Draft guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals

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Abstract

This draft Guidance document describes harmonised risk assessment (RA) methodologies for combined exposure to multiple chemicals for all relevant areas within European Food Safety Authority's (EFSA) remit, i.e. human health, animal health and ecological areas. First, a short review of the key terms, scientific basis for mixtures risk assessment and approaches to assessing (eco)toxicology of chemical mixtures is given, including existing frameworks for these risk assessments. This background was evaluated, resulting in a harmonised framework for risk assessment of mixtures of chemicals. The framework is based on the risk assessment steps (problem formulation, exposure assessment, hazard identification and characterisation, and risk characterisation including uncertainty analysis), with tiered and stepwise approaches for both whole mixture approaches and component-based approaches. Specific considerations are given to component-based approaches including the grouping of chemicals into common assessment groups, the use of dose addition as a default assumption, approaches to integrate evidence of interactions and the refinement of assessment groups. Case studies are annexed in this guidance document to explore the feasibility and spectrum of applications of the proposed methods and approaches for human and animal health and ecological risk assessment. The Scientific Committee considers that this Guidance is fit for purpose for risk assessments of chemical mixtures and should be applied in all relevant areas of EFSA's work. Future work and research are recommended.

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Summary

This Guidance document describes the harmonised application of risk assessment (RA) methods for combined exposure to multiple chemicals to all relevant areas within European Food Safety Authority's (EFSA) remit, i.e. human health, animal health and ecological areas.

The Terms of Reference (ToR) refer to 'risk assessment of combined exposure to multiple chemicals'. For ease of readability, this document uses the term 'chemical mixtures' which is defined as 'any combination of two or more chemicals that may jointly contribute to real or potential effects regardless of source and spatial or temporal proximity' and 'mixture risk assessment'.

In developing the Guidance, the Scientific Committee (SC) has taken into account other EFSA activities and related European and international activities to ensure consistency and harmonisation of methodologies and to avoid duplication of the work for the provided framework.

On this basis, a flexible overarching framework aiming to harmonise human health, animal health and ecological risk assessment of mixtures is presented (Chapter 2, General principles). The principles of mixture risk assessment for farm and companion animals generally apply the principles and tools used for human risk assessment; when this is not the case, this aspect is addressed separately. The harmonised framework consists of problem formulation, exposure assessment, hazard identification and characterisation, and risk characterisation including uncertainty analysis, for both the whole mixture and component-based approaches, describing the steps involved in each of these. The harmonised framework can be applied using the principles of tiering in both approaches. Tiering can avoid unnecessary expenditure of resources, by offering the possibility of discontinuing the analysis on the basis of simple assumptions on exposure and hazard estimates when the then resulting risk metrics do not flag potential risk (e.g. sufficient margins of Exposure). In the whole mixture approach, the mixture is essentially evaluated in the same way as for a single substance. Specific considerations are given to component-based approaches, including the grouping of chemicals into assessment groups, refinement of assessment groups, the use of dose (or concentration) addition as a default assumption, the use of response addition, and approaches to integrate evidence of interactions. The different steps of the mixture assessment framework are elaborated and discussed in more detail in the following chapters of this guidance:

- Problem formulation (Chapter 3)

Problem formulation is an iterative process involving risk assessors and risk managers during which the need for and the extent of a risk assessment are determined. The problem formulation step takes on a particular importance in the context of chemical mixtures because the demarcation of the problem generally is more complex than for single substances. A dialogue between (eco)toxicologists and exposure assessors is recommended. This step results in an analysis plan.

- Exposure assessment (Chapter 4)

Combined exposure assessment to multiple chemicals generally uses similar concepts and methods as for single chemicals, but can be more complex as chemical exposure may occur through multiple sources and sequential exposures. Exposure is typically assessed by combining occurrence data on chemicals with consumption data for human and animal health and using concentration data for the ecological area. A common challenge in the component-based approach relates to differing quantity and quality of the data for different components. Stepwise approaches are presented for the whole mixture approach and the component-based approach, respectively.

- Hazard assessment (Chapter 5)

Hazard assessment (i.e. hazard identification and characterisation) of chemical mixtures aims to derive quantitative metrics reflecting the combined toxicity to the exposed entities defined in the problem formulation. An initial decision on whether to apply a whole mixture approach or a component-based approach will have been made depending on the purpose of the assessment, data availability, time and resource constraints. If the component-based approach is to be used, then an initial decision on the chemicals to be included will also have been made. Following data collection and evaluation, this decision might need to be revised.

- Risk characterisation and uncertainty analysis (Chapter 6)

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Risk characterisation of chemical mixtures generates a ratio of combined exposure to the quantitative metric for combined toxicity for a defined species, subpopulation or the whole ecosystem. If this comparison indicates that there is no safety concern, the assessment can be stopped. Alternatively, it indicates a signal to proceed to a higher tier, with the possible need for additional data, or an indication of a risk that is transferred to the risk management step. Risk characterisation requires careful interpretation and communication, particularly if the data used in the evaluation are varying in quality, quantity or relevance. Uncertainties are identified in each stage of the framework and an overall uncertainty analysis has to be integrated in the risk characterisation. The different tools and methods that are applicable to the tiers are described for the human health, animal health and ecological areas.

The Guidance also provides a reporting table (Chapter 7) to enable summarising consistently and completely the results of a mixture risk assessment for each step of the process. Recommendations are made with particular reference to research needs in the mixture risk assessment area (Chapter 8).

Annexes include: 1) important aspects of uncertainty analysis for each step of the risk assessment process; and 2) three generic case studies using the reporting table to explore the feasibility and spectrum of applications of the proposed methods and approaches by showing diverse examples, covering human health (contaminants in food), animal health (essential oil used as feed additives) and ecological areas (impact of binary mixture interactions on hazard characterisation in bees).

The Scientific Committee considers that this Guidance is fit for purpose for mixture risk assessment and should be applied unconditionally in all relevant areas of EFSA's work.

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1. Introduction

1.1. Background and Terms of Reference as provided by EFSA

1.1.1. Background

Human and ecological risk assessment of combined exposure to multiple chemicals ('chemical mixtures') poses a number of challenges to researchers, risk assessors and risk managers, particularly because of the complexity of the problem formulation, the large numbers of chemicals involved, and the amount of data needed to describe the toxicological profiles and exposure patterns of these chemicals in humans, companion and farm animals and species present in the environment. The development of harmonised methodologies for combined exposure to multiple chemicals in all areas of EFSA's remit has been identified by EFSA's Scientific Committee as a key priority area (EFSA, 2016b).

Some EFSA panels and units have initiated activities to assess combined exposures, expanding on the approaches for single chemical risk assessments and to support harmonisation of risk assessment methods for the human health, animal health and the ecological areas.

In the human risk assessment field, recent examples include the Opinion of the Panel on Plant Protection Products and their Residues (PPR) dealing with an approach to group pesticides into 'cumulative assessment groups' based on the compounds' toxicological properties (EFSA PPR Panel, 2013a,b). The Panel on Contaminants in the Food Chain (CONTAM) published a number of Opinions involving case-by-case approaches to the human risk assessment of multiple contaminants using both whole mixture-based and component-based approaches (EFSA, 2005a, 2008a; EFSA CONTAM Panel, 2009; 2011, 2012; 2017a). Finally, the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) addressed the human risk assessment of rum ether [Flavouring Group Evaluation 500 (FGE.500)] as a complex mixture of 84 reported constituents using component-based approaches for 12 congeneric groups allocated based on structural and metabolic similarity (EFSA CEF Panel, 2017).

In the animal health area, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) recently published an Opinion on the safety and efficacy of a whole mixture of oregano essential oil when used as a sensory additive in feed for all animal species (EFSA FEEDAP Panel, 2017a).

In an ecological risk assessment of multiple chemicals, the PPR Panel in their 'Scientific Opinion on the Science Behind the Development of a Risk Assessment of Plant Protection Products on Bees (*Apis mellifera*, *Bombus* spp. and solitary bees)' discussed approaches for the risk assessment of multiple residues of pesticides in bees. Furthermore, the SCER unit recently published a scientific report 'Towards an integrated environmental risk assessment of multiple stressors on bees: review of research projects in Europe, knowledge gaps and recommendations' (EFSA PPR Panel, 2012a; EFSA, 2014b).

From a horizontal perspective, the SCER unit has published a scientific report in 2013 reviewing the available international frameworks dealing with human risk assessment of combined exposure to multiple chemicals (EFSA, 2013a). The report has also identified key needs for future work in the area of combined toxicity of chemicals from a consultation of EFSA Panels, Units and the Scientific Committee. A key recommendation was the need to collect data in the area of human, animal and environmental toxicology of mixtures for substances of relevance to EFSA (EFSA, 2013a). In response, the SCER unit launched two procurements on data collection on combined toxicity for the human health, animal health and ecological area (Quignot et al., 2015a, b). In 2014, the SCER unit organised a scientific colloquium on 'Harmonisation of human and ecological of risk assessment of combined exposure to multiple chemicals' (EFSA, 2015a). Finally, other procurements were launched to integrate new approaches in chemical risk assessment in the areas of human health, animal health and ecology, i.e. 1) integration of toxicokinetic tools (EFSA-Q-2014–00918; EFSA-Q-2015–00640), 2) modelling population dynamics of aquatic and terrestrial organisms for risk assessment of single and multiple chemicals (EFSA-Q-2015–00554), and 3) modelling human variability in toxicokinetic and toxicodynamic processes (EFSA-Q-2015–00641). Subsequently, the Scientific Committee of EFSA has identified this topic in 2015 as a priority for guidance development to support EFSA Panels to perform risk assessment of combined exposure to multiple chemicals in a harmonised manner.

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All these background activities support the development of this Guidance document, which aims to provide harmonised methodologies and case studies for the risk assessment of combined exposure to multiple chemicals for the human health, animal health and ecological areas.

1.1.2. Terms of Reference as provided by EFSA

The Terms of Reference for this Guidance document have been subject to public consultation between October 2016 and December 2016. A technical report presenting all comments from stakeholders and EFSA's replies is available online at: http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2017.EN-1189/pdf

After reviewing these comments from stakeholders, the Terms of Reference for developing this guidance were adopted as follows:

- EFSA requests the Scientific Committee to develop a Guidance document on harmonised risk assessment methodologies for combined exposure to multiple chemicals in the human health, animal health and ecological areas. The Guidance should be an overarching document aimed at the work of EFSA panels and relevant to scientific advisory bodies dealing with chemical risk assessment both within and across regulatory applications and sectors.
- The Working Group (WG) should review available definitions, methods and tools for different risk assessment contexts and develop harmonised framework(s) for human and ecological risk assessment of combined exposure to multiple chemicals supported by a consistent terminology.
- The Guidance document should start from first scientific principles for all relevant steps of the assessment i.e. problem formulation, hazard identification and characterisation, exposure assessment, risk characterisation and uncertainty analysis. For each step, the principles of tiering should be applied (purpose of the assessment, data availability, resources) and include decision points and associated assumptions (e.g. dose addition, response addition, deviation from dose addition including interactions).
- The Guidance should explicitly address both the whole mixture approach and component-based approach and the application of uncertainty factors in a mixture risk assessment context.
- Circumstances under which harmonisation between human and ecological risk assessment may not be possible or relevant (e.g. because of the state of science, regulatory framework) should also be discussed.
- In developing the Guidance, work should start from and build on European [e.g. European Commission, European Chemicals Agency (ECHA), EFSA] and international [e.g. US EPA, WHO, Organisation for Economic Co-operation and Development (OECD)] terminology, methods and frameworks, to ensure interagency co-operation, consistency and avoid duplication of the work.
- Case studies should be annexed in the Guidance to explore the feasibility and spectrum of applications of the proposed methods and approaches for human health, animal health and ecological risk assessment.
- In line with EFSA's initiative on Transparency and Engagement in Risk Assessment (TERA), the draft Guidance will be subject to public consultation. The published Guidance will be presented and discussed at an international event.

1.2. Interpretation of the Terms of Reference

When addressing the mandate, the Scientific Committee acknowledged that harmonisation of methodologies for human health, animal health and ecological risk assessments of combined exposure to multiple chemicals encompasses a number of regulatory and non-regulatory applications and a number of species including humans, farm animals, companion animals and the ecosystem.

For the human and animal health areas, the primary focus is dietary exposure as it is within EFSA's remit, and guidance on aggregate exposure assessment is currently lacking. For the ecological area,

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the primary focus is most often on exposure through water, soil or sediment, which typically covers multiple routes such as, e.g. ingestion and absorption through the skin. Under certain circumstances, the oral route may also be the focus of the assessment e.g. oral exposure in pollinators through pollen and nectar, oral exposure in fish through feed.

The Terms of Reference refer to 'risk assessment of combined exposure to multiple chemicals'. For ease of readability, this document uses the term 'chemical mixtures' and 'mixture risk assessment'. A 'mixture' is defined as 'any combination of two or more chemicals that may jointly contribute to real or potential effects, regardless of source and spatial or temporal proximity' (based on US Environmental Protection Agency, 1986, 1999; Agency for Toxic Substances and Disease Registry (ATSDR), 2004; EFSA, 2013b). The concept of spatial and temporal proximity is of more importance in the ecological area than in food safety. It is recognised that, as the focus of the mixtures risk assessment relates to the population of concern, it may be needed to take into account exposure from a number of events at several locations over broad and varied time periods (US EPA, 2007). This document aims to give guidance on when and how to assess the risk from combined exposure to chemical mixtures, to provide a basis for risk managers to protect the health of humans, animals and ecosystems (including specific target species).

It should be recognised that, although a binary choice between whole mixture and component-based approaches is presented, the cases are overlapping.

1.3. Existing EFSA regulatory mandates for mixture risk assessment

The Charter of the European Union obliges European governments to protect human health and the environment and provides a general basis to address concerns on combined exposures to multiple chemicals. Besides this general basis, there are several regulations within EFSA's remit that have specific provisions for mixtures.

For human health, Article 14 of EFSA's founding Regulation on general European Food Law [Regulation (EC) No. 178/2002], paragraph 4 states: 'In determining whether any food is injurious to health, regard shall be had…to the probable cumulative toxic effects.' However, the term 'cumulative toxic effects' is not defined, and because it is used with different meanings in the scientific literature, it is hard to interpret Article 14 as either a general legal requirement or as an operational basis for mixture risk assessments in EU Food Law.

More specific requirements for chemical mixture risk assessment in EU food-related regulations tend to focus on relatively narrow scenarios. On the use of pesticides, Regulation (EC) 1107/2009 requires that 'interaction between the active substance, safeners, synergists and co-formulants shall be taken into account' in the evaluation and authorisation of Plant Protection Products (Article 29). Commission Regulation (EU) No. 284/2013, further requests 'any information on potentially unacceptable effects of the plant protection product on the environment, on plants and plant products shall be included as well as known and expected cumulative and synergistic effects'. Regulation (EC) No. 396/2005 on maximum residue levels (MRLs) of pesticides in or on food and feed of plant and animal origin requires Cumulative Risk Assessment for pesticides to be performed. Recital 6 states: 'It is also important to carry out further work to develop a methodology to take into account cumulative and synergistic effects.' It further specifies that MRLs should be set in 'view of human exposure to combinations of active substances and their cumulative and possible aggregate and synergistic effects on human health'.

For animal health risk assessment, Regulation (EC) No. 429/2008 on the assessment of feed additives explicitly addresses risks that may arise from combined exposures if feed additives placed on the market contain more than one (active) ingredient. Annex II establishes the requirement that 'where an additive has multiple components, each one may be separately assessed for consumer safety and then consideration given to the cumulative effect (where it can be shown that there are no interactions between the components). Alternatively, the complete mixture shall be assessed.'

Legislation in relation to food additives, food contact materials and food contaminants does not have specific provisions requiring risk assessment of mixtures. However, this does not imply that mixtures are never addressed. For example, in Regulation (EC) 1881/2006 maximum levels for dioxins, polycyclic aromatic hydrocarbons and a number of mycotoxins are underpinned by mixtures risk assessment.

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1.4. Rationale for harmonising methods for mixture risk assessment across human health, animal health and ecological areas

Mixture risk assessment for human health, animal health and ecological areas is characterised by a plethora of terms, models and approaches. This can be explained by independent developments in the respective risk assessment fields and different jurisdictions. Close scrutiny, however, unveils substantial similarities with vast variation in terminology, providing a strong basis for harmonisation.

Examples of methodological similarities across the different areas of mixture assessment include the use of reference points, mechanistic data (i.e. mode of action and adverse outcome pathways), exposure and effect models, and similar risk metrics (i.e. the ratio between exposure and hazard). Using harmonised methods will support consistency, transparency and structured, reproducible risk assessments across all areas of EFSA's remit as well as further international cooperation between scientific advisory bodies across regulatory domains.

While there are many similarities, important differences between human/animal health risk assessment and ecological risk assessment exist that are not subject to harmonisation. Examples include differences in protection goals (effects on individuals within populations in animal/human risk assessment versus effects on populations and ecosystem integrity in ecological risk assessment), toxicological endpoints (community and/or ecosystem endpoints are unique for ecological risk assessment) and the exposure regime (each route is considered separately in animal/human risk assessment, whereas ecological risk assessment often considers integrated exposure regimes from water or soil).

EFSA has recognised the need to harmonise methods for mixture risk assessment across human health, animal health and ecological areas when possible at several occasions (EFSA, 2015a; EFSA Scientific Committee, 2016a). In general, harmonisation of methodologies is one of the key roles of the EFSA's Scientific Committee through providing horizontal guidance documents as specified in EFSA's founding Regulation [Regulation (EC) No. 178/2002], and these guidance documents provide means to develop consistent methodologies across EFSA panels (EFSA Scientific Committee, 2016b). Recent examples include the use of the weight of evidence approach in scientific assessments, assessment of biological relevance and uncertainty analysis (EFSA Scientific Committee, 2017a, b, 2018).

1.5. Audience and degree of obligation

This Guidance provides harmonised, but flexible stepwise procedures to assess the risk of chemical mixtures that are proposed to be used in EFSA's risk assessments. This guidance is unconditional for the EFSA panels and EFSA units performing mixture risk assessments. Acknowledging the variability in problem formulation and data availability, this document provides guidance on the general principles for risk assessment of chemical mixtures as well as on the different approaches that assessors may choose to apply the most appropriate methods that are available in their specific contexts. The Scientific Committee considers that the use of methods and data should be fit for the scientific assessment. Readers and users of the Guidance are assumed to be experienced in the risk assessment of single chemicals, and emphasis is on the specific aspects of mixture risk assessment.

2. Mixture risk assessment

This section gives a brief overview of key terms, state of the science and available frameworks used in human and ecological risk assessment of chemical mixtures. Based on this overview, a harmonised framework for human, animal and ecological mixture risk assessments is proposed at the end of this chapter. Details of the framework and support for its practical implementation are provided in the subsequent chapters.

2.1. Key terminology

Key mixture-related terms used in this Guidance are defined in Table 1, with further explanation in the relevant sections of the text and mathematical equations in Chapter 6. A full glossary is included at the end of this document. The terms are harmonised within the context of this Guidance, but this does not imply invalidation of terms used elsewhere.

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414 **Table 1:** Key mixture risk assessment terms used in this guidance

Term	Explanation
Assessment group (encompassing cumulative assessment group)	Mixture components, which are treated as a group by applying a common mixture assessment principle (e.g. dose addition) because these components have some characteristics in common (i.e. the grouping criteria)
Complex mixture	A mixture (e.g. extracts, protein hydrolysates, smoke flavourings) in which not all constituents are known or fully characterised.
Component-based approach	An approach in which the risk of a mixture is assessed based on exposure and effect data of its individual components.
Concentration addition	A component-based model in which the components are treated as if having a **similar action**. The components may vary in toxic potency. Components contribute to the mixture effect relative to the ratio between their concentration and toxic potency. Concentration is the exposure metric used as a proxy for dose in *in vitro* studies and ecological risk assessment
Dose addition	As above for concentration addition. Dose is the exposure metric used in human and animal health risk assessment. Dose addition is used as the generic term throughout this guidance document
Interaction	In risk assessment practice, the term interaction is used to refer to mixture effects that differ from an explicit null model, i.e. dose and/or response addition. Interactions are categorised as less than additive (**antagonism, inhibition, masking**) or greater than additive (**synergism, potentiation**)
Margin of Exposure	Ratio of (a) a reference point of (eco)toxicity to (b) the theoretical, predicted or estimated exposure dose or concentration
Mixture	Any combination of two or more chemicals that may jointly contribute to real or potential effects regardless of source and spatial or temporal proximity.
Mixture of concern	A mixture of chemicals that is the subject of a risk assessment because there are indications that the compounds in the mixture/of which the mixture is composed may jointly contribute to the real or predicted risk
Mode of action	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 compound with biological targets. It does not imply full understanding of mechanism of action at the molecular level
Reference point	Defined point on an experimental dose–response relationship for the critical effect. This term is synonymous to Point of departure (USA). 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 Margin of Exposure in human and animal health risk assessment. In the ecological area, these include lethal dose (LD_{50}), effect concentration (EC_5/ECx), no (adverse) effect concentration/dose (NOEC/NOAEC/NOAED), no (adverse) effect level (NEL/NOAEL), hazard concentration (HC_5/HCx) derived from a Species Sensitivity Distributions (SSD) for the ecosystem
Reference value	The estimated maximum dose (on a body mass basis) or the concentration of an agent to which an individual may be exposed over a specified period without appreciable risk. Reference values are established by applying an uncertainty factor to the reference point. Examples of reference values in human health include 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. For acute effects and operators, the acute reference dose (ARfD) and the acceptable operator exposure level (AOEL). In animal health and the ecological area, these include safe feed concentrations and the Predicted no effect concentration (PNEC) respectively
Response addition	A component-based mixture model in which the components are treated as if having **independent** or **dissimilar action**, i.e. by following the statistical concept of independent random events. Application of response addition requires toxicity data (e.g. mortality, target organ toxicity) to be expressed as a fraction (between 0 and 1), i.e. the percentage of individuals in a population, or species in an ecosystem affected by the mixture or exceeds a reference point (e.g. BDML, EC_{50}).

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Term	Explanation
	The term 'response addition' is a misnomer as responses are actually not added, but the unaffected fractions of the population are multiplied (see Chapter 6). However, the term is used in this guidance as it is commonly used in the area of mixture risk assessment
Similar mixture (also known as sufficiently similar mixture)	A mixture of chemicals that differs slightly from the mixture of concern, i.e. in components, concentration levels of components, or both. A similar mixture has, or is expected to have, the same type(s) of biological activity as the mixture of concern, and it would act by the same mode(s) of action and/or affect the same toxic endpoints
Simple mixture	Mixture whose components are fully chemically characterised, e.g. a group of defined substances with the potential to have combined effects
Whole mixture approach	A risk assessment approach in which the mixture is treated as a single entity, similar to single chemicals, and so requires dose–response information for the mixture of concern or a (sufficiently) similar mixture

2.2. Scientific basis of mixture assessment

Until relatively recently, the focus of human, animal and ecological risk assessment has been on single substances. During the last decades, however, good evidence has accumulated that chemicals can work together to produce combined effects that are larger or smaller than the effects of each mixture component applied singly. The literature shows that this applies to a host of different endpoints of relevance to human, animal and ecological risk assessments. It also holds true for a diverse set of chemicals that are subject to EU Food Law regulations (EC, 2002). The evidence for effects of combined exposure to multiple chemicals has been reviewed by scientific advisory bodies and experts in the field (e.g. US EPA, 2003, 2007; ATSDR, 2004; WHO, 2011; EFSA, 2008b, 2009, 2013a,b; Kortenkamp et al., 2009; SCHER, SCENIHR, SCCS, 2012; ECHA, 2014; OECD, 2017;). The overall evidence on combination effects indicates that combined effects can arise when each mixture component is present at doses around or above its no effect level and provides a strong basis for developing robust approaches to assess the risk of chemical mixtures to support decision making.

The risk of a chemical mixture can be assessed by testing the mixture of concern in toxicity tests. This is sometimes performed for common or poorly characterised mixtures, but it is practically unfeasible to test each and every mixture separately because of the sheer endless potential variation in mixture components and component concentrations. One of the key aspirations of mixture toxicology has therefore been to anticipate quantitatively the effects of mixtures of chemicals from knowledge about the toxicity of their individual components. Such predictions can be achieved by making the assumption that the chemicals in the mixture act in concert by exerting their effects without diminishing or enhancing each other's toxicity; the so-called **additivity** or non-interaction assumption. **Similar action** and **independent** action are distinct mechanistically defined concepts on the two types of interaction that can occur between chemical molecules and target molecules. These concepts form the basis for the two most commonly applied modelling approaches, often called 'null models': **dose addition** and **response addition**, respectively. **Synergisms** and **antagonisms** can then be defined in relation to this additivity assumption, as upwards or downwards deviations from the modelled predictions of the selected null model, respectively.

There is strong evidence that it is possible to predict the toxicity of chemical mixtures with reasonable accuracy and precision, when the toxicity of the components is known, both for human/animal and ecological effects (Kortenkamp et al., 2009; WHO, 2011; SCHER, SCENIHR, SCCS, 2012; Van Gestel et al., 2011; EFSA, 2013; OECD, 2017). This uniform insight provided the foundation for mixture risk assessment methods of the unified framework of this Guidance. An essential element underlying this framework is the recognition that there is no need for the experimental testing of each and every conceivable mixture, which would make mixture risk assessment unmanageable. Both dose addition and response addition provide reasonable approximations for the prediction of combination effects, although deviations from predicted additivity, indicative of synergisms or antagonisms, exist and have been reported in (eco)toxicological studies (Boobis et al., 2011; Cedergreen, 2014).Therefore, a specific assessment step that evaluates factors potentially leading to (toxicokinetic and/or toxicodynamic) interactions is required, with particular attention for synergisms in the context of the regulatory protection goals.

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The available empirical evidence and considerations from various EU committees and panels and international experts suggest that synergisms cannot be predicted quantitatively on the basis of the toxicity of individual components and are rare at dietary exposure levels in the **human health area**. Evidence for synergisms is available in the vast majority of cases for binary mixtures at biologically active concentrations/doses (SCHER, SENIHR, SCCS, 2012; EFSA, 2013b; ECETOC, 2012; Boobis et al., 2011).

For the **ecological area**, Cedergreen (2014) performed a systematic literature review for binary mixtures of three groups of environmentally relevant chemicals: pesticides (n = 194), metals (n = 21) and antifouling agents (n = 136) and found synergistic effects in 7, 3 and 26% of cases respectively. The author concluded from that review that true synergistic interactions between chemicals were rare, and often occurred at high concentrations with deviations from dose addition rarely above a factor of 10. Interactions (synergism and antagonism) may also occur due to indirect effects in the ecological context. An apparently higher impact than expected ('synergisms') may be observed as a result of the combined effects of different chemicals on different taxonomic groups and the indirect consequences on the structure and functioning of the European Union (SCHER, SCENIHR SCCS, 2012). For example, effects on a predator may induce indirect effects on a prey. It should be noted, that ecological interactions related to mixture exposures probably occur when there are direct effects of the chemicals such as mortality or effects on reproduction.

Other authors have proposed to derive extra uncertainty factors for interactions including an extra factor of 2 for biocidal mixtures (Backhaus et al., 2013). In the ecological area, Van Broekhuizen et al. (2016) and KEMI, (2015) proposed uncertainty factors of 5–20 to cover the large majority of potential coexposures, as analyses of environmental data suggested that mixture toxicity encountered in the environment is generally dominated by a limited number of compounds. National and international scientific advisory bodies have developed methodologies to incorporate concepts of (toxicological) interactions into guidelines and guidance with suggested methods to evaluate the possible influence of joint toxic action of chemicals on the overall toxicity (ATSDR, 2004; USEPA, 2007; WHO/IPCS, 2009; SCHER, SCENIHR, SCCS, 2012). ECHA (2014) published guidance for biocidal products and proposed that a deviation between dose addition predictions and measured mixture toxicities by a factor of 5 or more should be regarded as synergistic/antagonistic and should be explicitly addressed in the assessment of mixture risks (ECHA, 2014).

2.3. Approaches to risk assessment of chemical mixtures

The **whole mixture approach** is defined here as 'a risk assessment approach in which the mixture is treated as a single entity, similar to single chemicals, and so requires dose–response information for the mixture of concern or a (sufficiently) similar mixture'. In some instances, dose–response data might not be available for the mixture of concern itself, but may be obtained by read-across from **similar mixtures** (sometimes referred to as **sufficiently similar mixtures**). These are mixtures having the same chemicals but in slightly different proportions or having most chemicals in common and in highly similar proportions. Similar mixtures are expected to have similar fate, transport, and (eco)toxicological effects as the mixture of concern (see Chapter 5.2). Application of the whole mixture approach can be facilitated by the identification of marker substances, which are readily measurable prevalent components of the mixture and therefore can be used in the exposure assessment and the dose–response analysis.

Whole mixture approaches are particularly required with mixtures whose composition is unknown or difficult to characterise, sometimes referred to as **complex mixtures**.

If the components of the mixture and their exposure levels are largely known, which can be referred to as a **simple mixture**, then the **component-based approach** can be applied. This is defined as 'an approach in which the risk of a mixture is assessed based on exposure and effect data of its individual components' (EFSA, 2013a). Application of the component-based approach therefore requires exposure and effect data on the individual mixture components. These mixture components are often organised into chemical **assessment groups** (sometimes known as **cumulative assessment groups, Table 1**). Grouping of chemicals into assessment groups potentially: (1) reduces the potential for over estimating risks by combining impacts from compounds that are independent of each other; (2) minimises the need to collect and model correlations of doses; (3) focuses risk management on groups of chemicals that need to be tracked and controlled and so

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reduces management costs; and (4) minimises unnecessary impacts on regulated community. Examples of criteria for grouping chemicals into assessment groups include physicochemical properties, hazard characteristics, exposure considerations and practical criteria as described in Section 5.1.2. For chemicals in an assessment group, quantitative predictions of combined toxicity are derived from knowledge of the toxicity of the individual components, often using the dose addition model as a default.

