committee, 2017, 2019) and other scientific bodies including the WHO, US EPA, Joint Research Centre of the European Commission and the OECD (US Environmental Protection Agency, 2007; WHO/IPCS, 2009; Meek et al., 2011;

Meek, 2013; OECD, 2011, 2018; ECHA, 2012; SCHER, SCENIHR, SCCS, 2012; Bopp et al., 2015; Solomon et al., 2016). The components to be assessed are identified within the problem formulation, then available hazard data are collected and preliminary assessment groups can be formed (EFSA Scientific Committee, 2019).

Criteria for grouping chemicals can be classified into regulatory and scientific criteria. Regulatory criteria are most often set by risk managers in the ToR, based on legislative requirements and may provide a preliminary assessment group based on a common regulatory domain. Scientific criteria for grouping are hazard-driven and use similarity of toxicological properties for each individual chemical under consideration in a collection of multiple chemicals. Grouping based on hazard-driven criteria requires a weight of evidence (WoE) approach to assemble, weigh and integrate the available lines of evidence on toxicity (i.e. MoA, AOP, phenomenological effects, target organ/system toxicity, etc.) (EFSA Scientific Committee, 2017a). Hazard-based criteria including information on toxicity and toxicokinetics (TK) are described in Section 3.

Prioritisation methods are included to help risk assessors to filter the number of chemicals to be considered for grouping through pragmatic means, particularly when resources are limited. These methods are risk-based or exposure-driven and provide options to identify chemicals which contribute only marginally to the combined risk. In this guidance document, these chemicals are referred to as 'low-priority chemicals' and may be excluded from further grouping. Prioritisation methods are described in Section 4.

3. Hazard-driven criteria

Hazard-driven criteria use the evidence on hazard i.e. toxicological properties of chemicals from different levels of biological organisation to group chemicals into assessment groups using a WoE approach to assemble, weigh and integrate available lines of evidence on toxicity through a structured approach (EFSA Scientific Committee, 2017a,b; 2018a).

3.1. Grouping using toxicity information

Traditionally, common MoA information has been used as the scientific criteria to group chemicals into assessment groups using mechanistic information. For example, MoA information has been used by the US-EPA for organophosphates (i.e. methamidophos, acephate, bensulide, disulfoton, malathion, tetrachlorvinphos, trichlorfon) grouped on the basis of irreversible inhibition of acetylcholinesterase in the central and peripheral nervous systems as a common MoA (US-EPA, 2006). Another relevant example is the common MoA involved in the toxicity of polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls through binding and activation to the Aryl hydrocarbon receptor (EFSA CONTAM Panel, 2018).

Toxicological processes leading to an adverse outcome can be visualised as a continuum starting from external dose (exposure) to an internal dose at the target organ or tissue (i.e. biologically effective dose), leading to a first interaction with the molecular targets: the so-called molecular initiating event (MIE) under the AOP framework. This interaction triggers a downstream response consisting of a series of key events ultimately leading to an adverse outcome. International scientific advisory bodies have developed the MoA and AOP frameworks to describe the mechanistic basis of toxicity and the reader is referred to the WHO, US-EPA and OECD documents for a detailed account of these frameworks and to the glossary in this document for all definitions (WHO, 2007; Ankley et al., 2010; EFSA PPR Panel, 2013b; Meek et al., 2014; OECD, 2018; EFSA Scientific Committee, 2019). Figure 1 provides a simplified visualisation of the main differences between the MoA framework which, includes both the TK and toxicodynamic (TD) dimensions, whereas the AOP framework only covers the TD dimension. In an MoA analysis, TK can constitute a key event such as the metabolic bioactivation of a chemical to a toxic metabolite (e.g. acrylamide). However, recent attempts have considered the integration of the TK dimension within the AOP framework using the aggregate exposure pathway (AEP) framework (Teegarden et al., 2016). In addition, an integrated AOP-AEP framework has been proposed to organise mechanism-related information and to take into account interactions between chemicals (see glossary for definitions) (Price et al., 2020).

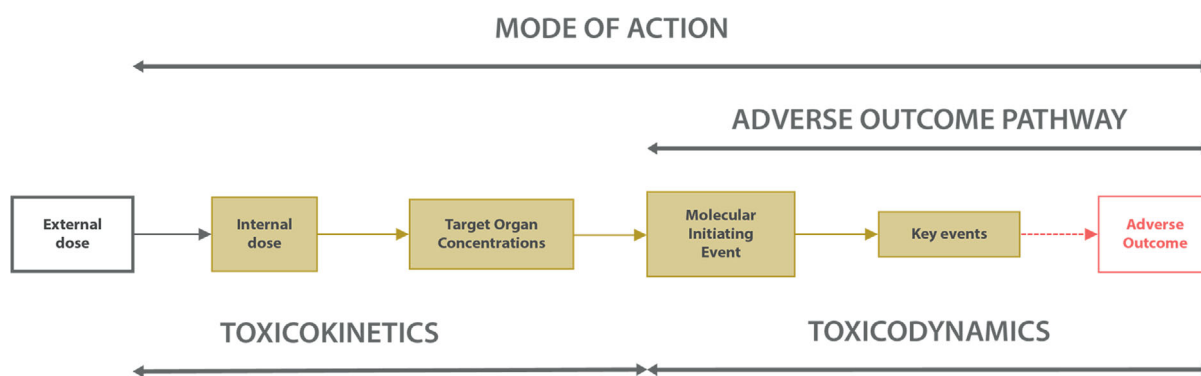


Figure 1: Conceptual representation of the mode of action and adverse outcome pathway frameworks under the exposure-response continuum

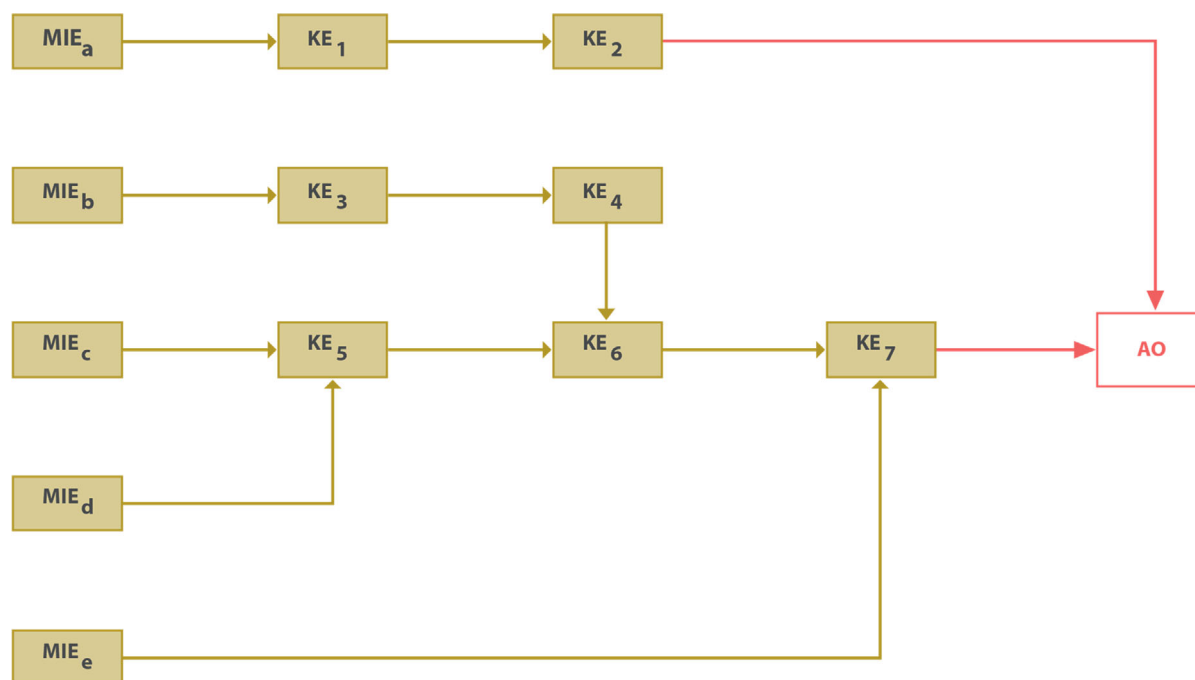
From the MIE, the individual key events, defined as an 'empirically observable precursor step that is itself a necessary element of the MoA or a biologically-based marker (e.g. biomarker of effect) for such an element', are then incorporated into the toxicity pathway and MoA eventually leading to an adverse effect. More details on AOPs are available in the OECD documents (Boobis, 2005; US-EPA, 2005; OECD, 2013, 2018). Such key events should be definable from physiological and biochemical perspectives and have a biological relevance in relation to a toxicity pathway. Risk assessors should be able to define, observe and measure changes associated with such KEs at the molecular, cellular, functional or morphological level to depict the physiological and biochemical basis of the toxicity pathway and use it as basis for defining assessment groups. However, the results from the Horizon 2020 funded project EuroMix have shown that chemicals with dissimilar MoA, or triggering different AOPs, but converging at the same adverse outcome or at downstream key events, should be included in the same assessment group (e.g. liver steatosis). The scientific basis for this is that combined toxicity has been best described using dose addition (Bopp et al., 2018; EFSA Scientific Committee, 2019).

Initially, AOPs have been described as a linear description of a toxicological process, leading from an MIE to an adverse outcome through one or several key events. In practice, however, each AOP is usually part of more complex networks (Figure 2). An AOP network provides a framework to better represent the complexity of biological processes by studying relationships among interconnected linear AOPs.

Indeed, whenever available, AOP information should be used to define assessment groups and for grouping chemicals (OECD, 2018). The Scientific Committee notes that AOP information is currently limited but in view of the international research activities through the AOP wiki (<https://aopwiki.org/>), as a repository platform for AOPs, it is foreseen that such information will be increasing in the future. Chemicals that share a common adverse outcome and their AOPs are known should be grouped together in the same assessment group. This approach is illustrated in Figure 2 as AOP networks which embraces a range of AOPs for different chemicals that may trigger:

- The same AOP by interacting with the same MIE (any MIE in Figure 2);
- Separate AOPs which then converge at any intermediate key event (e.g. MIEb to MIEe in Figure 2);
- An AOP which leads to the same adverse outcome without converging at intermediate key event from other AOPs (MIEa in Figure 2);

The Scientific Committee notes that these three categories include all chemicals with the same adverse outcome but distinct MIEs, thus having comprehensive mechanistic understanding.



AOP: adverse outcome pathway; AO: adverse outcome; KE: key event; MIE: molecular initiating event.

Figure 2: Schematic representation of adverse outcome pathway networks

Such mechanistic information anchored to an MoA, AOP or its related network allows the uncertainty of the chemical grouping to be reduced. However, if the available evidence indicates that chemicals with a common MoA do not contribute to the combined effects based on exposure and potency considerations, these may be excluded from the final assessment group (see prioritisation methods, Section 4). Recently, common AOPs have been used to group liver steatosis-inducing pesticides. An *in vitro* AOP-based assay toolbox provided a basis to measure MIEs and key events including nuclear receptor activation, gene and protein expression and triglyceride accumulation according to the proposed AOP for liver steatosis (Lichtenstein et al., 2020).

