CONSIDERATIONS FOR ASSESSING THE RISKS OF COMBINED EXPOSURE TO MULTIPLE CHEMICALS

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IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among **FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD**

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About the OECD

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This publication was developed in the IOMC context. The contents do notnecessarilyreflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Foreword

OECD member countries have been making efforts to expand the use of alternative methods in assessing chemicals. The OECD has been developing guidance documents and tools for the use of alternative methods such as (Quantitative) Structure Activity Relationship ((Q)SAR), chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches for Testing and Assessment (IATA).

The goal of this document is to overview the technical aspects of the various approaches and methodologies available with respect to the assessment of risks from combined exposures to multiple chemicals. The document draws from approaches applied and experience gained in the regulatory context and will therefore be most relevant to the regulatory authorities addressing chemicals, the regulated community and other interested stakeholders. The considerations are not presented as strict guidance but rather elements to recognise in assessing combined exposures to multiple chemicals. The concepts are often presented at a more general level as the aim is to address multiple potential assessment scenarios of different types of combined exposures.

This development of this document was led by Canada and the OECD secretariat. An initial draft was developed with contributions from Australia, Canada, the European Commission (Joint Research Centre), Sweden, the United States and the OECD secretariat, followed by revision based on reviews by a project team, the Working Party on Hazard Assessment, Working Party on Exposure Assessment and Working Group on Pesticides.

This report is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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List of Abbreviations

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism, Excretion
AO	Adverse Outcome
AOP	Adverse Outcome Pathway
BCF	Bioconcentration Factor
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower Confidence Limit
CA	Concentration Addition
CAS	Chemical Abstracts Service
CBA	Component Based Approach
DA	Dose Addition
DNEL	Derived No Effect Level
EC	Effect Concentration
ERA	Ecological Risk Assessment/Environmental Risk Assessment
EQS	Environmental Quality Standard
GHS	Globally Harmonised System (for the classification of chemicals)
HI	Hazard Index
HPV	High Production Volume
HQ	Hazard Quotient
HRA	Human Health Risk Assessment
HTS	High Throughput Screening
IATA	Integrated Approaches to Testing and Assessment
IC	Index Chemical
IRA	Integrated Risk Assessment
IA	Independent Action
KE	Key Event
KER	Key Event Relationship
Kow	Octanol-water Partition Coefficient
logKow	log of the Octanol-water Partition Coefficient
LC50	Concentration of a compound that causes 50% lethality

LD50	Dose of a compound that causes 50% lethality
LO(A)EL	Lowest Observable (Adverse) Effect Level
MAF	Mixture Assessment Factor
MCR	Maximum Cumulative Ratio
MIE	Molecular Initiating Event
MOE	Margin of Exposure
MOET	Combined Margin of Exposure
NO(A)EL	No Observable (Adverse) Effect Level
NOEC	No Observed Effect Concentration
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically based Pharmacokinetic
PBTK	Physiologically based Toxicokinetic
PEC	Predicted Environmental Concentration
PNEC	Predicted No Effect Concentration
POD	Point of Departure
PODI	Point of Departure Index
RA	Response Addition
RfD/RfC	Reference Dose/Concentration
RPF	Relative Potency Factor
RQ	Risk Quotient
SAR	Structure Activity Relationship
TK	Toxicokinetics
TK-TD	Toxicokinetic-Toxicodynamic
TTC	Thresholds of Toxicological Concern
QSAR	Quantitative Structure Activity Relationship
VOC	Volatile Organic Compound
WMA	Whole Mixture Approach
WoE	Weight of Evidence

Chapter 1. Introduction and Scope

Under an updated Cooperative Chemicals Assessment Programme, in 2014 the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology (Joint Meeting) identified exchange of experience on new hazard assessment methodologies, in particular for the assessment of risks from the combined exposure to multiple chemicals as a priority area of work. The overall objective of the Programme remains to support member countries and other stakeholders to improve the effectiveness of assessing and managing chemicals while finding efficiencies and saving resources. This contributes to meeting the objectives set out by the OECD Council Decision-Recommendation on the Co-operative Investigation and Risk Reduction of Chemicals, as well as those by the Strategic Approach to International Chemicals Management (SAICM) and the World Summit on Sustainable Development.

Harmonised methodologies and approaches for assessing chemicals ensure consistency in how information is interpreted by different stakeholders. They also generate confidence and support for integrating novel tools and approaches into the regulatory decision-making process. International cooperation and coordination is crucial for agreement on how novel methods can be used to refine, reduce and/or replace the conventional way of assessing and testing chemicals and concomitantly increase the mutual acceptance of hazard assessments. Consequently, this will result in the best use of all available resources and avoid duplication of efforts.

The goal of this document is to overview the technical aspects of the various approaches and methodologies available with respect to the assessment of risks from combined exposures to multiple chemicals to help identify where further alignment in scientific considerations can be made between member countries. The document draws from approaches applied and experience gained in the regulatory context and will therefore be most relevant to the regulatory authorities addressing chemicals, the regulated community and other interested stakeholders. The considerations are not presented as strict guidance but rather elements to recognise in conducting an assessment of risk of combined exposures to multiple chemicals. The concepts are often presented at a more general level as the aim is to address multiple potential assessment scenarios of different types of combined exposures - from intentional to environmental mixtures resulting from one or multiple sources of release and/or use(s).

Note that although aggregate exposure of individual chemicals is considered in the context of how to combine exposures from multiple chemicals; methodologies specific to aggregating exposure of individual chemicals are not included.

Both human health and environmental risk assessment aspects are considered. These may be further developed in one field or the other for various aspects. When possible, the document aims to be general and encompassing enough to address both aspects, however in certain parts of the document, specific considerations for human health or the environment are delineated.

In addition, while it is recognised that non-chemical stressors (such as disease state, nutritional status and diet, psychological stressors and many more), can affect an organism's response to chemical exposures, the focus of this document is only on chemical stressors.

This document builds upon the recommendations from the WHO OECD ILSI/HESI International Workshop on Risk Assessment of Combined Exposures to Multiple Chemicals (OECD, 2011) and other existing guidance and approach documents in order to examine the technical aspects of performing a hazard, exposure and risk assessment in this context and to provide further guidance in the following areas:

- Considerations for problem formulation providing guidance for prioritising and scoping of an assessment of combined exposures.

- Considerations regarding hazard characterisation to inform assessment of combined exposures.

- Considerations regarding co-exposure characterisation to inform assessment of combined exposures.

- Considerations regarding risk assessment of combined exposures using various approaches and capturing and communicating uncertainties in findings.

The document does not provide a strict schema to follow, due to the many legislative or regulatory questions that could be covered by the approaches; however a general framework is recommended and forms the organisation of the document. It is structured with problem formulation and scoping first in order to contextualise the combined exposure assessment being considered and outline the potential data availability and needs and the relevance for conducting a combined exposure assessment. This follows with considerations for hazard and co-exposure characterisation and the application of risk characterisation through a tiered approach.

This project is also linked to other work of the OECD Chemicals Programme such as the application of Integrated Approaches to Testing and Assessment (IATA), the generation, acceptance and exchange of data as implemented under the framework of OECD's Mutual Acceptance of Data (MAD) and information dissemination through tools such as the Global Portal to Information on Chemical Substances (www.echemportal.org). Further information on IATA related projects are available on the OECD website (http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm) and information on grouping and read-across in the OECD Guidance on Grouping of Chemicals (OECD, 2014).

Chapter 2. Terminology

In order to be consistent in wording throughout this document key terminology is based upon that developed through previous WHO/IPCS, OECD, ILSI/HESI, EU initiatives (WHO, 2009; Meek et al., 2011; OECD, 2011; EFSA, 2013; Kienzler et al., 2014). Further terminology can be found in the Glossary of Terms Related to this Document (page 98).

- **single chemical, all routes / aggregate exposure**: exposure to the same substance from multiple sources and by multiple routes

- **combined exposure**: exposure to multiple chemicals by a single route and exposure to multiple chemicals by multiple routes, from one or multiple sources of release and/or use(s)

- **combined hazard**: hazard from multiple substances by a single route or from multiple substances by multiple routes, from one or multiple sources of release and/or use(s)

- **risk from combined exposures**: risk from exposure to multiple substances by a single route and risk from exposure to multiple substances by multiple routes, from one or multiple sources of release and/or use(s)

- **risk assessment of combined exposures**: risk assessment of exposure to multiple substances by a single route and risk assessment of exposure to multiple substances by multiple routes, from one or multiple sources of release and/or use(s)

The terms **cumulative exposure**, or **cumulative risk**, have specific connotations in some national programmes, and 'combined exposures' is used preferentially in this document unless referring to a document or national programme using this specific terminology. For example, the US pesticide programme defines cumulative risk assessment as an evaluation of the potential for people to be exposed to more than one pesticide at a time from a group that share an identified common mechanism of toxicity.

The term **co-exposure** is used in the document as a synonym to combined exposure, defined above.

In addition, the following terminology is used in the context of this project, drawing from various references (UN, 2011; Bunke et al., 2014; Kienzler et al., 2014; EFSA, 2014; ECHA, 2017a):

- **substance:** a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent, which may be separated without affecting the stability of the substance or changing its composition

  (note that '**chemical**' and '**substance**' are used synonymously in this document)

- **mixture:** co-existing set of two or more substances in which they do not react

The word "mixture" has many connotations and interpretations. In the context of this document, the word mixture is not used when defining "risk assessment to combined exposures", but the term is used to describe scenarios where a target site is exposed to a discrete mixture within the "combined exposures" definition. Mixtures can be organised into the following categories, although they are not necessarily mutually exclusive (e.g. a coincidental mixture in an environmental compartment could be considered an environmental mixture). This document focuses equally on all of these categorised mixtures:

- **Intentional mixtures:** manufactured formulations e.g. commercial mixtures of industrial substances; technical mixtures; product formulations

- **Discharge mixtures:** substance combinations that are emitted by a single industrial site e.g. effluent of a production site

- **Coincidental mixture:** substances from different sources, occurring in a medium e.g. combination of substances applied dermally from use of two or more product formulations

- **Environmental mixtures:** substance combinations in one environmental compartment e.g. substances found in soil from various exposure sources (application of product formulation, deposition from air, water run-off, etc.).

Chapter 3. Background on Assessment of Risk from Combined Exposures to Multiple Chemicals

3.1. Introduction

There is a general recognition that the assessment of chemicals on an individual basis does not reflect conditions in the environment or in humans, where a target site is typically exposed to various chemicals at the same time. This includes natural and anthropogenic chemicals. Therefore, emphasis has been shifting from the traditional single-chemical risk assessment approach used historically, to consideration of risk scenarios that integrate multiple sources, stressors, pathways and effects on a community-relevant scale, in order to provide a more realistic, and possibly, a more protective approach (NEJAC, 2004; Hynes and Lopez, 2007; NRC, 2009).