Mechanistic concepts, such as mode of action, mechanism of action and the Adverse Outcome Pathway, can play an important role when grouping chemicals into assessment groups. In human risk assessment, the **Mode of Action (MoA, Table 1)** uses key events that include key cytological and biochemical events, that is 'those that are both measurable and necessary to the observed effect – in a logical framework and does not imply full understanding of mechanism of action at the molecular level' [EFSA (European Food Safety Authority), 2013b].

In the ecological area, MoA has a similar interpretation as in the human and animal health area, but the available evidence on plausible sequences of key events for MoA classification is often weaker. An example is the classification of chemicals in four very rough MoA classes: (1) narcosis, (2) polar narcosis, (3) reactive chemicals, and (4) specific toxicity (Verhaar et al., 1992; Segner, 2011). Beyond such basic distinctions, a suite of pragmatic approaches to grouping chemicals have been applied in ecotoxicology. In the pesticide arena, the MoA concept is used in a similar way as in the human and animal health area.

Related to the MoA concept, is the **Adverse Outcome Pathway (AOP)** concept, which is 'the mechanistic or predictive relationship between initial chemical–biological interactions and subsequent perturbations to cellular functions sufficient to elicit disruptions at higher levels of organisation, culminating in an adverse phenotypic outcome in an individual and population relevant to risk assessment' (Ankley et al., 2010). The AOP has potential applications in defining assessment groups but has so far found little practical application in mixture risk assessment.

An important consideration in applying component-based approaches is whether and how to account for potential **interactions** between mixture components. Interactions are defined as joint action between multiple chemicals that differ from dose addition or response addition categorised as less than additive or greater than additive'. In risk assessment practice, the term interaction is used to refer to mixture effects that differ from an explicit null model, i.e. dose and/or response addition. Interactions are then categorised as less than additive (antagonism, inhibition, masking) or greater than additive (synergism, potentiation).

2.4. Tiering in mixture risk assessment

This Guidance uses the principles of tiering described elsewhere (WHO, 2011; EFSA, 2008b; EFSA PPR Panel, 2013; EFSA, 2013; 2017; US EPA, 2007) for mixture risk assessment. Tiering principles allow for simple and conservative approaches at lower tiers, and more complex and precise approaches at higher tiers when needed. Appropriate application of tiering must exhibit decreased conservatism of final risk assessment results, so that predictions made at the highest tier most closely resemble true exposures and impacts. This principle implies that an assessment can be terminated as soon as there is clarity on sufficient protection. Alternatively, one progresses to risk management or a higher tier when clarity on sufficient protection is lacking. Generation of additional toxicity data, including relative potency, or exposure data can be necessary to progress to a higher tier. The assumptions applied in each tier must be specifically defined and refined with increasingly detailed data and approaches at higher tiers.

Because of the vast variety of problem formulations, approaches and data, the tiers applied in mixture risk assessment are not prescribed, e.g. by mapping data types or mixture models to tiers. Nor does the tiering principle imply that assessments necessarily proceed from lower to higher tiers. For example, in many assessments of regulated products, the tier(s) applied will be predetermined by the available data, the problem formulation and/or the regulatory context.

In practice, the tiers can be qualified as low, intermediate or high or using numerical attributes (0, 1, 2, 3, etc.). A low tier (tier 0) would typically describe a data poor situation, requiring conservative assumptions. At increasing tier levels (1, 2 and 3) more data become available, allowing assessments to become more accurate, with decreasing uncertainty (see Figure 1). The tier applied is not

necessarily symmetrical between exposure and hazard assessment or between the members of an assessment group, because availability of Exposure and effect data may vary and because of regulatory requirements under which the assessment is being performed.

Application of dose addition requires a decision on the grouping of chemicals into one or more assessment groups which, according to the underlying theory, have a 'similar action' (Section 2.1). In the conceptually correct, ideal situation, the application of dose addition is restricted to toxicity data on the same end-point and exposure route and duration (e.g. effects of multiple chemicals on one physiological process in toxicology). In practice, this criterion of similar action is often relaxed and the mixture components are grouped on more pragmatic grounds such as 'substances affecting the same target organ', 'substances originating from the same source' or 'substances found in the same mixture'.

Tiering and grouping relate in the following way. At a lower tier, the analysis may begin with all chemicals being grouped together, e.g. an exposure-driven grouping with neglect of modes of action. This approach is simple and conservative, particularly when the components are present below a supposed effect threshold, e.g. NOAEL, BMDL, HC_5 or NEL. If the outcome shows sufficient protection, the simplified and conservative approach yields sufficient information to stop the assessment. If not, it can be considered to create subgroups of chemicals, for example based on a common toxic effect. Grouping is discussed in more detail in Section 5.4.

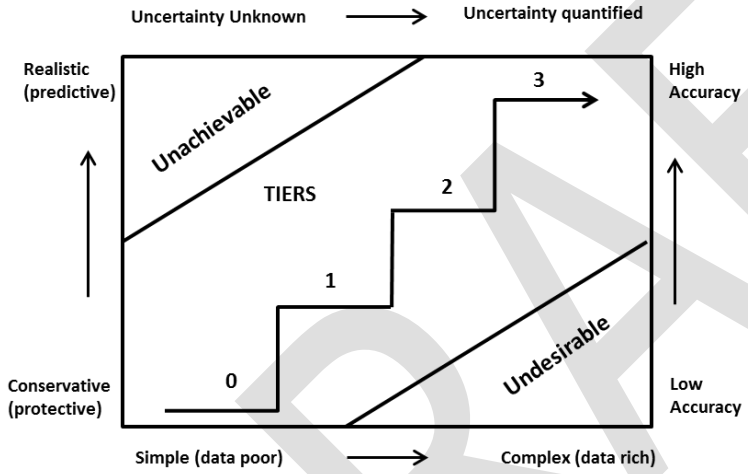

Figure 1: Tiering principles: relationships between tiers, data availability, uncertainty, accuracy and outcome of a risk assessment. From: Solomon et al. (2006).

2.5. Existing guidance for mixture risk assessment

The US EPA, WHO, OECD, EFSA, ECHA, and other national and international agencies have developed a number of guidance documents that deal explicitly with either or both human health and ecological risk assessment of multiple chemicals [US Environmental Protection Agency, 2007; EFSA, 2008b; Meek et al., 2011; OECD, 2011; EFSA CONTAM Panel, 2012; SCHER, SCENHIR, SCCS, 2012; EFSA, 2013b; EFSA PPR Panel, 2013b; Kienzler et al., 2014; ECHA, 2015; Bopp et al., 2015, 2016; Rotter et al., 2016; OECD, 2017]. Although terminology varies, all frameworks are based on the risk assessment paradigm and use the dose addition model as the default option for combined toxicity, while also considering options for dealing with interactions. Internationally, the dose addition model is considered the most relevant and conservative approach to support decision making in the chemical risk assessment remits of the US EPA, the Agency for Toxic Substances and Disease Registry (ATSDR), WHO, the EU non-food Scientific committees, The UK Interdepartmental Group on Health Risks from Chemicals, the Norwegian Scientific Committee for Food Safety (VKM), OECD and EFSA. The available frameworks covering human, animal and ecological risk assessment are briefly summarised to highlight the most important overarching commonalities.

2.5.1. Human and animal health risk assessment of mixtures

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Early frameworks for risk assessment of mixtures date back to publications of the US EPA, ATSDR, IGHRC and VKM (US EPA, 2000; ATSDR, 2004; IGHRC, 2008; VKM, 2008). These frameworks describe tools and decision trees, which provide guidance for dealing with multiple chemicals, based on the type of data available for the assessment. Reports of the EFSA PPR (EFSA, 2008b), the WHO/IPCS (Meek et al., 2011) and the BfR (Stein et al., 2014) propose tiered approaches, with simple deterministic (conservative/worst case) assessments at lower tiers and more complex and quantitative probabilistic (and realistic) assessments at higher tiers.

Scientific advisory bodies have not developed specific frameworks for mixture risk assessment in animal health (farm and companion animals), but in practice, these mostly apply the principles of human risk assessment.

In the approaches presented by CEFIC (Price et al., 2012) and (SCHER, SCENHIR, SCCS, 2012), the tiered framework proposed by WHO/IPCS was combined with a stepwise decision tree to guide practitioners through the assessment steps. Early evaluations of the potential for exposure (before any consideration of hazard potential) was considered essential in determining next steps and the use of the concept of Threshold of Toxicological Concern (TTC) was suggested as a first tier for the hazard assessment step (SCHER, SCENHIR, SCCS, 2012).

A common feature of many frameworks is the use of assessment groups based on phenomenological effects at the target organ level for compounds with similar MoAs (US Environmental Protection Agency, 2007; EFSA, 2008b; SCHER, SCENHIR, SCCS, 2012; OECD, 2017).

Risk characterisation is commonly performed through the calculation of risk metrics including Hazard Index, Reference Point Index or Margins of Exposure. The commonality of these methods, despite differences in terms and details, is that the assessment consists of comparing the predicted exposure to a reference point or reference value. A lower tier (using conservative defaults) supports the conclusion that there is either no cause for concern or that there are concerns. The latter can lead to refinement of the analysis in a higher tier, incorporating further case-relevant data and more accurate models (Van Gestel et al., 2011; OECD, 2017) or to risk reduction measures.

2.5.2. Ecological risk assessment

Early science-based frameworks date back to the US EPA (2003) framework for Cumulative Risk Assessment and to analyses of George et al. (2003), De Zwart and Posthuma (2005) and Posthuma et al. (2008), based on cross-sectoral expertise exchanges since 2003 lying at the basis of human and ecological mixture risk assessments (see Ragas et al., 2010). Expanding on the existing knowledge and approaches, the Non-food Scientific Committees of the European Commission adopted a tiered framework for ecological risk assessment of mixtures with the use of dose addition as the default assumption (SCHER, SCENHIR, SCCS, 2012). These committees furthermore concluded that the general principles used in human risk assessment of mixtures also provide a sound basis to predict effects at individual and population level in ecological risk assessments. However, ecological risk assessments have to deal with an additional level of interaction. That is, combined effects of different chemicals can operate on different taxonomic groups, having both direct and indirect consequences on the structure and functioning of the European Union, which e.g. impacts on prey species may cause an extra 'synergistic' effect via indirect effects on their predators (SCHER, SCENHIR, SCCS, 2012). This concept is further discussed in the Opinion on New Challenges for Risk Assessment (SCHER, SCENHIR, SCCS, 2013).

EFSA has developed several guidance documents dealing with pesticide residues and their effects on humans and organisms living in the environment. The combined effects of simultaneous exposures to several pesticide residues were first considered in relation to ecological risk assessments for birds and mammals (EFSA, 2009), and then in the context of risk assessment for pesticides on bees [EFSA PPR Panel, 2012a]. Both these pieces of guidance apply dose addition as the mixture risk assessment concept of choice, but do not draft details of the specific practical mixture risk assessment methods that should be applied.

This gap is filled in the Guidance on Tiered Risk Assessment for Plant Protection Products (PPP) for Aquatic Organisms in Edge-of-Field Surface Waters [EFSA PPR Panel, 2013a]. A detailed tiered decision scheme is proposed based on checking data availability for exposure and effect assessments. It filters out situations in which mixture risk assessments are not necessary for decision support

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because a single chemical already dominates the overall effect. The guidance acknowledges the need for considering possible unacceptable effects that may arise due to chemicals already present in the environment, but methods for dealing with this issue are not developed in detail. Dose addition is the recommended default, i.e. Toxic Unit summation based on single chemical chronic toxicity data for the same endpoints within three taxonomic groups, i.e. algae, daphnids and fish. If experimental testing with the formulated product can be conducted, the guidance recommends comparing the results with the dose addition predictions. Comparisons between measured and predicted mixture toxicity are recommended to decide on possible synergisms.

EFSA's Guidance on Effect Assessment of Pesticides on Sediment Organisms in Edge-of-Field Surface Waters [EFSA PPR Panel, 2015] builds on the principles developed in the Guidance for water dwelling organisms. It applies tiering principles to exposure assessment, by first adopting a screening approach in which 'worst case' maximum Predicted Environmental Concentrations (PECs) are entered into the analysis, to be replaced by more detailed exposure assessments, if needed. Methods for validating the predicted mixture toxicity by measurement are not recommended or elaborated, due to the practical difficulties of achieving this in sediment matrices.

ECHA's Transitional Guidance on Biocidal Products (ECHA, 2014) advocates the use of dose addition and rejects independent action on the grounds of insufficient conservatism. It proposes screening steps to determine whether a mixture risk assessment is necessary, e.g. when exposure to components in biocidal products is unlikely, or when a product contains only one relevant active substance. A tiered assessment scheme is recommended, which begins with the summation of ratios of Predicted Environmental Concentrations (PEC) and Predicted No Effect Concentrations (PNEC) at the lowest, most conservative tier. This simplified calculation approach encompasses some aggregations that have no meaningful scientific interpretation in terms of expected effects, as a consequence of the pragmatic mixing of toxicity endpoints, species and assessment factors in the aggregated PEC/PNEC ratios. However, it is applied as an efficient conservative approach, i.e. to enable stopping the assessment if the summed PEC/PNEC ratio is <1. If not, this is followed by more refined forms of toxic unit summation, in which ecologically meaningful approaches replace the simplified approaches. At the final, highest tier, experimental testing of the mixtures of concern is proposed (ECHA, 2015).

2.6. Harmonised overarching framework

Figure 2 summarises the proposed harmonised framework for human, animal and ecological risk assessment of chemical mixtures. It consists of problem formulation and the risk assessment steps namely exposure assessment, hazard assessment and risk characterisation (EC, 2002, WHO, 2009, US EPA, 2007; Ragas et al., 2010; Van Gestel et al., 2011). Some aspects require specific attention in all steps of a mixture risk assessment.

The problem formulation step is an iterative process between risk assessors and risk managers, describing the food safety problem and its context to identify those items of hazard, exposure or risk associated with a chemical that are relevant to potential risk management decisions (WHO, 2009). The problem formulation step takes on particular importance in the context of chemical mixtures because the demarcation of the problem (e.g. the exposure routes and substances to be included) is more complex than for single substances (Chapter 3).

The harmonised framework can be applied in a tiered manner. The tiers are implemented in this framework to avoid unnecessary expenditure of resources by offering the possibility of discontinuing the analysis on the basis of crude and simple assumptions about exposures and hazards when the outcome of the assessment is judged to be sufficiently protective, as described above.

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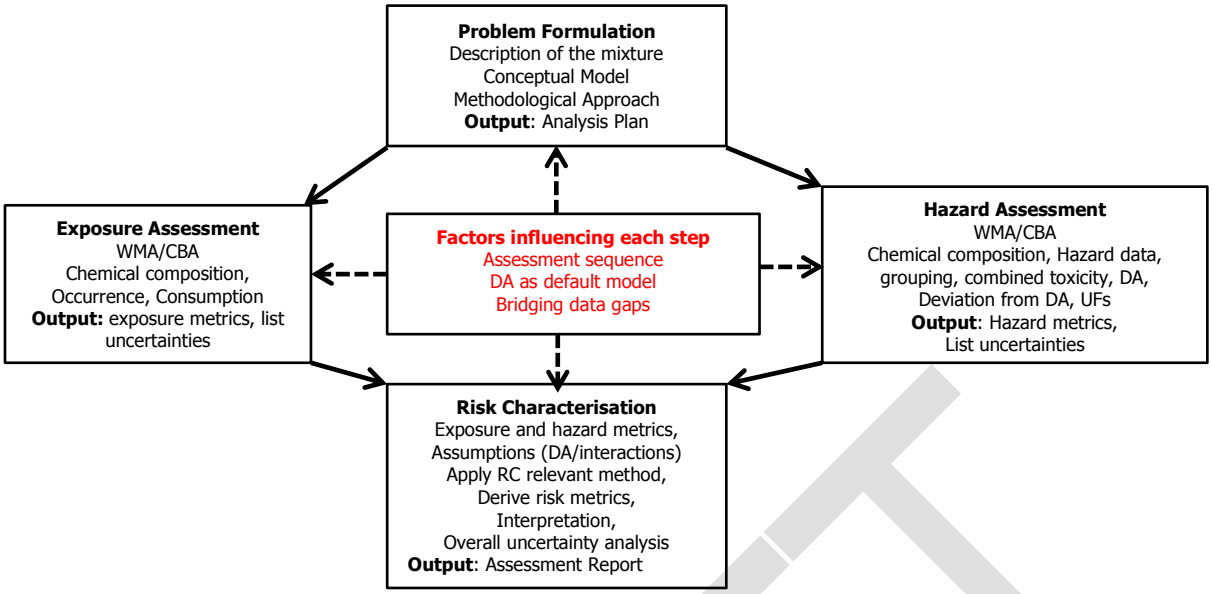

Central in red are specific aspects required for mixture risk assessment; these factors require attention and/or decisions for all assessment steps in an iterative way.
WMA: whole mixture approach; CBA: component-based approach; UF: Uncertainty factors; RC: risk characterisation; DA: dose addition.

Figure 2: Overarching framework for human, animal and ecological risk assessment of chemical mixtures with characterisation of specific mixture aspects and inputs and outputs for each step

The different steps of the mixture assessment framework are elaborated and discussed in more detail in the following chapters of this guidance, including practical stepwise approaches and iterations to support implementation:

- problem formulation (Chapter 3)
- exposure assessment (Chapter 4)
- hazard assessment (Chapter 5)
- risk characterisation (Chapter 6)

The specific aspects of mixture risk assessments that have a bearing on several of the risk assessment steps are discussed below with a focus on EFSA's food safety context.

2.6.1. Assessment sequence

After the problem formulation, it is possible to first pursue either the exposure or the hazard assessment steps, or both of these steps in parallel. There is no *a priori* or scientific reason to start with either of the two assessment steps and a decision should be driven by the context and problem formulation. In some cases, quantitative exposure assessment may be easier to conduct (given an exploration of available data in the context of the problem formulation), when it is first established whether the assessment problem indeed implies relevant coexposures to multiple chemicals within a relevant time frame. In other cases, the assessor could start with the hazard assessment to see whether the chemicals under consideration exhibit a common toxicity profile that might lead to combination effects. Iteration between exposure assessment and hazard assessment will be necessary to ensure that common dose metrics are used, for example if one substance is used as a marker of a whole mixture, or if Relative Potency Factors are established.

2.6.2. Dose addition as the default model

As noted in Section 2.2, the two commonly applied component-based assessment concepts have a similar action, with the associated assessment approach of dose addition, and independent joint

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action, with the associated assessment approach of response addition. For binary mixtures, both concepts often provide equally good approximations of observed mixture effects. For multicomponent mixtures, the two models often predict mixture toxicities of differing strength, with varying (dis)similarity to observed mixture effect levels (Faust et al., 2001; 2003; Altenburger et al., 2005). Dose addition usually produces the most conservative prediction, and therefore this approach is preferred in decision-making processes in the context of health or environmental protection, and selected as the default model. The practical advantage of applying dose addition as default is that it can be readily applied by comparing exposure doses or concentrations with reference values derived from toxicity data (such as no effect or effect concentrations) often available in public databases. In contrast, the use of response addition requires knowledge on the precise effect magnitude that each component would provoke if present individually at the concentration found in the mixture. This information is only accessible through comprehensive dose–response analysis of each mixture component. Such data are not readily available in practice, neither for human nor ecological assessments. Dose addition is therefore adopted as the default assessment approach, unless there is evidence that response addition is more appropriate and the necessary data to apply response addition are available or can be easily gathered (SCHER, SCENHIR, SCCS, 2012; EFSA, 2013b).

2.6.3. Bridging data gaps

Data gaps may be highly variable across problem formulation, and – for mixtures – across chemicals within one assessment. They may pertain to missing data on exposure or on hazards, and the gaps may pertain to few or many chemicals in the assessment. Methods developed for single chemical assessments can be applied to fill data gaps, such as read-across based prediction of hazard characteristics of chemical(s) in the assessment and in silico models. When read-across and *in silico* models agree, this reinforces the assessment, and such methods can help in decisions to attribute chemicals to common assessment groups. The suite of methods to fill data gaps is not specific to mixture risk assessments (and are therefore not described here), apart from the element of filling data gaps relevant for grouping, i.e. to make evaluations of the mode of action assumptions.

The set of assumptions, including approaches to fill data gaps, gives rise to specific uncertainties in mixture risk assessments, warranting specific attention to avoid potential interpretation pitfalls. For example, when hazard data gaps are bridged by a conservative approach, or when large assessment factors are applied to lowest observed effect levels to derive a reference value, the results of the mixture risk assessment may result in (extremely) high values for the aggregated mixture risk metrics. For example the summed PEC/PNEC ratio may have values >1,000, which – at first sight – might be interpreted as being indicative for extremely risky mixture exposures. Therefore, mixture risk assessment outcomes should always be scrutinised for interpretation bias, especially by evaluating the identities of and underlying data for chemicals that contributed most to such high risk characterisation values. Situations under which the high value is attributable to compounds for which the hazard assessment is based on a low tier assessment, with the use of a large assessment factor because of lack of compound-specific data, the final outcome should be interpreted as an indication of lack of knowledge, which can either be used for a risk management decision or for collecting additional data to feed into a higher tier (Price et al., 2009). A refined interpretation needs to state whether the outcome is interpreted as evidence for insufficient protection or as uncertainty caused by data gaps. The compounds for which the latter holds should be identified to avoid the derivation of biased conclusions.

3. Problem formulation

3.1. General considerations

Problem formulation is an iterative process involving risk assessors and risk managers during which the need for, and the extent of, a risk assessment are determined (EFSA Scientific Committee , 2017a). In a mixture context, it involves the generation of a conceptual model that describes the sources of the combined exposure, the exposure pathways, the populations and life stages exposed, the endpoints to be considered, and their relationships (EFSA PPR Panel, 2014). In the design of the conceptual model, assessors need to take the regulatory context into account to provide fit for purpose advice. The outcome of the problem formulation is an analysis plan describing how to

proceed with the assessment, and may include aspects such a specification of the study design, methodology, data requirements and uncertainty analysis (EFSA, 2015b).

The implementation of a problem formulation step within the context of combined exposure to multiple chemicals has been thoroughly discussed by a number of scientific bodies including 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; OECD, 2011; SCHER, SCENIHR, SCCS, 2012; EFSA, 2013b; Meek, 2013; Bopp et al., 2015; Solomon et al., 2016; EFSA Scientific Committee et al., 2017; OECD, 2017). The reader is referred to the cited references for a comprehensive overview.

Key issues to be considered in the problem formulation for risk assessment of chemical mixtures, including the development of the conceptual model and the analysis plan, are shown in Table 2 (see also OECD, 2017). Other aspects of the problem formulation, including selection of relevant endpoints is generally similar to the approach that would be taken for single chemicals, unless otherwise defined in the risk assessment request.

Table 2: Key issues to be considered in the problem formulation that are specific for risk assessment of chemical mixtures

Issues	Examples
On the basis of the assessment process:	
Is mixture assessment warranted?	Co-exposure and mixture effects are likely based on data in hand
Characterisation of the mixture	Origin: e.g. production process or emission sources Composition: e.g. components, stability (does the composition of the mixture change over time), variability (batch-to-batch differences) Reactivity
Whole mixture and/or component-based approach?	*Whole mixture*: e.g. an essential oil, for which not all components have been chemically identified *Component-based*: e.g. pesticide residues with potential for co-exposure
On the conceptual model:	
Approach to exposure assessment	Availability of data on components of the mixture or on a marker substance for the whole mixture
On grouping of chemicals:	
Criteria for inclusion in the assessment group?	Similar origin, similar Mode of Action (MoA), same target organ
What to do with chemicals belonging to different groups?	Consider applying response addition
On risk characterisation:	
What risk metrics to use?	Margin of Exposure, hazard or risk quotient

One of the first issues to be addressed is to decide, in communication with risk managers, whether a mixture assessment is warranted and, if so, which chemicals should be considered together. This is sometimes referred to as the 'gatekeeper' step (Solomon et al., 2016). This step can be based on the likelihood that chemicals co-occur in the scenario that is the topic of the assessment. With product-oriented assessments, the question might be limited to listing the chemicals that constitute a product, although it might also be appropriate to consider other relevant exposures. If co-occurrence/co-exposure within a relevant time frame is unlikely to be based on an initial assessment of the data in hand, a mixture assessment can be considered redundant. In the context of EFSA's responsibilities, the gatekeeper step has often been conducted by the European Commission in consultation with experts from Member States, before a request for a mixture risk assessment is sent to EFSA.

Another important issue to be addressed during the problem formulation is whether a whole mixture approach, a component-based approach or (parts of) both will be followed. The Scientific Committee recommends the component-based approach as the preferred option if the components are characterised analytically and sufficient exposure and toxicity data (reference points and reference values) on the mixture components are available. This recommendation particularly applies to regulated products and contaminants in the human and animal health area. This requires an initial

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assessment of the available information on mixture characteristics and composition (e.g. based on mass spectrometry data, detection limits and read-across), as well as of the available effect data. Due to the diversity in potential assessment questions and types of information needed to answer the questions, this Guidance does not specify the preferred characterisation level of mixtures to apply component-based approaches. This should be assessed on a case-by-case basis depending on the information at hand or information that can be generated readily.

A whole mixture approach is the preferred option for poorly characterised mixtures, typically consisting of many different components. Within this context, the term complex mixture is sometimes used. However, complexity and simplicity are neither sufficient nor necessary reasons for choosing between a whole mixture or component-based approach. A complex mixture should preferably be assessed following a component-based approach if sufficient exposure and effect data are available on the components governing its toxicity. Similarly, a mixture consisting of a few components may be assessed following a whole mixture approach if interaction between the components is considered likely.

Although resource intensive, a combination of component-based and whole mixture approaches may also be considered if interaction between the components is considered to be likely. After application of a component-based approach such as dose addition, a whole mixture test could for example be performed to test for potential interaction effects. Conversely, in subsequent tiers of a whole mixture approach, information on components in the mixture may become available, which allows for component-based approaches to be applied.

As a final step in the problem formulation, an analysis plan is generated (OECD, 2017), which includes: (1) the specific question to address; (2) the rationale for selecting specific pathways/chemicals and excluding others (conceptual model); (3) the design of the assessment (e.g. order of the assessment steps); (4) the description of data/methods/models to be used in the analyses and assessment steps (including uncertainty and intended outputs of the assessment, e.g. exposure, hazard and risk metrics for risk characterisation) and including tiering principles and decision points; (5) approach to evaluate the uncertainties in the assessment resulting from data gaps and limitations; (6) plans for stakeholder consultation and peer review; and (7) value of additional data collection. For specific EFSA methodologies, dealing with problem formulation and the analysis plan, the reader is referred to Section 3.2.

It is stressed that problem formulation is an iterative process and needs to be refined as relevant data are identified and evaluated, and key data gaps emerge during the process of a mixture assessment. This in principle could include identification of a need for mixture risk assessment in the course of risk assessment of a single substance.

3.2. Problem formulation under EFSA's remit

Many of the types of assessments relevant to EFSA are described within the specific legislation of a food or feed safety area (e.g. regulated products) and are dealt with in guidance documents published by EFSA panels or the Scientific Committee. In the context of EFSA's work, problem formulation is usually outlined in the Terms of Reference (ToR) provided by risk managers from the European Commission. The ToR contextualises the problem formulation for a specific risk assessment, which is often refined through a dialogue between risk managers and risk assessors to clarify the scope of the requested risk assessment (EFSA, 2015c). The exact question to be addressed is then described within EFSA opinions in the 'Interpretation of the Terms of Reference section'.

Three broad categories of risk assessments performed by EFSA could potentially require consideration of chemical mixtures:

Regulated products: This relates to the evaluation of regulated products proposed to enter the market, or already on the market for which important new data have emerged, and in some instances these require mixture risk assessments. For pesticides and feed additives, human risk assessment is also performed for non-dietary exposure to mixtures of operators, workers, bystanders and residents, but consideration of non-oral exposure is beyond the scope of this Guidance.

Contaminants in the food and feed chain: For human and animal risk assessment, these include environmental contaminants (e.g. brominated flame retardants, dioxins, heavy metals), compounds resulting from food and/or feed processing and natural toxins produced as undesirable substances in

865 food and feed by plants, fungi and other microorganisms (e.g. alkaloids, mycotoxins, marine
866 biotoxins).

867 **Chemicals under the remit of more than one panel** are evaluated by the Scientific Committee of
868 EFSA. So far, the Scientific Committee has not been asked to consider combined effects of specific
869 chemical mixtures.

870 3.3. Stepwise approach to problem formulation

871 Figure 3 summarises the iterative stepwise approach for problem formulation as follows:

872 *Step 1. Description of the mixture*

873 Does the problem formulation or the Terms of Reference specify that mixture risk assessment is
874 required? Is the mixture poorly or well characterised? For a well characterised (simple) mixture, the
875 components should be listed and quantified. For a poorly characterised (complex) mixture, describe
876 what is known about its composition, based on e.g. the production or manufacturing process (if
877 applicable), any compositional data, the stability and the specifications (if applicable) of the mixture.
878 How consistent is the mixture composition (i.e. stability over time and variability from different
879 batches or production processes or in different environmental matrices)? Is the exposed population
880 directly exposed to a discrete mixture or is the exposure pathway between source and exposed
881 population complex? Is hazard information available on the mixture of concern, its components or is
882 there information on a similar mixture that could be used as proxy for the mixture of concern? Are co-
883 exposure and/or potential combined effects likely to be based on an initial assessment of the problem
884 formulation, (preliminary) conceptual model and available data? Proceed with the mixture risk
885 assessment if the answer is yes.

886 *Step 2. Conceptual model*

887 The next step of the problem formulation is the development of the conceptual model to frame the
888 risk assessment. This can include identification of:

889 a) the origins/sources of the chemicals involved in the assessment;

890 b) the pathways along which those chemicals are transferred from the source to the target
891 organism(s) or ecological receptors (species of ecological relevance or ecosystem);

892 c) the temporal exposure pattern;

893 d) the human (sub)population(s), animal species or ecological receptor.

894 The conceptual model is the basis for deriving the data needs and the specific approaches for the
895 subsequent assessment steps. It is also the basis for the assessment plan, including a literature and
896 data search strategy, and for the mathematical formulations of the models involved in the exposure
897 and hazard assessment steps which are directly derived from the source–pathway–receptor
898 combinations shown in the conceptual model.