Overall, this approach allows assessment groups to be set based on a common subcellular or molecular target (MoA or AOP) (EFSA Scientific Committee, 2019).

When the grouping is based on incomplete mechanistic information, the exclusion of chemicals from an assessment group may lead to an underestimation of the risk of combined toxicity. In this context, grouping may nevertheless have to be based using other hazard criteria, e.g. on common adverse outcome. The rationale that supports this approach is that different AOPs can converge on the same adverse outcome even if they do not have any key event in common (see Figure 2, MIE_a vs. MIE_b-e).

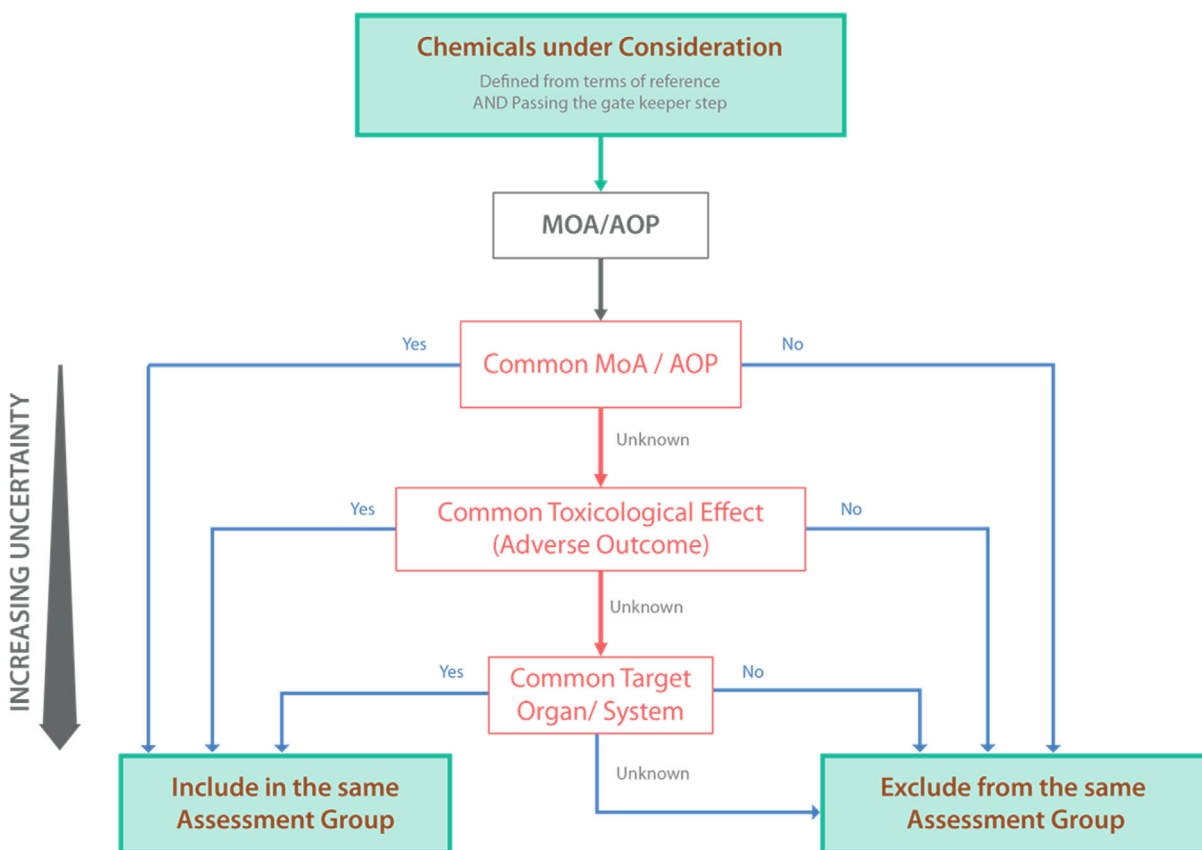
When the grouping is based on a common target organ/system toxicity, many chemicals may be included in an assessment group and may not share the same MoA. This may result in an overestimation of the risk of combined toxicity. The Scientific Committee notes that if the chemicals produce different adverse outcomes, there is no empirical evidence that combined toxicity would exceed that from the individual components when chemicals are present at doses around or below their respective no-observed adverse effect levels (NOAELs) (SCHER, SCCS, SCENIHR, 2012).

Data-poor chemicals (i.e. no or scant *in vivo* toxicological information) may be included in an assessment group with data-rich members if there are '*in vitro* or *in silico* bridging data' as part of NAMs, including similar physico-chemical properties and chemical structures, as described in the MIXTOX guidance document (EFSA Scientific Committee, 2019). For multiple chemicals, structural similarity can also be used as criteria for grouping of chemicals into assessment groups (ECHA, 2008; 2012; EFSA FAF Panel, 2021). The consideration of more than one feature, including chemical class, common functional groups, common precursor or breakdown products, usually increases the confidence in the assessment of similarity of the components (ECHA, 2012). There are also several software tools available to help in identifying structurally related substances, such as the OECD QSAR Toolbox.

The Scientific Committee notes that *in silico* models are also available which can be used for two main purposes: to predict the effect (such as toxicity) or to group substances within a same family, which can be used within the approach of dose addition. The availability of large collections of data related to MIE, such as within the ToxCast and Tox21 initiatives, boosted the development of *in silico* models to identify potential MIE (Gadaleta et al., 2018; Allen et al., 2020). Many *in silico* methodologies and other NAMs can be used for this purpose, such as molecular docking and different machine learning tools (Mansouri et al., 2016, 2020).

However, it is essential to assess the applicability domain of each model and integrate the prediction results of multiple models for the prediction of toxicological properties using WoE methods. In addition, the use of prediction results from multiple *in silico* models and read across are recommended to increase the confidence and the reliability of the results for the chemicals under consideration (EFSA Scientific Committee, 2017a; Benfenati et al., 2019). It is important to evaluate not only similarities between chemicals but also dissimilarities, particularly for the presence of specific chemical moieties or structural features, which may impact on MoA or toxicity. Specific open source software for this purpose includes ToxWeight (available open source within VEGA (www.vegahub.eu)).

Figure 3 provides a hierarchical framework to apply hazard-driven criteria for the grouping of chemicals into assessment groups using available mechanistic information (MoA or AOP) as the gold standard (EFSA Scientific Committee, 2019). The top-down hierarchical framework supports generating such mechanistic data and reducing uncertainty in the grouping process.



The thickest arrow indicates the gold standard hazard-driven criteria (MoA/AOP) with the lowest uncertainty.

Figure 3: Top-down hierarchical process for grouping chemicals into assessment groups using hazard-driven criteria

If the application of the hazard-driven criteria (Figure 3) results in an unmanageably large assessment group, the assessor can reduce the number of chemicals by applying prioritisation methods described in Section 4. If the assessor concludes that the application of such methods is needed, a rationale should be provided, accessibility of hazard data should be assessed and prioritisation methods should be applied accordingly.

In data-rich situations, when the MoA or AOP is known for the group of chemicals under assessment, this can result in (a) grouping chemicals sharing the same MoA/AoP into the same assessment group, (b) exclusion of chemicals from the same assessment group when they do not share the same MoA/AOP. When MoA/AOP information is scarce, lacking or unknown, the next tier is to resort to other lines of evidence, such as whether the multiple chemicals elicit a common phenomenological effect (e.g. impairment of immune response, cognitive development, sperm viability) or target organ toxicity. Decreasing the level of biological organisation in this way increases the uncertainty in the assessments and the likelihood for overestimation of the risk of combined toxicity. Indeed, grouping using phenomenological effects and, even more, target organs as a whole is considered a low tier approach with its inherent large uncertainty and it may imply the inclusion of many chemicals in an assessment group. In addition, when considering the target organ toxicity, it is important to note that not all cell populations in an organ play the same physiological role and chemicals may target different cell subpopulations (i.e. may have different adverse outcome related to the same organ). Hence, most organs and organ/systems exert different functions, as a result of the specialised role of their cell subpopulations. For example, the thyroid has follicular cells and C-cells, which show distinct features and functions, that can be targeted by different chemicals. The liver is another example of a single organ showing multiple functions: Chemicals may selectively affect one of these functions, depending on the type of chemical involved and its potency. Overall, the range of adverse effects in target organ/systems as a result of chemical exposures is based on chemical interference with key cellular functions, and depends on dose-related intensity of the chemical insults, the cell population affected and the duration of the exposure (acute or chronic), which are key determinants of the nature of the potential adverse outcome.

Evaluation of the hazard information is performed using a WoE approach for which the different lines of evidence (LoEs) are assembled, weighed and integrated according to their reliability, relevance and consistency, while considering biological relevance of the observed effects and reporting uncertainties, as described in the relevant EFSA Guidance documents (EFSA Scientific Committee, 2017a,b; 2018a,b). For each chemical under consideration, the process initiates with collection and organisation of the hazard information into lines of evidence (i.e. MoA, AOP, adverse outcome, critical effect, target organ, etc.) at different levels of biological organisation (molecular, cellular, organ level, whole organism). Methods for weighing and integrating the evidence can include qualitative approaches (simple description), semi-quantitative methods (low, moderate, high) or quantitative methods (probabilistic scale) (EFSA Scientific Committee, 2017a). The WoE assessment results in grouping chemicals into assessment groups and can be expressed as a simple qualitative description or as a probability based on quantitative assessment. Recent examples include establishment of cumulative assessment groups of pesticides for specific effects on the nervous system or the thyroid using quantitative weights to assemble and integrate the lines of evidence combined with expert knowledge elicitation and uncertainty analysis (EFSA, 2020a,b). This approach led to a probability distribution for the total number of substances in the assessment group that actually cause the specific effect on the nervous system or on the thyroid.

Appendix C provides an example of a generic WoE approach for the application of hazard-driven criteria to the grouping of five contaminants into assessment groups based on MoA information.

3.2. Grouping using toxicokinetic information

The main feature that separates the MoA and AOP frameworks is that the former also accounts for TK (Figure 1). This entails the consideration of absorption, distribution, metabolism and excretion (ADME) particularly when these processes are relevant for eliciting chemical toxicity within a known MoA (e.g. metabolic bioactivation to a toxic metabolite) in target organs and therefore governs the biologically effective dose on which the adverse outcome at the molecular level depends. While TK information should not be used in isolation for defining assessment groups and grouping, the combination of TK and TD properties would provide a robust basis for depicting toxicity and grouping chemicals, as proposed in the modified WHO MoA framework (Meek et al., 2014). Toxicokinetic data of importance for grouping chemicals into assessment groups include: (a) chemicals that are substrates of the same transporters; (b) chemicals producing the same metabolite(s) or are substrates of the same enzyme isoforms (e.g. phase I or phase II xenobiotic metabolising enzymes). An example of using toxicokinetic data is to group all 1,2-unsaturated pyrrolizidine alkaloids and their N-oxides, because they can be metabolically converted into pyrrole metabolites, which have a genotoxic and carcinogenic MoA on the liver as the primary target organ (EFSA CONTAM Panel, 2011). Finally,

available toxicokinetic data (e.g. body burden, clearance, half-life, elimination rate) and models are increasingly available and include physiologically based Kinetic (PB-K) and PB-K-dynamic (PB-K-D) models for test species or humans. A recent guidance document has been published by the OECD to support their use in risk assessment (OECD, 2021). These can also be used to refine grouping, if needed, or to compare risk metrics based on internal dose (EFSA Scientific Committee, 2019) (see Section 4, prioritisation methods).