Assessment of toxicity and risks of combined exposure to multiple chemicals is a concept that has been evolving over decades. First, attempts were made to assess the combined effects of chemicals before assessing the risks. In 1926, the German pharmacologist Loewe formulated the concept of additivity of chemicals (Loewe and Muischnek, 1926) and as early as 1939, Bliss published an article discussing the joint action of substances (Bliss, 1939). Risk assessments have been performed for various types of combined chemical exposures such as classes of substances (pesticides, asbestos fibres, dioxins, metal moieties) and for the evaluation of contaminated sites, surface waters and indoor air (see Chapter 4.4). These assessments can be retrospective (e.g. contaminants already released into the environment) or prospective (e.g. predictive of future combined exposures). At the international level, there have been numerous evolving frameworks put forward to capture the considerations, uncertainties and methodologies for use in the risk assessment of combined exposure to multiple chemicals. One of the main recurring themes in many of these frameworks is the need for a well-developed problem formulation in order to define the priority and the scope of the assessment.

3.2. Brief overview of key initiatives regarding risk assessment of combined exposure to multiple chemicals

Over the last decade, there have been significant advances in the field of combined exposure assessment internationally, accompanied by the publication of various guidance documents, frameworks and position papers. A common theme linking many of the approaches is the recommendation of a tiered approach and "fit for purpose" strategy for the risk assessment of combined exposures to multiple chemicals. Summarised below is a non-exhaustive selection of more recent and significant international and national initiatives, grouped geographically. Key historical milestones in the evolution of risk assessment of combined exposures can be found in several review articles such as MacDonell et al. (2013) and Sexton (2012).

The World Health Organisation (WHO) and the International Program on Chemical Safety (IPCS) human health-focussed initiatives have been ongoing for almost a decade in the

form of workshops and position papers (WHO, 2009; Meek et al., 2011; Meek et al., 2013), including the WHO OECD ILSI/HESI International Workshop on Risk Assessment of Combined Exposures to Multiple Chemicals (OECD, 2011). The objective of the Framework for Risk Assessment of Combined Exposures to Multiple Chemicals (Meek et al., 2011) (see Figure 1) was to develop a "fit for purpose" assessment strategy that uses only the resources necessary to support a decision. This framework outlines a tiered approach that can be applied based on available data, in consideration of the methods and the level of refinement possible, for conducting the hazard and exposure assessments and subsequent risk characterisation. This document builds upon this framework, in addition to considering aspects from other frameworks and advances in assessment approaches for chemicals.

Figure 1. A conceptual representation of the WHO/IPCS framework for assessing risk from combined exposure to multiple chemicals
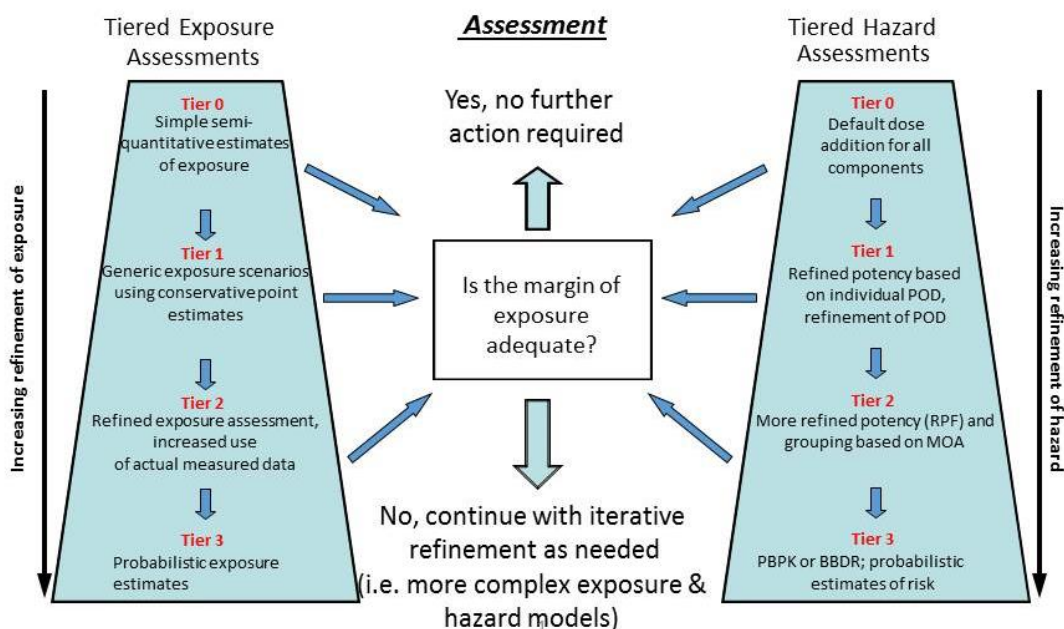
Note: POD=Point of Departure; RPF=Relative Potency Factor; the Margin of Exposure of a substance is the ratio between an effect level and the predicted exposure.
Source: Adapted from Meek et al., 2011

More recently, the WHO has focused efforts on chemical mixtures in source-water and drinking-water, publishing a document which provides an overview of available tools and practical recommendations to support the assessment and management of risks to human health associated with chemical mixtures in drinking-water and its sources, including through use of case studies (WHO, 2017).

The European Chemicals Agency (ECHA) has developed guidance documents for the intentional use of biocidal products to support cumulative risk assessment to the environment (2014) required by the Biocidal Products Regulation, which regulates the placing of intentional mixtures on the market. In line with most other agencies, a tiered approach for mixture assessment in the environmental risk assessment of biocidal products was developed with corresponding decision trees applied at the initial screening step as well as at the assessment level (ECHA, 2014). ECHA has also developed guidance for the risk assessment from combined exposure to multiple biocidal substances (ECHA, 2015) that follows the principles outlined within the WHO/IPCS Framework on Combined Exposures (Meek et al., 2011). The guidance explores how a component based risk assessment may be performed when it includes substances with varying levels of hazard and fate information. Uncertainties are introduced from the use of reference values derived based on a variety of endpoints (NOEC/EC50), species, trophic level and assessment factors.

The European Food Safety Authority (EFSA) has also made efforts in developing tiered approaches for risk assessment of combined exposures. In the area of human health, EFSA has proposed approaches for evaluation of pesticides with similar and dissimilar MOAs as well as different methodologies for the human risk assessment of combined exposures to multiple contaminants (EFSA, 2013a,b). EFSA highlighted the potential applications of physiologically-based models, 'omics and *in silico* tools for the hazard assessment of combined exposure to multiple chemicals (EFSA, 2014a). In the area of environmental risk, methodologies to deal with combined toxicity of pesticides on different non-target organisms such as birds and mammals, bees, aquatic organisms and terrestrial plants have been presented (EFSA, 2009a, 2012a, 2013c, 2014b,c). EFSA is supporting the development of technical tools such as Monte Carlo risk assessment software made scalable for large cumulative assessment groups (van der Voet et al., 2016) and has also increased international dialogue and provided recommendations for future work in the area to move towards harmonisation of methodologies in the form of workshops and published reports (EFSA, 2014c, 2015). In 2018, EFSA launched consultations on draft documents for MIXTOX, a draft Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA, 2018a) and on a draft statement "Genotoxicity assessment of chemical mixtures" (EFSA, 2018b).

Three scientific committees of the European Commission also summarised the general principles and methodologies of mixture toxicology and assessment for humans and the environment (SCHER, SCCS, SCENIHR (2012)). The committees emphasized the importance of screening, i.e. the application of filters to allow a focus on mixtures of potential concern. As mentioned above the committees also proposed a decision tree for evaluating risk assessment of combined exposure.

A report by the Joint Research Centre (JRC) of the European Commission provides a review of methodologies, current legislation in the EU that address the assessment of chemicals in different matrices, an overview of the extent to which the current legislation addresses the risk of mixtures, as well as a summary of other international work in both an ecological and human health context (Kienzler et al., 2014). Efforts continued with JRC conducting a survey to collect information on experiences and opinions on methodologies currently used for the risk assessment of chemical mixtures from various fields in order to develop a consistent assessment approach (Bopp et al., 2015). This report highlighted the need for more guidance on the use and application of these tools in the hazard assessment of mixtures from both an environmental and human health perspective. A review of case studies on the human and environmental risk assessment of combined exposures to multiple

chemicals has also been published in 2016 aiming at identifying priorities, methodologies, data gaps and future needs (Bopp et al., 2016; Kienzler et al., 2016b).

A multi-partner EU project called EuroMix (European Test and Risk Assessment Strategies for Mixtures; https://www.euromixproject.eu) was launched in May 2015 that aims to develop a tiered test strategy, bioassays and models to perform future risk assessment of chemical mixtures, including case studies. The results of the project will be made available on the website and in scientific publications. Other projects include EDCMix Risk (http://edcmixrisk.ki.se/), an EU project focusing on the effects of mixtures of endocrine disruptive chemicals on children; Denamic (http://www.denamic-project.eu/), which focused on developmental neurotoxicity of mixtures in children; and Acropolis (http://acropolis-eu.com/), which focused on the aggregate and cumulative risk of pesticides. Acropolis is the predecessor of EuroMix.

Also in the EU context, the research project SOLUTIONS and the European monitoring network NORMAN has analysed challenges that the European Union Water Framework Directive faces in regards to chemical assessment and management in European surface water resources and recommends more holistic chemical assessments (Brack et al., 2017). An overview of current activities in the area of combined exposure to multiple chemicals at European level can be found in Bopp et al., 2018.

Another tiered component-based approach for risk assessment of mixtures was proposed on behalf of the German Federal Environment Agency (Bunke et al., 2014). It links the current mixture risk assessment methodology with data requirements and the assessment philosophy according to REACH and proposes options for an assessment of technical mixtures under REACH, including prioritisation approaches. In addition, the Federal Office of Consumer Protection and Food Safety (BVL), the Federal Institute for Risk Assessment (BfR) and the German Federal Environment Agency (UBA), developed German guidance on cumulative risk assessment for plant protection products in order to provide: (1) the current scientific understanding of the regulatory requirements; (2) the available options for implementation; (3) guidance on the current practice in cumulative risk assessment (CRA) (Solecki et al., 2014; Stein et al., 2014; Frische et al., 2014).

The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) and the European Chemical Industry Council (Cefic) contributed to the efforts in the field in the form of workshops and presented a framework, which retrospectively allowed the evaluation of the potential impact of chemicals or combined exposures to multiple chemicals in the environment (ECETOC, 2011a,b). Cefic also initiated the development of a decision tree for mixtures that builds on the work of the committees of the European Commission and the tiered frameworks of the WHO/IPCS (Price et al., 2012a,b). More recently, the Health and Environmental Sciences Institute (HESI) has developed a framework for cumulative risk assessment in the 21st century in the context of its RISK21 work (Moretto et al., 2016) and also problem formulation guidance for assessment of risk to combined exposures (Solomon et al., 2016).