899 When the mixture assessment is performed under a specific regulatory framework (e.g. a Commission
900 Regulation within EFSA's remit) or the combined exposure scenario is otherwise defined, the ToR may
901 already pre-define the (sub)population/taxa/species of concern, the co-exposure scenario (acute,
902 chronic) and the whole mixture or known components. In this case, consider if additional chemicals
903 should be included in the mixture assessment. This may require dialogue between risk assessors and
904 risk managers. Any choices made (e.g. to take background contamination into account or not) should
905 be made explicit in the analysis plan and ultimate risk assessment report.

906 *Step 3. Methodological approach*

907 Here, the methodological approach for the mixture assessment is defined, based on an overview of
908 the available data and exploration of the assessment options. The outcomes of this exploration lead to
909 a decision on using a whole mixture and/or a component-based approach, which is a major
910 determinant of approaches to be subsequently used. A key consideration is the extent to which the
911 components of the mixture are unknown or toxicologically uncharacterised, and whether the
912 composition is expected to vary over time, e.g. with different batches or production methods, or in the

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environment. If a component-based approach is adopted, then this step may also include initial consideration of the chemicals to be included in an assessment group (see Section 5.3). It is also possible that a mixture risk assessment evolves from a whole mixture approach to an approach involving known chemicals, when the first assessment outcome suggests insufficient protection, and increasingly identifies compounds causing this. Partial identification of the compounds results then in a shift from a whole mixture to a mixed approach, with increasingly specific information on the relative importance of specific chemicals in the whole mixture.

The outcomes of the exploration of the conceptual model, approaches and data also help to decide to go first to either the hazard assessment step, the exposure assessment step, or to proceed with both in parallel.

Step 4. Analysis Plan

The outcome of the problem formulation is an analysis plan that encompasses the ToR (when applicable), the conceptual model, the strategy for the risk assessment, the initial tiers, the decision points to stop the assessment when information to support decision making is considered sufficient, the (probable) approaches and data needs when more refined and accurate tiers are triggered, the decisions taken on the specific mixture aspects and the anticipated approach to interpretation and communication of the risk assessment outcome. The analysis plan may be revisited and revised during the course of the assessment in an iterative manner.

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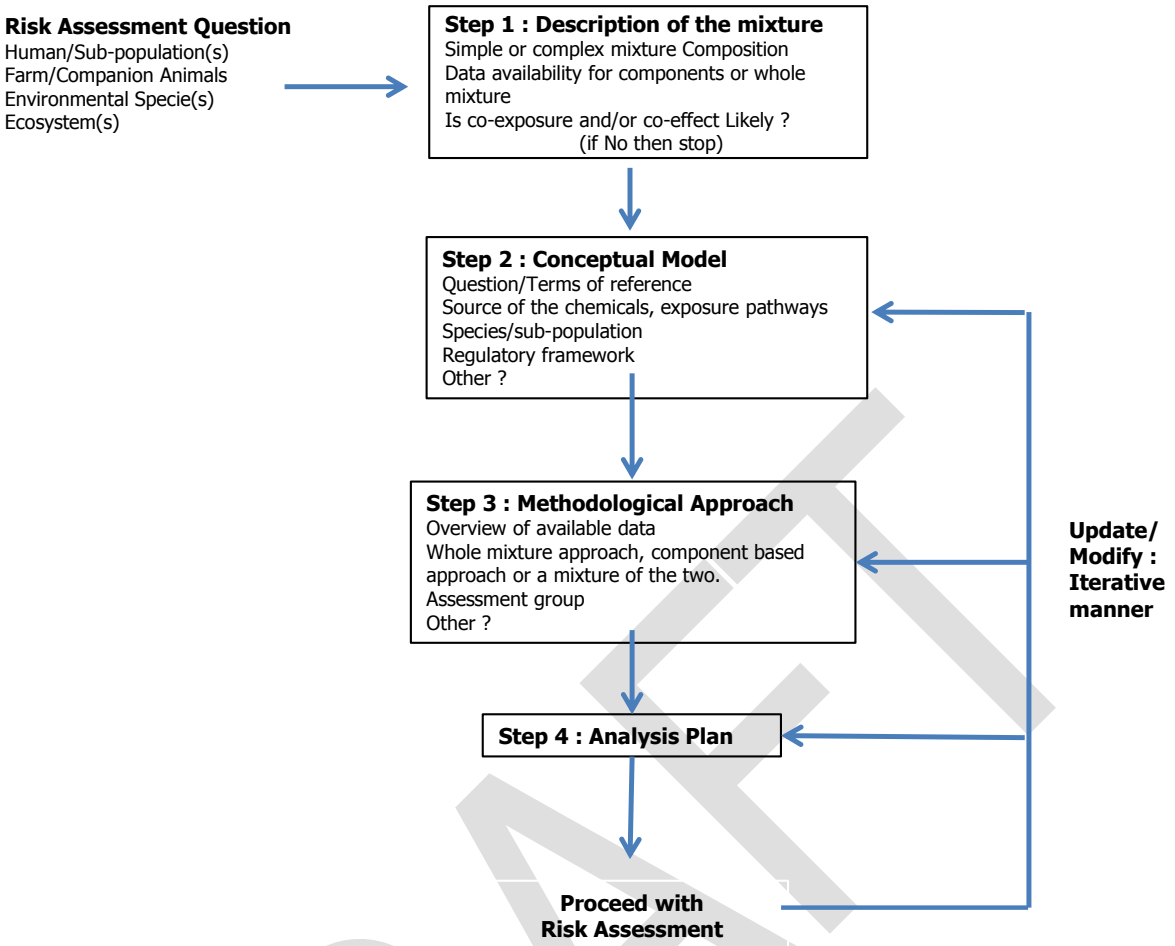

Figure 3: Problem formulation for human health, animal health and ecological risk assessment of chemical mixtures

4. Exposure assessment

4.1. General considerations

The purpose of the exposure assessment is to provide the exposure metrics findings to be used in the risk characterisation part of the assessment. In performing such an exposure assessment, the assessor addresses questions related to the source, exposure pathway, exposed population, variation of doses over the exposed population, and the uncertainty in the exposure estimates. While an assessment of combined exposure to multiple chemicals generally uses similar concepts and methods as an assessment for individual single chemicals, there are additional issues to consider that are unique to mixture risk assessment. As a result of these issues, the mixture exposure process can differ from single chemical assessments. Assessment of combined exposure to multiple chemicals generally uses similar concepts and methods as for single chemicals.

Figure 4 illustrates how the principles of tiering are applied in exposure assessment. While the principles of tiering are used for single substance and mixture exposure assessments, there are differences. For mixtures, the correlation of doses across the assessed chemicals is now part of the refinement addressed by the tiers. At a low tier, a component-based approach might assume that an individual might be exposed to an upper bound estimate of Exposure for each chemical as a conservative approach. At higher tiers, real correlations of chemical-specific doses for the exposed individuals are determined using monitoring or modelling data.

The selection of the tier and the specific approach that are used in the initial stage of the exposure assessment depends on the legal framework along with the data, time and resources available.

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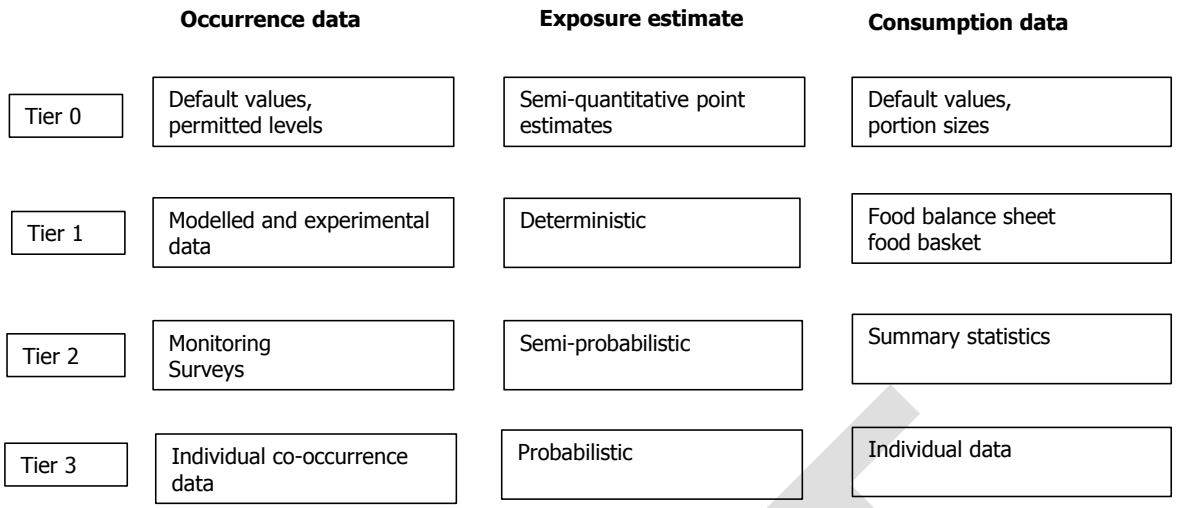

Note: Occurrence and consumption data ranges from default values (tier 0) to individual co-occurrence data and individual data respectively (tier 3) and consequently exposure estimates range from semi-quantitative point estimates (tier 0) to probabilistic (tier 3). Occurrence and consumption tiers do not necessarily match.

Figure 4: Examples of tiers in exposure assessments

In the human and animal health area, dietary exposure is typically obtained by combining occurrence data of the chemicals in food or feed with consumption data for those items. Available tools for assessment of human dietary exposure have been reviewed by EFSA (2011). Additional tools that were developed after this review include EFSA's guidance on the use of probabilistic methodology for modelling dietary exposure to pesticide residues (EFSA PPR Panel, 2012b), the EFSA pesticide residue intake model (PRIMO) (EFSA, 2018), the Food Additives Intake Model (FAIM) and the Feed Additive Consumer Exposure (FACE) calculator. For the animal health area, consumption values for farm and companion animals (i.e. cats and dogs) have been recently published by the FEEDAP panel (EFSA FEEDAP, 2017b).

Exposure assessment in the ecological area is usually less complex than in the human and animal health area, as the population, species or community under assessment often is in continuous contact with the exposure medium, e.g. fish living in polluted waters. Hence, occurrence data only, i.e. the concentration(s) in the dominant exposure medium, are often used as a proxy for exposure. In the absence of measured concentration data, the occurrence of substances in the environmental media are often predicted based on emission data using fate models (Di Guardo et al., 2018). Alternatively, conservative occurrence concentrations can be estimated assuming that all released substances reach the environment medium with no diminution by absorption, degradation or other physical or chemical processes.

So different exposure metrics are used in human/animal and ecological risk assessment, usually being dose and concentration, respectively. For humans, farm animals and companion animals, exposure estimates are usually expressed as a dose on a body-weight basis within a relevant timeframe for the (sub)populations of interest, e.g. mg substance per kg body weight per day (EFSA Scientific Committee, 2012a). For the ecological area, the concentration of the substance in the environmental medium (water, sediment or soil,) is generally used as a proxy for exposure.

In higher exposure assessment tiers, the internal dose is sometimes the preferred exposure metric. In the human and animal health area, biomonitoring data and/or toxicokinetic models may be used to estimate internal doses integrating all exposure routes (SCHER, SCENIHR, SCCS, 2012), although the Scientific Committee noted that such approaches are rarely used in practice (with the exception of pesticides and certain contaminants) because of the amount of data and resources required (EFSA, 2013b). In the ecological area, accounting for internal exposure can be complex because of the environmental fate of the substances and the diversity of species and associated species-specific traits such as toxicokinetic differences (EFSA Scientific Committee,2016a).

Compared with the exposure assessment of single compounds, the assessment of combined exposures is typically more complex. The central question is whether co-exposure is likely within the

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timeframe considered, and how this co-exposure can be adequately quantified. Co-exposure can be caused by co-occurrence (i.e. the presence of multiple substances in the same exposure medium within the time frame considered) and by co-incidence (i.e. exposure to multiple exposure media within the timeframe considered, each containing one or multiple substances of concern). Co-occurrence of chemicals in a particular exposure medium may vary both in space and time, which is further discussed in Sections 4.2 and 4.3. Co-incidence is mainly relevant in the human and animal health area. An example is exposure to a combination of different pesticide residues present in different food products which are consumed together. This co-incidence is typically captured by combining consumption (and occurrence) data of different food products. Like with single substances, correlations between the consumption of different food products are of importance. For example, if the consumption of two food products is negatively correlated (e.g. fish and meat), co-exposure will be much less likely than if it is positively correlated (e.g. meat and vegetables). Ignoring negative correlations in occurrence results in an overestimation of the co-exposure, whereas ignoring positive correlations results in underestimation. These correlations are of particular relevance for mixture assessment because of the many substances and exposure media involved. With many correlated parameters, a default assumption of independence will result in a bias towards assessing a situation as if average exposure occurs. Ignoring correlations can so result in a failure of identifying high exposure situations and here, probabilistic approaches may provide useful to identify such situations and correct such bias.

In the ecological area, the situation is different as it is often assumed that organisms are in continuous contact with the exposure medium, e.g. water, sediment or soil. Hence, the assessment generally focuses on co-occurrence, and co-incidence is less frequently addressed. An exception applies to spatially and temporally varying exposures such as for mobile organisms, organisms experiencing mixture exposures in 'mobile' compartments, such as surface water (rivers). These organisms may be exposed to different exposure media or pulse exposure with varying chemical levels as the organisms or water masses move through space (Loos et al., 2010).

It is recommended that panels and other expert bodies continue to use the exposure assessment approaches originally developed for single substances to estimate combined exposure to multiple substances. However, when assessing mixture exposure, specific attention should be paid to the likelihood of co-exposure, i.e. co-occurrence data, co-incidence data and their mutual correlations.

4.2. Whole mixture approach

Application of a whole mixture approach implies availability of toxicity data on the whole mixture of concern or a sufficiently similar mixture. The metric used for quantifying exposure should match that used for toxicity. This requires coordination between exposure and effect assessors.

Whole mixture approaches are usually limited to assessments in which there is direct exposure to the mixture by a single route of Exposure. The reason for this is when the exposure pathway for a whole mixture is complex, mixture components tend to separate and the exposed individuals may be exposed to only a few components and the ratios of the components could change as well. As a result, the hazard characteristics of their exposures are likely to differ from that of the whole mixture. For example, a whole mixture approach may be appropriate for a mixture of contaminants in a food item, but not for a plant protection formulation in which formulation components such as solvents or surfactants would not be expected to persist in the diet in the same way as the active ingredient.

Different methods are available to quantify the exposure to a whole mixture. The suitability of these approaches depends on the availability of knowledge on the mixture, i.e. data on composition and occurrence, and the variability and stability of the mixture. In the most extreme case, composition and occurrence data are completely lacking and a sample of the mixture of concern (which can be the mixture as it is added to a food product, but also an environmental sample) is directly tested in the laboratory for toxicity. These toxicity tests will typically be performed at different dilution and/or concentration levels of the sample. In such cases, toxic potency can be expressed as the dilution or concentration factor needed to reach a toxicity benchmark such as the LD_{50}, LC_{50} or NOEC. This means the exposure must also be expressed in a dilution (or concentration) factor, i.e. how many times is the mixture of concern diluted before exposure takes place? Risk can subsequently be quantified as the inverse of the ratio between both dilution (or concentration) factors. This approach is based on the assumption that the mixture composition (i.e. the relative concentration ratios

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between the mixture components) remains the same during the dilution process. This assumption will hold for the assessment of simple and swift processes such as the dilution of effluent in surface water, or the addition of a food additive to the dye, but may be inadequate if preferential processes such as absorption and degradation act differently on the various mixture components. The potential influence of such processes should always be critically assessed when applying a whole mixture approach.

As an alternative for dilution or concentration factors, the total mass of the mixture components may be used as an exposure and effect metric. This is an option if the mixture of concern is available in its pure form or if its components can be extracted from the environmental or test medium. Alternatively, occurrence values for the whole mixture may be estimated by using the concept of a marker substance. This concept is particularly useful when the composition of the mixture is only partially characterised or when occurrence data are not available for all components of the mixture. In these cases, one or more marker substances are selected if possible. Total concentrations of the marker substances are then used as a proxy for the whole mixture concentration. As the marker substances will only constitute a part of the mixture of concern, occurrence data obtained for the marker substances may need to be adjusted by an additional correction factor to account for potential variability in the composition.

As a final option, occurrence data for mixtures from similar sources, use patterns, life cycles of Exposure or physicochemical properties (including molecular weight, water solubility, density, vapour pressure, organic carbon and octanol/water partition coefficient, melting and boiling points) may be used as a proxy to estimate exposure for the mixture of concern. This approach requires explicit description of assumptions made, as those will contribute to the uncertainties of the risk assessment.

4.3. Component-based approach

As opposed to the whole mixture approach, a component-based exposure assessment accounts for the variability of the mixture's composition in the different exposure media and, when applicable, the (eco)toxicological potency of the individual components. The collection and analysis of occurrence data for the individual mixture components is therefore a prerequisite.

Data on the co-occurrence of the individual components may be used to understand how they are related, i.e. the likelihood of two or more substances to occur at the same time within a given time frame. The timescale of interest depends on the toxicokinetics and toxicodynamics of the chemicals (human, animal and ecological), the dispersal of the chemicals in the environment (ecological), the nature of the toxic effect (e.g. reversibility) in the target organism(s), and the time required for 'recovery'. Therefore, the co-occurrence assessment is critical, and should be determined by consultation between exposure assessors and (eco)toxicologists.

Under the dose addition model, the time course of interest for exposure is the same time course as for the chemicals individually. For chronic and subchronic exposure assessments, the timeframe when chemicals need to co-occur for eliciting combined toxicity may be very broad. In these cases, substances do not need to coexist in the same food, water or air sample. Potency-adjusted concentrations can be calculated at a high level of aggregation (e.g. based on the average concentrations of the individual components within a given matrix).

For acute exposure, however, the relevant timescale required for two or more substances to elicit combined toxicity may be as narrow as a single eating occasion for humans or animals or a single environmental release of chemicals (EFSA PPR Panel, 2012b; EFSA, 2013a). Under these circumstances detailed information on co-occurrence of the individual chemicals is required at sample level, and preferably potency-adjusted concentrations should also be calculated at the sample level before proceeding with the exposure calculations. However, such requirements cannot always be met, and difficulties may arise when the analysed components differ between samples. That would lead to e.g. missing occurrence values for certain substances in the different samples and a possible underestimation of the exposure. This uncertainty may be addressed by analysing the available dataset for ratios and correlations between components, and filling the missing values with an estimated concentration. This approach may use concentrations measured in other samples or derived from a known distribution, and include additional assumptions that will depend very much on the area, type of chemical and regulatory framework. A complex and probabilistic imputation technique was for example elaborated in the area of pesticide residues (EFSA PPR Panel, 2012b). All aspects on

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co-occurrence of individual components must be noted, and handled in the final interpretation and communication.

If the dose addition model is assumed, the occurrence data for each component within an exposure medium are summed and obtained media concentrations can subsequently be used for calculating total exposure using the same principles as for a single compound, e.g. by multiplication with consumption data for dietary exposure. When the toxicological potencies of the individual components are sufficiently understood and reliable Relative Potency Factors (RPF) or Toxic Equivalence Factors (TEFs) have been identified by toxicologists (see Chapter 5), these factors should be incorporated to obtain total potency-adjusted exposure estimates. In ecological risk assessment, the concept analogous to RPF is known as toxic units (TU; see Section 2.5.2) which are concentrations of individual substances standardised by dividing the concentration of each chemical in a mixture by its concentration eliciting a defined effect (e.g. EC10, EC50). Alternatively, exposure will be reported for the individual components and impact of their potencies will need to be considered at the level of risk characterisation.

The considerations above are all based on the assumption that the individual components can be assessed using dose addition. In the case interactions are likely, it is appropriate to calculate exposure for each individual component separately and deal with potential synergies or antagonisms in the hazard assessment step (see Chapter 5).

As discussed above, conservative assumptions that are appropriate for individual chemicals may cause problems for mixture assessments. Exposure estimates frequently address uncertainties in data and modelling by the adoption of conservative assumptions. When these assumptions are made for multiple chemicals in a component-based mixture assessment it is possible to bias the risk predictions. An example of this complex step in the component-based approach is the handling of concentration data reported to be below the limit of detection (LOD) or quantification (LOQ), which leads to left-censored exposure data distributions. The use of data substitution methods has been evaluated, from which it was concluded that the degree of censoring has a large impact on the uncertainty of the exposure assessment (EFSA, 2010). When assessing exposure to multiple substances with left-censored data, this uncertainty is further magnified (EFSA PPR Panel, 2012b). Hence, while for single compound assessments this uncertainty can usually be reduced through the application of cut-off values for the LOQ and/or LOD, exposure assessment for mixtures may require more sophisticated modelling in which left-censored results are replaced by a numerical value (equal to zero, to LOQ/LOD, or to any value in between) according to a certain probability. This probability may be based on more realistic assumptions such as the authorisation status of a chemical, usage data or its likelihood to co-occur with another chemical. This issue also should be kept in mind in the design of the analytical chemistry of a monitoring survey. Detection limits may need to be lower when the data are to be used to support mixture risk assessment. In all cases, observations on compound-related censoring data and assumptions applied should be reported, to support the final interpretation of the risk assessment.

4.4. Stepwise approaches

4.4.1. Whole mixture approach

Figure 5 summarises the steps of Exposure assessment for whole mixtures.

Step 1 - Characterisation of the whole mixture

In line with the problem formulation and analysis plan, characterise the whole mixture based on what is known about its source, origin, kinetics and composition. If exposure data are not available for the mixture of concern, are there data for a similar mixture that can be used? If the mixture can be reliably quantified by using just one or a few components as marker substances, then list the concentration ratios for these along with an estimate of their variability as components of the whole mixture. By using marker substance(s) in this way, it must be known or assumed that the mixture composition does not change, e.g. by environmental degradation or during processing of food or feed.

Step 2 - Assembling the chemical occurrence (concentration) data

Assemble chemical occurrence (concentration) data for the mixture of concern which may be estimates from predictive models, or measured data in the relevant samples. If appropriate, consider the analytical method(s) used and assess the extent to which the method allows quantification of the whole mixture or marker substances described at step 1. When specific occurrence data are not available, consider using usage levels or data from mixtures with similar sources, use patterns, life cycles of Exposure or physico–chemical properties.

Step 3 - Combining occurrence data and consumption data

Combine occurrence data with the consumption data to estimate exposure using the same tools and assumptions as are used for a single substance. This step is generally not required in ecological risk assessment as consumption data are usually not available and environmental concentration is taken as a proxy for exposure.

Step 4 - Report exposure data

Summarise the exposure results, associated assumptions, uncertainties and consequences for risk characterisation. In case of uncertainty because of limitations in the data or the analytical method used, provide comparative data and/or a rationale for consideration by (eco)toxicologists, who may wish to propose an additional assessment factor in the risk characterisation (especially for lower tiers, as a method to ascertain the characteristic of lower-tier conservatism).

If any identified component of the mixture is subject to an existing risk assessment and/or legal restriction, this should be reported in summary form. It may also be appropriate to estimate exposure to that chemical(s) from all sources.

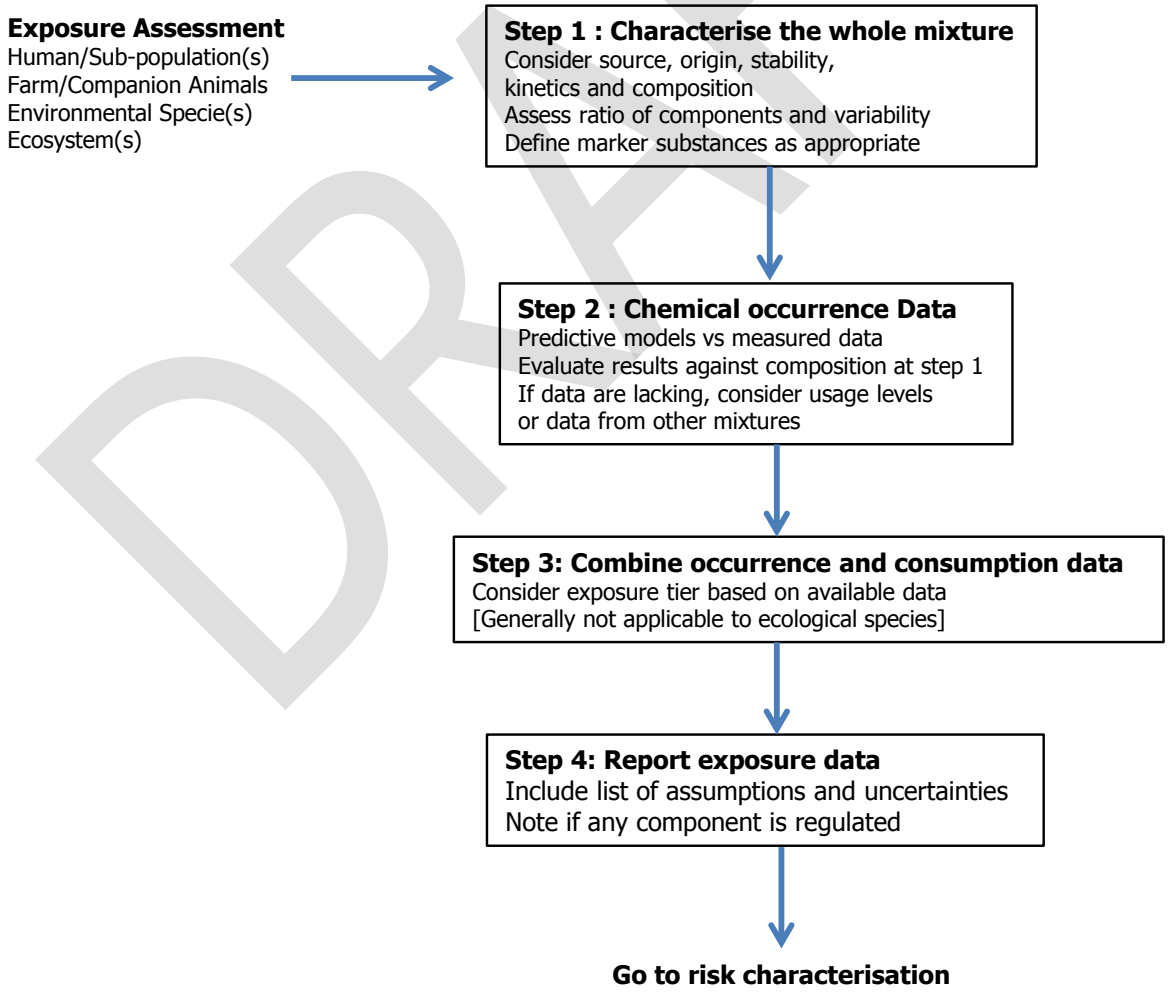

Figure 5: Exposure assessment using the whole mixture approach

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4.4.2. Component-based approach

Figure 6 summarises the steps of Exposure assessment using the component-based approach.

Step 1 – Components of the assessment group

According to the problem formulation, analysis plan and input from (eco)toxicologists, list the chemicals in the assessment group(s) depending on the criteria used for grouping (exposure-based or hazard-based, etc., with the option of treating all chemicals as if in one group as a lowest-tier grouping method). Consult (eco)toxicologists to obtain information on relative potencies of the individual components of the assessment group, if available, and to understand the timeframe that is required for those compounds which could potentially elicit combined toxicity.

Step 2 – Assembling chemical occurrence data

Assemble occurrence data considering plausibility of the individual components to co-occur, taking into account advice from (eco)toxicologists on the relevant timescale (see Step 1). When estimating acute toxicity use only data sources that provide information on the co-occurrence of components of the assessment group within a narrow timescale (e.g. a single eating occasion or a single environmental release). If occurrence data are not available for all components of the assessment group in all of the samples analysed, evaluate ratios and correlations between components with the available dataset and decide if the missing data can be imputed.

Consider the precision and accuracy of the analytical method(s) used for each component and the consequence of the detection limits for the exposure estimates. When necessary, apply appropriate corrections, assumptions or methods for left-censored data.

If occurrence data for individual components are available and relative potencies were provided by (eco)toxicologists (e.g. RPFs, see Step 1) potency-adjusted concentrations can be calculated.

Step 3 – Combine occurrence and consumption data

Combine occurrence data for all components with consumption data, taking into account advice from (eco)toxicologists on the relevant timescale (see Step 1), and estimate exposure using suitable tools depending on data availability and the selected approach for risk characterisation. When the toxicological potencies of the individual components are sufficiently understood and reliable factors have been identified by toxicologists (e.g. RPFs, see step 1) calculate potency-adjusted exposure.

This step is generally not required in ecological risk assessments as consumption data are usually not available and environmental concentrations are taken as proxies for exposure.

Step 4 – Report exposure data

Summarise the exposure results, associated assumptions, uncertainties and consequences for risk characterisation. Report the aggregated exposure estimates for the whole assessment group indicating the contribution of each individual component and each source, as this can help risk managers and guide the collection of new data and/or providing a mitigation plan.

Also report whether any of the individual chemical components of the assessment group is subject to an existing risk assessment and/or legal restriction. It may also be appropriate to estimate exposure to that substance(s) from all sources and describe the contribution coming from the mixture under assessment.