4. Prioritisation methods for grouping chemicals into assessment groups

4.1. Introduction

For a given risk assessment of multiple chemicals, chemicals under consideration are predefined in the ToR and problem formulation (Section 2) mainly through regulatory or pragmatic criteria. When the number of chemicals under consideration is *a priori* vast and resources are limited, the assessor has the option to filter these chemicals to be considered for grouping. In addition, identifying chemicals of priority could be useful to risk managers to mitigate risk. This can be achieved using the prioritisation methods described in this section.

Prioritisation methods can thus be deployed to reduce the number of chemicals to be considered further, within an already formed assessment group. Therefore, chemicals which contribute only marginally to a combined risk can be considered of low-priority for grouping. The marginal contribution to a combined risk can be quantified with the identification of a cut-off value which can be applied for defining low-priority chemicals. The different cut-off values will depend on the context of the assessment, the prioritisation method used and should be documented and justified. Because the prioritisation methods rely on different metrics and use different statistical methods, it is not possible to propose a generic cut-off value suitable to all contexts. Options for different cut-off values are proposed for each prioritisation method below. In practice, when hazard metrics are available for a common effect or target organ, low-priority chemicals with a marginal contribution to the combined risk can be identified and excluded from grouping using a combined risk-based approach. When hazard metrics are only accessible for the respective critical effect, a risk-based approach for single chemicals can be used as another prioritisation method to identify low-priority chemicals. Finally, if hazard information is not readily accessible, an exposure-driven approach aiming at assessing co-exposure to chemicals can be applied.

These prioritisation methods are summarised as follows:

1) Combined risk-based approach. This method can be used when hazard metrics for a common effect or target organ are already accessible. Combined risk metrics are determined using hazard metrics for a common effect or target organ and exposure metrics of the individual chemicals using dose addition as the default assumption (e.g. modified hazard index, reference point index, combined margin of exposure (MOE)). The relative contribution of each individual chemical to the combined risk (including the uncertainty in estimates) can then be used to identify low-priority chemicals (see Figure 4). As a starting point, the Scientific Committee recommends that any chemical contributing more than 10% to the combined risk (cut-off value) is retained for refinement of the assessment group using hazard-driven criteria (Section 3). However, this cut-off value might not perform well under all circumstances, e.g. when a high number of chemicals have a contribution slightly below the cut-off value. In this case, it is recommended to reduce the cut-off value for the individual chemicals, ensuring that the total contribution of retained chemicals accounts for at least 90% of the combined risk.

Furthermore, even when individual chemicals contribute to the combined risk below the cut-off value, these contributions may be strongly correlated (i.e. when contribution of chemical A is at its highest, the contribution of chemical B is also at its highest). When such correlations are identified between chemicals, it is recommended to retain those chemicals for refinement of the grouping, regardless of their individual contributions. Several methods are available for multivariate analysis and correlation calculations (Appendix C). One of these methods has been applied in the HORIZON 2020 EuroMix project for excluding low-priority chemicals in the assessment of multiple pesticides, with liver steatosis as a common adverse outcome (Crépet et al., 2019; Van Voet et al., 2020). This method has also been applied using the Chemical Mixture Calculator using an underlying hazard and exposure database for over 200 chemicals, a range of assessment groups and several levels of risk characterisation refinement depending on the question to be addressed (Boberg et al., 2021).

2) Risk-based approach for single chemicals. This method aims to determine risk metrics for each chemical under consideration and can be used when hazard metrics for the respective critical effect are available. Individual risk metrics are calculated (e.g. hazard quotient or MOE). This approach allows to identify low-priority chemicals which can be excluded from further assessment, when their individual risk metric falls below a predefined cut-off value (Te Biesebeek et al., 2021)

Recently, the FAO/WHO Expert Consultation on Dietary risk assessment of chemical mixtures has proposed a predefined cut-off value below 10% of the relevant health-based guidance value or a calculated MOE that is above 10-fold of the adequate MOE for each individual chemical. These predefined cut-off values have been recently explored by JECFA for the risk assessment of multiple veterinary drug residues (diflubenzuron and halquinol) and for neither of these compounds did the estimated dietary exposure from veterinary use exceeded 10% of the upper bound of the ADI in any population or subpopulation (FAO/WHO, 2020). The Scientific Committee recommends the use of this proposed cut-off value as a starting point, when experience and information for the chemicals under consideration are limited. However, this cut-off value can be lowered on a case-by-case basis, depending on the context of the assessment and the experience gained. The rationale for deviating from the proposed cut-off value should be documented. Furthermore, the cut-off value needs to be considered in relation to the protection goals defined by the risk managers. This means that when combined risks need to be characterised at a given percentile of the exposure distribution, the cut-off value needs to be applied to the same percentile of the exposure distributions for the individual chemicals.

3) Exposure-driven approach. This method aims to determine the probability of combined exposure to identify and exclude low-priority chemicals for which the probability of co-exposure is low. This method can be used in situations under which (i) hazard metrics are not available to prioritise chemicals with methods 1 and 2; (ii) large number of chemicals have to be evaluated in a short time frame and hazard metrics should be collected or generated subsequently. The Scientific Committee notes that exposure-driven approaches currently have limited applications in the risk assessment conducted by EFSA panels. This method has been so far mostly applied by national agencies (e.g. ANSES) using probability of co-exposure patterns to identify low-priority chemicals present in (a) breast milk (Crépet et al., in press) and total diet (Béchaux et al., 2013; Crépet et al., 2013a,b; Traoré et al., 2016); (b) biomonitoring data in body fluids (blood and urinary) providing correlations of internal exposure between multiple chemicals from Horizon 2020 research projects Helix and HBM4EU (Tamayo-Uria et al., 2019; Ottenbros et al., 2021)

As for method 1, multivariate analysis and correlation calculations and their corresponding proposed cut-off values are presented in Appendix C. This method has a drawback since potent compounds with low co-exposure might not be considered for grouping. Therefore, the Scientific Committee recommends its use only when methods 1 and 2 cannot be applied and associated uncertainties should be assessed and documented.

A workflow for the application of these prioritisation methods is provided below.

4.2. Workflow for the prioritisation of multiple chemicals using risk-based and exposure-driven approaches

When applying a prioritisation approach, exposure metrics for each chemical are required. Typically, exposure metrics result from combining occurrence data of each chemical in different foods with consumption data for the food items. Exposure metrics can be extracted also from previous assessments and, depending on data availability, can range from default values (tier 0) to individual co-occurrence data and individual consumption data (tier 3) (EFSA Scientific Committee, 2019; FAO/WHO, 2019). It is noted that the tiers for occurrence and consumption data do not necessarily match.

Exposure metrics can also be expressed on an internal dose basis when biomonitoring data, TK data (i.e. body burden) or TK models are available for individual chemicals in body fluids (e.g. plasma, milk etc.). Such exposure estimates based on internal dose can be applied to each chemical under consideration for the combined risk-based approach, the risk-based approach for single chemicals and the exposure-driven approach (EFSA Scientific Committee, 2019).

It is important to consider the time frame of exposure and the TK of the substances to decide whether they would co-occur and would have the potential for eliciting combined toxicity. If the chemicals are eliminated fast from the body, the likelihood of internal co-exposure decreases with non-concomitant exposure events. In contrast, co-exposure is very likely if persistent chemicals with long biological half-lives such as Persistent Organic Pollutants (POPs) are within an assessment group. For

further details, the reader is referred to Section 4 (exposure Section) of the MIXTOX guidance document (EFSA Scientific Committee, 2019).

Figure 4 describes the workflow for the three prioritisation methods described above.

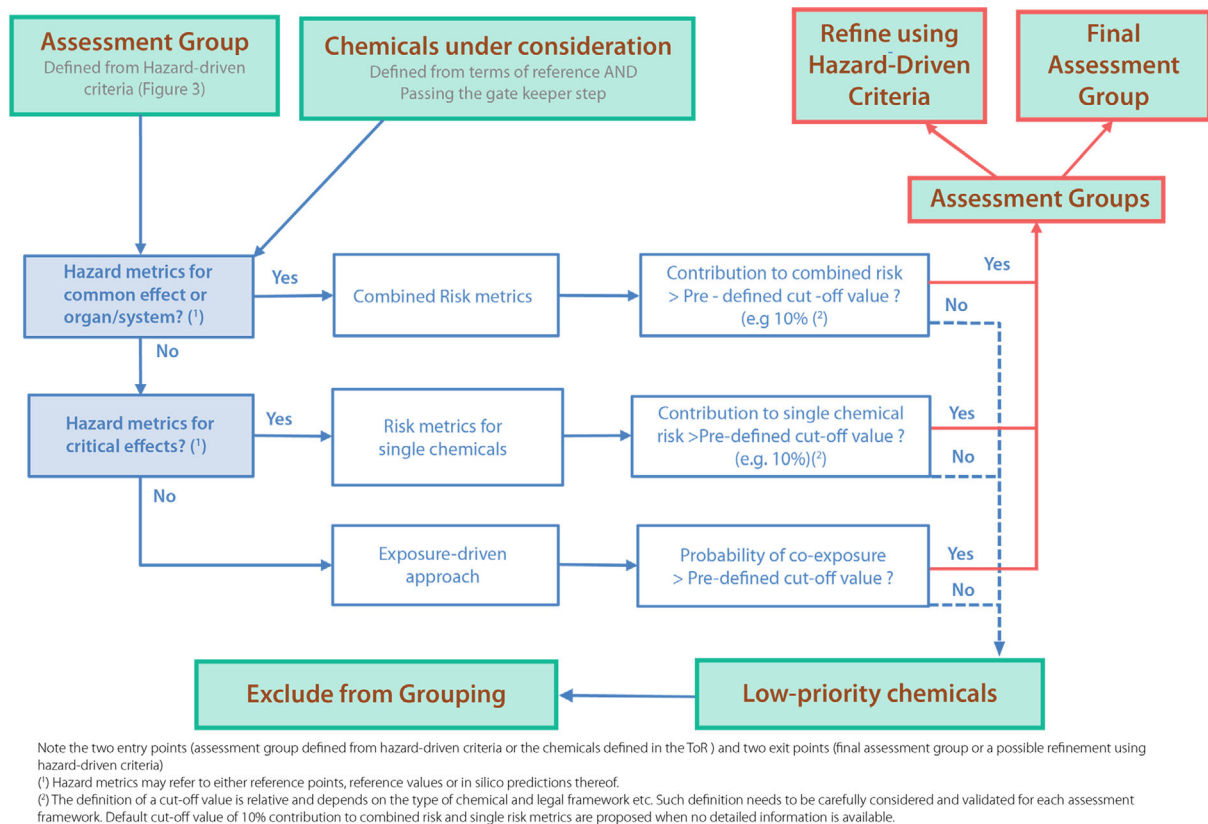


Figure 4: Workflow for risk-based and exposure-driven prioritisation methods applied to the grouping of chemicals into assessment groups

The starting point is either the assessment group defined using hazard-driven criteria (Section 3, Figure 3) or the multiple chemicals defined in the ToR and passing the gate-keeper step (EFSA Scientific Committee, 2019):

1) Combined risk metrics

Assess whether hazard metrics are available for common effect or common target organ/system for each chemical in the assessment group or each chemical under consideration.