Several North American governmental organisations including the United States Environmental Protection Agency (US EPA), US Food and Drug Administration (FDA), the US Agency for Toxic Substances and Disease Registry (ATSDR), Health Canada (HC) and Environment and Climate Change Canada (ECCC) have been involved in the assessment of combined exposures to multiple chemicals. The US EPA's "'Framework for Human Health Risk Assessment to Inform Decision Making" highlights the important roles of planning and scoping, as well as problem formulation, in designing a risk assessment (US EPA, 2014). Similar to the WHO/IPCS framework, it emphasizes the importance of

the "fit for purpose" concept in risk assessment. Although assessment of multiple chemicals was not a main component of this more recent framework, it summarises the efforts made by this agency to advance the understanding and application of this concept from both an ecological and human health perspective. The US EPA has committed to further develop methods on assessment of cumulative risk, in particular the environment, as part of their 2016-2019 strategic research action plan (US EPA, 2015). In 2016, the US EPA published the Pesticide Cumulative Risk Assessment: Framework for Screening Analysis (US EPA, 2016) which provides guidance on how to screen groups of pesticides for cumulative evaluation including establishing common mechanism groups. Also the 2017 report by the US National Academy of Sciences "Using 21st Century Science to Improve Risk-Related Evaluations" considers the assessment of cumulative exposure and exposure to mixtures and the integration of different types of information (NAS, 2017). In 2018, ATSDR published a "Framework for Assessing Health Impacts of Multiple Chemicals and Other Stressors" (ATSDR, 2018).

The Canadian Environmental Assessment Review Office published a guide on addressing cumulative environmental effects in relation to the combined effects of human activities on the ecosystem (CEARO, 1994). This guide briefly outlined a framework for addressing environmental effects from combined exposures, which included early thinking on the level of effort required and effective scoping. More recently, Health Canada and Environment and Climate Change Canada proposed a tiered approach following the WHO/IPSC framework for assessing the potential ecological and human health risk from combined exposures to certain phthalates in Canada, including key considerations for assessment (Health Canada and Environment Canada, 2015). This was followed by a proposal from Health Canada outlining a cumulative risk assessment framework for the health assessment of pesticides (Health Canada, 2017); this framework also mirrored that put forth by WHO/IPCS.

3.3. General concepts for risk assessment of combined exposure to multiple chemicals

Several methods are available for assessing risks from combined exposure to multiple chemicals. These are further outlined in this document. In general, these methods can be applied in a tiered approach ranging from hazard and exposure assessment with a large number of assumptions, to the use of more complex methods requiring large amounts of data. Each approach has its own strengths and limitations and the most appropriate one to use depends largely on the purpose of the assessment and the amount of data available for the substances being evaluated. A general framework to consider these aspects is outlined in Figure 2. This framework also applies to the structure of the chapters of this document focusing on problem formulation and scoping, hazard and exposure assessment, risk characterisation and documentation of uncertainties.

Figure 2. Overall framework for assessment of combined exposures to multiple chemicals including key elements that are further described within each chapter of the document.
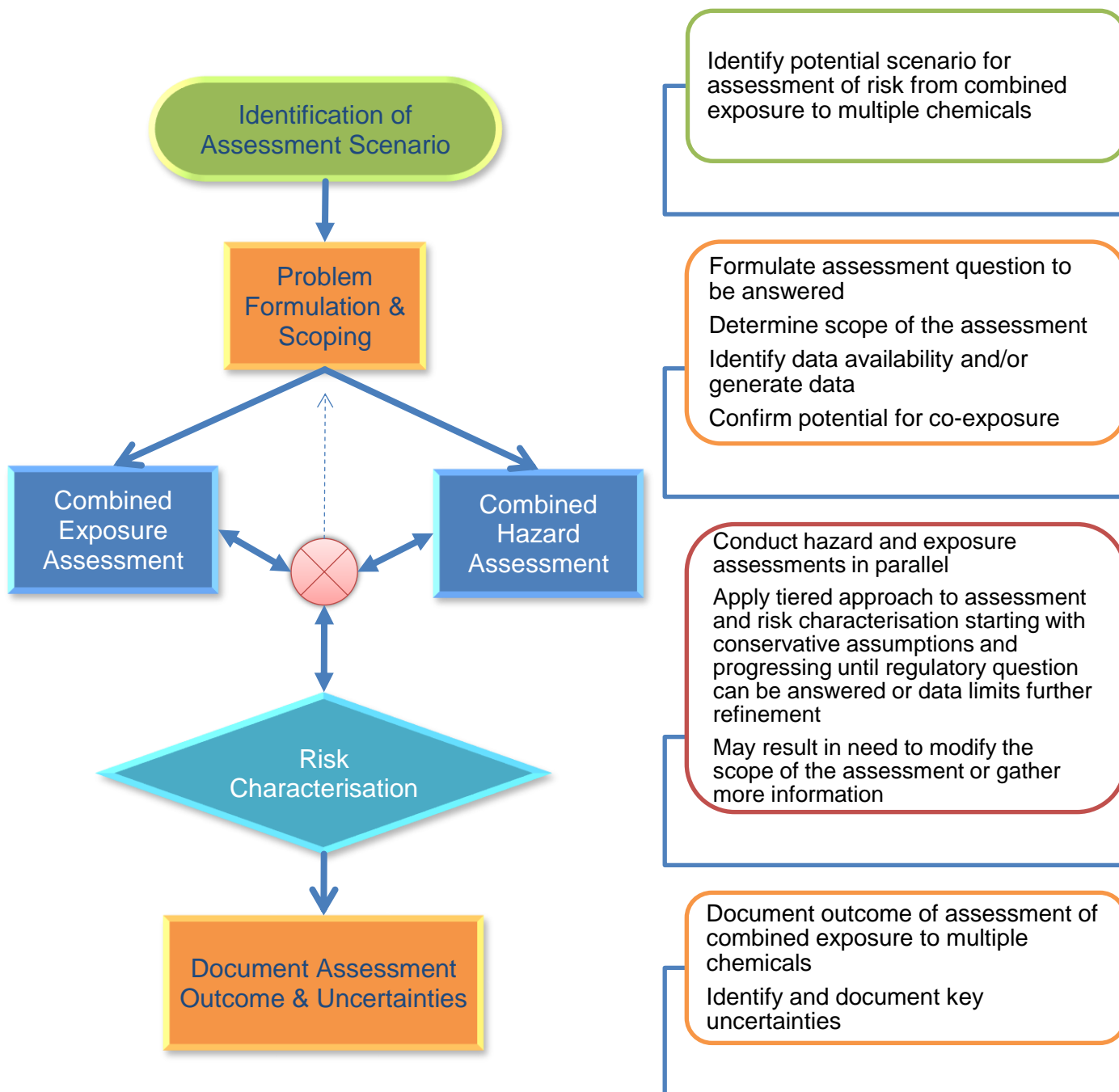
Prior to considering an assessment of combined exposures to multiple chemicals it is important to recognise that the approaches for risk assessment can be grouped into two fundamentally different classes, whole mixture approaches (WMAs) and component based approaches (CBAs). The approach chosen will necessarily alter the elements of hazard, exposure and risk characterisation as well as the data needs. Chapter 7 provides additional information, with the concepts briefly introduced here as they arise throughout other sections of the document.

The whole mixture approach to combined exposure assessment considers a group of substances as if they were a single unit, with the assumption and limitation that the components and concentrations of the mixture do not vary significantly across individuals, over time, or between exposure routes, and that toxicity studies are conducted on the whole mixture (NAS, 2008; US EPA, 2007; ECETOC, 2011b). This approach considers any unidentified components as well as any interactions among components, but it cannot identify the individual substance responsible for any interactions and it does not provide any information on the toxicity of individual components.

In a component-based approach (US EPA, 2007) substances are considered as a group of separate components, and effects of the substance group are assumed to be based on the individual components. As such, when applying this approach, it is necessary to determine the relative proportion of the components and their contribution to the overall toxicity of the substance group. Interactions between components that influence their toxicities need to be taken into account, however it is considered that the components do not chemically react.

For component-based approaches, an appropriate mathematical model for calculating the toxicity of the effects of the combined exposure needs to be selected. These are based on what is known about the mode of action (MOA) or adverse outcome pathway (AOP) of the substances. Three types of approaches are defined conceptually as: (1) Dose Addition (DA) / Concentration Addition (CA); (2) Response Addition (RA) / Independent Action (IA); and (3) models taking into account interactions between substances where a substance influences the toxicity of another substance, i.e. approaches that deviate from either DA/CA or RA/IA models (see Chapter 7 for more details).

DA/CA assumes a similar AOP/MOA for a given endpoint and that the components of the group of chemicals are dilutions of one another scaled for their potencies. Specifically, one chemical in the group can be replaced with a fraction of an equally effective concentration of another chemical in the same group without changing the overall combined effect (NAS 2008). The approach also assumes no chemical interaction between the co-occurring chemical components. The DA/CA model is based on the pharmacological concepts of ligand binding site theory, affinity, potency and receptor occupancy (Bliss, 1939; Kienzler et al., 2014; EFSA, 2013). DA/CA has found widespread acceptance as an assessment concept for combined exposures to multiple chemicals, if synergistic or antagonistic effects between the components are not expected and is extensively used by regulatory authorities as a protective default approach. The most frequently used methods based on this approach are the hazard index (HI), the reference point index (RfPI, or Points of Departure Index (PODI)), the toxic equivalency factor (TEF) and the Toxic Unit (TU) approach.

RA/IA is used for chemicals that have dissimilar MOA (toxicologically independent components) and assumes one chemical does not influence the toxicity of another and that a combined effect can be calculated using the statistical concept of independent random effects (US EPA, 2007; Kienzler et al., 2014). RA/IA is appropriate for groups of substances affecting different, inherently independent, apical endpoints and/or where the

MoA/AOPs can be sufficiently discriminated and are different and independent from each other.

Interactions may occur between chemicals that have either similar or dissimilar mechanisms of toxicity or MOA. This refers to a situation where the combined effect of two or more substances is either greater (e.g. synergistic, also referred to as potentiating, supra-additive) or less (e.g. antagonistic, also referred to as inhibitive, sub-additive, infra-additive) than that predicted based on DA/CA or RA/IA (Kienzler et al., 2014). Accordingly, interactions may vary depending on the ratio of substances, dose, route(s), timing of exposure (frequency and duration) and the biological target(s); examples include toxicokinetic, metabolic and toxicodynamic interactions (ECETOC, 2012; SCHER, 2012; Kienzler et al., 2014). Various agencies have incorporated concepts of 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; US EPA, 2007; WHO, 2009a; SCHER, 2012). The issue of interactions is further addressed in Section 5.4.3 and Section 7.2.2.