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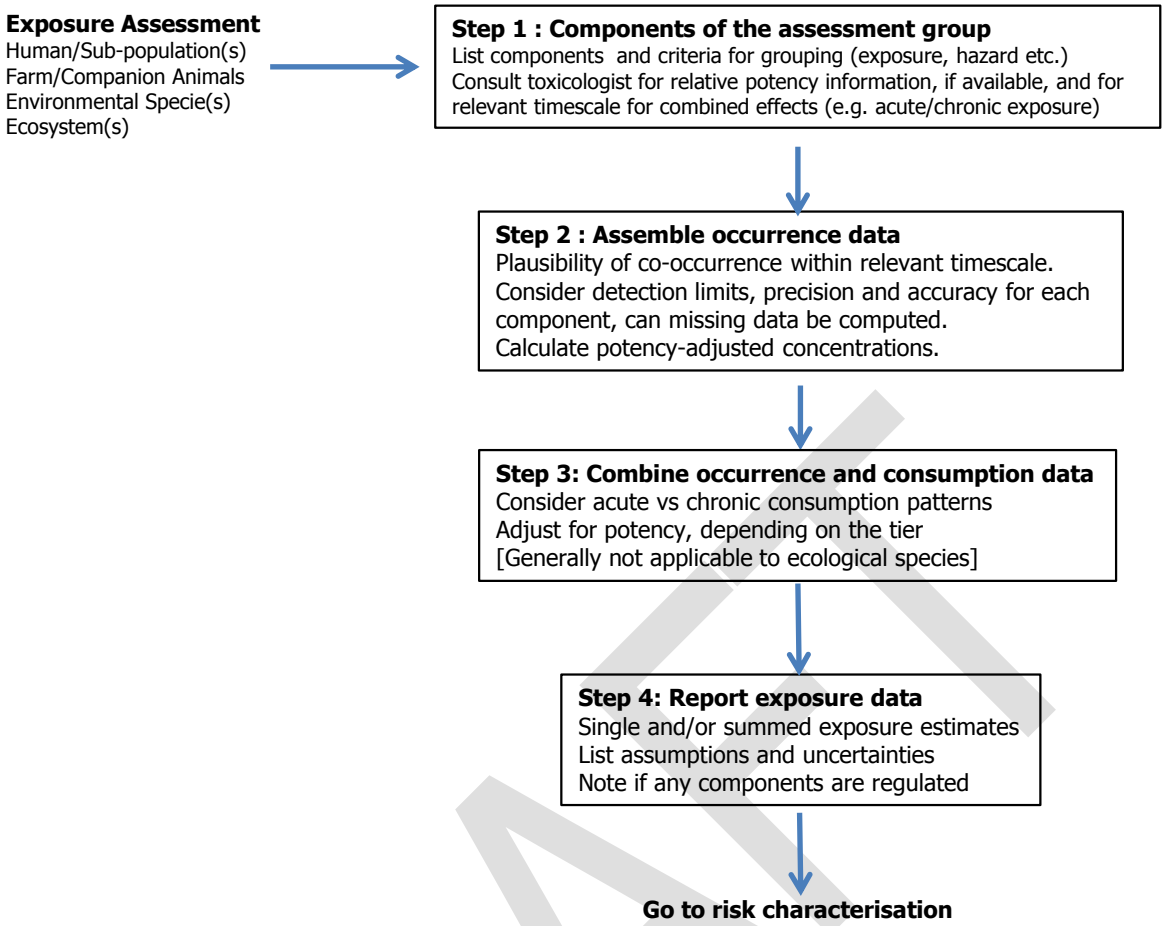

Figure 6: Exposure assessment using the component-based approach

5. Hazard identification and characterisation

5.1. General considerations

Hazard identification and characterisation (referred to as hazard assessment in some contexts) of chemical mixtures aim to derive quantitative metrics reflecting the combined toxicity of the mixture to the (sub)populations, species or the ecosystem of interest.

An initial decision on whether to apply a whole mixture approach and/or a component-based approach will have been made in the problem formulation step. Following data collection and evaluation, these might need to be revised. It will also become possible to select the appropriate entry tier for the assessment.

Hazard identification is a qualitative process, e.g. determining whether a chemical is neurotoxic; this plays an important role in grouping chemicals into e.g. a neurotoxic assessment group (see Section 5.4). Hazard characterisation is a quantitative process resulting in identification of reference points for the whole mixture or its components. Unlike other toxicological endpoints, genotoxicity, which is of relevance for human and companion animal health, is not used for hazard characterisation as there is currently no consensus on quantitative hazard characterisation even for single chemicals. Genotoxicity data are, however, used in a qualitative way to decide on the type of risk characterisation to be used in the assessment (i.e. whether a health-based guidance value is drafted or a Margin of Exposure approach is chosen)(EFSA, 2005b). Genotoxicity can be assessed for a whole mixture, or for components of an assessment group. Consideration of genotoxicity of mixtures is the subject of a specific EFSA statement in preparation (EFSA Scientific Committee, 2018b).

For the whole mixture approach, the hazard assessment might follow the approach commonly taken for single chemicals using toxicity data (i.e. reference points and reference values) of the whole

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mixture of concern or similar mixtures (see definitions and examples for human health, animal health and ecological in Chapter 2).

5.2. Characterisation of mixtures and their similarities

The characterisation of the level of similarity between two or more substances (i.e. of the chemicals belonging to a group), or of the similarity between mixtures, is very important to define the successive (tiered) steps of the risk assessment. Table 3 illustrates factors that can be used, as pragmatic lower-tier options or as higher tiers, to assess similarity. The list gives examples and is not exhaustive.

Table 3: Factors and tools for assessing similarity of mixtures and groups of chemicals

Aspect assessed	Factors for assessment	Procedures/Software
Factors for mixtures		
Related biological or toxicological activity	Quantitative or semi-quantitative evaluation based on similar biological activity; require experimental values of the compounds	Often manual strategy; software: CBRA, CIIPro using data from bioassays
Variability of the relative abundance of components	Quantitative analytical threshold identified	Classification Labelling Packaging; product composition information e.g. Plant Protection Products, products under REACH
Factors for substances/group component		
Chemical structure	Whole structure, identified assessed with different approaches	Software: VEGA, OECD Toolbox, ToxRead, AMBIT, AIM, ToxRead, ToxMatch, ToxDelta, IstSimilarity
Common structural alert(s)	Qualitative assignment to a group if chemicals have alert(s) in common	Software: VEGA, OECD Toolbox, ToxRead, AMBIT, AIM, ToxRead, ToxMatch
Common metabolite(s)	Qualitative assignment if chemicals have metabolite(s) in common as toxic moieti(es)	Software: OECD Toolbox, METEOR, MetabolExpert
Related physicochemical properties	Quantitative evaluation (eco)toxicological properties including toxicokinetics, based on similar physicochemical properties	Software: EPISuite, VEGA, OECD toolbox, ChemProp, ToxRead
Related toxicokinetics	Quantitative evaluation based on similar toxicokinetics (fast elimination, persistence, bioaccumulation factor, etc.)	Software: Cyprotex, Simulation plus, PharmPK
Same mechanism/AOP	Qualitative or semi-quantitative	Several AOP and toxicity mechanisms are defined

Abbreviations: CBRA: Chemical–Biological Read-Across, CIIPro: Chemical *In Vitro–In Vivo* Profiling, REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals.

Similarity of chemicals to compose assessment groups: The criteria for similarity used to assign a chemical to a common assessment group need to be clearly stated, to make the grouping transparent and allow for reproducible assessments. Recently, software has been developed to provide similarity evaluations in a quantitative way (see Table 3). It is recommended to consider more than one of the factors listed in Table 3 to provide a robust evaluation of similarity as each of criteria may only provide a partial assessment. For instance, the tools for similarity developed within the VEGA platform (www.vegahub.eu) have been optimised considering four million chemicals for selected properties, providing a multicriteria evaluation of chemical similarity. In some instances, two chemicals may be similar for specific properties, such as bio-concentration, but different for others, e.g. mutagenicity and structural differences i.e. epoxide ring and ether moiety. The tools to identify similarity should therefore be selected for the key information they provide for a mixture risk assessment, including key structural moieties of the chemicals, as defined in the problem formulation step.

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On the characterisation of mixtures, there may be limited information available to evaluate the similarity of two mixtures such as spectroscopic data (infrared, ultraviolet or visible spectroscopy) with no other analytical results. In this case, only a very coarse assessment of the whole mixture similarity can be performed, based on the overlap of the spectra of the two mixtures. On the other hand, the composition of two mixtures may be known and include quantitative measurements of individual components. The Classification, Labelling and packaging (CLP) Regulation provides guidance on the comparison of two mixtures, based on the percentage of variability of the abundance of the components. Similarly, for botanical preparations, compliance with the specifications defined by the European Pharmacopoeia for selected components can be used to identify criteria for similarity of plant extracts obtained by applying standardised methods (European Pharmacopoeia, 2017).

5.3. Whole mixture approach

5.3.1. Data availability and tiering

Methods for hazard identification and characterisation in a whole mixture approach depend on the nature of the mixture, what is known about its composition, variability and stability over time. The whole mixture approach is frequently used for poorly characterised mixtures. If little information is known about the composition of such a mixture, it might be possible to use data on the mixture itself for the hazard assessment step, provided there is evidence that the composition will not change substantially from batch to batch or over time, e.g. based on knowledge of the source or production process. Otherwise, it will be necessary to have at least partial characterisation of the composition (e.g. using marker substances), to confirm that the material tested in the suite of (eco)toxicological studies was sufficiently similar, or to identify similar mixtures that could be used for read-across to fill data gaps for the mixture of concern.

In some cases, it may be possible to evaluate separate fractions of a mixture, in which the fractions are mixtures themselves (e.g. mixtures of petroleum hydrocarbons can be split into aliphatic and aromatic fractions). The toxicities of the fractions could then be assessed. When only partial characterisation is available, an additional possibility is the selection of one component, for which toxicological data are available, as an index chemical for the whole mixture.

The whole mixture approach is applicable to **simple** (e.g. formulated pesticide or biocide products) and **complex mixtures** (e.g. wastewater effluents, natural flavouring agents, fermentation products, mixtures of contaminants), and these are assessed as if they were a single chemical. The Whole Mixture Testing Approach is, for example, used for assessing so-called UVCB substances (Substances with Unknown or Variable Composition, or of Biological Origin) under REACH, Biocides Regulation (Fisk, 2014) and for classification, labelling and packaging (CLP) (CEFIC, 2016).

One of the **advantages of the whole mixture approach** is its holistic nature, as the different components are taken into account as contributors to the overall toxicological activity of the mixture, including any potential synergistic or antagonistic interactions (Kortenkamp et al., 2009; Backhaus et al., 2010;Boobis et al., 2011; OECD, 2017). **Limitations of the whole mixture approach** include its applicability only to mixtures that that are not variable in composition and are not expected to change over time. Therefore, the three Non-Food Committees of the European Commission did not recommended its use as a general approach for human and ecological risk assessments (SCHER, SCENIHR, SCCS, 2012). However, the whole mixture approach may be needed in food and feed safety assessments; particularly for certain contaminants (e.g. mineral oil mixtures) or food and feed additives used as whole mixtures such as essential oils from botanical extracts.

For hazard assessment purposes, the whole mixture is treated like a single compound, and therefore the concept of tiering is less relevant than in the component-based approaches. However, there will be different levels of characterisation and completeness of the (eco)toxicological data for different mixtures.

For poorly characterised mixtures, options to generate hazard information for hazard characterisation are extremely limited as, in general, *in silico* and read-across methods require information on the chemical structures of components to establish the degree of similarity between mixtures. However, for **human and animal hazard assessments**, if information on the source of the mixture provides reassurance that certain types of chemicals (e.g. potent carcinogens or

accumulating substances) are not present, then it might be possible to use tools such as the Threshold of Toxicological Concern (TTC) approach. The TTC approach is described elsewhere (EFSA Scientific Committee, 2012b) and a revised guidance on this will be published in 2019.

Situations in which **data increasingly become available**, either for the mixture of concern or for similar mixture(s), may allow for the identification of reference points using the same methods as would be used for single chemicals [e.g. NOAEL, or no effect concentration (NEC), lethal concentration (LC_{50}), dilution/concentration factor for species of ecological relevance, applied either to the whole mixture, or to the marker substance]. Reference values may be derived by applying uncertainty/assessment factors, the size of which should be determined using expert judgement taking into account the data gaps.

For the ecosystem, when reference points are available for several species, Species Sensitivity Distributions (SSD) can be derived (Kooijman et al., 1987; Posthuma et al., 2002; Ragas et al., 2010) and applied to characterise expected mixture effects on most or all species that exist in a particular habitat (species assemblages)). As more data become available, hazard characterisation is more refined and quantitative, with more realistic estimates, which may include a full dose–response modelling for hazard characterisation and/or application of data-driven uncertainty factors.

When comprehensive ***in vitro*** and ***in vivo*** **toxicity data** are available, and possibly also **epidemiological and clinical data**, the BMDL is the preferred higher-tier reference point for human health and animal health area (EFSA Scientific Committee, 2017c). A biologically based model linking the external dose with the internal dose may be applied as well as either default uncertainty factors or data-driven assessment factors.

For **species of ecological relevance or the ecosystem**, under data rich conditions, the database for hazard characterisation may provide sound data for dose–response modelling and to derive reference points for single species (NEC or BMDL), data from field or mesocosm studies or SSDs derived from single species data for the whole ecosystem.

5.4. Component-based approach

5.4.1. Grouping chemicals into assessment groups

Setting up assessment groups can be based on the pragmatic aspects from the regulatory domain, from co-occurrence data or from common properties, as described in Table 4. The specific approach to be used for grouping will be determined by the context of the assessment and the problem formulation. Guidelines for grouping are available from ECHA (http://echa.europa.eu/support/grouping-of-substances-and-read-across) and OECD (2014; 2017).

5.4.1.1. Grouping based on regulatory criteria

Grouping of chemicals into assessment groups may be legally required for chemicals that belong to a common regulatory domain (e.g. biocides, pesticides). In such instances, the assessment group will often be defined in the ToR.

Grouping based on exposure scenarios can be used for chemicals that occur together in a common source. For example, this can be a first step in an evaluation of the combined toxicity of different active substances and co-formulants in the same biocide or pesticide formulations. It can also be relevant when assessments require analysis of the effects of groups of chemicals in a particular source/environmental media or an ecological receptor.

Grouping based on physicochemical similarities can be applied to co-occurring chemicals with similar chemical structures and similar steric and physicochemical properties. These can include common functional group(s) (e.g. aldehyde, epoxide, ester, specific metal ion) or similar structure (e.g. dioxins, phthalates) or similar carbon range numbers (e.g. mineral oils). Further refinements can be made by developing subgroups based on the nature of the chemical reactions (e.g. a specific electrophilic reaction mechanism leading to protein adduct formation) or providing common structural alerts. Grouping can also be based on formation of metabolites/degradation products with physicochemical similarities. Tools such as the OECD (Q)SAR Application Toolbox can be used for this purpose.

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5.4.1.2. Grouping based on biological or toxicological effects

MoA and AOP data ideally provide a strong scientific basis to group chemicals, but as these are rarely available, risk assessors rely often on toxicity studies in test species to group chemicals using less specific data (e.g. target organ, mortality, growth, reproduction). Dose addition modelling may then be applied to assess combined toxicity, as recommended by EFSA's PPR Panel (EFSA PPR Panel, 2013b). MoA and AOP data are most likely to be applied and required at a higher tier.

In addition to toxicological similarities, chemicals may also be **grouped into assessment groups using toxicokinetic similarities.** These can include common metabolic routes (e.g. oxidation, hydrolysis, specific phase I enzymes; e.g. cytochrome P450 isoform) or phase II enzymes [e.g. glucuronosyl-transferases (EFSA, 2013a)], fast or slow elimination (e.g. clearance, half-life, elimination rate, bio-concentration factor) or common bioactive or toxic metabolites. In these cases, the possibility of metabolic interactions should also be addressed.

Table 4: Examples of approaches for grouping chemicals

Grouping approach	Overarching common feature	Example	Comments
Common regulatory domain	Regulatory requirements	Biocides, pesticides, food additives, flavourings	
Common source	Exposure	Multiple biocidal and pesticidal active substances in a formulation in a mixture, feed and drinking water contaminants	A lower-tier method when assessing the common occurrence for specific exposure scenarios
Environmental media	Exposure	Exposure through presence in common medium (e.g. river, soil)	Grouping driven by common exposure through a particular medium
Common functional group(s)	Physicochemical characteristics	Aldehyde, epoxide, ester, specific metal ion	
Common constituents or chemical classes, similar carbon range numbers	Physicochemical characteristics	Substances of unknown or variable composition, complex reaction products or biological material (UVCB substances)	Frequently used with complex mixtures
Groups of chemicals with incremental or constant change across the category	Physicochemical characteristics	Mixtures of polyolefins	e.g. a chain-length category or boiling point range
Common breakdown products	Physicochemical characteristics	Related chemicals such as acid/ester/salt	Likelihood of common bioactive breakdown products via physical or biological processes that result in structurally similar chemicals
Common 'critical' target organ(s)	Toxicological or biological properties	Cumulative assessment groups used for pesticides	EFSA 2013 (EFSA PPR Panel, 2013b)
Common MoA or AOP	Toxicological or biological properties	Acetylcholine esterase inhibitors, AhR agonists, metabolism to similar bioactive parent	Chemicals acting via same pathways that converge to common molecular

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Grouping approach	Overarching common feature	Example	Comments
		compound or metabolite(s)	target
Similar toxicokinetics	Toxicological or biological properties	Common metabolic route, elimination patterns (slow, fast) or bioactive metabolites	Relevant to assess the likelihood of metabolic or toxicokinetic interactions

5.4.2. Refinement of grouping

When more hazard data become available, risk assessors have the option to refine the grouping of chemicals using weight of evidence approaches, dosimetry (TK) or mechanistic data (MoA, AOP, etc.). In this context, when an assessment group has been set up based on hazard considerations (e.g. phenomenological effects, target organ toxicity), it may be deemed necessary to refine the grouping, if the risk characterisation suggests insufficient protection (i.e. exposure exceeds the reference point). For this purpose, a more rigorous weight of evidence and uncertainty analysis needs be conducted, to find approaches relevant for higher-tier grouping. The approach to be taken should be determined by the available data and expert judgement.

5.4.2.1. Refinement using weight of evidence

The example below, for the cumulative assessment group (CAG) for pesticides hazard assessment (EFSA PPR Panel, 2013b), provides an indication of a possible approach.

Based on unambiguous and well defined effects in terms of site and nature of toxicity, pesticides have been grouped into cumulative assessment group (CAG) (EFSA PPR Panel, 2013b). However, the level of evidence supporting the allocation of a substance into the CAG can differ substantially for different pesticides. As an example, ataxia caused by an acetylcholinesterase inhibiting substance can, with reasonable certainty, be considered as an unambiguous and well defined effect, whereas an adverse effect not supported by any other findings/parameters would be more uncertain.

So, in practice, the question often remains as to whether the substances included in a proposed CAG truly causes the type of effect that allocates it to the designated group. A transparent and reproducible assessment would ask for inquiries on various aspects, such as:

- the dose–response relationship,
- the consistency throughout studies and species,
- the robustness of the evidence (if the effect was defined only at one level),
- the understanding of the effect as supported by a MoA/AOP knowledge.

These aspects can be attributed relative weights for each substance included in the CAGs by providing a scoring system. For example, knowledge on MoA has a higher relative weight compared with end-point-related toxicity data for the same effect from another species. To weigh these lines of evidence, expert knowledge elicitation can generate probabilities to be allocated as to whether a substance (or a group of substances having equal level of evidence) actually causes a specific type of effect. The result can then be summarised in a probability distribution for the range of substances in the CAG having the specified effect; i.e. a probabilistic output for hazard identification.

5.4.2.2. Refinement using Dosimetry

When grouping chemicals into an assessment group, it is important to recognise that toxic effects on different target organs are dose dependent and the most sensitive end-point may not be the one used for grouping the compounds into an assessment group. In such situations, input from toxicokinetics (TK) and the use of Physiologically based toxicokinetic (PB-TK) models can be valuable to refine the grouping of chemicals. This can be especially valuable when grouping is carried out on the basis of results from *in vitro* studies for some components.

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Although the target organ or organ system may be the same, the nature of the toxicity and functional impairment may not necessarily be the same. In such cases, the effects of the chemicals within a group based on target organ may need to be considered independent of each other.

5.4.2.3. Refinement using mechanistic data

Mechanistic data from OMIC technologies (transcriptomics, metabolomics, proteomics, etc.) and *in vitro* assays including high throughput screening (HTS) data may support the refinement of grouping chemicals in assessment groups (EFSA, 2014a).

Hazard end-points for the ecology area may be complex due to the diversity of taxa ranging from plants, invertebrates and vertebrates. Therefore, the concept of 'common MoA' for the components of an assessment group may have a different meaning in ecotoxicology in comparison with human toxicology as it may refer to broader end-points such as reproduction impairment, population growth and mortality (SCHER, SCCS, SCENIHR, 2012). In addition, a specific taxonomic group may be identified as the most sensitive (e.g. insects for insecticides). Specific considerations of such a sensitive taxon may be a relevant basis for grouping chemicals into an assessment group using a common MoA. In ecological risk assessment, knowledge of MoA/AOP is often limited and when no data are available on MoA, chemicals are often grouped using 'narcosis' as the default MoA. In contrast with the human area, in ecotoxicology narcosis is defined as a reversible non-specific disruption of cell membranes that may result in progressive lethargy, unconsciousness and, ultimately, death. When more data for more specific effects are available either from observation or from *in silico* predictions (e.g. relevant QSAR models), a specific MoA can be considered (e.g. effect on specific receptors) (SCHER, SCCS, SCENIHR, 2012). It should be acknowledged that narcosis as a default MoA to group chemicals is not a conservative assumption. This is particularly relevant when specific MoAs have been identified, as those may drive potent toxicity through specific receptors (e.g. acetylcholine or phosphatase inhibition) compared with narcosis-based toxicity.

5.4.3. Data availability and tiering

The choice of the tier is driven by the purpose of the assessment and the data available for the components of the assessment group. Harmonised tiering principles based on the frameworks of WHO/IPCS ad OECD are discussed below (Meek et al., 2011; OECD, 2017).

The reference points may be derived from *in silico*, read-across, *in vitro* and/or *in vivo* studies, and observations in the population of interest, but the data for different components of the mixture are likely to be variable and incomplete in many cases. It is, therefore, necessary to make assumptions that aim to be conservative and are based on expert judgement for lower tiers. When mechanistic information and data on relative potency of the different components are limited it may have to be assumed that all are as potent as the component for which the most toxicological data are available, and for which there is evidence that this is likely to be the most potent in the group. Exposure to the group is summed on a weight basis (i.e. mg per kg body weight) and dose addition is assumed. This approach is commonly taken for a group of structurally related contaminants (e.g. ergot alkaloids) (EFSA CONTAM, 2012). **Available options to fill data gaps** in data poor situations (**tier 0**) include: 1. *Read-across* using data for similar compounds from existing databases, 2. *In silico* models and non-testing tools to predict toxicity such as QSARs, 3. Use expert judgement through a structured expert elicitation.

In the **human and animal health area**, from **tier 1** onward, hazard data on the relative potency of the components increasingly become available: reference points such as NOAELs, BMDLs or a defined level of the common critical effect can often be identified: the toxicity of combined exposures of toxicologically similarly acting chemicals can be predicted from the sum of the doses/concentrations, taking into account the relative toxicity of each component. Beside a Hazard Index (HI), the Target Organ Toxicity Dose (TTD) or the Reference Point Index/Point of Departure Index can be applied. In tier 1, the quality of potency data are likely to vary for the different components. Typically, the richer the database, and the more mechanistic and toxicokinetic information is available, then the greater the confidence and the lower the uncertainty in the derived reference points. **For ecological hazard assessment**, in **tier 1** the assessment of combined toxicity requires ecotoxicological data for each component of the assessment group. These data are obtained in laboratory assays with test species, providing reference points for acute or chronic effects relevant to populations (e.g. EC_{50} NEC, HC_5,

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etc.). If the dose addition model can be assumed, the model frequently used in ecological risk assessment is the toxic units (TUs) approach.

At **tier 2**, for the **human and animal health area**, greater understanding of toxicity/mode of action can lead to refinement of the assessment groups. It might be possible within the assessment group to identify an index compound, which is often the compound for which the toxicological data are most robust and calculate the Relative Potency Factors (RPF) of each component by dividing the toxicity reference point of the individual component by that of the index compound, or using a weight of evidence approach if individual reference points cannot be established due to lack of data. The RPFs are used to estimate potency-related exposure (see Section 4.3). The health effect of the mixture is assessed using the dose–response curve of the index chemical, which is typically the most toxic member of the assessment group. TEFs are a type of RPF used in food chemical risk assessment in for comparing potency-adjusted exposure to a group reference value (e.g. group TDI) expressed as toxic equivalents or as equivalents of the index compound. Dioxins are the most common example of this approach, for which the TEFs are internationally established (Van den Berg et al., 2006); the EFSA CONTAM Panel has also used the toxic equivalents approach for various groups of marine biotoxins including okadaic acid and analogues, deciding on the TEF values *de novo* (EFSA, 2008a) as well as the Relative Potency factors for zearalenone and its modified forms (EFSA CONTAM Panel, 2017b).

At **tier 2 for the ecological area**, sublethal or chronic effects (e.g. NEC, NEL, LC10, LC50 for reproduction) are applied, whereas mesocosm studies can be available for assessment in tier 3. Commonly, these data sets are summarised for each component of the assessment group as individual reference points for each species from which SSD models can be built to quantitatively predict the effect magnitude of a given (mixture) exposure on the ecosystem. Commonly, to verify whether ecosystems are sufficiently protected, the exposure data are compared with these reference points (individual species) or SSDs (ecosystem) (see Chapter 6 – Risk characterisation). For acute effects on the ecosystem, effect-based test end-points for each species yield an SSD_{EC50} model, while chronic no effect-based end-points yield an SSD_{NOEC} model.

At **tier 3**, knowledge of underlying MoA/AOPs in **animals or humans** based on *in vivo* and *in vitro* mechanistic information, epidemiological data and toxicokinetic studies, may allow refinement of grouping if necessary and enable the derivation of reference points and the use of Relative Potency Factors or TEF based on internal dose in a probabilistic manner using biologically based models (PB-TK or PB-TK-TD).For the **ecological area**, biologically based models, e.g. toxicokinetic–toxicodynamic (TK-TD) and Dynamic Energy Budget model (DEB) models, may be applied for a given species to provide hazard parameters for each component of the assessment group (elimination rate and killing rate or NEC) for individuals and/or populations (Baas et al., 2010, 2018; Cedergreen et al., 2017).

5.4.4. Response addition

Applying response addition requires evidence of independent action between individual substances or assessment groups, and models for its application are not widely applied (see risk characterisation section). Response addition has added value only if the underlying hazard data quantify a response level, i.e. the percentage of individuals in a population, or species in an ecosystem, that shows a predefined effect (e.g. mortality, immobility or cancer) or exceeds a certain critical effect level (e.g. NOEL, ADI, EC50). The response values can then be combined using the rule for independent random events (see Chapter 6). **Response addition is rarely used in the human and animal health area** as the reference points (i.e. NOAELs) reflect a response level below the detection limit. Experimental NOAEL have been shown to often represent a 1–10% response of level remaining undetected due to methodological constraints. In principle, the dose–response curve used in BMDL modelling could be used in the response addition model if evidence of independent action indicated that the default assumption of dose addition is not appropriate. If inter-individual variability in exposure is quantified, and reference values for multiple substances are exceeded for part of the population, response addition can be used to quantify the fraction of the population at risk, i.e. the fraction exceeding one or multiple reference values (Ragas et al., 2011). However, as exposures to multiple substances often correlated, it can be more realistic to perform an individual-based exposure and risk assessment (Loos et al., 2010).

In the ecological area, response addition is used on a regular basis to assess the combined impact of multiple substances having a dissimilar mode of action and showing no interactions. This can be

attributed to the fact that the reference values used in ecological risk assessments often reflect some response level (e.g. an EC_{10}, EC_{50} or the potentially affected fraction (PAF) of species). If response levels of different substances are to be combined for one species, this requires the availability of the dose–response data for each substance. Risk is then no longer expressed as a PEC/PNEC ratio, but as the population fraction showing a predefined effect, e.g. mortality. For metals, response addition has recently been shown to be a better predictor of mixture risk at the species level than dose addition (Nys et al., 2018). At the ecosystem level, the fractions of species potentially affected by substances or CAGs showing dissimilar action can also be combined using response addition, i.e. the rule for independent random events (De Zwart and Posthuma, 2005). The multisubstance PAF is conceptually similar to the 'population fraction at risk', but reflects a higher level of biological organisation. When the population fraction at risk is an indicator for the relative number of individuals exceeding a reference value within a population, the potentially affected fraction indicates the relative number of species in an ecosystem exceeding a reference value.

5.4.5. Dealing with interactions

In the food safety area, it is also important to consider potential for interactions in hazard assessment, including chemical–chemical interactions, toxicokinetic and toxicodynamic interactions with synergy being of greater concern for decision making than antagonism (EFSA, 2013a).

The methods for **hazard assessment of mixture interactions** should be selected considering the nature (toxicokinetics, toxicodynamics or both) and the quality of the evidence available on such interactions (*in vitro*, *in vivo*, single dose or full dose–response). As discussed above, dose–response information for such interactions for single components and the mixture at exposure levels below reference points or reference values are not available and risk assessors have the option to derive and apply an **extra uncertainty factor derived from mixture interaction data at higher doses,** if available**.** This option could constitute a conceptually harmonised approach across the human, animal and ecological area (Ragas et al., 2010), although the value of the UF may be selected differently, because of the different protection end-points.

Risk assessors may address toxicokinetic or toxicodynamic interactions and derive an extra uncertainty factor resulting from:

- qualitative indications of interactions
- data-driven derivation of a interaction factor
- understanding of the mechanism-based approach:
  - Toxicokinetics

In some instances, synergistic effects have been reported to have a toxicokinetic basis often through inhibition or induction of metabolism or transport. The toxicological consequence then depends on whether the toxic moiety is the parent compound or a metabolite. The magnitude of the interaction (e.g. enzyme inhibition) can be determined *in vivo* as the dose-dependent ratio between the toxicokinetic parameters for the single chemical and the binary mixture (e.g. ratios of clearance for chronic exposure). *In vitro* data can also be used to develop toxicokinetic models to refine changes in internal exposure (e.g. constant of inhibition) (Haddad et al., 2001; Cheng and Bois, 2011).

  - Toxicodynamics

In some instances, interactions can have a toxicodynamic basis (i.e. interactions between the different MoA or AOP triggered by each mixture component). The toxicological consequence is translated by an effect differing from additivity based on the dose–response relationship of the individual components. These may vary according to the relative dose levels, the route(s), timing and duration of Exposure, and the biological target (Kienzler et al., 2014).