If *No*, assess the accessibility of hazard metrics for critical effects and proceed with risk metrics for single chemicals.

If *Yes*, proceed with the combined risk-based approach to determine combined risk metrics, on an external or internal dose basis, and determine the relative contribution of each chemical to the combined risk in the assessment group as a probability. Chemicals showing an estimated contribution to the combined risk above the predefined cut-off value, will remain in the assessment group (Figure 4) and can either constitute the final assessment group or the assessment group can be refined using hazard-driven criteria (Figure 3 in Section 3). In contrast, low-priority chemicals can be excluded from the assessment group (EFSA Scientific Committee, 2019).

2) Risk metrics for single chemicals

Assess the accessibility of hazard metrics for the critical effect for each chemical in the assessment group or each chemical under consideration.

If *No*, proceed with the exposure-driven approach.

If *Yes*, proceed and collect the available hazard metrics reflecting the critical effects for the single chemicals and determine risk metrics as follows:

Risk metrics for the single chemicals are typically expressed as hazard quotient (HQ), on an external or internal exposure basis, divided by the health-based guidance value for the effect (EFSA Scientific Committee, 2019). In the absence of a health-based guidance value, an MoE approach can be applied as the ratio of individual reference points to the estimated human exposure. Chemicals with a risk metric above a predefined cut-off value remain under consideration for grouping. As for the combined risk-based approach, Figure 4 shows that these chemicals can either constitute the final assessment group or additional hazard data may need to be collected to refine the assessment group using hazard-driven criteria (Figure 3 in Section 3). In contrast, when the risk metric for the single chemical is demonstrated to be low, the chemical is considered as a low-priority chemical and may be excluded from the assessment group. The cut-off value represents a protection goal and therefore needs to be defined by risk managers.

Appendix D provides an example of the use of risk metrics for single chemicals as a prioritisation method for grouping pesticides with acute neurotoxic effects into assessment groups. In addition, the example illustrates the impact of excluding low-priority compounds on the combined risk assessment using a combined margin of exposure approach (MoE_T).

3) Exposure-driven approaches

Hazard metrics may not be readily accessible for all chemicals within an assessment group or for the chemicals under consideration. This can be an obstacle, when the risk assessment question deals with a large number of chemicals (e.g. all contaminants in human blood or breast milk) or when the collection or generation of hazard data for a number of chemicals is needed. This exposure-driven approach method allows to identify chemicals that have a likelihood of co-exposure, expressed as probability. Chemicals that have a probability of co-exposure above a predefined cut-off value would remain under consideration for grouping. In contrast, chemicals with a low probability of co-exposure would be considered as of low-priority for combined risk assessment and can be excluded. As for methods 1 and 2, for chemicals remaining under consideration, Figure 4 provides two options: final assessment group or refinement of the assessment group using hazard-driven criteria for which hazard data will need to be retrieved or generated (Figure 3 in Section 3). A similar approach, as proposed for the combined risk-based method, can be used for combined exposure.

An example of application of this method has been illustrated from the ANSES Pericles project under which dietary co-exposure of the French general population to 79 pesticide residues was first assessed using the exposure-driven approach and the pesticides contributing most to the co-exposure were identified (Crépet et al., 2013a,b). Appendix E illustrates the use of this exposure-driven approach as a prioritisation method for multiple contaminants from human breast milk and results are compared with risk metrics for single chemicals (ANSES, in press).

5. Recommendations

The Scientific Committee recommends that the applicability and implementation of the proposed scientific criteria for grouping chemicals into assessment groups as described in this guidance document should be assessed through a testing phase in relevant EFSA panels using specific case studies. In addition, inter-agency, Member State and international cooperation in this area are recommended to facilitate data exchange and harmonisation of methods and tools.

Recommendations for future work to support further harmonisation of methodologies for grouping chemicals into assessment groups using scientific criteria include:

Hazard-driven criteria

- Further update the OpenFoodTox database with systematic data collection for individual chemicals reporting hazard metrics for specific effects, target organs, MoA, AOPs and related properties, whenever possible. The database will support the implementation of the grouping of chemicals into assessment groups in an efficient way.
- The use of OECD international harmonised standards to structure data on chemical properties (i.e. OECD harmonised templates (OHT)) is recommended to:
 - a) Develop structured means for WoE approaches and avoid divergence for grouping chemicals into assessment groups across EFSA Panels in the different assessments;

- b) Support integration of high throughput, *in vitro* and omics data generated from NAMs as currently investigated worldwide (OECD, US EPA, EFSA) and Horizon 2020 and Horizon Europe programmes (EuroMix, EUTOXRISK, HBM4EU, PARC, etc.). For this purpose, the existing OHT 201 template for intermediate effects can be updated and will also provide means to further integrate data from NAMs and improve the mechanistic basis for setting assessment groups using data on MoA, Key Events and AOPs for multiple chemicals.
- Further develop and implement generic *in silico* approaches that could support grouping of chemicals for combined toxicity (i.e. QSARs) and TK properties (i.e. 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).

Prioritisation methods

- The appropriateness of cut-off 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. This is particularly applicable to the default cut-off values of 10% for contribution to combined risk or to single risk metrics recommended here.
- Develop user-friendly open source tools to implement the use of prioritisation methods for risk assessment of combined exposure to multiple chemicals. The tools would include risk-based and exposure-driven approaches (Section 4) which can include simple deterministic as well as probabilistic methods for which further implementation as recommended in EFSA MIXTOX guidance (EFSA Scientific Committee, 2019).

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Abbreviations

AChE	acetylcholinesterase
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AEP	aggregated exposure pathways
AOP	adverse outcome pathways
ARfD	acute reference dose
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
CAG	cumulative assessment group
CONTAM	EFSA Scientific Panel on Contaminants in the Food Chain
ECHA	European Chemicals Agency
HBGV	health-based guidance value
HI	hazard index
HQ	hazard quotient
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOE	line of evidence
LOQ	limit of quantification
LOR	limit of reporting
JRC	Joint Research Centre of the European Commission
MCR	maximum cumulative ratio
MIE	molecular initiating event
MoA	mode of action
MOE	margin of exposure
MOE _T	combined margin of exposure
mRPI	modified Reference Point Index
NAMs	New Approach Methodologies
NOAEL	no observed adverse effect level
PPR	EFSA Scientific Panel on Plant Protection Products and their Residues
PRAS	EFSA's Unit on Pesticides
QSAR	Quantitative Structural Activity Relationship
RAC	raw agricultural commodity
RP	references point
RV	reference value
SCER	EFSA's Scientific Committee and Emerging Risks Unit
SNMU	Sparse non-negative matrix under-approximation
TDI	tolerable daily intake
TTC	threshold of Toxicological Concern
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
WoE	Weight of Evidence

Appendix A – Glossary

Acceptable daily intake (ADI): The estimate of the amount of a chemical in food or drinking water, expressed on a body weight basis that can be ingested daily over a lifetime without appreciable health risk to the consumer. It is derived on the basis of all the known facts at the time of the evaluation (WHO, 2009).

Adverse effect: Change in the morphology, physiology, growth, reproduction, development or lifespan of an organism that results in impairment of functional capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences (EFSA PPR Panel, 2013a).

Adverse outcome pathway (AOP): Conceptually, an AOP can be viewed as a sequence of events commencing with initial interactions of a stressor with a biomolecule in a target cell or tissue (i.e. molecular initiating event), progressing through a dependent series of intermediate events and culminating with an adverse outcome. AOPs are typically represented sequentially, moving from one key event to another, as compensatory mechanisms and feedback loops are overcome (OECD, 2018).

Aggregate exposure: Exposure to the same chemical from multiple sources and by multiple routes (OECD, 2018).

Aggregate exposure pathways (AEP): An AEP is the assemblage of existing knowledge on biologically, chemically and physically plausible, empirically supported links between introduction of a chemical or other stressor into the environment and its concentration at a site of action, i.e. target site exposure as defined by the National Academy of Sciences, USA. It may be relevant to exposure assessment, risk assessment, epidemiology or all three. The target site exposure (the terminal outcome of the AEP), along with the molecular initiating event from the AOP, represents the point of integration between an AEP and an AOP (Teeguarden et al., 2016).

Assessment group: Chemicals that are treated as a group by applying a common risk assessment principle (e.g. dose addition) because these components have some characteristics in common (i.e. the grouping criteria).

Component-based approach: An approach in which the risk of combined exposure to multiple chemicals is assessed based on exposure and effect data of the individual components.

Cumulative assessment group (CAG): A type of assessment group in which the active substances could plausibly act by a common mode of action, not all of which will necessarily do so (EFSA PPR Panel, 2013a).

Dose addition: Dose is the exposure metric used in human health risk assessment. All components in a mixture behave as if they were dilutions of one another.

Health-based guidance value (HBGV): A numerical value derived by dividing a point of departure (a no observed adverse effect level, benchmark dose or benchmark dose lower confidence limit) by a composite uncertainty factor to determine a level that can be ingested over a defined time period (e.g. lifetime or 24 h) without appreciable health risk (WHO, 2009).

Mode of action (MoA): biologically plausible sequence of key events in an organism leading to an observed effect, commonly supported by robust experimental observations and mechanistic data. It refers to the major steps leading to an adverse health effect following interaction of the chemical with biological targets. It does not imply full understanding of mechanism of action at the molecular level (EFSA PPR Panel, 2013a).

NAMs: New approach methodologies are taken in a broad context to include *in silico* approaches, *in chemico* and *in vitro* assays, as well as the inclusion of information from the exposure of chemicals in the context of hazard assessment. They also include a variety of new testing tools, such as 'high-throughput screening' and 'high-content methods' e.g. genomics, proteomics, metabolomics; as well as some 'conventional' methods that aim to improve understanding of toxic effects, either through improving toxicokinetic or toxicodynamic knowledge for substances. (ECHA, Proceedings of a scientific workshop Helsinki, 19–20 April 2016).

Probability: defined depending on philosophical perspective (1) the frequency with which samples arise within a specified range or for a specified category; (2) quantification of uncertainty as degree of belief on the likelihood of a particular range or category (EFSA Scientific Committee, 2018a). The latter perspective is implied when probability is used in a weight of evidence assessment to express relative support for possible answers.

Problem formulation: In the present document, problem formulation refers to the process of clarifying the questions posed by the terms of reference, deciding whether and how to subdivide them, and deciding whether they require weight of evidence assessment.

Reference point (RP): defined point on an experimental dose response relationship for the critical effect (i.e. the biologically relevant effect occurring at the lowest dose level). This term is synonymous to point of departure. Reference points include the lowest or no observed adverse effect level (LOAEL/NOAEL) or benchmark dose lower confidence limit (BDML), used to derive a reference value or MOE in human and animal health risk assessment.