In order to determine what type of assessment approaches should be used, different organisations have developed decision trees. One such example is a Decision Tree for the Risk Assessment of Mixtures developed by Scientific Committees within the EU (SCHER, SCCS, SCENIHR (2012)). In addition, various component-based approaches for evaluating the risk to multiple chemicals have been described in a graphical scheme by the US EPA (US EPA, 2007).

Chapter 4. Considerations for Problem Formulation and Scoping for an Assessment of Combined Exposure

Problem Formulation and Scoping	• Identify regulatory question to be answered and its scope • Determine need to conduct combined exposure assessment
Hazard and Exposure Characterisation	• Conduct in parallel • Apply tiered approach to both elements progressing until regulatory question can be answered or data limits further refinement
Risk Characterisation	• Answer regulatory question • Document and communicate uncertainties

4.1. Introduction

The initial stage in conducting any risk assessment includes planning, scoping and problem formulation. Chapter 4.1 and 4.2 describe problem formulation more generally, with considerations specific to combined exposures detailed in Chapter 4.3 and onward. Planning and scoping involves consideration of the specific issue/problem to be addressed, the legal framework under which any action will be taken, the risk management options, and any public-, stakeholder-, or community-specific issues.

Based on the information developed during planning and scoping, problem formulation is then conducted. It requires some preliminary consideration of the hazard identification, hazard characterisation and exposure assessment and usually proceeds in an iterative fashion. Problem formulation for a combined exposure assessment should consider questions such as the following:

- What is the nature of the hazard?

- What is the nature of the exposure?

- Is there a likelihood of co-exposure within a relevant timeframe?

- What is the rationale for considering compounds in an assessment group?

When the problem formulation is completed, the scope of the assessment (populations to be evaluated, chemicals to be considered, boundaries of the analysis, projected level of complexity of the assessment) and the specific questions that the risk assessment seeks to answer in order to meet the needs of the risk manager should be clearly defined. A technical approach/plan for analysing and characterising risk is determined, which can be changed as the risk assessment progresses.

4.2. General Concept of Problem Formulation

Conceptual models are used to plan the risk assessment and associated data collection activities and they may be periodically revised as additional data become available. This section describes general approaches applied while considerations for combined exposure assessment follow in the next section. This section is largely summarised from "Framework for Human Health Risk Assessment to Inform Decision Making" (US EPA, 2014) where the reader can access further detail. A general conceptual model includes:

- Types of stressor(s) considered in the assessment, including physical, chemical and biological stressors; in this document, chemicals are the focus.

- Exposure pathways, including fate and transport processes by which chemicals move from the original point of release through the environment, and the routes/interaction(s) through which populations or individuals are exposed (oral/ingestion, inhalation, dermal) via various media (water, sediment, soil, air, food/feed, products).

- Exposed groups to be considered: These may be groups of individuals or populations identified by common characteristics (e.g. trophic levels, general populations, populations located near site of concern, adult workers, consumers), or particular populations with unique exposures and/or susceptibilities to chemicals, or specific types of ecosystems (e.g. aquatic, terrestrial) and include life stages (e.g. infants or woman of childbearing age, early life/larval stages for ecological receptors such as fish).

- Types of endpoints to be considered: For individuals the effects include systemic effects (e.g. cancer, non-cancer, developmental) or local effects (e.g. irritation, corrosivity, sensitisation). For ecological assessments, the effects are typically on the population level (e.g. growth, or survival, reproduction) or on a particular species.

- The scientific implications of additional data gathering (e.g. the value of additional data gathering with respect to the available science).

- Risk metrics or lines of evidence (e.g. cases of disease or disease incidence, hazard quotient, magnitude of effect, or margin of exposure, risk quotients, critical body residues, bioaccumulation potential and biodegradation potential/persistence) and protection goal.

The complexity of the conceptual model depends on the complexity of the problem the assessment seeks to address. This may be related to the number of chemicals, exposure pathways or assessment endpoints, the nature of the effects, and/or the characteristics of the exposed populations or life stages. Generally, the conceptual model identifies factors and endpoints that will be analysed in the risk assessment. It also addresses those aspects that might not be analysed in the risk assessment but could be important in the overall decision-making process.

The analysis plan is the final portion of the problem formulation. It is developed with attention to the conceptual model and the needs for the risk assessment. The analysis plan describes the intentions of the assessment, which may have been developed during the planning and scoping process, and it provides details on technical aspects of the risk assessment.

The analysis plan may include these components:

- The specific questions the assessment is intended to address.

- The assessment design and rationale for selecting specific pathways to include in the risk assessment and the exclusion of others.

- A description of the data, information, methods and models to be used in the analyses (including uncertainty analyses), as well as intended outputs (e.g. exposure and risk metrics).

- The extent or aspects of the assessment that are qualitative rather than quantitative are also described in the analysis plan.

- The approach that will be used to address the uncertainty introduced from data gaps and limitations and plans for stakeholder consultation and peer review.

- The value of additional data collection. This could include a sensitivity analysis to prioritise the needs of data generation in case of a higher tier assessment.

Analysis plans may be brief or extensive, depending on the assessment and its level of complexity. The analysis plan should also address the quality of the data to be used; assessments of exposure, hazard and dose-response; and risk analyses. The plan should also include a discussion of the analyses of uncertainty and variability and associated limitations and assumptions.

4.3. Determining whether to conduct a risk assessment of combined exposures

The decision to conduct a risk assessment of combined exposures needs to take into consideration the potential for relevant co-exposures to the chemicals in the populations and/or ecosystems of interest. The need for a combined exposure assessment is increased when it involves exposure to substances exhibiting the same or similar effects, although non-similar effects can also be integrated. Therefore, both the evidence regarding co-occurrence/co-exposure and common hazard can be taken into account in the determination to conduct an assessment and these elements are further described in the following sections.

If there is no relevant co-exposure of a human or ecological target, then there is no need for a combined assessment. Thus, it is critical that an initial finding on co-exposure be made as part of the scoping of the assessment. The level of evidence required to justify a combined assessment will vary with the purpose of the assessment. As discussed below, co-exposures can also be considered even if applied (external) exposure is not simultaneous.

4.3.1. Evidence regarding co-occurrence/co-exposure

Evidence for the existence of combined exposures can come from many types of data. Chapter 6 provides more detailed information on considerations for co-exposure characterisation. Direct evidence of co-exposed individuals will be readily available for some assessments (e.g. chemicals in a common source). Assessments that address combined risks from exposure to chemicals in a common source (a consumer product, a combination of chemicals in an environmental media, etc.) will have data on the specific chemicals present in a source, and in these cases, the target population of interest is defined by the source. Thus, this type of assessment is based on an actual finding of co-occurrence of exposures. However, other types of assessments (e.g. endpoint-based, chemical class, disease-based, as further described in Chapter 4.4.1) will need to consider additional exposure data in order to identify co-exposure.

Evidence for co-occurrence that can be used to identify if an assessment of risk from combined exposures should be considered can include:

- Actual measurement of substances in the same media (e.g. monitoring data).

- Data on likeliness of finding co-occurring substances (e.g. release or fate information, market penetration information, use information).

- Intentionally produced mixtures/products containing several components (mostly with known composition) such as pesticide/biocide formulations, cosmetic products, commercial mixtures of industrial chemicals, mixtures of food/feed additives etc.

- Information on intended uses for regulated substances, under potentially multiple legislations.

Timing of exposure is an important factor in evaluating the potential for co-exposure. Co-exposures that occur as the result of exposure to multiple sources can occur on the same day but not necessarily at the same time or may occur on several sequential days. Such exposures can result in internal co-exposures when the chemicals persist in the body.

Information on toxicokinetics and monitoring data can be used to characterise this potential for internal co-exposures, or alternatively to justify that co-exposure is unlikely, when external exposure does not overlap in time. The need to consider such exposures is greater for compounds that persist in an organism over time. Combined exposures to such compounds could be the result of exposures that occur over decades (Demond et al., 2012). In addition, even if a chemical does not persist in the body long enough to overlap with a latter dose of a second chemical, if the effects of the initial chemical persist over time a combined effect may still occur.

At the problem formulation and scoping step, it should be noted that a finding of co-exposure is not the same as a finding that the co-exposures are, or are likely to be, toxicologically meaningful. This evaluation will occur during the assessment. Nor does the finding of co-exposure at this stage need to detail all chemicals, or populations or ecosystems of interest. This occurs in the exposure and hazard steps. At this point, it is sufficient to determine whether there is enough evidence to conclude that there exists at least a single target potentially co-exposed to two or more chemicals of interest.

4.3.2. Evidence regarding common hazard

An assessment may be prioritised based on a finding of co-exposure to multiple chemicals, as outlined above, and screening assessments of risks from combined exposures can be performed in the absence of data on a common hazard (Tier 0 and Tier 1 hazard assessments as defined by WHO/IPCS framework (Meek et al., 2011), see also Chapter 5 for more detailed information on considerations for hazard characterisation. However, information on a common hazard can also be a factor that raises the priority of performing an assessment since it suggests an increased probability that combined exposures could produce effects that would be missed by single chemical assessments. In addition, the WHO/IPCS framework suggests that when such data are available, they should be incorporated in an assessment (Meek et al., 2011).

If there is a concern that multiple chemicals are causing the same effects (e.g. same target organ or endpoint) then there may be interest in determining the combined effects on the population even if the chemicals are assumed to operate by independent mechanisms or MOAs.

Questions that should be considered regarding common hazard include:

- Are the chemicals causing the same or similar adverse effects on the same target organs (i.e. is the biological outcome the same)?

- Are they known to follow the same AOP/MOA? Alternatively, do they have different AOPs/MOAs but the same target organ? Or do they share one or more key events (KE) between AOPs?

- Is there evidence suggesting that the compounds may interfere with relevant metabolic pathway(s)?

4.4. Defining the Scope of the Risk Assessment of Combined Exposures

4.4.1. Considerations in defining the scope of a risk assessment of combined exposures to multiple chemicals

At the problem formulation stage, it is critical to define the scope of the assessment of combined exposure to multiple chemicals. From a purely scientific viewpoint, a comprehensive assessment would consider all substances acting on all organisms, by all routes and possibly at different times, taking into account all effects on the health and well-being of the organisms. In practice, assessors establish specific boundaries to limit the scope of the assessment in order to take into account regulatory considerations, to respond to specific risk-management questions and to make the analysis manageable.