The direction (synergism or antagonism) and characterisation of the magnitude of deviation from dose or response addition (i.e. model deviation ratio) is performed by comparing the available dose–response for the single chemicals and the mixture with reference models. This can be performed both for single dose–response curves of mixtures of any number and mixture ratios at any effect level and for whole dose–response data of binary mixtures (Jonker et al., 2005; Cedergreen, 2014; EFSA Scientific Committee, 2017).

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In **human and animal toxicology**, full dose–responses for chronic effects of mixtures *in vivo* are not often reported and are most often reported either as a single dose of the mixture or *in vitro* studies using cell systems. The slope of the dose–responses between the single chemicals and the mixtures can be compared using benchmark dose modelling and a **magnitude of interaction** can be derived (EFSA Scientific Committee, 2017c). A well-known example of synergism in toxicity resulting from chemical–chemical interactions with full dose–response data include melamine and cyanuric acid forming a covalent complex being several fold more nephrotoxic than melamine alone (7 and 28 days studies) (EFSA CONTAM and CEF Panel, 2010; Jacobs et al., 2011; da Costa et al., 2012).

In ecotoxicology, the dose–response for acute population endpoints such as mortality, growth and reproduction are more often reported and a full assessment of the dose–response can be performed. The **model deviation ratio** can be determined through comparison of the experimental data with models (e.g. MIXTOX model) or concentration-response surfaces in data-rich situations [see review by Greco (1995),Jonker et al., 2005, Sørensen et al., 2007, White et al., 2004]. Relevant synergistic effects with full response data include piperonyl butoxide and a number of pesticides in bees measured as acute mortality (LD_{50}) (Johnson et al., 2009; EFSA PPR Panel, 2012).

The experimentally observed **magnitude of interactions** or **model deviation ratios** can be used to derive an extra uncertainty factor to cover relevant percentiles of the species or population under assessment, depending on the protection goals (e.g. 95th centile). These UFs may then be applied in risk characterisation (see Risk characterisation Section 6.3.3).

If there is evidence for possible interaction of substances, the Scientific Committee recommends applying an additional uncertainty factor. The size of the factor should be determined on a case-by-case basis depending on: (1) the strength of the evidence for the presence or absence of interactions; (2) the expected impact of the interactions; and (3) the level of conservativeness in the assessment. For example, no additional uncertainty factors are deemed necessary if (binary) mixture tests with the mixture components do not show any interactions and/or when the assessment already includes a high level of conservativeness (e.g. because a large number of substances are grouped into one assessment group). A factor higher than 1 may be appropriate in cases in which the assessment has a low level of conservativeness, and there are indications for potential interactions (e.g. based on metabolic interaction data). If information on interactions is completely lacking, the application of an interaction factor should be considered within the context of the level of conservativeness of the assessment. An interaction factor above 10 should only be applied if there is clear evidence for interactions exceeding a factor of 10.

5.5. Stepwise approaches

5.5.1. Whole mixture approach

Figure 7 summarises the steps of hazard assessment for whole mixtures.

Step 1. Hazard data collection

Collect toxicity data on the mixture of concern, or on a similar mixture(s) considered to be relevant for read-across.

Step 2. Reference points

Identify or derive a reference point for the mixture or for the similar mixture, using the tier for which data are available.

Step 3. Reference values

If data are limited, or read-across is required from a similar mixture, then consider whether an additional uncertainty factor is required in establishing reference values or applying a Margin of Exposure approach to the reference point.

Step 4. Report

Summarise hazard metrics, associated assumptions and list uncertainties.

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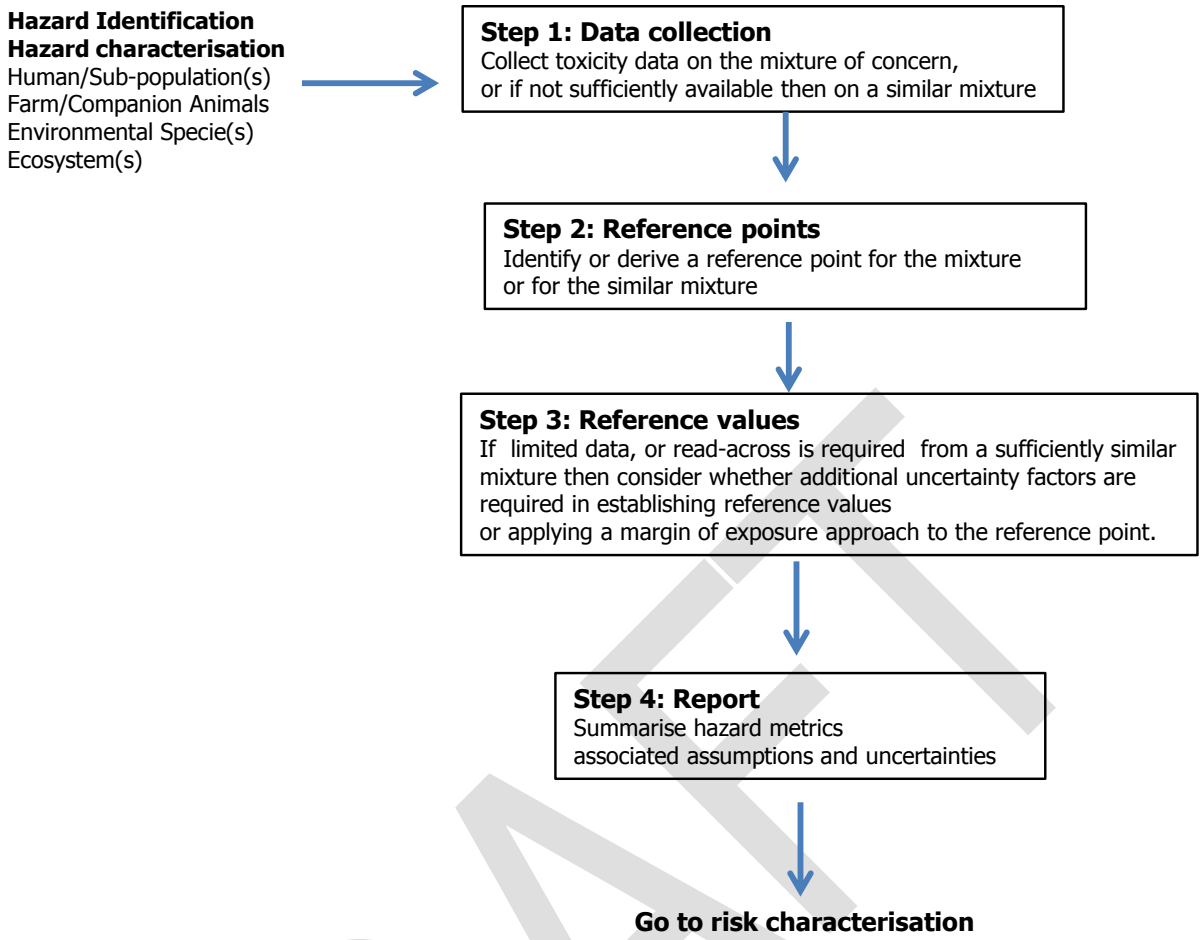

Figure 7: Stepwise approach for hazard identification and characterisation using a whole mixture approach

5.5.2. Component-based approach

Figure 8 summarises the steps of hazard assessment in the component-based approach. These steps do not necessarily need to occur in the sequence presented and may need to be conducted in an iterative way.

Step 1. Confirm chemicals and establish components of the assessment group

Prepare the chemicals in the mixture. Review and, if necessary, weigh the evidence for proposing and handling the assessment groups as described in the problem formulation, taking into account the approaches described in Table 3.

Step 2. Collect available hazard information

Collect the available hazard information for each chemical in the assessment group. This includes toxicity data, reference points, reference values, mode of action, toxicokinetic information, and relative potency information, if available. Identify the relevant entry tier for the assessment depending on the data available.

Step 3. Evidence for combined toxicity

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Assess evidence available for combined toxicity and the possibility of deviation from dose addition (interactions). Consider exposure to assess the possibility of interactions. Identify the most appropriate method(s) for risk characterisation, which determines the approach in Step 4 and generates the input for the risk characterisation (Chapter 6).

Step 4. Hazard characterisation

Derive reference points for each component of the assessment group, identify appropriate uncertainty factors and derive reference values as appropriate, using the relevant tier. Depending on the data and the selected approach, reference values might be used for individual components, or for the group expressed as equivalents of an index compound, based on potency data.

Step 5. Summarise hazard metrics

Summarise hazard characterisation for components of the assessment groups, associated assumptions (relative potency, dose addition, interaction), and list uncertainties.

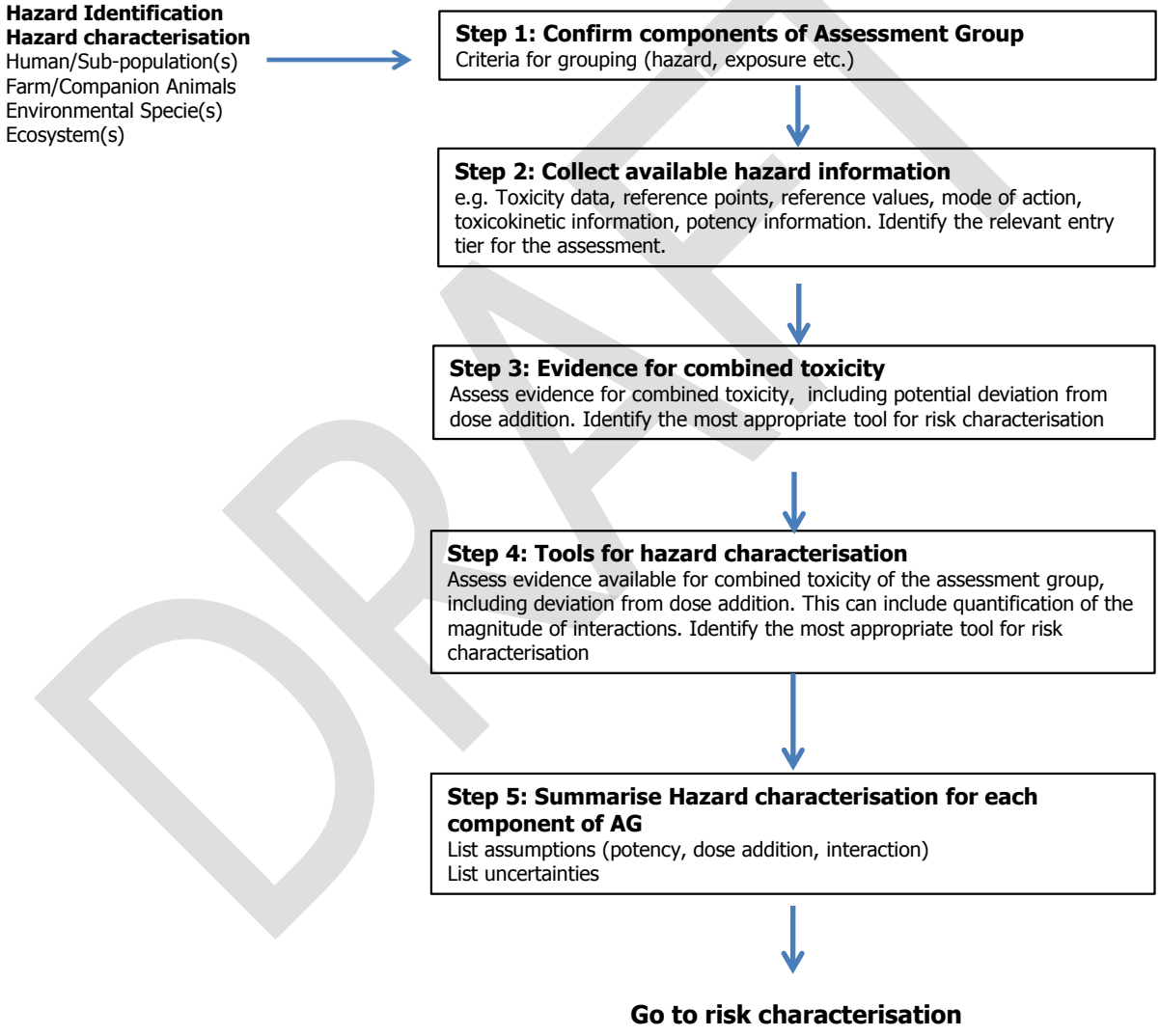

Figure 8: Stepwise approach for hazard identification and characterisation of multiple chemicals using a component-based approach

6. Risk characterisation

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6.1. General considerations

Risk characterisation of chemical mixtures aims to:

1) Calculate the ratio of Exposure to hazard, using the metrics defined in the problem formulation, to determine whether there is a possible concern for a defined species, subpopulation or the whole ecosystem.

2) Identify the components in an assessment group that represent particularly important risk drivers for the component-based approach.

This assessment will support risk management conclusions (EFSA, 2013b, 2015c). Many mixture risk characterisation methodologies are available (see Table 5). However, for all areas, they compare the sum of individual chemical exposures and the reference points or reference values to characterise the risk.

In mixture risk assessment, the tiering can bring together highly divergent types of data, for example, when all compounds are pragmatically handled as if sharing the same MoA, e.g. when the risk characterisation data for an insecticide are aggregated with those for a photosynthesis inhibitor (in ecological risk assessment) in lower tiers. Although it is mechanistically unjustified to apply the dose addition model in this case, it is pragmatic to evaluate whether this simple approach leads to sufficient protection (after which an assessment can be terminated).

6.2. Whole mixture approach

From a risk characterisation perspective, the **whole mixture is essentially treated as a single substance**. In the **human and animal health area**, if a reference point or a reference value has been decided on, then the aim is to identify whether, taking into account uncertainties, the estimated exposure exceeds that reference value or results in an inadequate Margin of Exposure or Hazard Quotient.

In the **ecological area**, risk characterisation in the EU uses the PEC/PNEC ratio for the whole mixture (or similar exposure to hazard ratio) as a risk score to quantify adverse effects that may occur at specific (predicted) environmental concentration (EC 2003). Similarly, in the USA, the risk quotient (RQ) is used and defined as the quotient of Exposure over toxicity, where exposure is the estimated environmental concentration (EEC), analogous to PEC, and toxicity is expressed as LC_{50} or EC_{50} for acute toxicity or as the NOAEC for chronic toxicity. For multiple species or the whole ecosystem, an SSD can be generated based on whole mixture toxicity data as the HC5 (hazardous concentration for $\leq$5% of each species) with the aim to identify whether the estimated exposure exceeds the HC_5, as the lower limit of the 95% confidence interval for 5% species affected in the SSD.

If the **toxicity data are insufficient to decide on a reference value**, then, in **human and animal risk assessments**, a Margin of Exposure can be calculated as the ratio between the estimated exposure and the reference point. As noted above, the value of the resulting Margin of Exposure (MoE) has to be interpreted taking into account the uncertainties and the nature of the toxic effect (see Section 6.4). In either situation, the exposure data may identify specific subgroups of humans, animals or species of ecological relevance for which the calculated metric has the highest values to help inform the type and focus of risk management action that is most likely to be effective.

6.3. Component-based approach

6.3.1. Dose addition

Methodologies and associated calculations for risk characterisation of mixtures using dose addition are summarised in Table 5. In **tier 0**, the **Hazard Index** (HI) is commonly applied in the human and animal health area and the analogous **Risk Index** (RI) in the ecological area. The HI is defined as the sum of the hazard quotients of the individual components of an assessment group, in which each of the hazard quotients is calculated as the ratio between exposure to a chemical and the respective reference values (i.e. ADI, TDI). If reference values are not available for all components, the lowest available reference value (i.e. for the most potent chemical in the mixture) can be used, assuming that the components with missing reference values are equally potent, which is likely to be

conservative. Major advantages of the HI approach include its relatively easy and rapid application, its comparatively broad empirical foundation and the fact that it often provides a conservative risk estimate for combined exposures (Kortenkamp et al., 2009; Meek et al., 2011; SCHER, SCENIHR, SCCS, 2012). In the ecological area, the RI is calculated as the sum of the risk quotients of the individual components of an assessment group, in which the risk quotient is calculated as the ratio between the predicted exposure concentration and the predicted no effect concentration. The major limitation is that uncertainty factors are applied to decide on reference values for each component to account for intrinsic uncertainties, which are combined when calculating the HI; in addition, reference values may have been derived from different study types, with differing end-points and differing quality.

In **tier 1**, for the **human and animal health area**, the HI can be applied as well, using the respective reference values, but when the database is richer an additional possibility could be the **Target Organ Toxicity Dose (TTD)** in a **refined Hazard Index approach** taking into consideration that not all the components have the same adverse effect/target organ and is derived for each end-point to estimate an end-point-specific Hazard Index (EFSA, 2013a); Kienzler et al., 2014). Alternatively, the **Reference Point Index (RPI; also known as the point of departure index)** can be used. The RPI has the advantage over HI in that it sums the exposures to the different components in relation to their relative potencies, expressed as the reference point (RP) (i.e. NOAEL, BMDL) and that a single group assessment factor (either a default or chemical-specific assessment factor) can be applied as the last step in the process, avoiding the potential interpretation bias introduced by a combination of individual but different uncertainty factors (Wilkinson et al., 2000; EFSA, 2013b; Kienzler et al., 2014). The **reciprocal** of the Reference Point Index is the **combined Margin of Exposure,** representing the reciprocal of the sum of the Margin of Exposure for all compounds in the assessment group (referred to as the MOET). In the ecological area, the sum of **toxic units (TUm)** approach is similar to the RPI. The TUm is the sum of concentration ratios of the individual chemicals in a mixture and their toxic units (TU) i.e. the concentration eliciting a defined effect (such as the EC50 or LC50) (Kienzler et al., 2014; (SCHER, SCCS, SCENIHR, 2012; EFSA,2013b; OECD, 2017). When the TU model is applied to Predicted Environmental Concentrations (PECs) it is conceptually comparable with the Hazard Quotient (HQ) with the reference value being the PNEC.

In **tier 2**, the potency-adjusted exposure determined using **Relative Potency Factors (RPF)** is compared with the reference point for the index compound to calculate a Margin of Exposure. With **Toxic Equivalency Factors (TEF)**, if available, a single reference value can be established for the most studied, and generally most potent member of the group, which is then expressed as a group reference value (such as a group TDI), expressed as toxic equivalents, and the risk characterisation is a comparison of Exposure to the group reference value. **For the ecosystem**, quantitative impact metrics can be derived in higher-tier assessments using Species Sensitivity Distributions (SSDs). The exposure levels of the mixture components belonging to the same assessment group are first summed based on their relative potency (ΣTU approach), and then the impact metric is derived from the SSD: the multisubstance probably affected fraction (msPAF) (Posthuma et al., 2002). The msPAF has been proposed as a method for assemblage-level mixture risk assessment in ecotoxicology, and has been used for various purposes including analyses of (bio)monitoring data combined in the study of site-specific impacts on species assemblages with toxic mixture modelling (see e.g. Mulder et al., 2005, 2006; De Zwart et al., 2006; Harbers et al., 2006).

A prioritisation method applicable to **all areas** is the **Maximum Cumulative Ratio (MCR)**, which identifies the specific chemicals that are drivers of toxicity in an assessment group and can be applied in combination with any of the methods described above. Originally developed by Price and Han (2011), the MCR is the ratio of the combined toxicity (i.e. Hazard Index) to the highest toxicity [Hazard Quotient (HQ)] from a single component of the assessment group (i.e. maximum Hazard Quotient (HQ)] to an individual in the target population. The maximum MCR-value is equal to the number of compounds in a mixture, and the lowest value is 1 (Price and Han, 2011).

At **higher tiers**, the risk metrics become **more quantitative and probabilistic** with increasing consideration of internal dose using either TK data or PB-TK or PB-TK-TD modelling. In the **human and animal health area**, the **internal dose HI** corrects exposure for internal dose taking into account TK parameters such as absorption or body burden (e.g. clearance). In the **ecological area**, the **internal dose sum of toxic units (IDTUm)** aims to derive internal concentrations for each compound in the assessment group as the product of the occurrence in the biological medium and the

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bioaccumulation factor (OECD, 2017). All these methods that integrate internal dose can be applied to compare with the hazard benchmark, i.e. the RPI, PODI, MOET, RPFI or TEQI (US EPA, 2005; EFSA, 2013b; Bopp et al., 2016; OECD, 2017).

The most refined methods include the application of probabilistic methods such a probabilistic sum of Margin of Exposure derived from PB-TK-TD models and probabilistic exposure estimates for the mixture components. However, as these methods require full dose–response data for each substance in the assessment group (toxicokinetic parameters including absorption, clearance, etc.; mechanistic data on MoA or AOP), they are rarely used in mixture risk assessment (EFSA, 2013a; 2014b; Cedergreen et al., 2017; OECD, 2017).

Table 5: Risk characterisation methodologies applied to component-based approaches using the dose or concentration addition assumption

Method	Area	Calculation
HI	Human, animal Ecological	$\sum_{i=1}^{n} \frac{\text{Exposure}_i}{\text{RV}_i}$
RI	Ecological	$\sum_{i=1}^{n} \frac{\text{PEC}_i}{\text{PNEC}_i}$
RPI/PODI	Human, animal, Ecological	$\sum_{i=1}^{n} \frac{\text{Exposure}_i}{\text{RP}_i} * \text{UF}$
MOE	Human, animal	RP_{index}/potency-adjusted exposure
MOET	Human, animal, ecological	$MOET = 1/[\Sigma(1/MOE_i)]$
ΣTU	Animal, ecological	$TU = [Concentration_i]/[ECx_i]$
Internal dose HI	Human, animal, ecological	$IHQ_i = (Internal\ Exposure_i/RV_i)$ $IHI_i = \Sigma$ Internal exposure/ΣHQ
Internal dose sum TU (IDUTUm)	Animal, ecological	IDTU = [concentration]*BAF/[Critical body residue] IDTUm = Σ IDU

HI: Hazard Index; $Exposure_i$: exposure of the individual substance in the mixture; RV_i: reference value of the individual substance in the mixture (e.g. ADI or TDI); RI: Risk Index; PEC_i: predicted effect concentration of the individual substance in the mixture; $PNEC_i$: predicted no effect concentration of the individual substance in the mixture; RPI/PODI: Reference Point Index/Point of departure Index; RP_i: reference point of the individual substance in the mixture (e.g. NOAEL or BMDL); UF: uncertainty factor; MOE: Margin of Exposure; RP_{index}: reference point of the index chemical; MOET: sum of margin of Exposures; MOE_i: Margin of Exposure for compound i in the mixture; TU: Toxicity unit; $Concentration_i$: concentration in media of compound i in mixture; EC_{xi}: Effect concentration of substance i in the mixture (e.g. LD_{50}, LC_{50}, EC_{50}, $EC_{X)}$; RPFi: relative potency factor of the individual substance in the mixture; Internal Dose HI: HI corrected for internal dose; Internal $exposure_i$: internal exposure for compound i as a correction of the external dose (absorption, body burden etc); IHQ_i: Internal Hazard Quotient; IHI_i: Internal Hazard Index; HQ: Hazard Quotient; IDUTUm: Internal dose sum TU: TU corrected for internal dose; BAF: bioaccumulation factor.

6.3.2. Response addition

Application of response addition for risk characterisation becomes an option if the following conditions are met:

- The substances considered are likely to act by independent action or mechanisms.
- No interactions between the substances are expected, either in the exposure medium or the exposed organisms.
- Response points and ideally the full dose–response should be available for all or at least two substances in the mixture.

The combined response can then be calculated using the equation for independent random events (Bliss, 1939):

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$$R_{mix} = 1 - \prod_{i}^{n} (1 - R_i)$$

R_{mix} is the toxicological response elicited by the mixture where R_i represents the response level as a consequence of Exposure to substance *i*. The response values represent probabilities or fractions and can take values between 0 and 1. It is important to realise that the outcome of an assessment using response addition is conceptually different from a risk quotient (e.g. PEC/PNEC ratio). It indicates the population fraction or fraction of species at risk, the acceptability of which has to be decided on a case-by-case basis.

At very low response values, e.g. tumour risks in the range of 1–10 in a million, responses are sometimes summed under the assumption of response addition, producing virtually the same results as application of the equation for independent random events. This probably explains the use of the term 'addition', which is not in line with the fact that (non-)responses are multiplied in the equation of independent random events. While applying response addition, particularly in the ecological area, summing responses should be discouraged as it is conceptually wrong and produces erroneous results at higher response levels.

6.3.3. Interactions

Methods for risk characterisation of chemical mixtures deviating from dose addition, i.e. 'interaction', have been developed by a number of international scientific advisory bodies and are reviewed elsewhere (US EPA, 2000, 2007; ATSDR, 2004; Pohl et al., 2009; EFSA, 2013b; OECD, 2017). In all areas, ideally the hazard assessment step will allow the assessment of interactions and the magnitude of the interaction which then can be taken into account in the risk characterisation. As discussed in the hazard assessment chapter (Section 5.4), toxicologically relevant interactions are uncommon at low levels of Exposure and the methods to be applied will depend on the nature and the quality of the evidence available on such interactions.

To take into account interactions in the risk characterisation step, risk assessors can use a number of methods. At a low tier, the **HI modified by binary interactions** provides a method to evaluate hazard data for possible pairs of compounds to determine the binary weight of evidence for each of these pairs, determining the expected direction of an interaction (EFSA, 2013a). An **interaction-based HI (HI_{int})** allows translating the available information about interactions by means of an algorithm into a numerical score, based on expert judgement. The numerical score takes into account: (1) the nature of the interaction; (2) the quality of the available data; (3) the biological/toxicological plausibility of the interaction under real exposure conditions; and (4) the relevance for human health (Mumtaz and Durkin, 1992; US EPA, 2000, 2007; ATSDR, 2004; Sarigiannis and Hansen, 2012; EFSA Authority, 2013b). Recently, the three Non-Food EU Committees have discussed the limitations of the approach as: (1) providing only a numerical score of potential risk related to a chemical mixture exposure; (2) being strongly affected by 'subjective evaluation'; and (3) as for HI, also in HI_{int} derivation, intrinsic uncertainties affecting reference values, are combined and amplified (SCHER, SCENIHR, SCCS, 2012).

In Ecological risk assessment, an interaction is demonstrated to occur, its magnitude should be taken into account in the risk characterisation using a **modified interaction-based toxic unit approach** [EFSA PPR Panel, 2012].

At high tiers and for all areas, dosimetry can be taken into account using PB-TK-TD modelling and either an internal dose Hazard Index modified by binary interactions or an MOET can be calculated on an internal dose basis. Such data are currently rarely available but large research efforts are ongoing at EFSA (EFSA-Q-2015–00554, EFSA-Q-2015–00641) and internationally to increasingly apply these methods for human health, animal health and ecological risk characterisation of mixtures (Cedergreen, 2014; Cedergreen et al., 2017; JRC, 2016; OECD, 2017).

6.4. Uncertainty analysis

Like in any other risk assessment, it is important to consider the uncertainties involved in assessing the risks of combined exposure to multiple chemicals when interpreting the assessment results. In

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general, there are more sources of uncertainties, and uncertainties will be larger than in assessments of single substances, as the assessment has to deal with more complex situations.

EFSA recently adopted a guidance document on uncertainty analysis in EFSA's scientific assessments, which is supported by a more extensive Opinion providing an assessment of the underlying principles and a toolbox of reviewed quantitative and qualitative methods (EFSA Scientific Committee, 2018). The guidance is aimed at all types of scientific assessment undertaken at EFSA and therefore should also be followed when conducting a mixtures risk assessment. The individual uncertainties should be listed throughout the risk assessment process. The most important uncertainties involved in the different assessment steps of combined exposure to multiple substances are discussed in Annex I.

6.5. Interpretation of risk characterisation

The uncertainty in an appropriate risk metric will primarily be determined by the nature of the mixture, the approach used and the respective tiers for exposure and hazard.

6.5.1. Whole mixture approach

Risk characterisation for the whole mixture is not different from that used for individual chemicals, as the mixture is treated as a single entity. So, if the estimated exposure exceeds the reference value, there is a potential risk. In human and animal risk assessment, in general a Margin of Exposure of at least 100 (applied when extrapolating between and within species) is generally considered not to represent a case for which health risks would exist. However, a larger Margin of Exposure might be required if there are important data gaps, or a smaller Margin of Exposure may be considered appropriate if relevant human or animal data indicate that a lower factor is appropriate for interspecies extrapolation (EFSA Scientific Committee, 2012c). For substances that are genotoxic and carcinogenic, the EFSA Scientific Committee advises that a Margin of Exposure ≥10,000, when comparing estimated exposure with a BMDL10 from a rodent carcinogenicity, would be of low concern from a public health point of view and might be considered a low priority for risk management (EFSA Scientific Committee, 2005). Such a judgement is ultimately a matter for risk managers and a Margin of Exposure of that magnitude should not preclude risk management measures to reduce human exposure (EFSA Scientific Committee, 2005). This also applies to whole mixtures that are genotoxic and carcinogenic, both for humans and companion animals. Genotoxicity and carcinogenicity are not considered to be of similar concern for farm animals and the ecological area because of lifespan.

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6.5.2. Component-based approach

In general, when the **HI** approach is used, a Hazard Index ≤ 1 indicates that the combined risk is acceptable, whereas when it exceeds 1, that there is a potential concern. When the value of 1 is exceeded, it is important to take into consideration both the over-conservative nature of HI (due to combining multiple uncertainty factors used for the individual components) and the quality and nature of the underlying data and assumptions, especially at lower-tier assessments that may even relate to different endpoints. In such cases, risk characterisation may need to be refined including exposure and hazard assessment particularly when assuming similarity and no interaction.

The **Reference Point Index (RPI)** often incorporates the default (100-fold) uncertainty factor to account for the uncertainties and the RPI value multiplied by this uncertainty factor should be ≤ 1. If it exceeds 1, a potential concern may be identified but needs to be interpreted in the light of the biological relevance of the effect, the likelihood of under- or overestimation of risk. Alternatively, if the combined (total) Margin of Exposure (**MOET)** is greater than 100 or another alternative value specified for the MOET, depending on the nature of the effect on the target population, the combined risk is considered acceptable. For a Maximum Cumulative Ratio **(MCR)**, the value obtained reflects whether a single chemical is the overall contributor to the risk estimate (MCR ~ 1) or whether each chemical contributes equally to the risk estimate (MCR ~ the number of chemicals present).