Reference value (RV): the estimated maximum dose (on a body mass basis) or concentration of an agent to which an individual may be exposed over a specified period without appreciable risk. Reference values are established by applying assessment factor(s) to the reference point. Examples of reference values in human health include the acceptable daily intake (ADI) for food and feed additives, and pesticides, tolerable upper intake levels (UL) for vitamins and minerals, and tolerable daily intake (TDI) for contaminants and food contact materials. Examples for acute effects and operators are the acute reference dose (ARfD) and the acceptable operator exposure level (AOEL).

Refinement: one or more changes to an initial assessment, made with the aim of reducing uncertainty in the answer to a question. Sometimes performed as part of a 'tiered approach' to risk or benefit assessment.

Relevance: The contribution a piece or line of evidence would make to answer a specified question, if the information comprising the line of evidence was fully reliable. In other words, how close is the quantity, characteristic or event that the evidence represents to the quantity, characteristic or event that is required in the assessment. This includes biological relevance (EFSA Scientific Committee, 2017b) as well as relevance based on other considerations, e.g. temporal, spatial, chemical, etc.

Reliability: The extent to which the information comprising a piece or line of evidence is correct, i.e. how closely it represents the quantity, characteristic or event to which it refers. This includes both accuracy (degree of systematic error or bias) and precision (degree of random error).

Specific effects: Specific effects have been defined by the PPR Panel in 2013 to identify pesticides which may be grouped into cumulative assessment groups (CAG) and further considered for cumulative risk assessment (EFSA PPR Panel, 2013b). These specific effects have to be observed at statistically significant and/or biologically relevant level in at least one whole organism toxicity test and are identified based on information analysis and expert judgement aiming at excluding local effects, non-adverse effects, effects not relevant to humans and non-specific effects (e.g. age-related or occurring at or above the maximum tolerated dose). The unambiguous nature of the specific effects should be evaluated as well. When the specific effect is to be used under the adverse outcome pathway (AOP) approach as an apical outcome, it can be referred to as 'adverse outcome'. It should be noted that the 'specific effect' or the 'adverse outcome' not necessarily consist of a single toxicity endpoint observed or measured in regulatory toxicology studies (e.g. acute or chronic acetylcholinesterase inhibition), but rather sometimes may be a set of endpoints that make up an effect of greater biological complexity (e.g. hypothyroidism or functional alterations of the motor division of the nervous system).

Toxicodynamics: Process of interactions of toxicologically active substances with target sites in living systems, and the biochemical and physiological consequences leading to adverse effects (EFSA PPR Panel, 2008).

Toxicokinetics: (1) Process of the uptake of substances by the body, the biotransformation they undergo, the distribution of the parent chemicals and/or metabolites in the tissues and their elimination from the body over time. (2) Study of such processes (EFSA PPR Panel, 2008).

Uncertainty: A general term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question. Available knowledge refers here to the knowledge (evidence, data, etc.) available to assessors at the time the assessment is conducted and within the time and resources agreed for the assessment. Sometimes uncertainty is used to refer to a source of uncertainty (see separate definition), and sometimes to its impact on the conclusion of an assessment (EFSA Scientific Committee, 2018a).

Uncertainty analysis: A collective term for the processes used to identify, characterise, explain and account for sources of uncertainty (EFSA Scientific Committee, 2018a).

Variability: Heterogeneity of values over time, space or different members of a population, including stochastic variability and controllable variability (EFSA Scientific Committee, 2018a,b).

Weight of evidence assessment: A process in which evidence is integrated to determine the relative support for possible answers to a scientific question.

Weighing the evidence: The second of three basic steps of weight of evidence assessment that includes deciding what considerations are relevant for weighing the evidence, deciding on the methods to be used and applying those methods to weigh the evidence.

Weighing: Weighing refers to the process of assessing the contribution of evidence to answering a weight of evidence question. The basic considerations to be weighed are identified in this Guidance as reliability, relevance and consistency of the evidence.

Weight of evidence: The extent to which evidence supports one or more possible answers to a scientific question. Hence, 'weight of evidence methods' and 'weight of evidence approach' refer to ways of assessing relative support for possible answers.

Appendix B – Generic Weight of Evidence Methodology for grouping multiple chemicals into assessment groups using hazard-driven criteria

This appendix proposes a generic example to apply the WoE approach for grouping chemicals into assessment groups using hazard-driven criteria. For full details, the reader is referred to the WoE Guidance document which also provides an example for setting cumulative assessment groups for pesticides (Appendix C.2) (EFSA Scientific Committee, 2017a). Here, a generic example applicable to most EFSA Panels dealing with chemical risk assessment is provided.

Problem formulation

EFSA is required to produce a risk assessment of combined exposure to five contaminants (A, B, C, D, E) with common adverse outcome using a component-based approach. Each contaminant has been previously assessed individually by EFSA and individual hazard metrics and exposure metrics are available for risk characterisation. As described in the MIXTOX GD, the problem formulation requires a description of the mixture, conceptual model and methodological approach to produce an analysis plan and proceed with the risk assessment (EFSA Scientific Committee, 2019). Here, the question focuses on the application of hazard-driven criteria for the grouping of the five contaminants into assessment groups and does not address the whole risk assessment process.

Weight of evidence assessment

A generic approach for grouping chemicals into assessment groups using a WoE assessment is illustrated in Figure B.1:

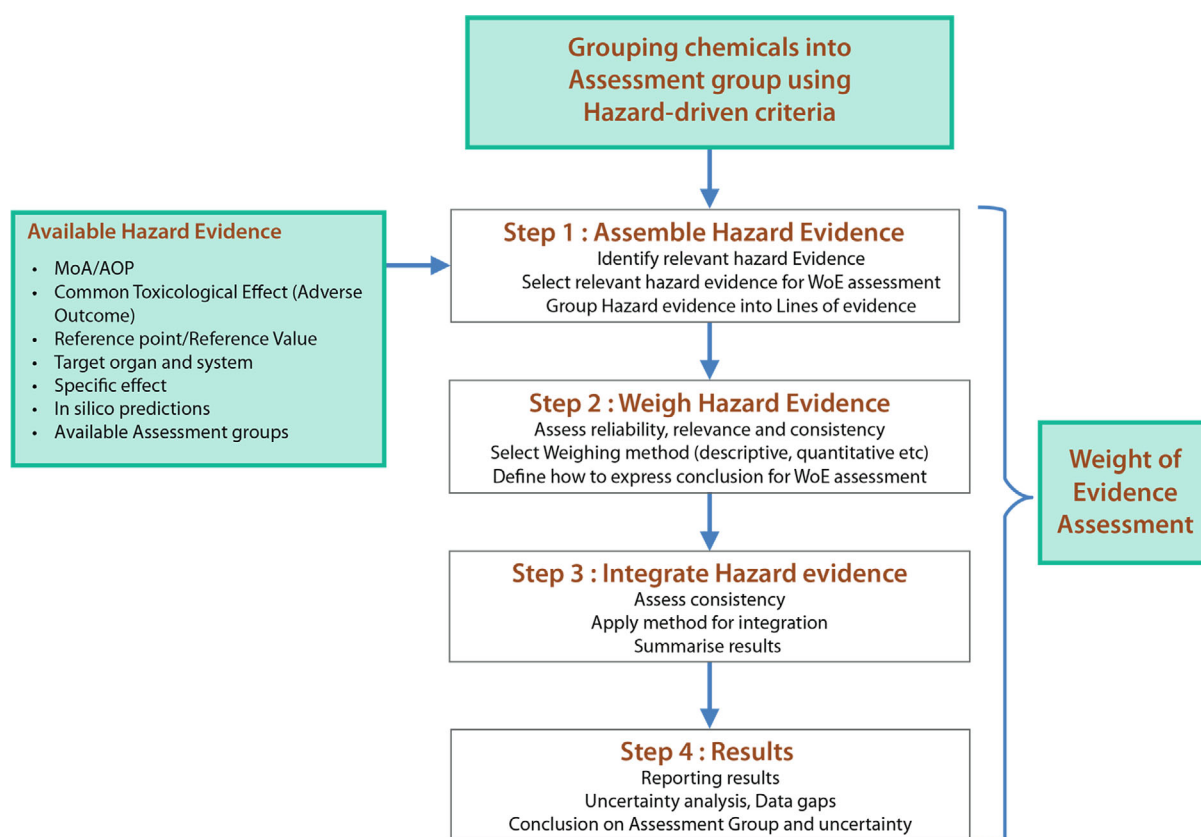


Figure B.1: Generic approach for grouping chemicals into assessment groups using a WoE assessment

Assembling the evidence

Hazard data for chemicals A, B, C, D and E are collected from previous EFSA assessments, available open source databases (i.e. OpenFoodTox, US-EPA Chemistry dashboard, OECD E-chem portal, JECFA assessments, etc.) and the peer-reviewed literature. Such data are then assembled into pieces of evidence and lines of evidence¹ including:

- Critical effect from subchronic toxicity, associated target organ and reference point (dose response).
- Specific effects and associated target organ from subchronic toxicity studies.
- MoA information (i.e. information on key events, dose response, biochemical changes and adverse outcome)
- From this analysis, four lines of evidence (LOEs) can be assembled:

LOE1: Dose response relationships for specific effects; LOE2: Clinical evidence for the effect; LOE3: Biochemical evidence for the effect; LOE4: Mode of action supporting the effect.

Weighing and integrating evidence

Methods for weighing and integrating hazard evidence have been described elsewhere and include qualitative methods (listing, best professional judgement, semi-quantitative methods (causal criteria, logic); quantitative methods (scoring, indexing and quantification) (Linkov et al., 2009; EFSA Scientific Committee, 2017a). The methods of choice to be applied will depend on data availability, context of the assessment, complexity of the method, time constraints and resources and the assessor should provide a rationale for choosing a particular method. A key aspect for weighing and integrating the evidence is the assessment of the reliability, relevance and consistency of the evidence and the iterative nature of the process (EFSA Scientific Committee, 2017a).

For each chemical A, B, C, D and E, a semi-quantitative scale was applied to the weighing and integration of the four LOEs while assessing reliability, relevance and consistency of each LOE as low (*), moderate (**), and high (***). Expert judgement was then applied to conclude on the probability of membership to the assessment group (Table B.1).

Table B.1: Semi-quantitative WoE analysis for the grouping of chemicals A, B, C, D and E in assessment groups

Chemical	LOE ₁ : specificity and dose response	LOE ₂ : clinical	LOE ₃ : biochemical	LOE ₄ : MoA	Assessment group level	Probability of membership to assessment group
A	*** (AO1)	NA	***	*** (MOA ₁)	MoA	Extremely likely (99–100%)
B	*** (AO1)	NA	***	*** (MOA ₁)	MoA	
C	*** (AO1)	***	***	*** (MOA ₁)	MoA	
D	*** (AO2)	NA	**	** (MOA ₂)	MoA	Likely (66–90%)
E	** (AO2)	NA	**	** (MOA ₂)	MoA	

AO1: adverse outcome 1; AO2: adverse outcome 2; relative weights: Moderate (**), High (***); NA: not available; Probability scale (EFSA Scientific Committee, 2017a): Extremely likely (99–100%), Very likely (90–99%), Likely (66–90%), as likely as not (33–66%), Unlikely (10–33%), Very Unlikely (1–10%), extremely unlikely (0–1%).