The risk management questions that assessments of combined exposures are asked to address vary greatly. Many different disciplines deal with combined exposures and pose distinctly different questions. These questions result in very different scopes for the assessment of combined exposures, which can be grouped into the following categories for a combined exposure assessment (see also Table 1):

- Endpoint-based assessments. These assessments start with a specific (eco)toxicological endpoint and address the question "What is the combined impact of multiple chemical exposures on the occurrence of the endpoint in a population?" Some examples of endpoint-based assessments include assessment of chemicals that affect the endocrine, nervous or immune systems, or algal growth.

- Mechanism-based assessments. These assessments often start with considering chemicals from the same class or chemicals that produce a common endpoint but are refined to consider concurrent exposure to only those that have a similar mechanism of action. An example of this type of assessment can be found with organophosphate pesticides that act through phosphorylation of the acetylcholinesterase enzyme thus affecting neurotransmission.

- Chemical-class assessments. These assessments start with a specific group of chemicals and address the question "Which targets are exposed to more than one of the chemicals in a group and what are the toxicological effects of the combined exposures?" Some examples of chemical-class assessments include assessments on phthalates, dioxins and dioxin-like PCBs, and polyaromatic hydrocarbons.

- Source-based assessments. These assessments define the exposed population in terms of a specific source or pathway of chemical exposure that results in concurrent exposures to two or more chemicals. Some examples of source-based assessments include exposures to combinations of chemicals from industrial effluents contaminating surface water or releases to air from metal smelters and refineries. Site-specific emissions include discharges from wastewater treatment plants or landfill leachates. The chemicals involved are defined as those that are emitted by or are present in the source.

- Formulation-based assessments. This is a subset of source-based assessments. Here the source is a commercial product that is an intentional mixture composed of multiple chemicals. Some examples of these assessments include chemicals that are present in personal care products, pesticide formulations and household cleaners.

- Population-based assessments. These assessments define the exposed population using temporal and geographic information. For example, all individuals who live within 1 km of a specific source or a population living in a specific city. This could also include assessment of specific environmental receptors such as assessment of a lake, a river stretch, a coastal area. These assessments include all of the chemicals that reach the assessed population.

- Disease-based assessments. These assessments begin with a specific disease (e.g. breast cancer, autism, or asthma) in the population and look for combined exposures that could influence the occurrence of the disease or symptom. From the environmental perspective, this could be a disease observed in a wildlife species that may be caused by exposure to multiple chemicals.

These examples of defining the scope of a risk assessment of combined exposure are not limiting. In fact, some of the above could be combined. For example, an assessment of a single endpoint may be conducted for a specific chemical class if this is the question that requires answering. Further examples of case studies can be found in Bopp et al., 2016 which reviews case studies on the human and environmental risk assessment of chemical mixtures.

An assessment of risk to combined exposures could also examine human health or ecological receptors/endpoints, or both. In the consideration of both, it is conceivable that the boundaries and scope for the combined exposure assessment would differ for human health and the environment.

Table 1 presents a summary of how assessments in each of the examples of categories of scoping define the chemicals of interest and the populations to be assessed. The table also lists example assessments that fall into each of the categories.

Because of these different starting points, the problem formulation process will not be the same in each case. In some scenarios, the problem formulation will need to focus on refining the scope of the chemicals to be included. At other times, the process will need to focus on refining the scope of population to be assessed. In all cases, the issues that drive the assessment will need to be translated into a testable hypothesis that can define the design of an assessment of co-exposures.

4.4.2. Defining the starting boundaries for sources/uses for inclusion

At the outset, the sources of chemicals that will be included in the assessment of risks from combined exposures need to be defined, depending on the starting point, as described in the examples in Table 1. These could be used to frame the scope of the assessment as a whole (e.g. source-based or formulation-based assessment) or could be delineated in the context of an assessment that has been identified based on an endpoint or chemical class. Although the sources and uses are also important for ecological assessment, the environmental media of interest for the combined exposure assessment need to be clearly defined.

In the problem formulation stage, one can define the "exposure scenarios" to be considered within the assessment. Exposure scenarios specify the routes of exposure (e.g. oral, dermal, inhalation), pathways of exposure (atmospheric or surface water transport) and final media

of exposure (air, water, food, soil and sediment). In addition, for human health assessment, one should determine at the outset the extent to which the assessment will consider direct (e.g. product) versus indirect exposure (i.e. via environment) scenarios and which population should be considered (occupational, consumer, general population etc.). For ecological endpoints it will need to be determined whether to examine near-field (local) or far-field (regional) exposures and to determine which ecological targets/endpoints should be considered.

Table 1. Examples of the varying scope of risk assessment of combined exposure to multiple chemicals including the characteristics for defining the initial scope of chemicals and populations considered

	Basis for selection of chemicals included	What is the initial scope of the exposed population?	Example assessments
Endpoint-based assessments	Chemicals that affect the same endpoint. The chemical list may be open ended[1].	Defined by the sources of the chemicals selected based on hazard.	- Junghans et al., 2006 - pesticide mixture effects on the reproduction of the freshwater alga - Moretto et al., 2015 - teratogenic conazoles - Health Canada and Environment Canada, 2015 - developing male reproductive system, phthalates - Schmidt et al., 2016 – effect of azole fungicides on hepatotoxicity, with other effects examined - Rieke et al., 2017 – effect of azole fungicides on the adrenal gland
Mechanism-based assessments	Chemicals that affect the same endpoint by a similar mode of action.	Defined by sources of the chemicals selected based on hazard.	- US EPA, 2006a - Organophosphorus Pesticide Cumulative Risk Assessment - US EPA, 2006b - Triazine Cumulative Risk Assessment - USE EPA, 2011a - Pyrethrins/Pyrethroid Cumulative Risk Assessment
Chemical - class assessments	Chemicals in a specific class.	Defined by sources of the chemicals selected based on chemical class.	- Borg et al., 2013 - perfluoroalkylated and polyfluoroalkylated substances (PFASs) - Environment Canada and Health Canada, 2013; - Aromatic Azo and Benzidine-based Substance Grouping. Certain Azo Disperse Dyes - CPSC, 2014 - phthalates - Health Canada and Environment Canada, 2015 - phthalates - ECHA, 2017b - phthalates
Source-based assessments	Chemicals present in a specific source. The chemical list may be open ended	Defined by the source.	- Environment Canada and Health Canada, 1991 - effluent from pulp mills using bleaching - Environment Canada and Health Canada, 1993 - chlorinated wastewater effluents

	depending on the source.		• Environment Canada and Health Canada, 1994 - waste crankcase oils • Environment Canada and Health Canada, 2001a - textile mill effluents • Environment Canada and Health Canada, 2001 b - releases from primary and secondary copper smelters and copper refineries and releases from primary and secondary zinc smelters and zinc refineries • Teuschler et al., 2004 - drinking water disinfection by-product mixtures • Price et al., 2011 - surface water • Price et al., 2012b - surface water and waste water effluents • Backhaus and Karlson, 2014 - pharmaceuticals in sewage treatment plant effluents • Boon et al., 2015 - dietary exposure • De Brouwere et al., 2014 - residential indoor air • Vallotton and Price 2016 - surface water monitoring • Malaj et al., 2014 - 233 chemicals in 4000 EU surface water monitoring sites
Formulation-based assessments	Chemicals present in a specific formulation.	Defined by the use of the formulation.	• Coors et al., 2012 - wood preservative products • Bunke et al., 2014 - technical mixtures for leather tanning and ecological risk assessment (see Annex 5 of document)
Population-based assessments	Chemicals that reach a specific population. The chemical list may be open ended.	Defined by demographics, location and time.	• Han et al., 2012 -biomonitoring data and occupationally exposed populations • CPSC, 2014 - biomonitoring • Qian et al., 2015 - biomonitoring
Disease based-assessments	Chemicals that affect the occurrence of a specific disease. The chemical list may be open ended.	Defined based on presence of disease.	• UNEP and WHO, 2012 - thyroid acting compounds

Note: [1]Lists may be open ended when certain chemicals are known to be included in the assessment but when other chemicals could be added as part of the assessment. The population indicated is that which is driving the initial scoping; however, this would be further defined during the assessment phase.

Factors for determining which routes, sources of exposure and substances to incorporate into the assessment include:

- Evidence of or potential for co-exposure or co-occurrence, evidence of extent of use, particularly widespread use, direct release to the environment and consideration of how substances/product use may be correlated (e.g. seasonal or waves of releases of the chemicals to the environment including continuous versus batch releases).

- Because some chemicals may have the ability to affect an organism's response to other chemicals, consideration of the time sequence of exposure may take on an additional layer of complexity in multiple-chemical combined risk assessments (EPA, 2003; Ashauer et al., 2017).

- The ability of sources/uses to potentially contribute to toxicologically significant exposures.

  - While the potency of the chemicals would influence the definition of "significant", it may be possible to exclude compounds using concepts such as the Thresholds of Toxicological Concern, developed for human health endpoints (SCHER, SCCS, SCENIHR, 2012; Price et al., 2009; Annex B in Meek et al., 2011) and ecological Thresholds of Toxicological Concern for environmental risk assessment which are under development (Belanger et al., 2015). However, it should also be considered that low exposure levels of many chemicals may contribute to overall combined toxicity, if they follow the same AOP/MOA (Backhaus, 2014) and potential body burden from other compounds could influence this approach.

- If the dominant sources of exposure are known to be associated with a limited range of sources/uses, one could focus on these and expand to other sources across the product's life cycles, only if necessary (Csiszar et al., 2016).

- If the hazard is the primary driver of the assessment (e.g. endpoint-based assessment in Table 1), the type of hazard can be used to define the route, source and duration of exposure that pairs with hazard of interest (acute, chronic, route specific toxicity etc.).

- Potential levels of background exposure and sources/uses that change the environmental background. For example, if an assessment is supporting an epidemiological finding of differences across communities, then background exposures that align with these differences should be the focus.

- Consumer, industrial and/or commercial products (e.g. pest control products, personal care products, household products, cleaners) containing multiple substances including substances with known common MOA and/or targets.

4.4.3. Defining the starting boundaries for hazard identification

As with defining the sources/uses for inclusion, the endpoints that will be evaluated in an assessment of risks from combined exposure need to be defined. As discussed above, certain assessments of combined exposures include the consideration of all potential effects, while others define a limited number of the hazards of interest for the assessment:

- A specific (eco)toxicological endpoint or disease.

- Effects observed in specific target organs, systems or species, regardless of a common mode of action.

- Effects that result from a specific AOP or common/similar MOA.

Once the endpoints have been defined, the specific endpoints can be used to prioritise the substances on which the assessment will focus.