When applying **Relative Potency Factors,** the health effect of the mixture is assessed using the dose–response curve of the index chemical and then divided by the exposure to derive an MOE. Again an MOE of 100 or more is generally considered acceptable, unless indications exist that it should be adjusted (EFSA Scientific Committee, 2012c).

If one or more components of a mixture are **genotoxic and carcinogenic**, then the MOET for the mixture should be larger than 10,000, as for a single substance (EFSA Scientific Committee, 2012c). In the event that a mixture of genotoxic substances is assessed at a low tier, it may be assumed that all components have equal carcinogenic potency, and the MOET is calculated about one BMDL10 (assumed to be the most potent carcinogen of the mixture). The exposure to the components of the mixture is summed and the value of 10,000 would again be applied. A recent application of this approach is illustrated in the Opinion of the Scientific Panel on Contaminants in the Food Chain on human risk assessment of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements (EFSA CONTAM, 2017a). Alternatively, if the **genotoxicants** in the mixture are structurally diverse the combined Margin of Exposure (MOET) can be calculated as the reciprocal of the sum of the reciprocals of the MOE of the individual substances. If the MOET is higher than 10,000, then the exposure to the mixture would be of low concern from a public health point of view.

F**or ecological risk assessment**, the sum of toxic units is often used as a risk metric. If LC_{50} values are used as the basis for the toxic unit, an acute lethal sum of toxic units of 1 (**ΣTU** = 1) for a mixture means that the mixture would cause 50% lethality. For communities and ecosystems the SSD approach can be used to identify the reference point, usually as the HC_5–NOEC (Hazardous Concentration for 5% of the species against exceedance of their no effect level, see Chapter 6.2).

For the **response addition approach**, as long as the doses/concentrations of each individual independently acting component remain below the (true) no effect values, they theoretically do not contribute to mixture toxicity. However, as the NOAEL(C)s and NOECs derived from experimental studies are often associated with effect levels in the range 5 to 20% (EFSA PPR Panel, 2009, Kortenkamp et al., 2009), although unlikely, exposures equal to these levels may contribute to mixture effects also for dissimilarly acting substances (SCHER, SCENIHR, SCCS, 2012) and an additional uncertainty factor may be considered when the exposure of two or more components of the mixture are close to their respective reference points.

If the information of the combined exposure and hazard characterisation does not indicate a concern, the assessment can be stopped. Alternatively, the outcome of the risk characterisation may indicate a potential risk and may indicate a need for a risk management decision, or a trigger to proceed to a higher tier that offers sufficient information for risk management, in which assumptions and uncertainties are reduced in an iterative way (US EPA, 2007; OECD, 2017).

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6.6. Stepwise approach

These steps do not necessarily need to occur in the sequence presented and may need to be conducted in an iterative way. The step wise approach is illustrated in Figure 9.

- **Step 1.** Collate the exposure and hazard metrics determined in the exposure assessment and the hazard characterisation, and the decision points for the risk characterisation from the analysis plan of the problem formulation.
- **Step 2.** Confirm or revise the approach for the risk characterisation metric and its interpretation, starting with a fit for purpose methodology (Hazard Index, Margin of Exposure, relative potency factor index, etc).
- **Step 3.** Summarise risk characterisation results, associated assumptions (exposure, potency, dose addition, interaction), list uncertainties.
- **Step 4.** Interpret the risk characterisation results, i.e. whether the combined risk is acceptable or not, based on established procedure or risk management protection goals and quantify uncertainties, whenever possible. If the combined risk is not acceptable, advise on the types of data that would be of value for potential refinement of the assessment.

The stepwise approach is summarised below in Figure 9.

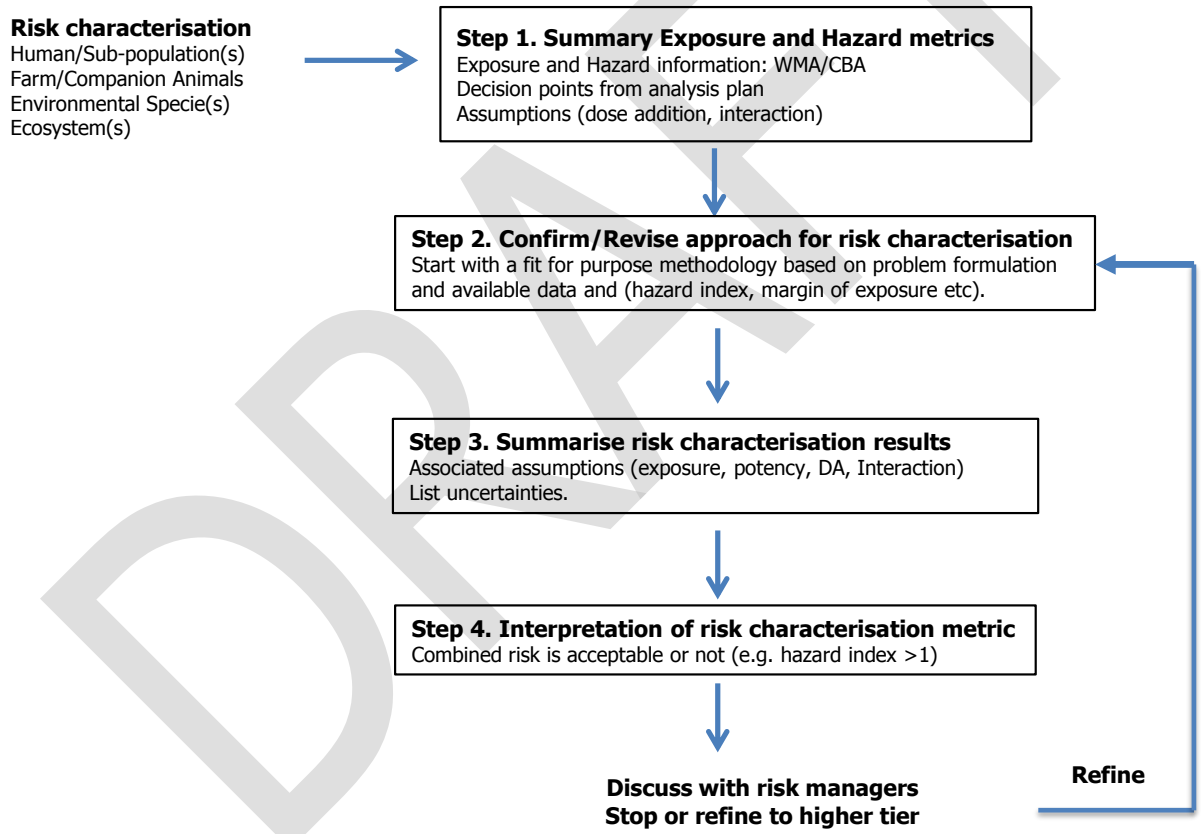

Figure 9: Stepwise approach for risk characterisation of chemical mixtures

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7. Reporting a mixture risk assessment

Reporting should be consistent with EFSA's general principles on transparency (;) and reporting (EFSA, 2015c), including the use of the weight of evidence approach, assessment of biological relevance and reporting of uncertainties (EFSA Scientific Committee, 2017a,b; EFSA Scientific Committee, 2018). In a mixture assessment, this should include justifying the choice of methods used, documenting all steps of the procedure in sufficient detail for them to be repeated, and making clear where and how expert judgement has been used (EFSA, 2015b). Where the assessment used methods that are already described in other documents, it is sufficient to refer to those. Reporting should also include referencing and, if appropriate, listing or summarising all evidence considered; identifying any evidence that was excluded; detailed reporting of the conclusions; and supplying sufficient information on intermediate results for readers to understand how the conclusions were reached.

To aid transparency and accessibility for readers it may be useful to also summarise a mixture assessment in a tabular form, and to use the tabular format as a trigger to check on reporting completeness. A suggested format is shown in Table 6. Whether or not a tabular format is used, all the information listed in Table 6 must be included in the mixture risk assessment report, in a location and format that can easily be located by the reader (e.g. identifiable from section headings in the table of contents). If the information is presented in tabular form it should be concise (ideally not more than one page per table) and refer the reader to the text of the mixture risk assessment for details.

Table 6: Optional tabular format for summarising a mixture risk assessment

Problem formulation	Description of the mixture	Simple or complex mixture, Composition, Data availability for components or whole mixture
	Conceptual model	Question/Terms of Reference, Source, exposure pathways, Species/subpopulation, Regulatory framework, Other?
	Methodology	Overview of available data Whole mixture or component-based approach or a combination of the two. Assessment group
	Analysis plan	
Exposure Assessment	Characterisation of the mixture Components of the assessment group	
	Summary occurrence (concentration) data	
	Summary exposure	Assumptions, Exposure metrics
		Identify uncertainties
Hazard identification and Hazard characterisation	Mixture composition WMA/CBA	
	Reference points/Reference values	
	Summary hazard metrics	Assumptions combined toxicity (DA, RA), hazard metrics
		Identify uncertainties
Risk characterisation	Summary exposure and hazard metrics	
	Risk characterisation approach	
	Summary risk metrics	Associated Assumptions (DA, RA, interactions), Risk metrics
		Overall uncertainty analysis
	Interpretation	

DA, dose addition.

To illustrate the applicability of the Guidance and reporting table to human health, animal health and the ecological area, three case studies are reported in Annexes I, II and III (to be added before public consultation):

1) Human health risk assessment of combined exposure to hepatotoxic contaminants in food.

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2) Animal health risk assessment of botanical mixtures in an essential oil used as a feed additive for fattening in chicken.

3) Quantifying the impact of binary mixture interactions on hazard characterisation in bees.

8. Way forward and recommendations

Mixture risk assessment is a field that has often followed a independent development pathway in various disciplines, for which even within-discipline differences have been evolving for e.g. different chemical groups. This means that the available mixture exposure, effect and risk information is not only scattered in literature, but also apparently diverse in nature and in their definitions, models and metrics used. However, the apparent divergences mask an underlying high degree of similarity, as recognised from the review of the concepts, models, data and practical approaches of mixture risk assessment. Based on that review, not only these similarities were recognised and used for this guidance, but also various remaining gaps were identified. Recommendations for future work to support closing these gaps include the following:

Evaluate the applicability of the guidance document through a testing phase and the development of specific case studies relevant to the different EFSA panels:

- Exposure assessment
  - Further implement probabilistic exposure assessment methodologies for mixture components.

Develop guidance for aggregate exposure assessment methodologies for mixture components.

  - Further assess the use of non-target chemical analysis (broad scope chemical screening) for exposure assessment of chemical mixtures.
- Hazard assessment
  - Further development and implementation of methodologies to take into account deviations from dose addition using both biologically based and statistical modelling:
    - Investigating dose-dependency for specific interactions of toxicokinetic or toxicodynamic nature [e.g. cytochrome P450 (CYP) induction or inhibition, inhibition of repair mechanisms].
    - Investigating specific scenarios under which the application of an extra uncertainty factor for interactions is justified.
    - Investigating when binary interaction data provide a basis for predicting effects of mixtures with more components.
  - Provision of better integration of high throughput, *in vitro* and 'omics data generated from modern methodologies as currently investigated world-wide in translational research (OECD, US EPA, EFSA), horizon 2020 programmes (EUROMIX, EUTOXRISK, etc.). These will provide the means to improve the mechanistic basis for setting assessment groups using data on mode of action, Aggregated Exposure Pathways (AEPs) (see Glossary for definition) and AOPs for multiple substances.
  - Further support the establishment of big data through the development of large and curated databases capturing historical toxicokinetic and toxicity data for specific human subpopulations and different taxa for animal health and the ecological area. These will improve the integration of inter-individual and interspecies differences in the risk assessment process.
  - Towards the implementation of generic *in silico* approaches for mixture toxicity (i.e. refinement of TTC, specific QSARs) integrating mechanistic data and different types of evidence (*in vivo*, *in vitro*, *in silico*, 'omics, etc.) to support component-based approaches.
  - Move towards the implementation of generic pharmacokinetic (PK) tools and pharmacodynamic pharmacokinetic (PB-PK) models in human health, animal health and the ecological area integrating internal dose in component-based approaches.

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These are currently under development at US EPA, JRC, EFSA and under other research programmes and will enable risk assessment based on internal doses of multiple chemicals.

- Risk assessment
  - Further implement the use of landscape modelling in ecological risk assessment of mixtures to integrate taxa-specific hazard information, exposure information, eco-epidemiological information in a spatial explicit fashion for different habitats and ecosystems.
  - A potential activity in the longer term includes the development of methodologies for risk assessment of Exposure to multiple chemicals combined with other stressors (e.g. biological hazards, physical agents).

References

Agency for Toxic Substances and Disease Registry (ATSDR), 2004. Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures. US Department of Health and Human Services, 107.

Altenburger R, Schmitt H and Schuurmann G, 2005. Algal toxicity of nitrobenzenes: Combined effect analysis as a pharmacological probe for similar modes of interaction. Environmental Toxicology and Chemistry, 24, 324–333.

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE and Villeneuve DL, 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry, 29, 730–741.

Baas J, Augustine S, Marques GM and Dorne JL, 2018. Dynamic energy budget models in ecological risk assessment: from principles to applications. Science of the Total Environment, 628–629, 249–260.

Baas J, Jager T and Kooijman B, 2010. A review of DEB theory in assessing toxic effects of mixtures. Science of the Total Environment, 408, 3740–3745.

Backhaus T, Brooks BW and Kapustka L. 2010.Chemical risk assessment: pressures, perceptions and expectations. Integr Environ Assess Manag. 2010 Jul;6(3):323-4.

Backhaus T, Faust M and Kortenkamp A. 2013. Cumulative risk assessment: a European perspective on the state of the art and the necessary next steps forward. Integr Environ Assess Manag. 2013 Oct;9(4):547-8. doi: 10.1002/ieam.1475.

Backhaus T, 2016. Environmental risk assessment of pharmaceutical mixtures: demands, gaps, and possible bridges. AAPS Journal, 18, 804–813.

Béchaux C, Zeilmaker M, Merlo M, Bokkers B and Crepet A, 2014. An integrative risk assessment approach for persistent chemicals: A case study on dioxins, furans and dioxin-like PCBs in France. Regulatory Toxicology and Pharmacology, 70, 261–269.

Bliss CI, 1939. The toxicity of poisons applied jointly. Annals of Applied Biology, 26, 585–615.

Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D and Farland W, 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Critical Reviews in Toxicology, 36, 781–792.

Boobis A, Budinsky R, Collie S, Crofton K, Embry M, Felter S, Hertzberg R, Kopp D, Mihlan G, Mumtaz M, Price P, Solomon K, Teuschler L, Yang R and Zaleski R, 2011. Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment. Critical Reviews in Toxicology, 41, 369–383.

Bopp S, Berggren E, Kienzler A, van der Linden S and Worth A 2015. Scientific methodologies for the assessment of combined effects of chemicals – a survey and literature review. JRC Technical Reports, 64.

DRAFT

Bopp SK, Kienzler A, van der Linden S, Richarz A-N, Triebe J and Worth A 2016. Review of case studies on the human and environmental risk assessment of chemical mixtures: Identification of priorities, methodologies, data gaps, future needs. JRC Technical Reports. Available online: https://ec.europa.eu/jrc/en/publication/review-case-studies-human-and-environmental-risk-assessment-chemical-mixtures

Cedergreen N, 2014. Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. PLoS One, 9, e96580. doi:10.1371/journal.pone.0096580

Cedergreen N, Dalhoff K, Li D, Gottardi M and Kretschmann AC, 2017. Can toxicokinetic and toxicodynamic modeling be used to understand and predict synergistic interactions between chemicals? Environmental Science and Technology, 51, 14379–14389.

Cheng S and Bois FY, 2011. A mechanistic modeling framework for predicting metabolic interactions in complex mixtures. Environmental Health Perspectives, 119, 1712–1718.

da Costa GG, Jacob CC, Von Tungeln LS, Hasbrouck NR, Olson GR, Hattan DG, Reimschuessel R and Beland FA, 2012. Dose–response assessment of nephrotoxicity from a twenty-eight-day combined-exposure to melamine and cyanuric acid in F344 rats. Toxicology and Applied Pharmacology, 262, 99–106.

De Zwart D, Dyer SD, Posthuma L and Hawkins CP, 2006. Predictive models attribute effects on fish assemblages to toxicity and habitat alteration. Ecological Applications, 16, 1295–1310.

De Zwart D and Posthuma L, 2005. Complex mixture toxicity for single and multiple species: Proposed methodologies. Environmental Toxicology and Chemistry, 24, 2665–2676.

Di Guardo A, Gouin T, MacLeod M and Scheringer M, 2018. Environmental fate and exposure models: advances and challenges in 21st century chemical risk assessment. Environmental Science-Processes and Impacts, 20, 58–71.

Dorne J-L, Richardson J, Kass G, Georgiadis N, Monguidi M, Pasinato L, Cappe S, Verhagen H and Robinson T, 2017. OpenFoodTox: EFSA's open source toxicological database on chemical hazards in food and feed. EFSA Journal, 15, e15011.

ECHA (European Chemicals Agency) 2014. Transitional Guidance on mixture toxicity assessment for biocidal products for the environment. 45.

https://echa.europa.eu/documents/10162/23492134/tg_mixture+toxicity_superseded_en.pdf/de8eede9-45f0-4be3-538e-8e922d21e8dd

ECHA (European Chemicals Agency) 2015. Guidance on the Biocidal Products Regulation: Volume III Human Health, Part B Risk Assessment. https://echa.europa.eu/documents/10162/23036412/biocides_guidance_human_health_ra_iii_part_bc_en.pdf/30d53d7d-9723-7db4-357a-ca68739f5094

EFSA (European Food Safety Authority), 2005a. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the European Parliament related to the safety assessment of wild and farmed fish. EFSA Journal 2005;3(6):236, 118 pp. doi:10.2903/j.efsa.2005.236

EFSA (European Food safety Authority), 2005b. A harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA Journal 2005;3(10):282, 31 pp. doi:10.2903/j.efsa.2005.282

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the European Commission on marine biotoxins in shellfish – okadaic acid and analogues. EFSA Journal 2008;6(1):589, 62 pp. doi:10.2903/j.efsa.2008.589

EFSA (European Food Safety Authority), 2008a. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on polycyclic aromatic hydrocarbons in food. EFSA Journal 2008;6(8):724, 114 pp. doi:10.2903/j.efsa.2008.724

DRAFT

EFSA (European Food Safety Authority), 2008b. Scientific Opinion of the Panel on Plant Protection Products and their Residues (PPR Panel) on a request from the EFSA evaluate the suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005. EFSA Journal 2008;6(5):705, 85 pp. doi:10.2903/j.efsa.2008.705

EFSA (European Food Safety Authority), 2010. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. doi: 10.2903/j.efsa.2010.1557

EFSA (European Food Safety Authority), 2011. Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances EFSA Journal 2011;9(12):2490, 33 pp. doi: 10.2903/j.efsa.2011.2490

EFSA (European Food Safety Authority), 2013a. International framework dealing with human risk assessment of combined exposure to multiple chemicals. EFSA Journal 2013;11(7):3313, 69 pp. doi: 10.2903/j.efsa.2013.3313

EFSA (European Food Safety Authority), 2013b. EFSA Guidance Document on the risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp. doi: 10.2903/j.efsa.2013.3295

EFSA (European Food Safety Authority), 2014a. Modern methodologies and tools for human hazard assessment of chemicals. EFSA Journal 2014;12(4):3638, 87 pp. doi: 10.2903/j.efsa.2014.3638

EFSA (European Food Safety Authority), 2014b. Towards an integrated environmental risk assessment of multiple stressors on bees: review of research projects in Europe, knowledge gaps and recommendations. EFSA Journal 2014;12(3):3594, 102 pp. doi: 10.2903/j.efsa.2014.3594

EFSA (European Food Safety Authority) 2015a. Harmonisation of human and ecological risk assessment of combined exposure to multiple chemicals. EFSA supporting publication, 76 pp. Available online: https://publications.europa.eu/en/publication-detail/-/publication/df252bd7-0aa7-11e6-b713-01aa75ed71a1/language-en

EFSA (European Food Safety Authority), 2015b. Scientific report on Principles and process for dealing with data and evidence in scientific assessments. EFSA Journal 2015;13(5):4121, 35 pp. doi: 10.2903/j.efsa.2015.4121

EFSA (European Food Safety Authority), 2015c. Editorial: Increasing robustness, transparency and openness of scientific assessments. EFSA Journal 2015;13(3):e13031, 3pp. doi:10.2903/j.efsa.2015.e13031

EFSA (European Food Safety Authority), 2018. Guidance on use of EFSA Pesticide Residue Intake Model (EFSA PRIMo revision 3. EFSA Journal 2018;16(1):5147, 43 pp. https://doi.org/10.2903/j.efsa.2018.5147

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760, 60 pp. doi: 10.2903/j.efsa.2012.2760.

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), Silano V, Bolognesi C, Castle L, Cravedi J-P, Engel K-H, Fowler P, Franz R, Grob K, Gurtler R, Husøy T, Karenlampi S, Milana MR, Penninks A, Tavares Pocas MF, Smith A, Tlustos C, Wölfle D, Zorn H, Zugravu C-A, Beckman Sundh U, Benigni R, Brimer L, Mulder G, Oskarsson A, Svendsen C,Martino C and Mennes W, 2017. Scientific Opinion of Flavouring Group Evaluation 500 (FGE.500): rum ether. EFSA Journal 2017;15(8):4897, 53 pp. https://doi.org/10.2903/j.efsa.2017.4897

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009. Scientific Opinion on marine biotoxins in shellfish – Palytoxin group. EFSA Journal 2009;7(12):1393, 38 pp. doi: 10.2903/j.efsa.2009.1393

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2011. Scientific Opinion on Pyrrolizidine alkaloids in food and feed. EFSA Journal 2011;9(11):2406, 134 pp. doi: 10.2903/j.efsa.2011.2406.

DRAFT

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012. Scientific Opinion on Ergot alkaloids in food and feed. EFSA Journal 2012;10(7):2798, 158 pp. doi: 10.2903/j.efsa.2012.2798.

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vleminckx C, Vollmer G, Wallace H, Ruiz Gomes JA and Binaglia M, 2017a. Statement on the risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements. EFSA Journal 2017;15(7):4908, 34 pp. https://doi.org/10.2903/j.efsa.2017.4908

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vleminckx C, Vollmer G, Wallace H, Dall'Asta C, Dänicke S, Eriksen G-S, Altieri A, Roldán-Torres R and Oswald IP, 2017b. Scientific opinion on the risks for animal health related to the presence of zearalenone and its modified forms in feed. EFSA Journal 2017;15(7):4851, 123 pp. https://doi.org/10.2903/j.efsa.2017.4851

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain) and EFSA CEF Panel (EFSA Panel on Food Contact Materials E, Flavourings and Processing Aids), 2010. Scientific Opinion on Melamine in Food and Feed. EFSA Journal 2010;8(4):1573, 145 pp. doi: 10.2903/j.efsa.2010.1573.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayao B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Dusemund B, Van Beelen P, Westendorf J, Gregoretti L, Manini P and Chesson A, 2017a. Scientific Opinion on the safety and efficacy of an essential oil from *Origanum vulgare* subsp. *hirtum* (Link) letsw. var. Vulkan when used as a sensory additive in feed for all animal species. EFSA Journal 2017;15(12):5095, 16 pp. https://doi.org/10.2903/j.efsa.2017.5095

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017b. Guidance on the assessment of the safety of feed additives for the target species. EFSA Journal 2017;15(10):5021, 19 pp. https://doi.org/10.2903/j.efsa.2017.5021

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues, 2009. Guidance Document on risk assessment for birds and mammals on request from EFSA. EFSA Journal 2009;7(12):1438. doi: 10.2903/j.efsa.2009.1438.

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2012a. Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2012; 10(5) 2668, 275 pp. doi: 10.2903/j.efsa.2012.2668.

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2012b. Guidance on the Use of Probabilistic Methodology for Modelling Dietary Exposure to Pesticide Residues. EFSA Journal 2012;10(10):2839, 95 pp. doi: 10.2903/j.efsa.2012.2839.

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013a. Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile (2014 update). EFSA Journal 2013;11(7):3293, 131 pp. doi: 10.2903/j.efsa.2013.3293

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013b. Guidance on tiered risk assessment for Plant Protection Products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi: 10.2903/j.efsa.2013.3290.

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013c. Scientific Opinion on relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides residues in food. EFSA Journal 2013;11(12): 3472, 40 pp. doi: 10.2903/j.efsa.2013.3472

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2014. Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of Plant Protection Products. EFSA Journal 2014;12(3):3589, 92 pp. doi: 10.2903/j.efsa.2014.3589

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2015. Scientific Opinion addressing the state of the science on risk assessment of Plant Protection Products for non-target arthropods. EFSA Journal 2015;13(2):3996, 212 pp. doi: 10.2903/j.efsa.2015.3996

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2015. Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface water. EFSA Journal 2015;13(7):4176, 145 pp. doi: 10.2903/j.efsa.2015.4176

EFSA Scientific Committee, 2012a. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. doi: 10.2903/j.efsa.2012.2579

EFSA Scientific Committee, 2012b. Scientific Opinion on Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). EFSA Journal 2012;10:103EFSA Scientific Committee, 2012c. Scientific Opinion on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed. EFSA Journal 2012;10(2):2570. [12 pp.] doi: 10.2903/j.efsa.2012.2570

EFSA Scientific Committee, 2016a. Scientific opinion on coverage of endangered species in environmental risk assessments at EFSA. EFSA Journal 2016; 14(2):4313. 85 pp. doi: 10.2903/j.efsa.2016.4313

EFSA Scientific Committee, 2016b. Scientific opinion on priority topics for the development of risk assessment guidance by EFSA's Scientific Committee in 2016–2018. EFSA Journal 2016;14(6):4502, 9 pp. doi: 10.2903/j.efsa.2016.4502

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Benfenati E, Chaudhry QM, Craig P, Frampton G, Greiner M, Hart A, Hogstrand C, Lambre C, Luttik R, Makowski D, Siani A, Wahlstroem H, Aguilera J, Dorne J-L, Fernandez Dumont A, Hempen M, Valtuena Martınez S, Martino L, Smeraldi C, Terron A, Georgiadis N and Younes M, 2017a. Scientific Opinion on the guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp. https://doi.org/10.2903/j.efsa.2017.4971

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Younes M, Bresson J-L, Griffin J, Hougaard Benekou S, van Loveren H, Luttik R, Messean A, Penninks A, Ru G, Stegeman JA, van der Werf W, Westendorf J, Woutersen RA, Barrizone F, Bottex B, Lanzoni A, Georgiadis N and Alexander J, 2017b. Guidance on the assessment of the biological relevance of data in scientific assessments. EFSA Journal 2017;15(8):4970, 73 pp. https://doi.org/10.2903/j.efsa.2017.4970

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen KH, More S, Mortensen A, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Aerts M, Bodin L, Davis A, Edler L, Gundert-Remy U, Sand S, Slob W, Bottex B, Abrahantes JC, Marques DC, Kass G and Schlatter JR, 2017c. Update: Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 41 pp. doi: 10.2903/j.efsa.2017.4658

EFSA Scientific Committee, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Younes M, Craig P, Hart A, Von Goetz N, Koutsoumanis K, Mortensen A, Ossendorp B, Martino L, Merten C, Mosbach-Schultz O and Hardy A, 2018a. Guidance on Uncertainty Analysis in Scientific Assessments. EFSA Journal 2018;16(1):5123, 39 pp. https://doi.org/10.2903/j.efsa.2018.5123.

DRAFT

EFSA Scientific Committee, Tony Hardy, Diane Benford, Thorhallur Halldorsson, Mike Jeger, Helle Katrine Knutsen, Simon More, Hanspeter Naegeli, Hubert Noteborn, Colin Ockleford, Antonia Ricci, Guido Rychen, Vittorio Silano, Roland Solecki, Dominque Turck, Maged Younes; Gabriele Aquilina, Riccardo Crebelli, Sandro Grilli, Rainer Guertler, Karen Ildico Hirsch-Ernst, Pasquale Mosesso, Elsa Nielsen, Jan Van Benthem, Christiane Vleminckx; Maria Carfi, Carla Martino, Daniela Maurici, Juan Parra Morte and Josef Schlatter, 2018b. Statement on genotoxicity assessment of chemical mixtures. EFSA Journal 2018;volume(issue):NNNN, 81 pp. doi:10.2903/j.efsa.2018.NNNN

European Pharmacopoeia, 2017. 9.0, Council of Europe. Available online: https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-9th-edition

Faust M, Altenburger R, Backhaus T, Blanck H, Boedeker W, Gramatica P, Hamer V, Scholze M, Vighi M and Grimme LH, 2001. Predicting the joint algal toxicity of multi-component s-triazine mixtures at low-effect concentrations of individual toxicants. Aquatic Toxicology, 56, 13–32.

Faust M, Altenburger R, Backhaus T, Blanck H, Boedeker W, Gramatica P, Hamer V, Scholze M, Vighi M and Grimme LH, 2003. Joint algal toxicity of 16 dissimilarly acting chemicals is predictable by the concept of independent action. Aquatic Toxicology, 63, 43–63.

Fisk P, 2014. Chemical Risk Assessment: a manual for REACH. 418pp. Publisher: Wiley, Editor: Peter Fisk, Oliver Warwick, ISBN: 978-1-119-95368-5.

Galic N, Ashauer R, Baveco H, Nyman AM, Barsi A, Thorbek P, Bruns E and Van den Brink PJ, 2014. Modeling the contribution of toxicokinetic and toxicodynamic processes to the recovery of *Gammarus pulex* populations after exposure to pesticides. Environmental Toxicology and Chemistry, 33, 1476–1488.

George TK, Liber K, Solomon KR and Sibley PK, 2003. Assessment of the probabilistic ecological risk assessment-toxic equivalent combination approach for evaluating pesticide mixture toxicity to zooplankton in outdoor microcosms. Archives of Environmental Contamination and Toxicology, 45, 453–461.