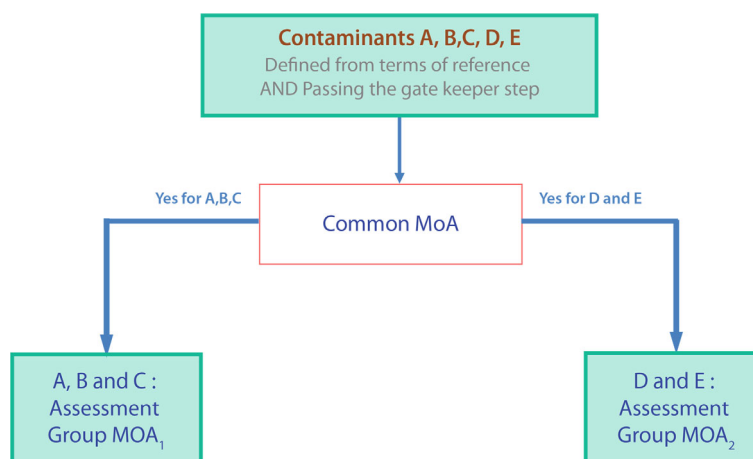
Conclusion and summary of results

Table B.2 and Figure B.2 summarise the WoE assessment for the grouping of chemicals A, B, C (associated with adverse outcome 1) into common assessment group MOA₁ and D and E (associated with adverse outcome 2) into common assessment groups MOA₂.

¹ Piece of evidence: A broad term used to refer to distinct elements of evidence that may be combined to form a line of evidence, e.g. a single study, expert judgement or experience, a model or even a single observation. Line of evidence: set of evidence of similar type (EFSA, 2015).

Table B.2: Proposed summary table of the weight of evidence assessment to group chemicals into common assessment groups using MoA information

Question		Can contaminants A, B, C, D, E be grouped in common assessment groups?
Assemble evidence	Select evidence	Previous EFSA assessments, open source databases and open literature
	Lines of evidence	LOE1: Dose response relationships for specific effects LOE2: Clinical data for effect; LOE3: Biochemical evidence for the effect; LOE4: Mode of action supporting the effect
Weigh the evidence	Methods	Semi-quantitative scale (low, moderate, high)
	Results	Tabular forms for the weighing of each LOE (see Table B.1)
Integrate the evidence	Methods	Semi-quantitative scale/Expert judgement/Probability scale
	Results	The WoE assessment concludes that: <ul style="list-style-type: none"> – Chemicals A, B, C share a common MoA (MOA₁), adverse outcome (AO1) and can be grouped into assessment group MOA₁. Expert judgement concludes that membership to this group for A, B and C is extremely likely (99–100%). – Chemicals D and E share a common MoA (MOA₂), adverse outcome (AO2) and can be grouped into assessment group MOA₂. Expert judgement concludes that membership to this group for D and E is likely (66–90%). Clinical evidence was scarce for most chemicals and no information was available on AOPs for A, B, C, D or E.



MoA₁ and MoA₂ are different modes of action (MoAs) which produce different adverse outcomes.

Figure B.2: Hazard-based criteria for grouping contaminants A, B, C, D and E in assessment groups using MoA information

Appendix C – Statistical methods to study the probability of combined risk or combined exposure

The combined risk-based approach (method 1) allows to prioritise multiple chemicals for grouping into assessment groups (Section 4) and to identify low-priority chemicals through considering the relative contribution of each individual chemical to the combined risk. Hence, the contribution of risk quotient of each chemical to the combined risk can be calculated and chemicals with a contribution to a combined risk below a predefined cut-off value can be removed from the assessment group. In addition, relationships between chemicals with regards to combined risk can also be analysed using correlation and multivariate analyses. These statistical analyses can also be applied to an exposure-driven approach (method 3) and are described thereafter.

A straightforward method to identify chemicals with high probability of combined risk or combined exposure is to assess the respective correlations between the risk metrics or the exposures metrics. Thus, those chemicals showing no or low correlations can be excluded from an assessment group. Spearman and Pearson correlation coefficients¹ are commonly used to assess the strength and direction of association between two variables. A positive correlation coefficient indicates that when the first variable increases, the second variable increases too. Likewise, a negative correlation coefficient indicates that when the first variable decreases, the second variable decreases too. The closer the correlation coefficient to 1 (or to -1), the strongest the dependencies between the variables.

As a rule of thumb, one can say that, for identifying relevant co-exposures, a correlation of magnitude $r = 0.4$ or greater would usually be of relevance, with an r value above 0.6 or 0.7 being considered strong. In that case, exclusion of chemicals from the assessment group with no or low correlation ($r < 0.4$) may be justified. However, the r value to exclude chemical must be fixed on a case-by-case basis, as for example correlations from biomonitoring data are generally lower (highest values around 0.5) compared to those from external exposures.

Correlation analysis has been applied previously together with a clustering method to identify multiple pesticides in the highest exposed groups of individuals (Crépet et al., 2013a,b). If for a particular pesticide, more than 90% of the analytical results for each commodity were left-censored i.e. lower than the limit of reporting (limit of detection (LOD) or limit of quantification (LOQ)), it was considered not important for co-exposure calculation. Thus, a total of 79 pesticides out of over 300 were selected for the analysis. Residues of the selected pesticides were analysed in 120 raw agricultural commodities (RACs) and in drinking water consumed by the INCA2 population (second French national cross-sectional dietary survey). A total of 306,899 analytical results for pesticides in different commodities corresponding to 8,364 food/residue combinations were modelled using a histogram distribution for each combination. A clustering model was applied to divide individuals into groups with similar patterns of co-exposure. It resulted that two groups of children and three of adults were highly exposed compared to the other groups. After screening the correlations between exposures to 79 pesticides, 25 pesticides with at least one correlation above 0.7 were selected. A correlation cut-off of 0.7 was fixed by the authors to identify low-priority pesticides from the co-exposures of the five subpopulations. Thus, seven mixtures of two to six pesticides each were prioritised.

More recently, Pearson correlations have also been applied to study the relationships between 87 environmental exposures during pregnancy and 122 during the childhood period from biomonitoring data of six European birth cohorts containing a total of 1,301 mother–child (6–11 years) pairs. In this case, correlation coefficients were plotted using network visualisation and makes it possible to group substances in 19 exposure groups (atmospheric pollutants, PBDEs, water DBPs, etc.). Then correlations between exposure groups were studied for the overall data set and within cohorts. The highest correlation between exposure groups was found to be 0.45. An important limitation of such correlation methods lies in the fact that they can only detect pairwise correlations between two chemicals (Nguyen et al. 2014).

Thus, more advanced statistical methods using multivariate analysis have been adapted to identify prevalent chemical combinations from combined exposure (Béchaux et al., 2013; Traoré et al., 2016; Chazelas et al., 2021; Crépet et al., in press) or combined risk (Crépet et al., 2019; Van der Voet et al., 2020). The authors proposed to combine dimension reduction and classification/clustering techniques to analyse interdependencies between risk or exposure for several chemicals and to define population clusters in relation to combined risk or combined exposures to multiple chemicals. For example, the Sparse non-negative matrix under-approximation (SNMU) has been applied to decompose the

combined risk or combined exposure matrix into two non-negative matrices W and H , such that the product of the two is as close as possible to the original matrix (Gillis and Plemmons, 2013). Matrix W contains weights of chemicals per mixture, and matrix H contains the coefficients of the presence of the mixture per individual. Chemicals with non-zero entries in each column of W indicate that the chemicals have a positive probability to be found together. The closer to 1 the W weight, the higher the probability of combined risk or exposure. A classification or clustering method to the matrix H is then applied to identify cluster of individuals for which exposure to the multiple chemicals is either low or high. With these methods, chemicals with zero or low entries in W or multiple chemicals for which exposures were found as the lowest in clusters can be considered as low-priority chemicals. The chemical combinations that remain under consideration for further refinement of the grouping into assessment groups are the ones with high exposure for one or several clusters of the population or the ones contributing to a high proportion of the variance in the overall data set.

With regard to other methods, Su et al. (2014) proposed to use copulas² to characterise dependency structures between multiple chemicals in personal exposure measurements of volatile organic compounds. Other methods based on frequency of co-occurrence have been applied to identify chemical combinations. These include frequent itemset mining³ which has also been applied by Kapraun et al. (2017) to exposure and allows to count combinations of chemicals present and to identify the most prevalent ones using the US National Health and Nutrition Examination Survey (NHANES). Prado et al. (2019) used co-occurrence network to study frequency of combinations of pesticides in pollen samples.

The maximum cumulative ratio (MCR) developed by Price and Han (2011) is also a common method to prioritise chemicals as described in the MIXTOX guidance document (EFSA Scientific Committee, 2019). MCR allows the categorisation of mixtures according to whether or not they are of concern for toxicity and, if so, whether this is driven by one substance or multiple substances (De Brouwere et al., 2014). The MCR is the ratio of the combined risk estimate (e.g. HI) to the highest risk calculated for a single chemical within the assessment group (e.g. maximum HQ) and provides a measure of whether combined risks are dominated by a single chemical or from the contribution of multiple chemicals. An MCR of 1 for a chemical in an assessment group indicates that the combined risk metric is dominated by a single chemical and that a combined risk assessment is not needed. When the MCR is higher than 1, it indicates that more than one chemical contributes to the risk. At its maximum value, the MCR equals to the number of chemicals assessed where all chemicals have an equal contribution to the combined risk and all chemicals should be prioritised for further/refined assessment (EFSA Scientific Committee, 2019).

² Copulas are functions that enable us to separate the marginal distributions from the dependency structure of a given multivariate distribution. <http://www.columbia.edu/~mh2078/QRM/Copulas.pdf>

Appendix D – Risk-based approach for single chemicals as a prioritisation method for grouping pesticides into assessment groups

Section 4 and Figure 4 describe prioritisation methods for grouping chemicals into assessment groups using a combined risk-based approach, a risk-based approach for single chemicals and an exposure-driven approach. The example presented here illustrates the application of a risk-based approach for single chemicals to identify low-priority pesticides with acute effects on the nervous system.

The pesticides under consideration have been defined from the terms of reference, passed the gate keeper step and enter the workflow (Figure 4) for prioritisation. 'Assess the accessibility of hazard metrics for the critical effect for each chemical in the Assessment Group or each chemical under consideration' was answered with 'YES' (see description next paragraph).