When there are indications of common hazard, an important consideration is data availability and data quality on the hazard of the relevant co-occurring chemicals to provide sufficient rationale to serve as the basis to conduct the combined exposures assessment, as well as inform the most appropriate method to use. One of the key questions is on the sufficiency and quality of the data to form assessment groups for the chemicals. The decision to form an assessment group is based on the available information from different sources and levels of biological organisation to support a hypothesis that specific chemicals act similarly. The types of information that can serve to support groupings of common hazard include predictive information on chemical structure (e.g. structure-activity relationships (SARs) and quantitative structure-activity relationship (QSAR) modelling), structural alerts and toxicological criteria such as toxicokinetic and toxicodynamic properties, toxicological and epidemiological studies, developed reference values and hazard classifications from the Globally Harmonised System (GHS) of Classification and Labelling (see Chapter 5).

4.4.4. Regulatory program considerations in defining the scope

It is important that the risk assessment is well-tailored to the problems and decisions at hand so it can adequately inform risk management decisions, hence 'fit-for-purpose'. This ensures that the nature and/or scope of the risk assessment is aligned with the decision to be made and requires early engagement of risk assessors, risk managers and implicated stakeholders to define needs, scope, data availability and resources.

Legal requirements

If the combined exposure assessment is conducted in a regulatory context, the scope of the assessment will also depend on legal obligations and regulatory requirements. This may influence the flexibility concerning the scope of the risk assessment of combined exposures by either limiting the scope (to the scope of the regulation) or expanding the scope (to check the effectiveness of a proposed risk management measure). In addition, under certain regulations specific legal requirements for certain classes of compounds need to be followed (e.g. cumulative risk assessment for pesticides required in EU pesticide regulation (EC, 2005; EC, 2009); US guidance specifying consideration of aggregate exposures to

pesticides (diet, water and residential use) (US EPA, 2016)). See also Kienzler et al. (2014) for other examples. Legislation may also define who is responsible for data collection and assessment and define who has the ability to request the generation of data necessary to address data gaps/needs.

Regulatory program priorities

Regulatory program priorities can also influence the scope of a combined exposure assessment. There may be a need to focus on substances that have previously been prioritised based on risk to public health or the environment. Sometimes, these prioritisations focus on particular endpoints of potential concern from a hazard perspective. Alternatively, if a group of substances are restricted in other jurisdictions based on combined exposure assessment and no current national risk management measures are in place, these could be identified as a priority for assessment. This may narrow the initial scope for a combined exposure assessment.

It is also possible at the problem formulation stage to preliminarily examine the scope of the assessment from an economic analysis perspective to determine what the overall costs, benefits and impacts of potential actions would be, and if investing in a broader combined exposure assessment is merited.

The National Research Council (NRC, 2009) recommends the consideration of new approaches to facilitate cumulative risk assessments including using biomonitoring, epidemiological, and biomarker and surveillance data. New tools are also being developed that utilise aspects of geographic information systems (GIS) methods to assess chemical, physical and social exposures and sometimes health outcomes for identifying environmentally relevant chemical co-exposures. These and other new approaches, such as genomics, may help to better understand, identify and characterise high-priority communities, populations, or locations where a combined exposure assessment would be most relevant.

Risk management frameworks or capabilities

The type of risk management framework and its associated capabilities will influence the potential scope of a risk assessment of combined exposure to multiple chemicals as the feasibility and range of risk management options can be delineated by the framework, along with the level of uncertainty that is acceptable in a particular regulatory context. For example, a particular regulatory program might be focussed on chemicals management or on watershed or site management in ecological assessments. Often food, work safety and industrial chemicals are handled by separate regulatory programs. Risk management frameworks for public health/environment may be different between jurisdictions, which could result in a different focus for assessments. In addition, these programs may have different authorities for information gathering to address data needs along with varying data demands and data confidentiality.

In examining risk assessments of combined exposure to multiple chemicals within and across jurisdictions, it is important to keep these potential differences in mind in order to minimise duplication of effort. There may be opportunities to harness information and approaches used in different jurisdictions during data collection, risk assessment and risk management.

4.4.5. When should the scope change/be reconsidered?

Although it is important to define the scope of a risk assessment of combined exposure to multiple chemicals at the outset, there should also be flexibility in changing the scope once the assessment is underway. This could occur when the nature of the use or exposure conditions change such that they would result in a significantly increased/decreased level of exposure and/or new hazard information becomes available that could influence the points of departure of the chemicals included in the assessment. Alternatively, if the AOPs/MOAs are further defined for substances, this may demonstrate that compounds operate by different mechanisms and may not belong in the same assessment group.

There also could be a change in the legal framework or it could be determined that an imprecise question was asked within the problem formulation. These may merit changes in the scope of the assessment. In addition, when the cost outweighs the benefit of conducting an assessment to inform the regulatory decision and risk management, then the scope could be examined.

Price et al., (2012b) have shown how the determination of Maximum Cumulative Ratio (MCR) values in an initial tier of an assessment can focus the higher tiers of the assessment on specific combinations of chemicals of concern that could not be recognised prior to performing the initial tier (Vallotton and Price, 2016; Reyes and Price, 2018). Such findings may justify additional collection of exposure information. MCR is further described in Chapter 7.

4.5. Context of problem formulation, what changes between data rich and data poor substances or groups of substances?

Data limitations pose a major challenge to the assessment of combined exposures. Data on toxicity and exposure are required for each of the substances included in the assessment or the mixture as a whole and the population exposed to the combined exposures may have unique characteristics that are difficult to assess. Data gaps are a concern since they could result in leaving certain risks unidentified or poorly quantified. However, one should not only perform assessments when there are high quality data on all chemicals since it could prevent the assessments of known risks. As a result, all assessments should clearly describe the data available for the assessment and any evidence that suggests the presence of key data gaps and varying levels of uncertainty between substances within a combined assessment. Such information will provide a valuable context for the findings of the assessments and any potential refinement.

Variation in data are also addressed by the WHO/IPCS and other frameworks for assessment of risks posed from combined exposures to multiple chemicals (Meek 2011, 2013; Bunke et al., 2014; Price et al., 2012a; ECHA, 2015). The frameworks provide an approach that addresses both data rich and data poor situations. The initial hazard and exposure assessment approaches (screening tiers) require minimal information on toxicity and exposure (Meek et al., 2011). Such assessments are designed to obtain initial findings that can be made under very data poor conditions (minimal toxicity and exposure information). Where such assessments are sufficient to meet the needs of risk managers, then the framework will be sufficient for both data rich and data poor assessments. In instances where the assessment does not meet the needs of the decision makers, the assessment's findings can be used to direct the data collection efforts for the assessment. In data poor assessments, such additional data would be required before completing the

assessment. In data rich assessments, the additional data would be used to proceed to the higher tier assessments.

The US EPA (2014) described the importance of data availability during problem formulation. The analysis plan phase of the framework includes a consideration of how the level of confidence (or precision) needed for the management decision compares with that expected from available analytical approaches; this comparison determines data needs and evaluates which analytic approach is best. When new data are needed, the feasibility of obtaining them is evaluated. This evaluation should include the timeframe needed to obtain further information and whether a delay can be accommodated for the assessment.

In order to ensure appropriate interpretation of the output, a clear description of the level of uncertainty at both early and later tiers and how the application of conservative assumptions was used to offset the uncertainties is necessary. This can be particularly important for exposure estimates where the level of variation between crude conservative estimates, rather than estimates that are probabilistic in nature, can be several orders of magnitude greater than respective differences in points of departure for hazard (Meek et al., 2011). Further discussion on uncertainty is available in Chapter 7.

The net impact of the frameworks is that assessments of risks posed by combined exposures for data poor situations will be restricted to screening assessments while data rich assessments can proceed to higher tiers. This can be reflected in the problem formulation when outlining potential tiers of an assessment.

4.6. Should problem formulation indicate possible tier/likely range of tiers for the combined assessment?

As outlined in the WHO combined exposures framework, tiering should be considered in the problem formulation phase (Meek et al. 2011; Meek, 2013). The selection of the initial tier is driven in part by the availability of the data. In many instances, available data are only capable of supporting lower tier exposure and toxicity assessments. The amount of effort increases for higher tiers; however, the confidence in the accuracy of the exposure and hazard assessments also increases. Thus, the additional effort required by higher tiered assessments may be warranted in certain cases. In other cases, even when high quality data are available, an assessor may wish to consider only performing a lower tier assessment. If a Tier 1 assessment is sufficient to reach a robust decision, then introduction of detailed data set and complex analyses may not be warranted and the resources could be better placed on other priority assessments of greater complexity and/or risk (Meek et al. 2011; Price et al., 2012a).

Chapter 5. Considerations for Hazard Characterisation to Inform Assessment of Combined Exposures to Multiple Chemicals
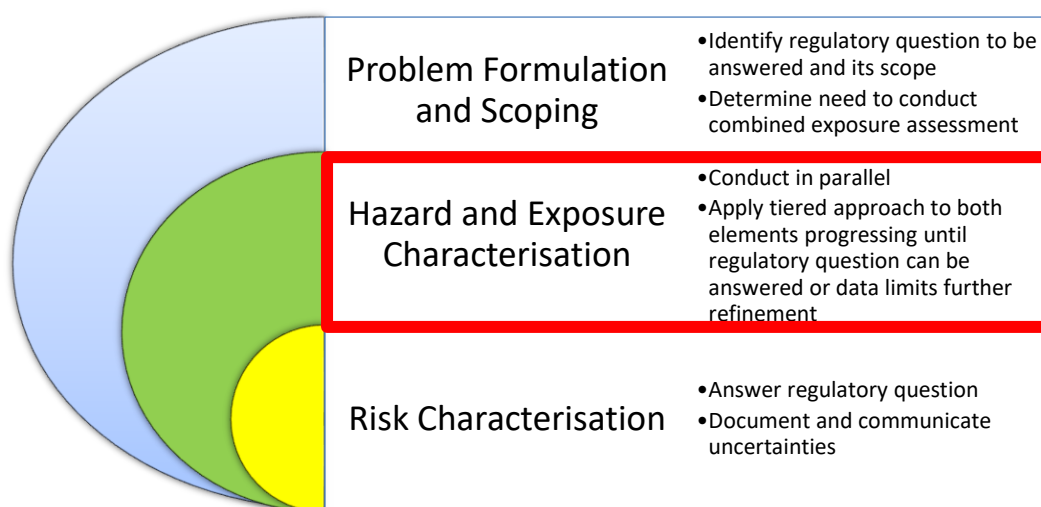
5.1. Introduction

The main aim of this Chapter is to provide considerations for hazard assessment in the context of assessment of combined exposures to multiple chemicals and in addressing the differences in potency of individual components using a tiered approach. It applies mainly to component-based approaches as whole mixture approaches would follow standard hazard assessment practice. This Chapter also covers in some detail the approaches to group chemicals into hazard categories and/or subcategories and different types of hazard information.