Greco WR, Bravo G, and Parsons, JC, 1995. The search for synergy: a critical review from a response surface perspective. Pharmacol. Rev. 47, 331–385

Haddad S, Beliveau M, Tardif R and Krishnan K, 2001. A PBPK modeling-based approach to account for interactions in the health risk assessment of chemical mixtures. Toxicological Sciences, 63, 125–131.

Harbers JV, Huijbregts MAJ, Posthuma L and Van de Meent D, 2006. Estimating the impact of high-production-volume chemicals on remote ecosystems by toxic pressure calculation. Environmental Science and Technology, 40, 1573–1580.

IGHRC 2008. Chemical Mixtures: A framework for assessing risks to human health (CR14). 95 pp. Available online: file:///C:/Users/Chris/Downloads/Risk-Assessment-Procedures_Levy_9.pdf

Jacob CC, Reimschuessel R, Von Tungeln LS, Olson GR, Warbritton AR, Hattan DG, Beland FA and da Costa GG, 2011. Dose–response assessment of nephrotoxicity from a 7-day combined exposure to melamine and cyanuric acid in F344 rats. Toxicological Sciences, 119, 391–397.Johnson RM, Pollock HS and Berenbaum MR.2009. Synergistic interactions between in-hive miticides in Apis mellifera. J Econ Entomol. 102 (2):474-479.Jonker MJ, Svendsen C, Bedaux JJM, Bongers M and Kammenga JE, 2005. Significance testing of synergistic/antagonistic, dose level-dependent, or dose ratio-dependent effects in mixture dose–response analysis. Environmental Toxicology and Chemistry, 24, 2701–2713.

KEMI (The Swedish Chemicals Agency), 2015. An additional assessment factor (MAF) – A suitable approach for improving the regulatory risk assessment of chemical mixtures?, 52.Kienzler A, Berggren E, Bessems J, Bopp S, Van der Linden S and Worth A, 2014. Assessment of Mixtures - Review of Regulatory Requirements and Guidance Luxembourg: Publications Office of the European Union. Scientific and Technical Research series – ISSN 1831-9424 (online). ISBN 978-92-79-64812-0 (PDF) doi:10.2788/138523

DRAFT

Kienzler A, Bopp SK, van der Linden S, Berggren E and Worth A, 2016. Regulatory assessment of chemical mixtures: Requirements, current approaches and future perspectives. Regulatory Toxicology and Pharmacology, 80, 321–334.

Kooijman S, 1987. A safety factor for LC50 values allowing for differences in sensitivity among species. Water Research, 21, 269–276.

Kortenkamp A, Backhaus T and Faust M 2009. State of the Art Report on Mixture Toxicity. Report. 391. http://ec.europa.eu/environment/chemicals/effects/pdf/report_mixture_toxicity.pdf

Loos M, Ragas AMJ, Plasmeijer R, Schipper AM and Hendriks AJ, 2010. Eco-SpaCE: An object-oriented, spatially explicit model to assess the risk of multiple environmental stressors on terrestrial vertebrate populations. Science of the Total Environment, 408, 3908–3917.

Loureiro S, Svendsen C, Ferreira ALG, Pinheiro C, Ribeiro F and Soares A, 2010. Toxicity of three binary mixtures to *Daphnia magna*: comparing chemical modes of action and deviations from conceptual models. Environmental Toxicology and Chemistry, 29, 1716–1726.

Meek ME, Boobis AR, Crofton KM, Heinemeyer G, Van Raaij M and Vickers C, 2011. Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. Regulatory Toxicology and Pharmacology, 60, S1–S14.

Meek MEB,2013. International experience in addressing combined exposures: Increasing the efficiency of assessment. Toxicology, 313, 185–189.Mulder C, Cohen JE, Setälä H, Bloem J and Breure AM. 2005. Bacterial traits, organism mass, and numerical abundance in the detrital soil food web of Dutch agricultural grasslands. Ecology Letters 8 (1), 80-90.

Mulder C, 2006. Driving forces from soil invertebrates to ecosystem functioning: the allometric perspective. Naturwissenschaften 93 (10), 467-479

Mumtaz MM and Durkin PR, 1992. A weight-of-evidence approach for assessing interactions in chemical mixtures. Toxicology and Industrial Health, 8, 377–406.

Norwegian Scientific Committee for Food Safety (VKM), 2008. Combined toxic effects of multiple chemical exposures. Vitenskapskomiteen for mattrygghet/Norwegian Scientific Committee for Food Safety, Oslo, No. 105.

https://vkm.no/download/18.d44969415d027c43cf1e869/1509708687404/Combined%20toxic%20effects%20of%20multiple%20chemical%20exposures.pdf

Nys C, Van Regenmortel T, Janssen CR, Oorts K, Smolders E and De Schamphelaere KAC, 2018. A framework for ecological risk assessment of metal mixtures in aquatic systems. Environmental Toxicology and Chemistry, 37, 623–642.

Oaks JL, Gilbert M, Virani MZ, Watson RT, Meteyer CU, Rideout BA, Shivaprasad HL, Ahmed S, Chaudhry MJI, Arshad M, Mahmood S, Ali A and Khan AA, 2004. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature, 427, 630–633.

OECD (Organisation for Economic Co-operation and Development), 2011. WHO OECD ILSI/HESI International Workshop on Risk Assessment of Combined Exposures to Multiple Chemicals Workshop Report. 77. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2011)10&doclanguage=en

OECD (Organisation for Economic Co-operation and Development), 2017. Guidance document: Consideration for assessing the risks of combined exposure to multiple chemicals. 104.

Pohl HR, Mumtaz MM, Scinicariello F and Hansen H, 2009. Binary weight-of-evidence evaluations of chemical interactions-15 years of experience. Regulatory Toxicology and Pharmacology, 54, 264–271.Posthuma, L Traas, TP and Suter, G. 2002. General Introduction to Species Sensitivity Distributions. Chapter 1 8 pp. In Posthuma L, Suter GW II, Traas TP, editors. Species sensitivity distributions in ecotoxicology. Boca Raton (FL): Lewis.

DRAFT

Posthuma L, Richards S, De Swart D, Dyer SD, Sibley PK, Hickey CH and Altenburger R, 2008. Mixture extrapolation approaches. In: Extrapolation Practice for Ecotoxicological Effect Characterization of Chemicals. Eds Solomon K, Brock T, De Zwart D, Dyer S, Posthuma L, Richards S, Sanderson H, Sibley PK and van den Brink PJ, CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 135–185.Price PS, Hollnagel, HM, and Zabik, JM, 2009. Characterizing the noncancer toxicity of mixtures using concepts from the TTC and quantitative models of uncertainty in mixture toxicity. Risk Analysis, 29, 1534–1548.

Price P, Dhein E, Hamer M, Han X, Heneweer M, Junghans M, Kunz P, Magyar C, Penning H and Rodriguez C, 2012. A decision tree for assessing effects from exposures to multiple substances. Environmental Sciences Europe, 24, 12.

Price P, Zaleski R, Hollnagel H, Ketelslegers H and Han XL, 2014. Assessing the safety of co-exposure to food packaging migrants in food and water using the Maximum Cumulative Ratio and an established decision tree. Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure and Risk Assessment, 31, 414–421.

Price PS and Han X, 2011. Maximum cumulative ratio (MCR) as a tool for assessing the value of performing a Cumulative Risk Assessment. International Journal of Environmental Research and Public Health, 8, 2212–2225.

Quignot N, Béchaux C and Amzal B 2015a. Data collection on toxicokinetic and toxicodynamic interactions of chemical mixtures for human risk assessment. EFSA supporting publication, 85 pp. Available online: https://www.efsa.europa.eu/en/supporting/pub/en-711

Quignot N, Grech A and Amzal B 2015b. Data collection on combined toxicity of multiple chemicals for animal health and ecological risk assessment. EFSA supporting publication, 112 pp. Available online: https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/sp.efsa.2015.EN-861

Ragas AMJ, Teuschler LK, Posthuma L and Cowan C, 2010. Human and ecological risk assessment of chemical mixtures. In: Mixture toxicity: linking approaches from ecological and human toxicology. eds van Gestel CAM, Jonker M, Kammenga JE, Laskowski R and Svendsen C, CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 157–215.Ragas AM, Oldenkamp R, Preeker NL, Wernicke J, Schlink U. 2011. Cumulative risk assessment of chemical exposures in urban environments. Environ Int. 2011 Jul;37(5):872-81.

Rotter S, Beronius A, Hanberg A, Zilliakus J, Nikolopoulou D, Machera K and Solecki R (EuroMix European Test and Risk Assessment Strategies for Mixtures), 2016. Report on mixtures and implementation strategy in Europe – Assessment of chemical mixtures under consideration of current and future regulatory requirements and scientific approaches. https://zenodo.org/record/1067586#.WykCpPlubIU

Sarigiannis DA and Hansen U, 2012. Considering the cumulative risk of mixtures of chemicals – a challenge for policy makers. Environmental Health, 11, S1–S12.

Schafer RB, Gerner N, Kefford BJ, Rasmussen JJ, Beketov MA, de Zwart D, Liess M and von der Ohe PC, 2013. How to characterize chemical exposure to predict ecologic effects on aquatic communities? Environmental Science and Technology, 47, 7996–8004.

SCHER (Scientific Committee on Health and Environmental Risks), SCCS (Scientific Committee on Consumer Safety) and SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), 2012. Opinion on the Toxicity and Assessment of Chemical Mixtures. 50 pp. Available online: http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf

SCHER (Scientific Committee on Health and Environmental Risks), SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks) and SCCS (Scientific Committee on Consumer Safety), 2013. Addressing the New Challenges for Risk Assessment. Available online: .

Segner H, 2011. Moving beyond a descriptive aquatic toxicology: The value of biological process and trait information. Aquatic Toxicology, 105, 50–55. http://ec.europa.eu/health/scientific_committees/docs/challenges_mandate_en.pdf

DRAFT

Sirot V, Fremy JM and Leblanc JC, 2013. Dietary exposure to mycotoxins and health risk assessment in the second French total diet study. Food and Chemical Toxicology, 52, 1–11.

Sirot V, Tard A, Venisseau A, Brosseaud A, Marchand P, Le Bizec B and Leblanc JC, 2012. Dietary exposure to polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls of the French population: Results of the second French Total Diet Study. Chemosphere, 88, 492–500.

Solomon KR, Brock T, De Swart D, Dyer SD, Posthuma L, Richards S, Sanderson H, Sibley PK and Van den Brink PJ, 2008. Extrapolation in the context of criteria setting and risk assessment. In: Extrapolation Practice for Ecotoxicological Effect Characterization of Chemicals. Eds Solomon K, Brock T, De Zwart D, Dyer S, Posthuma L, Richards S, Sanderson H, Sibley PK and van den Brink PJ, CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 1–31.

Solomon KR, Wilks MF, Bachman A, Boobis A, Moretto A, Pastoor TP, Phillips R and Embry MR, 2016. Problem formulation for risk assessment of combined exposures to chemicals and other stressors in humans. Critical Reviews in Toxicology, 46, 835–844.

Sørensen PB, Giralt F, Rallo R, Espinosa G, Munier B, Gyldenkaerne S and Thomsen M, 2010. Conscious worst case definition for risk assessment, part II A methodological case study for pesticide risk assessment. Science of the Total Environment, 408, 3860–3870.

Sørensen PB, Thomsen M, Assmuth T, Grieger KB and Baun A, 2010. Conscious worst case definition for risk assessment, part I A knowledge mapping approach for defining most critical risk factors in integrative risk management of chemicals and nanomaterials. Science of the Total Environment, 408, 3852–3859.

Stein B, Michalski B, Martin S, Pfeil R, Ritz V and Solecki R, 2014. Human health risk assessment from combined exposure in the framework of Plant Protection Products and biocidal products. Journal Fur Verbraucherschutz Und Lebensmittelsicherheit [Journal of Consumer Protection and Food Safety], 9, 367–376.

Teeguarden JG, Tan YM, Edwards SW, Leonard JA, Anderson KA, Corley RA, Kile ML, Simonich SM, Stone D, Tanguay RL, Waters KM, Harper SL and Williams DE, 2016. Completing the Link between Exposure Science and Toxicology for Improved Environmental Health Decision Making: The Aggregate Exposure Pathway Framework. Environmental Science and Technology, 50, 4579–4586.

US EPA (US Environmental Protection Agency), 1986. Guidelines for the Health Risk Assessment of Chemical Mixtures. Federal Register, 51, 34014–34025.

US EPA (US Environmental Protection Agency) (Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances), 1999. Guidance for identifying pesticide chemicals and other substances that have a common mechanism of toxicity. 14 pp. Available online: https://www.epa.gov/sites/production/files/2015-07/documents/guide-2-identify-pest-chem_0.pdf

US EPA (US Environmental Protection Agency), 2000. Supplementary guidance for conducting health risk assessment of chemical mixtures. 209 pp. Available online: https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=20533

US EPA (US Environmental Protection Agency) (Office of Pesticide Programs, US Environmental Protection Agency), 2002. Guidance on cumulative risk assessment of pesticide chemicals that have a common mechanism of toxicity. 90 pp. Available online: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-cumulative-risk-assessment-pesticide

US EPA (US Environmental Protection Agency), 2007. Concepts, methods, and data sources for cumulative health risk assessment of multiple chemicals, exposures and effects: a resource document (Final Report, 2008). 412 pp. Available online: https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=190187

US EPA (US Environmental Protection Agency), 2010. Development of a relative potency factor (RPF) approach for polycyclic aromatic hydrocarbon (PAH) mixtures. 574 pp. Available online: https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=158302

DRAFT

US EPA (US Environmental Protection Agency), 2016. Pesticide cumulative risk assessment: framework for screening analysis purpose. 17 pp. Available online: https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=158302

van Broekhuizen FA, Posthuma L and Traas TP (RIVM), 2016. Addressing combined effects of chemicals in environmental safety assessment under REACH – A thought starter. 36 pp. Available online: https://www.rivm.nl/en/Documents_and_publications/Scientific/Reports/2017/February/Addressing_combined_effects_of_chemicals_in_environmental_safety_assessment_under_REACH_A_thought_starter

Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N and Peterson RE, 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicological Sciences, 93, 223–241.

Van Gestel CAM, Jonker MJ, Kammenga JE, Laskowski R and Svendsen C, 2011. Mixture Toxicity. Linking Approaches from Ecological and Human Toxicology. SETAC Press, Pensacola, USA, 320 pp. ISBN 9781439830086

Verhaar HJM, Vanleeuwen CJ and Hermens JLM, 1992. Classifying environmental-pollutants.1. Structure-activity-relationships for prediction of aquatic toxicity. Chemosphere, 25, 471–491.

Wang NC, Rice GE, Teuschler LK, Colman J and Yang RS, 2012. An in silico approach for evaluating a fraction-based, risk assessment method for total petroleum hydrocarbon mixtures. Journal of Toxicology, 2012, 410143.

Whitehorn PR, O'Connor S, Wackers FL and Goulson D, 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science, 336, 351–352.

WHO/IPCS (World Health Organization/International Programme on Chemical Safety) (World Health Organization), 2009. Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria. Available online: http://www.who.int/foodsafety/publications/chemical-food/en/

Wilkinson CF, Christoph GR, Julien E, Kelley JM, Kronenberg J, McCarthy J and Reiss R, 2000. Assessing the risks of Exposures to multiple chemicals with a common mechanism of toxicity: How to cumulate? Regulatory Toxicology and Pharmacology, 31, 30–43.

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Acceptable daily intake (ADI)	Estimate of the amount of substance in food expressed on a body-weight basis, that can be ingested daily over a lifetime, without appreciable risk to any consumer on the basis of all known facts at the time of evaluation, taking into account sensitive groups within the population (e.g. children and the unborn) (EFSA, 2013).
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, 2013).
Adverse Outcome Pathway (AOP)	A sequence of events from the exposure of an individual or population to a chemical substance to a final adverse (toxic) effect at the individual level (for human health) or population level (for ecotoxicological end-points). The key events in an AOP should be definable and make sense from a physiological and biochemical perspective. AOPs incorporate the toxicity pathway and mode of action for an adverse effect. AOPs may be related to other mechanisms and pathways as well as to detoxification routes (OECD 2012; EFSA, 2014).
Aggregate exposure	Exposure to a single substance originating from different sources (Kienzler et al., 2014, JRC).
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, represent the point of integration between an AEP and an AOP' (Teeguarden et al., 2016).
Antagonism	Pharmacological or toxicological interaction in which the combined biological effect of two or more substances is less than expected on the basis of the simple summation of the toxicity of each of the individual substances (EFSA, 2013).
Assessment factor	See Uncertainty Factor Assessment Group; See Cumulative Assessment Group.
Assessment group (encompassing cumulative assessment group)	Mixture components that are treated as a group by applying a common mixture assessment principle (e.g. dose addition) because these components have some characteristics in common (i.e. the grouping criteria).
Consistency	The extent to which the contributions of different pieces or lines of evidence to answering the specified question are compatible (see Section 2.5).
Combined Margin of Exposure (MOET)	The reciprocal of the Reference Point Index is the combined Margin of Exposure.
Complex mixture	A mixture (e.g. extracts, protein hydrolysates, smoke flavourings) in which not all constituents are known or fully characterised. A qualitative and quantitative characterisation of the main constituents should be performed, at least via sum parameters. On the basis of these data, a mass balance should be calculated. The amount of unidentified components should be indicated and should be as low as possible.
Component-based	An approach in which the risk of a mixture is assessed based on exposure

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approach	and effect data of its individual components.
Components of concern	'Components of concern' have been defined as 'chemicals in a mixture that are likely contributors to health hazard either because their individual exposure levels exceed health guidelines, or because joint toxic action with other components, including additivity or interactions, may pose a health hazard (US EPA, 2000, ATSDR, 2001).
Concentration addition	A component-based model in which the components are treated as if having a similar action. The components may vary in toxic potency. Components contribute to the mixture effect relative to the ratio between their concentration and toxic potency. Concentration is the exposure metric used as a proxy for dose in *in vitro* studies and ecological risk assessment.
Conceptual model	Defined by EFSA (2016b) in the context of environmental risk assessment as 'Step of the environmental risk assessment problem formulation phase describing and modelling scenarios and pathways on how the use of a regulated product may harm a specific protection goal'. A form of conceptual framework, which is defined by PROMETHEUS (EFSA, 2015) as 'The context of the assessment; all subquestion(s) that must be answered; and how they combine in the overall assessment.' In the present Guidance, conceptual model refers to a qualitative description or diagram showing how pieces and lines of evidence combine to answer a question or subquestion, as well as any relationships or dependencies between the pieces and lines of evidence. The conceptual model could be presented as, for example, a flow chart or list of logical steps (see Chapter 3 problem formulation).
Cumulative Assessment Group (CAG)	Group of active substances that could plausibly act by a common mode of action, not all of which will necessarily do so (EFSA, 2013).
Cumulative exposure	Combined exposure to multiple chemicals by multiple routes or combined exposure to multiple chemicals by a single route.
Cumulative risk assessment	The combined risks from aggregate exposures to multiple agents or stressors.
Dissimilar action	Occurs when the modes of action and possibly, but not necessarily, the nature and sites of toxic effects differ between the chemicals in a mixture, and one chemical does not influence the toxicity of another.
Dose addition	As above for concentration addition. Dose is the exposure metric used in human and animal health risk assessment. Dose addition is used as the generic term throughout this guidance document. All components in a mixture behave as if they were dilutions of one another. One chemical can be replaced by an equal fraction of an equi-effective concentration (e.g. an EC_{50}) of another, without diminishing the overall combined effect. This implies that every toxicant in the mixture contributes to the combination effect in proportion to its dose and individual potency (EFSA, 2013).
Emergency assessment	Emergency procedures, in which the choice of approach is constrained by unusually severe limitations on time and resources. See also EFSA (2016) and Section 4.
Estimate	A calculation or judgement of the approximate value, number, quantity, or extent of something (adapted from OED, 2017). Some weight of evidence questions refer to estimates, while others refer to hypotheses (see Section 2.1).
Evidence	Information that is relevant for assessing the answer to a specified question. In PROMETHEUS, a piece of evidence for an assessment is defined as data (information) that is deemed *relevant* for the specific objectives of the assessment (EFSA, 2015b). In this Guidance, this is expanded to all *potentially relevant* information, i.e. all evidence identified by the initial

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	search process, to recognise that the assessment of relevance in the search process is necessarily a preliminary one (e.g. based on keywords and titles alone). 'Evidence' can refer to a single piece of potentially relevant information or to multiple pieces (see Section 2.1).
Expert judgement	EFSA (2014) defines an expert as a knowledgeable, skilled or trained person. An expert judgement is a judgement made by an expert about a question or consideration in the domain in which they are expert. Such judgements may be qualitative or quantitative, but should always be careful, reasoned, evidence-based and transparently documented. (see Section 4.4).
Hazard Index	Sum of Hazard Quotients, i.e. ratio between exposure and the reference value for the common toxic effect of each component in a mixture or a Cumulative Assessment Group (JRC and EFSA, 2013).
Hazard index modified for binary interactions	This evaluates hazard data for possible pairs of chemicals to determine qualitative binary WOE (BINWOE) taking into account effects of each chemical on their respective toxicity so that two BINWOEs are needed for each pair of chemicals.
Hazard Quotient	The ratio of the potential exposure to the substance and the level at which no adverse effects are expected.
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).
Hypothesis	One type of framing for weight of evidence questions. Defined by Suter (2016) as a proposition proposed to be a potential explanation of a phenomenon or a potential outcome of a phenomenon. Some weight of evidence questions refer to hypotheses, while others refer to estimates (see Section 2.1).
Identity of the mixture	Chemical composition The methods used for the analysis of the mixture shall comply with the quality criteria laid down in Commission Regulation 152/2009. Information should be provided on the batch-to-batch variability in all the measured parameters for chemical composition, along with information on the stability of the mixture during storage. The sample(s) of the mixture tested for chemical composition should be the same as or identical to the sample(s) tested toxicologically. This should be stated explicitly in the dossier. If the samples are not identical then an explanation should be provided.
Independent action	Occurs when the mode of action and possibly, but not necessarily, the nature and sites of toxic effects differ between the chemicals in a mixture, and one chemicals does not influence the toxicity of another. The effects of exposure to such a mixture are the combination of the effects of each component compounds (also referred to as response addition) (Kienzler et al., 2014, JRC).
Independent joint action	See simple dissimilar action.
Index chemical	The chemical used as the point of reference for standardising the common toxicity of the chemical members of the CAG. The index chemical should have a clearly defined dose–response, be well defined for the common mechanism of toxicity, and have a toxicological/biological profile for the common toxicity that is representative of the CAG (US EPA, 2000).

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Interaction	In risk assessment practice, the term interaction is used to refer to mixture effects that differ from an explicit null model, i.e. dose and/or response addition. Interactions are categorised as less than additive (antagonism, inhibition, masking) or greater than additive (synergism, potentiation). (ATSDR, 2004a; US EPA, 2007a; EFSA, 2008b).
Limit of detection (LOD)	Lowest concentration of a pesticide residue in a defined matrix in which positive identification can be achieved using a specified method (EFSA PPR, 2008).
Limit of quantitation (LOQ)	Lowest concentration of a pesticide residue in a defined matrix in which positive identification and quantitative measurement can be achieved using a specified analytical method (EFSA, PPR, 2,208).
Margin of Exposure (MOE)	Ratio of (a) a reference point of (eco)toxicity to (b) the theoretical, predicted or estimated exposure dose or concentration.
Marker substance	One or more prevalent components of a mixture that can be measured readily and therefore used in exposure assessment.
Mass balance	A mass balance is the percentage compilation of individual constituents or classes of constituents, in the ideal case summing up to 100%.
Mechanism of action	Detailed explanation of the individual biochemical and physiological events leading to a toxic effect (EFSA, 2013).
Mixture	Any combination of two or more chemicals that may jointly contribute to real or potential effects regardless of source and spatial or temporal proximity.
Mixture of concern	A mixture of chemicals that is the subject of a risk assessment because there are indications that the compounds in the mixture/of which the mixture is composed may jointly contribute to the real or predicted risk.
Mode of action	Biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. It refers to the major steps leading to an adverse health effect following interaction of the compound with biological targets. It does not imply full understanding of mechanism of action at the molecular level (EFSA, 2013).
Point of Departure (POD)	In the USA, a dose that can be considered to be in the range of observed responses without significant extrapolation. A POD can be a data point or an estimated point that is derived from observed dose–response data. A POD is used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposure. The dose–response point that marks the beginning of a low-dose extrapolation. This point is most often the upper bound on an observed incidence or on an estimated incidence from a dose–response model. (US EPA, 2003; EFSA PPR, 2008).
Probability	Defined depending on philosophical perspective1) 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 Commitee, 2018a). The latter perspective is implied when probability is used in a weight of evidence assessment to express relative support for possible answers (see Sections 2.3 and 2.6).
Problem formulation	In the present guidance, 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.
Production process	The process(es) employed to produce the mixture (e.g. chemical synthesis, enzyme catalysis, fermentation, pyrolysis or isolation from a natural source,

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	etc.) should be described. The description of the production process should be detailed enough to provide the information that will form the basis for the evaluation. For safety, the description should include, in particular, information on potential by-products, impurities or contaminants.
Quantitative assessment	An assessment performed or expressed using a numerical scale (see Section 4.1 in EFSA Scientific Commitee, 2018a).
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, 2017) 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).
Reference point (RP)	Defined point on an experimental dose–response relationship for the critical effect. This term is synonymous to point of departure (USA). 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 Margin of Exposure in human and animal health risk assessment. In the ecological area, these include lethal dose (LD_{50}), effect concentration (EC_5/ECx), no (Adverse) effect concentration/dose (NOEC/NOAEC/NOAED), no (adverse) effect level (NEL/NOAEL), hazard concentration (HC_5/HCx) derived from a Species Sensitivity Distributions (SSD) for the ecosystem.
Reference value (RV)	The estimated maximum dose (on a body mass basis) or the concentration of an agent to which an individual may be exposed over a specified period without appreciable risk. Reference values are derived by applying an uncertainty factor to the reference point. Examples of reference values in human health include acceptable daily intake (ADI) for food and feed additives, pesticides and food contact materials, tolerable upper intake levels (UL) for vitamins and minerals, and tolerable daily intake (TDI) for contaminants. For acute effects and operators, the acute reference dose (ARfD) and the acceptable operator exposure level (AOEL).In animal health and the ecological area, these include maximum tolerated dose (MTD) and predicted no effect concentration (PNEC) respectively.
Reference point index/Point of departure index	This differs slightly from the HI as the sum of the exposures to each chemical component is expressed as a fraction of their respective RP for effects of toxicological relevance (i.e. NOAEL, LOAEL, BMDL) rather than as a fraction of the HBGV.
Relative potency factor	Approach uses toxicity data for an index chemical in a group of multiple chemicals to 'to determine potency-adjusted concentration or exposure data for chemicals in the mixture' assuming similarity of MoA between individual chemicals in the mixture. Also known as potency equivalency factor (PEF).
Response addition	A component-based mixture model in which the components are treated as if having independent or dissimilar action, i.e. by following the statistical concept of independent random events. Application of response addition requires toxicity data (e.g. mortality, target organ toxicity) to be expressed

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	as a fraction (between 0 and 1), i.e. the percentage of individuals in a population, or species in an ecosystem affected by the mixture or exceeds a reference point (e.g. BDML, EC_{50}). The term 'response addition' is a misnomer as responses are actually not added, but the unaffected fractions of the population are multiplied (see Chapter 6). However, the term is used in this guidance as it is commonly used in the area of mixture risk assessment. See independent action or simple dissimilar action.
Specifications	The specifications define the key parameters that characterise and substantiate the identity of the mixture, as well as the limits for these parameters and for other relevant physicochemical or biochemical parameters. The specifications will be used as key parameters, among other compositional data, to evaluate whether the data provided to demonstrate the safety are relevant to the mixture intended to be placed on the EU market. In addition, the limits set in the specifications for toxicologically relevant components will be considered in the risk assessment.
Stability	The stability of the mixture should be evaluated to identify hazards which might arise during storage and transport. The nature of degradation products should be characterised.
Similar action	Occurs when chemicals in a mixture act in the same way, by the same mechanism/mode of action, and differ only in their potencies (EFSA, 2013).
Simple dissimilar action	Describes the modes of action and possibly, but not necessarily, the nature and site of the toxic effect, when they differ among the chemicals in the mixture. Note Also referred to as simple independent action or independent joint action or response additivity (EFSA PPR, 2008).
Similar mixture	(also known as sufficiently similar mixture). A mixture of chemicals that differs slightly from the mixture of concern, i.e. in components, concentration levels of components, or both. A similar mixture has, or is expected to have, the same type(s) of biological activity as the mixture of concern, and it would act by the same mode(s) of action and/or affect the same toxic endpoints.
Simple mixture	Mixture whose components are fully chemically characterised, e.g. a group of defined substances with potential to have combined effects and therefore subject to mixture risk assessment.
Simple similar action	Describes the mode of action when all chemicals in the mixture act in the same way, by the same mechanism/mode of action, and differ only in their potencies. The effects of exposure to a mixture of these compounds are assumed to be the sum of the potency-corrected effects of each component. Note also referred to as similar joint action or dose additivity or relative dose additivity (EFSA PPR, 2008).
Sum of toxic units	Toxic units (see definition below) can be added to predict mixture effects.
Synergy	The result of an interaction between two or more chemicals resulting in an effect that is more than dose additive or response additive (EFSA PPR, 2008).
Synergism	Pharmacological or toxicological interaction in which the combined biological effect of two or more substances is greater than expected on the basis of the simple summation of the toxicity of each of the individual substances (EFSA, 2013).
Toxic Equivalency Factor (TEF)	TEF expresses the toxicity of a mixture of congeners in terms of the most toxic congener. This approach has been used for e.g. mixtures of dioxins.
Toxic Equivalency Quotient (TEQ)	The total Toxic Equivalent Quotient (TEQ) is defined by the sum of the products of the concentration of each compound multiplied by its TEF value,

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	and is an estimate of the total e.g. 2,3,7,8-TCDD-like activity of a mixture.
Toxic units (TU)	A measure of toxicity as determined by the acute toxicity units or chronic toxicity units. Higher TUs indicate greater toxicity.
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, 2008).
Toxicokinetics	1) Process of the uptake of substances (e.g. pesticides), by the body, the biotransformations they undergo, the distribution of the parent compounds and/or metabolites in the tissues, and their elimination from the body over time. 2) Study of such processes. (EFSA PPR, 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, 2018).
Uncertainty analysis	A collective term for the processes used to identify, characterise, explain and account for sources of uncertainty (EFSA Scientific Committee, 2018). See Section 6.3.
Uncertainty factor	Reductive factor by which an observed or estimated no observed adverse effect level or other reference point, such as the benchmark dose or benchmark dose lower confidence limit, is divided to arrive at a reference dose or standard that is considered safe or without appreciable risk (WHO, 2009).
Variability	Heterogeneity of values over time, space or different members of a population, including stochastic variability and controllable variability (EFSA Scientific Committee, 2018).
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 (see Sections 2.4 and 4.3).
Weighing	In this Guidance, 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 (see Section 2.5).
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.
Whole mixture approach	A risk assessment approach in which the mixture is treated as a single entity, similar to single chemicals, and so requires dose–response information for the mixture of concern or a (sufficiently) similar mixture.