The assessment starts with 100 pesticides for which concentrations and occurrence patterns were available (Van Klaveren et al., 2019). For exposure to single pesticides, the concentration in drinking water was set at 0.1 µg (for further details, see Van Klaveren et al., 2019; EFSA, 2020a; Te Biesebeek et al., 2021). For 96 of the 100 pesticides, acute reference doses (ARfDs) were extracted from the OpenFoodTox database (Dorne et al., 2017) and EU-Pesticides Database.³ ARfDs were not available for azinphos-ethyl, fonofos, permethrin and tetramethrin. The EFSA MIXTOX guidance recommends using the lowest available ARfD of the particular cumulative assessment group (CAG) for imputation of missing ARfDs in a cumulative risk assessment (EFSA Scientific Committee, 2019) as a conservative approach. For pesticides without a health-based guidance values (HBGVs), Te Biesebeek et al. (2021) suggested three approaches:

- Set additional inclusion criteria based on e.g. occurrence patterns, authorised uses and expected consumption of foods containing the pesticides;
- Derive a tentative HBGV where possible;
- Use the lowest HBGV available as a surrogate as suggested by the Scientific Committee (2019)

Information on occurrence patterns, authorised uses was retrieved for azinphos-ethyl, fonofos, permethrin and tetramethrin. The occurrence data of fonofos only contained analytical values below the level of quantification, which would result into zero exposure using a cumulative exposure calculation according to EC Tier 2018 II (Van Klaveren et al., 2019). In addition, both azinophos-ethyl and permethrin are not authorised in the EU and showed very low percentages of potential presence in the occurrence data (max 0.32% presence). The presence of tetramethrin, which is authorised in the EU, was also very low in the occurrence data (maximum of 0.33% presence). Based on occurrence patterns, authorised uses and expected low consumption of foods containing these pesticides, pesticides with a missing ARfDs were assumed of low-priority and thus not further considered. The assessor proceeded with prioritisation method 2: Risk metrics for single chemicals using the hazard quotient (HQ) method.

Two prioritisation scenarios were applied for single pesticides: Hazard Quotients calculation at the 95th and 99.9th exposure percentiles, the former as a standard scenario in risk assessment and the later as the required percentile for the human risk assessment of combined exposure to multiple pesticides (see Te Biesebeek et al., 2021). HQs for each pesticide were calculated as the individual ratio between the exposure to a single pesticide observed at a particular exposure percentile and the ARfD of the relevant predefined cut-off values for identifying low-priority pesticides. The cut-offs have been set to 1% and 10% of the ARfD corresponding to HQ values of 0.01 and 0.1, respectively (EFSA Scientific Committee, 2019; FAO/WHO, 2019). Pesticides were prioritised and grouped according to four different criteria:

- $HQ(p95) \leq 0.01$: low-priority pesticides which are not considered based on the 95th HQ percentile ≤ 0.01 .
- $HQ(p95) \leq 0.1$: low-priority pesticides which are not considered based on the 95th HQ percentile ≤ 0.1 .
- $HQ(p99.9) \leq 0.01$: low-priority pesticides which are not considered based on the 99.9th HQ percentile ≤ 0.01 .
- $HQ(p99.9) \leq 0.1$: low-priority pesticides which are not considered based on the 99.9th HQ percentile ≤ 0.1 .

Table D.1 illustrates the results of the prioritisation exercise.

³ <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN>

Table D.1: Overview of pesticides in the assessment group low-priority pesticides and those remaining under consideration based on critical effects

Hazard-driven Criteria	All pesticides under consideration	Screening on 95th HQ percentiles		Screening on 99.9th HQ percentiles	
		Low-priority pesticides/remaining under consideration		Low-priority pesticides/remaining under consideration	
		HQ(P95) ≤ 0.01	HQ(P95) ≤ 0.1	HQ(P99.9) ≤ 0.01	HQ(P99.9) ≤ 0.1
Critical Effect	100	47/53	89/11	22/78	54/46

HQ: hazard quotient.

For single pesticides with HQ values below the predefined cut-off values, at the 95th percentile of exposure, 47 pesticides had a $HQ \leq 0.01$ and 89 pesticides had a $HQ \leq 0.1$. At the 99.9th percentile of exposure, 22 pesticides showed a $HQ \leq 0.01$ and 54 pesticides showed a $HQ \leq 0.1$. Those were identified as low-priority for combined risk assessment and thus not considered for further assessment.

Following the workflow of Figure 4, the pesticides were grouped using hazard-driven criteria (e.g. common effect or common MoA/AOP). All 100 pesticides were previously grouped as pesticides that can cause functional alterations of the motor division of the nervous system (CAG-NAM, Van Klaveren et al., 2019). Forty-seven of these 100 pesticides can cause brain and/or erythrocyte acetylcholinesterase (AChE) inhibition (CAG-NAN, Van Klaveren et al., 2019). Using the prioritisation criteria listed above, the CAG-NAM contained 53 high priority pesticides with a $HQ > 0.01$ at the 95th percentile, the CAG-NAN contained 28 high priority pesticides with an $HQ > 0.01$. For the $HQ(p95) \leq 0.1$, the criteria resulted into a CAG-NAM and CAG-NAN with 11 and 7 priority pesticides, respectively. See Table D.2.

Table D.3 shows that CAG-NAM and CAG-NAN contained 78 and 26 high priority pesticides with a $HQ > 0.01$ at the 99th percentile, while the CAG-NAM and CAG-NAN contained the number of 46 and 17 pesticides with a $HQ > 0.1$ at the 99th percentile.

Risk characterisation: impact of excluding low-priority pesticides on total margin of exposures (MOE_T)

The assessor then tested the impact of excluding low-priority pesticides on the combined risk assessment using a combined MOE_T . According to the risk management principles, exposure calculations are performed using a tiered approach. Tier 1 accounts for very conservative assumptions that are less resourceful regarding data and computational capacity. In contrast, tier 2 is more resourceful as it includes more refined assumptions (EFSA, 2021). Here, only the tier 2 approach was used for the refined assessment groups compiled after the prioritisation criteria ($HQ(p95) \leq 0.01$, $HQ(p95) \leq 0.1$, $HQ(p99.9) \leq 0.01$ and $HQ(p99.9) \leq 0.1$). The tier 2 cumulative exposure assessment used specific NOAELs for the refined assessment group (CAG-NAN and CAG-NAM; see Te Biesebeek et al., 2021). $MOETs$ of 100-fold are interpreted as of low concern as detailed in MIXTOX guidance (EFSA Scientific Committee, 2019). Table D.2 illustrates the $MOETs$ at the 95th percentile of exposure. Likewise, Table D.3 illustrates the $MOETs$ for the 99.9th percentile of exposure. For comparison, both tables also include the $MOETs$ based on cumulative exposure assessment for all 100 CAG-NAM and all 47 CAG-NAN pesticides, thus without exclusion of low-priority chemicals, as previously described by Van Klaveren et al. (2019).

Table D.2 shows that, compared to assessment groups containing all pesticides (thus without exclusion of low-priority pesticides), the combined $MOETs$ at the 95th percentile of exposure groups based on the prioritisation criteria $HQ(p95) \leq 0.01$ (i.e. excluding low-priority substances) were larger. For groups based on $HQ(p95) \leq 0.1$ (thus excluding low-priority pesticides), the combined $MOETs$ at the 95th percentile of exposure were much larger compared with assessment groups containing all pesticides.

In contrast, Table D.3 shows that the combined $MOETs$ at the 99.9th percentile of exposure for assessment groups $HQ(p99.9) \leq 0.01$ and $HQ(p99.9) \leq 0.1$ are equal to the assessment group containing all pesticides. Thus, for pesticides having acute effects on the nervous system, a prioritisation with HQs based on the 99.9th percentile did not have an impact on the cumulative exposure.

Table D.2: Total margin of exposure (MOE_T) and associated uncertainties from cumulative assessments (at the 95th percentile of exposure) for pesticides with acute effects on the nervous system CAG-NAN (acute AChE inhibition) and CAG-NAM (functional alterations of the motor division)

Specific effect	Total margin of exposure (MOE _T) median estimate and 95% CI at the 95th percentile of exposure		
	All pesticides under consideration	CAGs containing pesticides remaining under consideration	
European populations assessed	Tier 2 approach for NAN 47 pesticides	HQ(p95) ≤ 0.01 ^(a) for NAN 28 pesticides	HQ(p95) ≤ 0.1 ^(b) for NAN 7 pesticides
Belgium – Adults	1,160 [1,062–1,249]	1,514 [1,320–1,655]	2,533 [2,049–2,768]
Czech Republic – Adults	1,144 [1,030–1,235]	1,522 [1,273–1,659]	2,638 [2,028–3,002]
Germany – Adults	988 [948–1,025]	1,275 [1,197–1,325]	2,109 [1,915–2,296]
Italy – Adults	973 [626–1,261]	1,125 [654–1,647]	1,534 [856–2,247]
Bulgaria – Other Children	609 [576–636]	876 [820–903]	1,630 [1,504–1,748]
France – Other Children	735 [647–791]	968 [825–1,080]	1,505 [1,240–1,766]
Netherlands – Other Children	610 [578–647]	752 [700–799]	1,024 [948–1,092]
Denmark – Toddler	500 [481–521]	643 [599–688]	905 [834–970]
Netherlands – Toddler	459 [428–489]	556 [518–601]	720 [671–782]
United Kingdom – Toddler	589 [562–613]	792 [754–827]	1,371 [1,249–1,454]
European populations assessed	Tier 2 approach for NAM 100 pesticides	HQ(p95) ≤ 0.01 for NAM 53 pesticides	HQ(p95) ≤ 0.1 for NAM 11 pesticides
Belgium – Adults	1,306 [1,235–1,387]	1,659 [1,524–1,772]	5,800 [4,237–7,080]
Czech Republic – Adults	1,286 [1,190–1,370]	1,676 [1,540–1,806]	6,704 [4,127–8,474]
Germany – Adults	1,142 [1,106–1,178]	1,454 [1,398–1,513]	5,546 [4,608–6,291]
Italy – Adults	1,177 [866–1,402]	1,335 [995–1,650]	2,798 [1,326–4,039]
Bulgaria – Other children	636 [607–667]	797 [734–849]	3,635 [3,260–4,085]
France – Other children	763 [703–834]	905 [810–980]	3,430 [2,558–4,166]
Netherlands – Other Children	725 [680–784]	862 [808–923]	3,234 [2,648–3,632]
Denmark – Toddler	454 [407–511]	505 [435–577]	3,080 [2,685–3,454]
Netherlands – Toddler	566 [542–610]	678 [630–735]	2,468 [2,125–2,751]
United Kingdom – Toddler	578 [542–614]	694 [620–760]	3,905 [3,285–4,301]

AChE: acetylcholinesterase; CI: confidence interval; HQ: hazard quotient.

(a): HQ(p95) ≤ 0.01: low-priority pesticides which are not considered based on the 95th HQ percentile ≤ 0.01.

(b): HQ(p95) ≤ 0.1: low-priority pesticides which are not considered based on the 95th HQ percentile ≤ 0.1.