Approaches constitute potential tools for hazard assessment of combined exposure that are relevant to both human health and environment. However, there are some separate sections addressing specific methodology for ecological considerations.

5.2. Grouping chemicals into hazard categories and/or sub-categories

5.2.1. Key considerations for defining a group

The purpose of this section is to describe the criteria that could help assessors to decide which chemical substances to consider together from a hazard perspective. It would largely apply to groups that have been constructed using a hazard approach (endpoint or disease based) but can also be used to inform the combined hazard assessment of groups constructed from an exposure perspective (e.g. source-based assessments or formulation-based assessments) (see Chapter 4.4). This grouping will inform the hazard and risk assessment methods applied within the combined exposure assessment (i.e. using a DA/CA or RA/IA approach).

The main two concepts for creation of assessment groups, from a hazard perspective, are based on: (1) structural similarities or (2) similarities in toxicological or biological responses/effects (SCHER, SCCS, SCENIHR, 2012). A combination of these two concepts is also possible. Determining common or similar group characteristics is described in the OECD Guidance Document on Grouping of Chemicals and in general, a similarity hypothesis is typically based on one or more of the following criteria as a starting point (OECD, 2014):

- Common functional group(s) (e.g. aldehyde, epoxide, ester, specific metal ion).

- Common AOP/MOA.

- Common constituents or chemical classes, similar carbon range numbers.

- The likelihood of common precursors and/or breakdown products via physical or biological processes that result in structurally similar chemicals.

- An incremental and constant structural change in the chemicals across the category (e.g. a chain-length category such as a homologous series of alpha-olefins, where each category member differs by a methylene group).

The choice of how to group the chemicals (i.e. chemical structure or toxicological response) depends on the scope of the assessment and the availability of relevant information. Explanation of group choice should be explicit on what is included and excluded with a justification for boundaries. Generally, in lower tiers/screening assessments, larger chemical groups should be formed, to be inclusive in case of uncertainties about a common effect; further refinement should occur at higher tiers. The uncertainties, and to which compounds they relate, should be clearly documented in the assessment. Irrespective of the starting point for grouping, it is recommended to use all available information on the whole mixture and its components: physico-chemical properties, structural alerts, (Q)SAR and read-across information, evidence from omics, *in vitro* (high throughput screening (HTS) or other) or *in vivo* experimental data and epidemiological or field data, depending on availability.

If toxicological data are lacking, it is recognised that chemicals whose physico-chemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group or 'category' of chemicals in order to characterise potential health or ecological effects. The methodology of using data from (a)

similar chemical(s) to predict endpoint or property information for one or more substances that lack empirical data is generally referred to as the 'read-across' approach. General elements that should be considered when justifying the read-across approach between 'similar' chemicals to fill data gaps include chemical structure, composition, toxicokinetics, physico-chemical properties, mechanism/mode of action and responses found in alternative assays (e.g. toxicogenomics, *in vitro* cell systems, or other screening assays), combined with all available (eco)toxicological data (OECD, 2014). A strength of this methodology is that it supports health or ecological effects characterisation when one or more substances in a category lack data for one or more endpoint(s), or when there are challenges with data adequacy for some substances in a category (e.g. low quality studies). Additionally, for some categories there is a basis for establishing subcategories for further refinement in the combined exposure assessment.

5.2.2. Grouping on structural similarities

Grouping on structural similarities should be possible for cases where co-exposure occurs to chemicals that are known and similar. It should consider the chemical structures of the components as well as the related steric and physico-chemical properties. It can start from compound families/chemical classes with similar structure (such as e.g. dioxins, phthalates) and similar carbon range numbers (typically for UVCB substances); or it can start from different compound families sharing structural similarities, focusing on common functional groups or structural alerts. Commonalities in structure between group members might also help in identifying structural alerts and these can be used to infer toxic properties based on chemical reactivity. Under the OECD Cooperative Chemicals Assessment Programme hazard profiles were prepared for several categories of industrial chemicals. Documentation can be found via searching by CAS number in the OECD HPV database (http://webnet.oecd.org/hpv/ui/Default.aspx).

The chemicals could be further sub-grouped based on the nature of their reactions (e.g. covalent reactions by specific mechanisms of electrophilic-nucleophilic interaction; Enoch et al., 2010, 2011; Schwöbel et al., 2011). Molecular structural limits of the domains can then be defined using structural alerts or chemotypes, which are able to integrate information on physico-chemical properties of e.g. atoms, bonds and electron systems in addition to the structural connectivity information, and thus allow refining of the grouping taking into consideration reactivity (Yang et al., 2015). A software application for searching for and grouping by chemotypes is publicly available (ChemoTyper, https://chemotyper.org).

Apart from pure structural and physico-chemical properties, grouping could also be based on common transformation or metabolic processes. If different parent compounds are (bio)degraded to structurally similar breakdown products to a relevant extent, this could also be a reason for assigning these compounds to a common group (see for further information the OECD Guidance Document on Grouping of Chemicals, OECD, 2014).

5.2.3. Grouping based on similarities in toxicological or biological responses/effects

Information on toxic responses elicited by individual constituents allows groupings based on common toxic effects. Different stages of a continuum can be applied for grouping of

chemicals: (1) grouping of chemicals with common MOA or AOP(s) or (2) grouping of chemicals eliciting similar effects in the same target organ. Both approaches can be combined and refined as described in the following.

A report from the NRC (2008) proposes to focus on chemicals that share common adverse outcomes (AOs). The report acknowledges that moving beyond structural or mechanistic similarity may appear challenging because of the large number of chemicals that might cause common AOs and thus warrant risk assessment of combined exposure. However, the report concludes that such an approach is achievable and would be more directly relevant to the public health goals set by national and international agencies or organisations and better investigate the relationships between chemical exposures and human diseases and disorders. This would be a conservative approach suitable for a lower tier assessment.

Groupings based on similar/common AOP

An AOP provides mechanistic understanding of complex biological systems and pathways of toxicity that are responsible for adverse outcomes relevant to regulatory endpoints. The AOP framework captures, in a structured way, the causal relationships linking initial perturbation of a biological system resulting from chemical interaction with molecular biological target(s) to an adverse outcome through a sequential series of key events (KEs) at subcellular, cellular, tissue, organ, whole organism and population level (when required) of observation (OECD, 2017). The initial step, which represents direct interaction of a chemical with a biological target, that starts the overall sequence of events, is referred to as the molecular initiating event (MIE). The following steps are referred to as KEs along the pathway. The link between an upstream KE and a downstream KE is referred to as a key event relationship (KER). Individual AOPs can converge on the same AO, and together these comprise an AOP network. AOPs that share KEs can also be used to construct AOP networks that better represent the biological and toxicological complexity within organisms. For grouping chemicals for assessment of combined exposures, whole AOP networks might be of greater relevance. AOPs are published online (https://aopkb.oecd.org/, https://aopwiki.org/ and http://effectopedia.org/) and in the OECD Series on Adverse Outcome Pathways (http://www.oecd-ilibrary.org/environment/oecd-series-on-adverse-outcome-pathways_2415170x).

Ankley et al. (2010) illustrated how effects caused by multiple chemicals that act via the same MIE or affect pathways that converge at common KEs can be aggregated for risk characterisation. In order to group components based on their AOP, any available information on the individual components (MIEs, KEs and AOs) needs to be collected (although, it is not necessary to have information on the full pathway from initial MIE to the final AO). Sometimes information will be available on several KEs along a pathway, sometimes only on one or few KEs. Because there are both data rich and data poor KEs, the initial grouping could be based on existing information, which can then be refined when further information on additional KEs (upstream or downstream) becomes available (Figure 3). A similarity matrix can be developed and evaluated depending on available data derived after testing a number of chemicals using assays to assess KEs and AOs (Figure 4), which can be used to help grouping based on toxicological event, to identify data gaps or to guide further refined assessments if needed.

In this way, results from in silico modelling and from different types of experiments can be used, e.g. molecular screening, in vitro and in vivo studies, omics methods etc. For example, toxicogenomic studies may provide further information for the detection and distinction of similar and dissimilar joint responses in toxicological action.

Altenburger et al. (2013) note that DA/CA and RA/IA approaches have proven useful for the quantitative prediction and assessment of combined effects but have been primarily based on apical endpoints. Therefore, how molecular and cellular level information can be used in the context of these concepts requires further work.

Figure 3. Considering information at different levels of observation to support common effect grouping.

Level of Biological Organisation	Molecular	Cellular	Tissue	Organ	Organism	Population/ Species
Examples for effect types	Covalent binding, hydrogen bonding, electrostatic interaction with DNA or proteins	Activity of an enzyme, upregulation or downregulation of a gene, increased or decreased levels of a hormone or protein	Histopathological findings in a tissue	Structural or functional changes of an organ	Disease, reproductive failure	Impairment of population growth, mortality
Accuracy of grouping for common effects	The more information across different levels of observation is available and coherent for the components, the more accurate the common effect grouping will be.					
Lower accuracy	←→			←→		
Medium accuracy			←——————→			
Higher accuracy	←——→					

Figure 4. Example of a similarity matrix based on shared toxicological events and adverse outcome.

CHEMICALS	Molecular Initiating Event		Intermediate Events									Adverse Outcome				
	event 1	event 2	event 3	event 4	event 5	event 6	event 7	event 8	event 9	event 10	event 11	event 12	event 13	event 14	event 15	event 16
Chemical 1																
Chemical 2																
Chemical 3																
Chemical 4																
Chemical 5																
Chemical 6																
Chemical 7																
Chemical 8																
Chemical 9																
Chemical 10																
Chemical 11																

The EuroMix project (http://www.euromixproject.eu/) is developing a bioassay toolbox to test the combined effects of chemicals in vitro. The testing strategy will be based on the concept of AOPs. The potential of several existing in-vitro tests will be analysed regarding their suitability to present key events in the respective AOP in a quantitative manner leading to the in-vitro test battery.

Same phenomenological effect/target organ

Typically, there is more information that describes downstream chemical effects (e.g. on target organs) and less information about earlier upstream KEs or the whole AOP/MOA. Therefore, groupings can be done based on common target organ, which can be refined based on common phenomenological effects (e.g. common observed effects) and further refined when more upstream AOP/MOA information is available. Indeed, this approach was recommended for example by EFSA's PPR Panel for the cumulative risk assessment of pesticides (EFSA, 2014). When insufficient or no information is available, a conservative methodological choice will be to treat all chemicals with the same downstream effects as if they acted via a similar toxicological mechanism (even though they exhibit a wide range of chemical structural features) (EFSA, 2014). This approach is based on empirical evidence that chemically unrelated substances may have a common effect in target organs/organ systems, which can be well approximated by DA/CA (Kortenkamp et al., 2009). An additional example exists for effects for combined exposures to multiple chemicals caused by endocrine disrupting chemicals (EDCs) (Kortenkamp, 2007). The authors argue in this review that a strict focus on molecular mechanisms would be too narrow for estrogenic, anti-androgenic and thyroid-disrupting chemicals, and that the application of phenomenological similarity to grouping of these chemicals would be more appropriate. Similar argumentations are also discussed in Danish EPA, 2009.