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2492 **Abbreviations**

ADI	Acceptable daily intake
AG	Assessment Group
ARfD	Acute Reference Dose
AOP	Adverse Outcome Pathways
ATSDR	Agency for Toxic Substances and Disease Registry,
BINWoE	Binary weight of evidence
BMD	Benchmark dose
BMDL	Benchmark dose lower confidence limit
CAG	Cumulative Assessment Group
CSAF	Chemical-Specific Adjustment Factor
CONTAM	EFSA Scientific Panel on Contaminants in the Food Chain
DCM	EFSA's Unit on Dietary and Chemical Monitoring
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EMRISK	EFSA's Unit on Emerging Risks
ERA	Ecological/Environmental Risk Assessment
FIP	EFSA's unit on Food Ingredients and Packaging
HBGV	Health-based guidance value
HI BIN interaction	HI modified by binary interactions
HI	Hazard Index
HQ	Hazard Quotient
IPCS	International Programme on Chemical Safety
JRC	Joint Research Centre of the European Commission
LOAEL	Lowest observed adverse effect level
MEA	Mechanism of Action
MOA	Mode of Action
MOE	Margin of Exposure
NOAEL	No observed adverse effect level
NRC	National Research Council
PB-PK	Physiologically based pharmacokinetic models
PB-PK-PD	Physiologically based pharmacokinetic pharmacodynamic models
PB-TK	Physiologically based toxicokinetic models
PB-TK-TD	Physiologically based toxicokinetic–toxicodynamic models
POD	Point of departure
PPR	EFSA Scientific Panel on Plant Protection Products and their Residues
PRAS	EFSA's Unit on Pesticides

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QSAR	Quantitative Structural Activity Relationship
RfC	Reference concentration
RfD	Reference dose
RP	Reference point
RPI	Reference point index
RPF	Relative potency factor
RVs	Reference values
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCER	EFSA's Scientific Committee and Emerging Risks Unit
SCF	Scientific Committee on Food
SCHER	Scientific Committee on Health and Environmental Risks
TDI	Tolerable daily intake
TEF	Toxic equivalency factors
TEQ	Toxic equivalent quotient
TTC	Threshold of Toxicological Concern
TTD	Target Organ Toxicity Dose
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

WOE: Weight of Evidence

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Appendix A – Uncertainty analysis

A.1. Problem formulation

Two important types of uncertainty to consider during the phase of problem formulation are framing 1) uncertainty and 2) ignorance (EFSA Scientific Committee, 2018). Framing uncertainty refers to the situation in which different assessment questions may be obtained when different people are being asked to define the problem, e.g. due to varying problem perceptions or practical considerations (e.g. a lack of data). Framing uncertainty is of minor importance if the details of the assessment (e.g. substances, exposure routes and endpoints) have been specified in legislation or guidance documents such as the EFSA guidance on ecological risk assessment of Plant Protection Products (EFSA, 2013). But if detailed guidance is lacking, there is room for interpretation. It is then essential that the assessor clearly states what is included in the assessment and what is not.

A typical and practical response to complex tasks such as the assessment of combined exposure to multiple chemicals is to limit the scope of the assessment, e.g. to the substances, pathways and endpoints which can be easily assessed. Although there can be legitimate reasons to undertake this, it should be realised that this may mask the uncertainty involved in answering the original (broader) assessment question. The uncertainty of an assessment with a limited scope may be small, but the uncertainty in answering the original assessment question can be large because only part of the original question is being answered. For example, a risk assessment limited to the parent compounds in a pesticide formulation may be very accurate, but it may lack realism if plant metabolites cause the major part of the risk but are excluded because of a lack of data.

Besides framing uncertainty, ignorance may play a role in the problem formulation phase. It is by definition impossible to account for e.g. a particular substance or exposure route if it is not included in the conceptualisation of the problem. Ignorance can result in unanticipated risks, e.g. the exposure of bees through pollen polluted with neonicotinoids (Whitehorn et al., 2012). It is therefore essential that, when defining the problem, risk assessors keep an open eye for phenomena that may influence the risk but that have not been included in the problem formulation.

Typical questions that a risk assessor should ask to identify uncertainties in the problem formulation phase of combined exposures to multiple substances are:

- How well does the problem formulation cover the variation in problem perceptions by the stakeholders involved? (Note: this question is relevant only if the problem formulation has not been specified in detail in legislation or guidance documents.)
- How complete is the conceptual scheme that relates the 'mixture of assessment' to the 'endpoints of assessment'? Have all potentially relevant fate processes (e.g. transformation) and exposure routes been covered?
- Are there any differences between the 'mixture of concern' as defined in the problem formulation and the mixture that was actually addressed during the assessment? Were any exposure pathways, substances, metabolites or endpoints excluded during these assessment phases?
- It is generally not possible to quantify the uncertainty in the problem formulation phase. It is therefore recommended to describe the uncertainty qualitatively and discuss how this uncertainty might influence the conclusion of the original assessment question.

A.2. Exposure assessment

Uncertainties involved in exposure assessment of combined exposure to multiple substances are largely similar to those of single substances. Distinction can be made between exposure assessment for component-based approaches and whole mixture approaches. The main challenge for component-based approach is the completeness of the predicted or measured exposure levels. This is reflected in the following questions:

- Have all relevant substances been included in the exposure assessment? More specifically:
  - Were analytical methods available for all substances in the 'mixture of concern'?

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  - Were potential metabolites and transformation products adequately addressed?
- How were detection limits dealt with? What is the resulting uncertainty?
- Have all relevant routes been included? What is the resulting uncertainty?
- What level of uncertainty is associated with the estimated or measured concentration levels of the substances?

An important question in exposure assessment of whole mixtures is to what extent the concentration ratios between the different mixture components are constant; an implicit assumption of whole mixture approaches. Over time, changes in mixture level and composition may occur resulting in potential differences between the mixture that is being analysed and the mixture of Exposure. Such issues may be identified by answering the following questions:

- What uncertainties are involved in the dose metric used for assessing the exposure to the whole mixture?
- Are concentration ratios in the mixture fixed?
- How may transformation processes have influenced the mixture composition between the moment of analysis of the mixture and the moment of exposure?
- Were these transformation processes adequately accounted for?

A.3. Hazard assessment

Distinction is made between uncertainty in hazard assessment using a component-based approach or a whole mixture approach. The main uncertainties in a component-based approach result from:

- the choice for a particular mixture model, e.g. dose or response addition;
- the grouping of chemicals in cumulative assessment groups (dose addition);
- dealing with substances that have multiple modes of action;
- dealing with lacking data, e.g. lacking reference values, reference points or data on the mode of action of a substance;
- derivation of reference points, Reference values and/or application of uncertainty factors;
- lack of data on potential interaction, i.e. synergism or antagonism.

The default mixture model is dose addition because it generally results in relatively conservative predictions. The level of conservativeness depends on the compounds in the mixture and will be difficult to quantify in practice. The level of conservativeness also depends on the number of substances in an assessment group, i.e. the larger the number of substances in a group, the more conservative the results will be. Detailed information on the mode of action of the compounds is required to quantify the extent of the resulting uncertainty. If data on mode of action are lacking, a conservative assumption is to add these substances to the largest assessment group. A further source of uncertainty in relation to grouping is that a substance may have multiple MoAs. Ideally, the reference point or value that is being used for a compound should be derived for the effect that formed the basis of the grouping. Ignoring components that have several modes of action which fits the group may result in underestimation of the risk, whereas including these components based on their most critical MoA may result in overestimation.

If reference points are lacking, these may be estimated from QSARs or reference values using the TTC concept. The level of uncertainty in such estimates can usually be tentatively estimated based on meta-data of the QSAR and the data used for derivation of the TTC. If using reference points in an assessment, the resulting Margin of Exposure should be sufficiently high to account for uncertainty (e.g. interspecies extrapolation and inter-individual differences in sensitivity) in the reference points that drive the mixture risk. When using a combined Margin of Exposure approach, it should also be checked whether the risk ratios for the individual compounds for which also reference values are available do not exceed unity. When using reference values the uncertainty can be more difficult to address as each reference value has its own case-specific safety factor which may result in combining conservative and less conservative estimates.

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Finally, a potentially important source of uncertainty in the hazard assessment step of component-based approaches is the likelihood of interactions in the mixture. This likelihood may be assessed based on case-specific data. If these are unavailable the risk assessor may consider data from meta-analyses and the application of extra uncertainty factors should be considered on a case-by-case basis. Examples in the ecological area (see Chapter 2) include the analyses by Ross (1996) and Ross and Warne (1997) which indicated that 5 and 1% of mixtures deviated from concentration addition a factor above 2.5-fold by a factor above 2.5-fold and 5-fold respectively. Likewise, Cedergreen (2014) showed that synergy occurred in 7, 3 and 26% of the 194, 21 and 136 binary pesticide, metal and antifoulants mixtures analysed and the difference between predicted and observed effects was rarely more than 10-fold.

For whole mixture approaches, an important uncertainty involved in the hazard assessment is the representativeness of the mixture tested for the mixture of concern. If a mixture sample is tested in the laboratory, changes in mixture composition may occur during transport or in the laboratory. If the results of a sufficiently similar mixture are being used, an effort should be undertaken to assess the maximum deviation in toxicity between the mixture of concern and the sufficiently similar mixture. If safety factors are being applied, these should cover for these differences. Another important potential source of uncertainty is the full coverage of all relevant end-points in the toxicity tests particularly for the ecological area that are being performed with the mixture. For ecosystem protection, multiple species should be tested. Ideally, chronic endpoints such as cancer and food chain accumulation effects for the protection of the ecosystem should also be included in the assessment.

Typical questions that a risk assessor should so ask to identify uncertainties in the hazard assessment phase of combined exposures to multiple substances are:

Component-based approaches:

- What uncertainties are involved in the assumed mixture assessment model, i.e. dose addition, response addition or a combination of the two?
- What level of uncertainty is associated with the grouping of chemicals?
- How to deal with substances for which mode of action-specific endpoints are lacking? What are the associated uncertainties?
- What uncertainties are involved in dealing with substances for which toxicity data are lacking?
- What uncertainties are involved in dealing with potential synergism and/or antagonism?

Whole mixture approaches:

- How representative is the mixture tested for the mixture of concern?
- How well do the toxicity tests cover the endpoints of the assessment? Are chronic endpoints (e.g. cancer, bioaccumulation) sufficiently covered? What are the associated uncertainties?

A.4. Risk characterisation

In the risk characterisation phase, results of the exposure assessment are combined with those of the hazard assessment. Consequently, the overall risk in the risk ratio is a combination of the uncertainties involved in the exposure and hazard assessment steps. Some of these uncertainties probably can be quantified, whereas others cannot. An estimate of the impact of the individual quantifiable uncertainties on the risk estimate may be obtained by propagating these uncertainties through the mixture model that is being used, e.g. the Hazard Index or response addition.

A.5. Stepwise procedure

The insights outlined above result in the following stepwise procedure to analyse uncertainty in the risks of combined exposure to multiple chemicals:

1) Inspect the results of the risk characterisation phase and decide for which mixture components an uncertainty analysis is required.
2) Identify, describe and try to quantify all uncertainties involved in the exposure and hazard assessment.

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3) Propagate the quantifiable uncertainties into an overall uncertainty estimate of the predicted risk.

4) Identify and describe all uncertainties involved in the problem formulation.

5) Report and interpret the results of Steps 1–4.

It is suggested that the results of Steps 2 and 4 are reported in a table listing all identified uncertainties and adding a quantitative estimate of each identified source of uncertainty in a separate column, when possible. The report should conclude whether the calculated risk sufficiently covers the mixture of concern (i.e. uncertainty in problem formulation) and whether quantifiable and unquantifiable sources of uncertainty do not hamper an unambiguous conclusion, i.e. that the risk is acceptable or unacceptable.

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Appendix B – Case study 1: Human health risk assessment of combined exposure to hepatotoxic contaminants in food

B.1. Problem formulation

This case study deals with the application of the harmonised framework to the human risk assessment of a mixture of three hepatotoxic contaminants (C1, C2 and C3) from food sources on a chronic exposure basis. The Terms of Reference requires the mixture risk assessment to be performed for European consumers. The three compounds are well characterised in food including structure, toxicity (hepatotoxicity with likely common MoA) and exposure. On this basis, a component-based approach can be applied for the human risk assessment. The results of the problem formulation are summarised in table 7.

Table 7: Human risk assessment of a mixture of three hepatotoxic contaminants: summary results of the problem formulation

Mixture	Composition	Target species	Exposure patterns	Approach	Grouping criteria
Contaminants	Known	Human: adult European consumers	Chronic	Component-based	Common MoA as grouping criterion

B.2. Exposure assessment

1) Occurrence data were reported for C1, C2 and C3 originating from a number of food commodities in 17 member states in Europe. These compounds were found to occur mainly in rice (60% of the samples), seafood (30% of the samples) and bread (10% of the samples). The proportion of left-censored data (results below the limit of detection (LOD) or limit of quantification (LOQ)) was high and reached 90% for the three compounds in rice, seafood and bread. The LODs and LOQs ranged between 1-10 and 2-20 µg/kg respectively for all sources. Mean and P95 estimates were derived for each compound and food commodity, applying to each estimate lower bound, median bound and upper bound scenarios.

2) . Consumption data were retrieved from EFSA's comprehensive food consumption database which contains dietary consumption data at individual level. For each individual in the database, the average consumption of rice, seafood and bread was calculated.

3) Exposure assessment was performed combining, for each compound and for each commodity, the upper bound mean occurrence data with the corresponding average consumption for each individual in the comprehensive database. The estimates of mean chronic human exposure for all sources and each compound across Member State dietary surveys and age groups ranged from 12-200 ng/kg body weight (bw) per day for C1; 30-450 ng/kg body weight (bw) per day for C2 and 25-250 ng/kg body weight (bw) per day for C3. The estimates at the 95th percentile ranged from 150-500 ng/kg body weight (bw) per day for C1; 320-600 ng/kg body weight (bw) per day for C2 and 175-450 ng/kg body weight (bw) per day for C3. As a conservative scenario, the maximum exposure values for each compound are used as exposure metrics for the risk characterisation namely 500, 600 and 450 ng/kg body weight (bw) per day for C1, C2 and C3 respectively.

B.3. Hazard identification and characterisation

Review of available evidence confirmed that the three compounds likely caused hepatotoxicity by the same MoA, confirming the Assessment Group. For each compound, hazard characterisation was performed using benchmark dose modelling (BMD) from 90-day toxicity studies in rats (6 doses: 0, 10, 20, 30, 50 and 75 and 100 mg kg b.w per day) using Alanine Aminotransferase (ALT) activities as

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the most sensitive biomarker of liver toxicity in the studies. BMD modelling was performed for each compound to derive BMD limits for 10% of effect (BMDL10). BMDL10 for C1, C2 and C3 were 15, 25 and 60 mg/kg b.w per day respectively. No evidence of interactions between C1, C2 and C3 were available from the literature.

B.4. Risk characterisation

The individual exposure metrics and reference points for each compound were combined applying the Reference Point Index (RPI) method to generate a risk metric. The RPI method assumes dose addition between C1, C2 and C3 and is derived from the sum of the ratios of the exposure metrics and reference points on which an uncertainty factor of 100-fold is applied. A RPI below value of 1 is interpreted as not raising health concerns for human health. For the current human risk assessment of combined exposure to multiple contaminants in food, the RPI reflecting the combined risk is 0.006 and does not raise human health concerns for European consumers. The reporting table below summarises the exercise.

Table 8: Reporting Table : Human risk assessment of a mixture of three hepatotoxic contaminants in food

Problem formulation	Description mixture	Simple mixture. Composition: mixture of three contaminants fully characterised (C1, C2 and C3)
	Conceptual model	Exposure to C1, C2 and C3 mixture in European consumers through food. Exposure pattern: chronic. Occurrence available. food consumption available in European consumers Hazard data: reference point for C1, C2 and C3 based on 90 days rat study and hepatotoxicity by common MoA
	Methodology	Grouping compounds using liver toxicity by common MoA as the grouping criteria
	Analysis plan	Risk assessment of contaminant mixtures in food in European consumers
Exposure assessment	Mixture composition WMA CBA	Mixture of C1, C2 and C3 Component-based approach
	Summary occurrence data	Occurrence in food from 17 Member States in samples of rice (60%), seafood (30%) and bread (10%)
	Summary exposure	Mean occurrence in food for each component (95th centiles) combined with mean individual chronic consumption from EFSA comprehensive food consumption database for each MS (mean chronic)
	Assumptions	Maximum exposure used for chronic exposure assessment (conservative)
	Uncertainties	High proportion of left censored occurrence data. Maximum exposure used (overestimation of exposure)
Hazard identification and hazard characterisation	Mixture composition WMA/CBA	Component-based approach-assessment group and set using liver toxicity as grouping criteria
	Reference points	Reference point for each component as BMDL10 from 90-day studies in rats using alanine aminotransferase as the most sensitive biomarker of liver toxicity in the studies
	Combined toxicity	Dose addition
	Summary hazard metrics	$BMDL_{10}$ values for each component
	Uncertainties	Uncertainties in $BMDL_{10}$ values for each component particularly for interspecies extrapolation (rats to humans)
Risk characterisation	Decision points	Apply Reference Point Index (RPI) method
	Assumptions	Dose addition
	Summary risk metrics	RfPI
	Uncertainties	Uncertainties in exposure, hazard and RPI: Conservative approach
	Interpretation	An RfPI of 0.006 does not raise human health concerns

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Appendix C – Case study 2: Animal health risk assessment of botanical mixtures in an essential oil used as a feed additive for fattening in chicken

C.1. Problem formulation

An essential oil (a mixture of botanical origin) is used as flavouring feed additive in the diet of chickens for fattening (target animal species). Each substance in the mixture has been identified and the relative amount in the essential oil determined. Co-exposure to the components of the essential oil in chickens for fattening occurs on a daily basis from hatching to 35 days. Thirteen substances have been identified and account for 100% of the composition of the feed additive. A component-based approach can be applied for the risk assessment. The results of the problem formulation are summarised in table 9.

Table 9: Animal health risk assessment of botanical mixtures in an essential oil used as a feed additive for fattening in chicken : summary results of the problem formulation

Mixture	Composition	Target species	Exposure patterns	Approach	Grouping criteria
Essential oil	Known 13 components	Chicken for fattening	From hatching to 35 days	Component based	Assessment groups using Flavouring groups[1] as the grouping criteria

C.2. Exposure assessment

1) The maximum proposed use levels of the essential oil in feed (e.g. 20 mg/kg) is combined with the maximum percent amount of each component in the oil to provide their maximum occurrence in feed.

2) The maximum occurrence values are combined with feed consumption patterns in the chicken (default values: body weight (bw) 2 kg; feed intake 79 g/kg bw; EFSA FEEDAP Panel, 2017) to derive exposure metrics on a body weight basis (mg/kg bw per day).

C.3. Hazard identification and characterisation

All substances in the essential oil were characterised as flavourings and assessment groups (AG) are set for all components using flavouring groups (FL) as the grouping criteria. Reference points for each substance in each assessment group are collected from the open source EFSA OpenfoodTox Database[2] as NOAELs from sub-chronic rat studies (90 days) expressed on a body weight basis (mg/kg bw per day). In the absence of reference points for a specific substance, the reference point for a similar compound in the flavouring group (read across) is used or the 5th percentile of the distribution of the NOAELs of the corresponding Cramer Class is applied (threshold of toxicological concern approach). Combined toxicity is assessed using the dose addition assumption since no evidence for interactions is available.

C.4. Risk characterisation

[1]As defined in Annex I of Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council. OJ L 180,19.7.2000, p. 8

[2]https://zenodo.org/record/344883#.WquUCfnwbIU

Dose addition is applied to combine the exposure metrics and reference points for each assessment group and the method of choice is the combined (total) margin of exposure (MOET). The summary results for the exposure metrics, hazard metrics and the combined margin of exposure are given in the table below. A combined margin of exposure of 100-fold is interpreted as safe for the target species allowing for a 100-fold safety factor. The combined margins of exposure for FL-1, FL-2, FL-3 and FL-4 were 1389, 212, 380 and 632 and do not raise health concerns for chickens for fattening. Summary of the results are presented in the table 10 below and in the reporting table (table 11).

Table 10: Summary of the results for the animal health risk assessment of botanical mixtures in an essential oil used as a feed additive for fattening in chicken

AG	Compound	% compound in botanical mixture	Use level mg/kg	Feed [C] mg/kg	Exposure metrics mg/kg bw per day	Hazard metrics mg/kg bw per day	Risk metrics MOE	MOET
FL-1	A	0.5	20	0.10	0.0079	100	12,658	
FL-1	B	1	20	0.20	0.0158	100	6,329	
FL-1	C	5	20	1.00	0.079	200	2,532	
FL-1	D	0.5	20	0.10	0.0079	90	11,392	
FL-1								1,389
FL-2	E	36	20	7.20	0.5688	150	264	
FL-2	F	10	20	2.00	0.158	300	1,899	
FL-2	G	5	20	1.00	0.079	200	2,532	
FL-2								212
FL-3	H	25	20	5.00	0.395	150	380	
FL-4	I	5	20	1.00	0.079	170	2,152	
FL-4	J	2	20	0.40	0.0316	170	5,380	
FL-4	K	3	20	0.60	0.0474	170	3,586	
FL-4	L	5	20	1.00	0.079	170	2,152	
FL-4	M	2	20	0.40	0.0316	170	5,380	
FL-4								632

(a) Assessment groups (AG) as defined in Annex I of Commission Regulation (EC) No 1565/2000.

(b) MOE: margin of exposure

(c) MOET: combined margin of exposure, calculated as the reciprocal sum of the reciprocals of the MOE of the individual substances (MOET(1-n)= 1/[(1/MOE1)+…+(1/MOEn)])

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2749 **Table 11:** Reporting Table: Animal health risk assessment of botanical mixtures in an essential oil
2750 used as a feed additive for fattening in chicken

Problem formulation	Description mixture	Simple mixture. Composition: a fully characterised essential oil used as a flavouring feed additive with 13 components
	Conceptual model	Exposure to the components of the essential oil in chickens for fattening. Exposure pattern in chickens for fattening from hatching to 35 days at the maximum use. Hazard data collection: reference point for each component of the essential oil
	Methodology	Component-based approach. Assessment group set using flavouring groups as grouping criteria
	Analysis plan	Risk assessment of flavourings in an essential oil used as a feed additive for fattening in chickens for fattening - Ccomponent-based approach
Exposure assessment	Mixture composition CBA	13 compounds/4 flavouring groups. Component-based
	Summary occurrence data	Maximum proposed use levels of essential oil in feed combined with Maximum relative percentage of each component in the essential oil to derive maximum occurrence data in feed for each component
	Summary exposure	Maximum occurrence data in feed for each component combined with feed consumption in chickens for fattening (see table of results)
	Assumptions	Maximum used levels, occurrence and feed consumption in chickens for fattening
	Uncertainties	Uncertainties in exposure: conservative assumptions with maximum use levels and occurrence: Conservative overestimation
Hazard identification and hazard characterisation	Mixture composition WMA/CBA	Component-based approach-assessment group set using flavouring substance groups as grouping criteria: Four assessment groups (FL-1, FL-2, FL-3, FL-4)
	Reference points	Reference point for each component of each assessment group (using NOAEL 90-day studies in rats)
	Combined toxicity	Dose addition
	Summary hazard metrics	Range of NOAEL values for each FL group (mg/kg bw per day): FL-1 (4 compounds): 90–200; FL-2 (3 compounds): 150–300; FL-3 (1 compound): 150; FL-4 (4 compounds): 170
	Uncertainties	Uncertainties in reference points particularly for interspecies extrapolation (rat to–chicken)
Risk characterisation	Decision Points	Apply combined Margin of Exposure (MOET)
	Assumptions	Dose addition
	Summary Risk Metrics	Combined Margins of Exposure for each flavouring group: MOET values for FL-1:1389, FL-2: 212 FL-3:380 and FL-4:632
	Uncertainties	Uncertainties in exposure, hazard and MOET: Conservative (maximum use levels and occurrence, 100-fold uncertainty factor (rat to chicken)
	Interpretation	The combined Margin of Exposure does not raise health concerns for chickens for fattening

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Appendix D – Case study 3: Quantifying the impact of binary mixture interactions on hazard characterisation in bees

D.1. Problem formulation

This case study deals with the application of the harmonised framework to the risk assessment of a binary mixture of chemicals in adult honey bee workers. The two compounds are well characterised including structure and toxicity dose response and a component-based approach can be applied for hazard characterisation. The results of the problem formulation are summarised in table 12.

Table 12: Quantifying the impact of binary mixture interactions on hazard characterisation in bees : summary results of the problem formulation

Mixture	Composition	Target species	Exposure patterns	Approach	Grouping criteria
Binary mixture of chemicals	Known	Bee workers	Acute mortality	Component-based	Assessment groups using mortality end-point

D.2. Hazard identification and characterisation

For each compound, hazard characterisation is performed using available individual dose responses in adult bees for chemical A (chem A), chemical B (Chem B) and for a single ratio binary mixture (equitoxic at LC_{50}) using percentage of survival as the endpoint of interest as shown in the figure below. The experimental dose responses are given for each compound, the binary mixture (observed mixture) and the predicted effect of the mixture using the concentration addition model (CA predicted) and are plotted against a toxic unit adjusted-dose (toxic units with a toxic unit of 1 equal to the 50% survival or the reciprocal of the LD_{50}). The TU dose needed to cause an observed effect of interest (e.g. 50% mortality) in the mixture exposures are then compared to the TU dose expected from the combined toxicity prediction to determine the model deviation ratio (MDR) of the combined toxicity. The results in figure 10 demonstrate deviation from concentration addition and a synergy between the chemical A and B in the binary mixture with a model deviation ratio of 5 at the LD_{50} level (i.e. the mixture dose causes the expected effects at a dose that is 5 fold below the effect caused by the single compound). The MDR derived from the comparison of the modelled predicted data vs the observed experimental data can be applied as a mixture adjustment factor ($M_{IX}AF$)[3].

Summary of the results of this exercise quantifying the impact of binary mixture interactions on hazard characterisation in bees are presented in the reporting table (table 13).

[3] Risk assessors should note that the size of the MDR will depend on the relative toxic units applied and the relative potencies of chemical A and B. In some cases, the slopes of the observed and predicted effects for the binary mixtures may be very dissimilar and MDR values can be determined at lower doses of relevant environmental exposure. Accuracy of the results should be assessed and reported.

Figure 10: Hazard characterisation of a single ratio binary mixture in adult honey bee workers: Comparison of effect prediction using concentration addition and experimental data for the characterisation of model deviation ratio

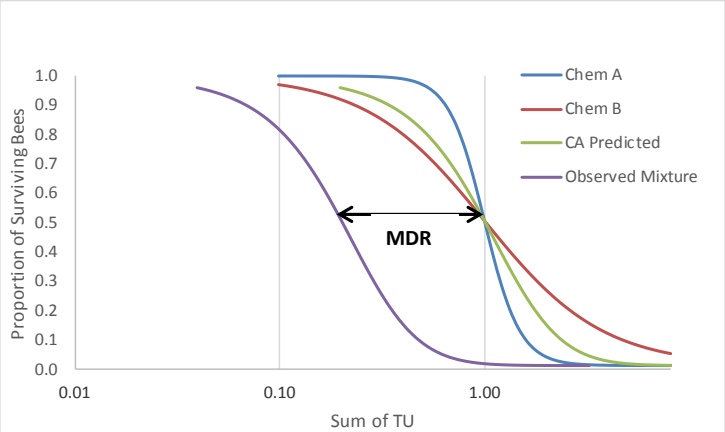

Table 13: Reporting Table: quantifying the impact of binary mixture interactions on hazard characterisation in bees

Problem formulation	Description mixture	Simple mixture. Composition: single ratio binary mixture (equitoxic at LC_{50}) fully characterised
	Conceptual model	Hazard characterisation of binary mixtures in bees through dose–response analysis Exposure pattern in bees is acute. Hazard data collection: dose–response data and reference point expressed as oral acute mortality in bees for each component of the binary mixture
	Methodology	Component-based approach. Grouping compounds using oral acute mortality end-point as the grouping criteria
	Analysis plan	Risk assessment of a binary mixture of chemicals in bee workers
Hazard identification and hazard characterisation	Mixture composition WMA/CBA	Component-based approach-assessment group and set using oral acute mortality
	Reference points	Full dose response and reference Points available for each component (A and B) and single ratio binary mixture (equitoxic at LC50)
	Combined toxicity	Interaction: Synergy with Model Deviation Ratio (MDR) of 5
	Summary hazard metrics	Dose response curve for compound 1, 2 and the single ratio binary mixtures. MDR of 5 can be applied as a mixture Assessment factor (MixAF) for the binary mixture to take into account synergistic effects. Application of the MixAF proposed for the risk characterisation step using the hazard index modified for binary interactions.
	Uncertainties	Uncertainties in acute lethal doses (LD_{50}) and maximum deviation ratio for the binary mixture

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