Table D.3: Total margin of exposure (MOE_T) and associated uncertainties from cumulative assessments (at the 99.9th percentile of exposure) for pesticides with acute effects on the nervous system CAG-NAN (acute AChE inhibition) and CAG-NAM (functional alterations of the motor division)

Specific effect	Total margin of exposure (MOE_T) median value and 95% CI at 99.9th percentile of exposure		
	All pesticides under consideration	CAGs containing pesticides remaining under consideration	
European populations assessed	Tier 2 approach for NAN 47 pesticides	HQ(p99.9) \leq 0.01 ^(a) for NAN 26 pesticides	HQ(p99.9) \leq 0.1 ^(b) for NAN 17 pesticides
Belgium – Adults	102 [72–162]	101 [71–166]	106 [75–178]
Czech Republic – Adults	120 [87–176]	122 [90–179]	130 [90–190]
Germany – Adults	95 [73–120]	95 [76–123]	99 [75–126]
Italy – Adults	96 [75–149]	96 [76–150]	97 [75–149]
Bulgaria – Other children	49 [36–63]	49 [36–63]	48 [35–63]
France – Other children	59 [46–74]	60 [47–75]	60 [47–75]
Netherlands – Other children	52 [45–62]	52 [45–63]	53 [45–65]
Denmark – Toddler	60 [50–69]	61 [50–73]	62 [49–73]
Netherlands – Toddler	40 [33–50]	41 [33–50]	41 [34–52]
United Kingdom – Toddler	61 [47–76]	62 [48–78]	62 [48–77]
European populations assessed	Tier 2 approach for NAM 100 pesticides	HQ(p99.9) \leq 0.01 for NAM 78 pesticides	HQ(p99.9) \leq 0.1 for NAM 46 pesticides
Belgium – Adults	176 [115–228]	183 [118–241]	186 [115–243]
Czech Republic – Adults	172 [131–236]	179 [128–229]	182 [137–246]
Germany – Adults	171 [127–211]	177 [125–215]	178 [134–215]
Italy – Adults	141 [109–185]	148 [115–183]	148 [118–197]
Bulgaria – Other children	63 [53–81]	65 [53–82]	67 [54–80]
France – Other Children	84 [65–102]	87 [67–109]	87 [70–110]
Netherlands – Other Children	89 [75–111]	92 [74–112]	90 [75–108]
Denmark – Toddler	80 [63–100]	82 [66–99]	81 [66–101]
Netherlands – Toddler	68 [56–85]	69 [57–83]	70 [54–81]
United Kingdom – Toddler	73 [61–89]	74 [62–87]	75 [58–87]

AChE: acetylcholinesterase; CI: confidence interval; HQ: hazard quotient.

(a): $HQ(p99.9) \leq 0.01$: low-priority pesticides which are not considered based on the 99th HQ percentile ≤ 0.01 .

(b): $HQ(p99.9) \leq 0.1$: low-priority pesticides which are not considered based on the 99th HQ percentile ≤ 0.1 .

Conclusions

The example presented here for the prioritisation of pesticides having acute effects on the Nervous System showed that the exclusion of low-priority pesticides based on $HQ(p99.9) \leq 0.01$ and $HQ(p99.9) \leq 0.1$ has no impact on the combined MOE_T at the 99.9th percentile of exposure, which is the preferred percentile for combined exposure to pesticides. For the prioritisation based on $HQ(p95) \leq 0.01$ and $HQ(p95) \leq 0.1$, the exclusion of low-priority pesticides has an impact on the combined MOE_T at the 95th percentile of exposure, which is the preferred exposure percentile in many other frameworks (e.g. contaminants). Therefore, this example highlights that the trigger value for chemicals other than pesticides needs to be carefully considered. Its effectiveness will depend on several factors (e.g. regulatory context, number of chemicals, etc.). An adequate validation of the trigger value is therefore recommended.

Appendix E – Exposure-driven approach as a prioritisation method for grouping multiple contaminants from breast milk and comparison with a risk-based approach for single chemicals

The example presented here illustrates the use of an exposure-driven approach to identify low-priority contaminants for grouping within human breast milk as described elsewhere (Crépet et al., in press).

The chemicals under consideration have been defined from the terms of reference, passed the gatekeeper step and enter the workflow (Figure 4) for prioritisation. The question 'are the hazard metrics for critical effects as defined for the Assessment Group available for all chemicals' was answered with 'NO'. The assessor proceeds with prioritisation method 3: 'exposure-driven approach'.

The assessment includes 32 chemicals with positive concentrations in 180 breast milk samples from six French lactariums (ANSES, in press; Crépet et al., in press). Using a lower bound scenario, censored data were replaced by zero values when not detected and by the limit of detection when not quantified (EFSA, 2010). Combined exposure for infants was calculated by multiplying each chemical concentration with a mean consumption of breast milk of 763 mL/day and a mean body weight of 6.1 kg (EFSA Scientific Committee, 2017c). In Crépet et al. (in press), prioritisation of chemicals was conducted by applying the Sparse non-negative matrix under-approximation (SNMU) method to the exposure matrix obtained (180×32) (Gillis and Plemmons, 2013). This resulted in a selection of 19 chemicals with high probability of combined exposure. To calculate the risk, Crépet et al. applied the modified Reference Point Index (mRPI), a method similar to the HI yet taking specific uncertainty factors into account for substances/effects for which no HBGVs are available (Vejdovszky et al., 2019).

In order to compare the results with the risk-based approach for single chemicals, HQs were calculated as the individual ratio between exposure and the HBGVs for the subsample of 26 chemicals with available HBGVs among the 32 chemicals under assessment (ANSES, in press). HBGVs were collected from WHO monographs (JECFA, JMPR), EFSA, US-EPA, ATSDR and ANSES, the reader is referred to the ANSES opinion for full details (ANSES, in press). The P95 of the HQs for each chemical was then calculated. Similar to the example presented in Appendix C, predefined trigger values for identifying low-priority chemicals were set at 1% and 10% of the HBGVs corresponding to P95 HQ values of 0.01 and 0.1, respectively (EFSA Scientific Committee, 2019; FAO/WHO, 2019).

The prioritisation methods led to the selection of 19, 20 and 17 chemicals using the combined exposure metrics, risk metrics for single chemicals (using a cut-off value of 1%) and risk metrics for single chemicals (using a cut-off value of 10%), respectively (Table E.1).

Table E.1: Overview of prioritised chemicals in the assessment group using an exposure-driven approach and risk-based approach for single chemicals using the trigger values of 0.01 and 0.1 for the P95 HQ

All chemicals under assessment	Chemicals with HBGV	High priority chemicals		
		Combined exposure	RSC 1% P95HQ > 0.01	RSC 10% P95HQ > 0.1
32	26	19	20	17

HBGV: health-based guidance value; HQ: hazard quotient; RSC: risk for single chemicals.

Risk characterisation: impact of excluding low-priority contaminants on hazard index

To study the impact of excluding low-priority chemicals, the hazard index was calculated as the sum of the HQs for all chemicals under consideration with an available HBGVs (26 chemicals) and for the prioritised chemicals obtained with the three prioritisation methods: combined exposure metric; risk metric for single chemicals using a cut-off of 1% ($HQ > 0.01$) and risk metric for single chemicals using a cut-off value of 10% ($HQ > 0.1$) (Table E.2).

More than 99.8% of the HI estimated with the 26 chemicals with available HBGVs was predicted with two risk metrics for single chemicals and 95.6% and 98.5% with the combined exposure for the mean and the P95, respectively. Thus, exclusion of the low-priority chemicals has a very limited impact on the HI values. Note that for the combined exposure, two chemicals with no HBGVs were identified as prioritised chemicals, thus for this group, the HI was calculated on 17 substances instead of 19.

Table E.2: Hazard index (HI) values for the multiple contaminants in breast milk and % of HI predicted by an exposure-driven approach and risk-based approach for single chemicals

HI	26 chemicals with HBGVs		Combined exposure		RSC 1% (HQ > 0.01)		RSC 10% (HQ > 0.1)	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95
		67.98	126.4	65	124.4	67.97	126.4	67.88
% of the 26 chemicals HI			95.6%	98.5%	99.98%	99.99%	99.85%	99.93%

HI: hazard index; HQ: hazard quotient; RSC: risk for single chemicals.

Table E.3 shows the relative contribution of each individual chemical, expressed as percentage of the HI for the multiple contaminants. For the three scenarios, the main contributors to the HI (i.e. indicator polychlorinated biphenyls, dioxins and furans, perfluorooctanoic acid; hexachlorocyclohexanes) were retained as prioritised chemicals and chemicals with low contribution were considered as low-priority chemicals.

Table E.3: Prioritised chemicals and their percentage contribution to the hazard index of multiple contaminants in breast milk

Chemicals	Contribution to HI			
	26 chemicals with HBGV	RSC 1% (HQ > 0.01) 20 chemicals	RSC 10% (HQ > 0.1) 17 chemicals	Combined exposure 19 chemicals
Indicator polychlorinated biphenyls (Σ PCBi)	36%	36%	36%	38%
Dioxins and furans (Σ PCDD/Fs)	36%	36%	36%	38%
Perfluorooctanoic acid (PFOA)	9%	9%	9%	10%
Hexachlorocyclohexanes (Σ HCHs)	9%	9%	9%	9%
Lead (Pb)	2.5%	2.5%	2.5%	–
Lindane (γ -HCH)	1.3%	1.3%	1.3%	–
Trichloroethanes/dichloroethylene/dichloroethane (Σ DDT/D/E)	1.2%	1.2%	1.2%	1.3%
Σ Aldrin-dieldrin	1.0%	1.0%	1.0%	1.1%
Chrome (Cr)	0.8%	0.8%	0.8%	–
Arsenic (As)	0.6%	0.6%	0.6%	0.7%
Perfluorooctanesulfonic acid (PFOS)	0.4%	0.4%	0.4%	0.4%
Inorganic mercury (inorganic Hg)	0.4%	0.4%	0.4%	0.4%
Hexachlorobenzene (HCB)	0.3%	0.3%	0.3%	0.3%
Σ Heptachlor	0.2%	0.2%	0.2%	0.2%
Polybrominated diphenyl ethers (Σ PBDEs)	0.2%	0.2%	0.2%	0.2%
Σ Chlordane-nonachlor	0.09%	0.09%	0.09%	0.09%
Brominated flame retardant (Σ HBCD)	0.08%	0.08%	–	–
Methylmercury (MeHg)	0.08%	0.08%	0.08%	0.09%
Polybrominated biphenyls (Σ PBBs)	0.04%	0.04%	–	0.04%
Aluminium (Al)	0.03%	0.03%	–	–
Nickel (Ni)	0.015%	–	–	–
Mirex	0.005%	–	–	0.01%
Polybrominated diphenyl ether 209 (PBDE 209)	0.0003%	–	–	0.0003%
Σ Endosulfan	0.00005%	–	–	–
Tetrabromobisphenol A (TBBPA)	0.00002%	–	–	–
Endrine	0.00002%	–	–	–

Chemicals	Contribution to HI			
	26 chemicals with HBGV	RSC 1% (HQ > 0.01) 20 chemicals	RSC 10% (HQ > 0.1) 17 chemicals	Combined exposure 19 chemicals
Pentachlorobenzene (PeCB)	–	–	–	Retained but no HBGV available
Perfluorohexanesulfonic acid (PFHxS)	–	–	–	Retained but no HBGV available

HI: hazard index; RSC: risk for single chemicals; HQ: hazard quotient; HBGV: health-based guidance value.

This example shows that low-priority chemicals within the assessment group with low probability of combined exposure can be excluded, e.g. the HI calculated only for prioritised chemicals was close to the HI obtained from the 26 chemicals under consideration (which have a HBGV) and for those obtained using single risk metrics.

Conclusions

The example presented here for the prioritisation of multiple chemicals in breast milk using an exposure-driven approach shows that the exclusion of low-priority chemicals has a very limited impact on the HI as results were close to the ones obtained with single risk metrics (1% and 10% cut-off values). In addition, the prioritised chemicals were similar across the three scenarios and were the main contributors to the HI. Overall, the exposure-driven approach allows to prioritise multiple chemicals, exclude chemicals with low correlations and is of particular interest to prioritise chemicals for which available reference values (i.e. HBGVs) have not been set.