Additional considerations for toxicological or biological responses/effect-based groupings:

- **Grouping along an AOP**: An effect-based grouping could start by identifying a common AO (identified from *in vivo* data, or epidemiological data, but also predicted from structural alerts and QSARs), which could be further refined based on shared upstream events. Alternatively, it could begin by grouping according to MIEs and earlier KEs, and refined further based on shared downstream events. This approach will largely depend upon what data is available. Since different MIEs can lead to the same AO (creating an AOP network), or the same MIEs to different AOs on the same target organ, it is recommended to not focus groupings exclusively on commonality at (sub)cellular level but to consider all information along an AOP for grouping purposes.

- **Relevance of exposure levels:** Another important aspect to keep in mind is the fact that most chemicals produce different effects at different doses and may cause multiple effects by different mechanisms. Consequently, it is important to address effects caused at the relevant exposure level (Borgert et al., 2004).

- **Consideration of multiple effects:** A chemical could be placed into more than one assessment group if it produces multiple effects by different mechanisms in one or multiple organs (e.g. causes neurotoxicity, as well as liver toxicity via two distinct AOPs). In such cases, the chemical could be placed in more than one group, and be subject to more than one assessment. Other situations that have been encountered for chemicals with multiple effects when forming assessment groups are

demonstrated in the following examples from an EFSA Opinion on the prospective
risk assessment for pesticides (EFSA, 2014):

- o In order to address chemicals that elicit different effects in the same organ, it is
  possible to group based on these target organ effects that can be refined using
  further available information derived from available AOP/MOA. It needs to be
  acknowledged that effects on the same organ do not necessarily lead to the same
  functional impairment and thus not always to common effects.

- o Alternatively, effects on different organs can result in the same overall effect
  (e.g. effects on kidney and heart affecting blood pressure in the same way),
  which could be considered together.

Apart from similarities in adverse effects, also similar metabolic pathways and metabolites
can be considered for grouping chemicals. Toxicokinetic data and physiologically based
toxicokinetic (PBTK) models can provide valuable input, when a metabolic route or
bioactivation to a toxic metabolite is important.

Considerations specific to environmental risk assessment in case of grouping based on toxicological similarities

Unlike human health assessments, the goal of ecological risk assessments is to protect the
population or ecosystem and not the "individual". As a result, the endpoints for ecological
risk assessments are broad and are population-specific. The non-food Scientific
Committees of the European Commission (SCHER, SCCS, SCENIHR, 2012) stated: "the
concept of "common mode of action" may have a different meaning in ecotoxicology in
comparison with human toxicology and should be referred to broader endpoints, such as
reproduction impairment, population growth, mortality etc." Effect-based grouping
approaches should also consider that chemicals exert different effects in different
organisms or at different concentration levels in the context of environmental assessment.

In ecological assessments, the AOP/MOA is often unknown and likely to be variable
among species (e.g. the same chemical may have a different toxicological mechanism in a
fish than in a plant). Narcosis, a reversible, non-specific disruption of cell membranes that
results in progressive lethargy, unconsciousness and subsequent death, is generally
considered to represent the baseline toxic effect for all chemicals. If chemicals produce
more severe effects than narcosis (e.g. as predicted by relevant testing or QSARs), a
specific mechanism can be inferred. An example of such an approach was presented in
Junghans et al. (2006), where based on such information components were categorised into
mechanistically defined subgroups, mainly distinguishing between non-polar narcotic
compounds and chemicals having an unknown (specific) mechanism of action in the algae
Scenedesmus vacuolatus.

When information on the AOP/MOA is available, chemicals could be grouped, for
example, based on effects elicited at different trophic levels. If a group has specific effects
on a certain sensitive trophic level and the risk is assessed for this group, the assessment
can be considered protective for other trophic levels. When AOP/MOA information is not
available and broad ecotoxicological endpoints are used to group chemicals, DA/CA may
be a conservative approach when chemicals have a different AOP/MOA leading to the
same outcome.

Combined exposures of potential concern can be identified by performing a Tier 1
combined assessment assuming additivity of the effects, irrespective of the toxicological
mechanism, for each trophic level (i.e. fish, invertebrates, plants) combined with a MCR

approach (Price and Han, 2011; Price et al., 2012b; Vallotton and Price, 2016). For general industrial chemicals, Backhaus and Faust (2012) recommend applying a DA/CA approach irrespective of the mode of action of the components in a first tier using the basic data set for algae, crustaceans and fish, making best use of data from single-substance assessments. Further investigations should be requested only if there are indications of unacceptable environmental risk. They recommend conducting MOA driven analyses only if error estimations indicate the possibilities of substantial differences between DA/CA and RA/IA -based assessments.

5.3. Considerations for incorporating chemicals with limited data

For chemicals with limited information regarding their toxicity or mode of action, a conservative or cautionary approach can be taken to incorporate them into combined exposure assessments by:

- Forming assessment groups on a wider basis, by using partial or predictive information.

- Using lower assessment tiers, which can then be refined (subcategorised) at higher tiers upon generation of additional data.

- Grouping together chemicals with similar functional use, as this may be indicative of similar toxicological profile.

- Grouping together chemicals with similar structures and physico-chemical properties.

The default assumption of a similar mode of action can be used as a protective approach. This concept is further illustrated in Chapter 5.4 and Tables 2 and 3.

5.3.1. Specific ecological considerations for incorporating chemicals with limited data

Aquatic risk assessment should comprise, as a minimum, acute toxicity data for crustaceans and fish and effects on algal growth for each component (unless the scope of the risk assessment were confined to a specific trophic level). However, obtaining all desired information for each component may be costly and time-consuming. Data gap filling for acute effects might be achieved using QSARs or read-across. Resulting EC/LC_{50} values can be sufficient to assess combined exposures using DA/CA based approaches. If experimental testing is done to meet the minimal data set, then the dose-response curve should be described so that also the RA/IA approach can be conducted.

Since mostly acute toxicity data are used for the combined exposure toxicity approaches in ecological risk assessments, chronic or sub-chronic effects might not be sufficiently addressed (e.g. endocrine disruption or reproductive effects). It is acknowledged that using acute-to-chronic ratios and application factors or interspecies correlations, might partly alleviate this situation (Kienzler et al., 2016a). A battery of AOP/MOA specific assays and tools could support the identification of alerts for specific effects even if focussing on known isolated targets, regardless of time scale effects. Results from such testing can be

put into context by mapping results on AOP networks, thus linking observed effects between different levels of biological organisation.

Filling of ecotoxicity data gaps for components can be achieved in different ways, applying e.g. QSARs or read-across apart from experimental testing. Another approach, which is however still under development, is the use of the ecoTTC (the threshold of toxicological concern concept developed further for environmental assessments based on data for aquatic organisms) (Belanger et al., 2015). Once available, its use in the context of assessment of combined exposures has to be further explored.

5.3.2. Considerations for use of AOP/MOA to facilitate the integration of data and the identification of data needs and subsequent targeted testing

As outlined above, when all available information on components is collected (regardless of whether the type of data are *in silico*, *in vitro*, *in vivo* etc.), the data can be further organised to derive information on their AOP and relevant information can be mapped to certain AOPs or AOP networks and patterns and similarities can be identified.

As already illustrated above (Chapter 5.2), the AOP framework allows existing data to be organised at various levels of biological organisation and to map relevant and different data types onto KEs in AOPs or AOP networks. In this way, the existing data can be integrated, providing an overview of toxicity profiles and identifying data gaps that can be further addressed with methods targeting specific KEs. Data from alternative methods (HTS data, in vitro, in silico) might be used to predict if and how perturbations are propagated at the molecular and cellular levels, to fill data gaps and inform decisions about the need for higher tiered in vivo data on specific chemicals. At the final stage, a weight of evidence (WoE) approach should be adopted to integrate the quantitative data coming from the different methods. The weight given to the individual tests should consider their relevance and their reliability.

A relevant conceptual framework is provided by AOP-informed Integrated Approaches to Testing and Assessment (IATA) (see Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA), OECD, 2016a). An IATA is an approach that integrates and weights all relevant existing evidence and guides the targeted generation of new data, where required, to build a hazard or risk assessment acceptable in regulatory decision-making. When estimates of exposure are included, the IATA can provide predictions of risk. Ideally toxicokinetic (TK) modelling should be used to estimate internal concentrations at the site of the MIE that result from external doses. These estimates of internal concentration can be linked to dose-response information for MIEs and ultimately to the dose-response of apical endpoints. By using tiered approaches in IATA, substances contributing less to combined effect(s), e.g. with high excretion rates, might be identified early in the process so that further efforts can be targeted on drivers of the overall risk of the assessment group.

Structured integration of different data types can be performed at different levels, including raw data and summarised level data. Different levels of data integration can then be used including Boolean combinations of categorised results, scoring approaches, decision trees, deterministic and probabilistic approaches (Tollefsen et al., 2014). As experience is gained, approaches to data integration can become standardised. Such approaches, called "defined approaches," can thus become core elements of IATA. A defined approach is a formalised decision-making approach consisting of a fixed data interpretation procedure (DIP) used to

interpret data from a defined set of information elements (OECD, 2016b). Defined approaches do not require WoE, which may be an advantage for some regulatory frameworks (WoE always includes some degree of expert judgement).

Where no clear AOP/MOA can be identified, such matrix can help to identify data needs and to design a targeted strategy to fill data gaps for confirming suspected pathways, e.g. by planning specific testing to investigate particular KEs or AOs. If modelled data are used and there are doubts regarding their reliability, any needs to substantiate the hypothesis by empirical data can be identified.

If components are first grouped based on common target organs, the information organised in this way and further generated to fill gaps, could help to refine groupings based on AOP/MOA information.

Modelled (*in silico*), empirical (*in vivo*) and analogue data (through read-across) can all be used in environmental combined exposure assessments. The quality and relevance of the information should be taken into consideration in a WoE context (i.e. substance was within the applicability domain of the model used; empirical data were robust and reliable, test species is geographically relevant, etc.), and reflect comparable exposure durations, endpoints, taxonomic groups, etc. *In vitro* data can be useful in examining modes of action, however, for using them in the hazard assessment a strong link needs to be plausible between the *in vitro* result and apical or population-level effects (e.g. growth, reproduction, survival).

5.4. Using a Tiered Approach and Considerations for Addressing Potency

When considering adjusting for differences in potency of individual chemicals in an assessment of combined exposure, one can utilise a tiered approach, refining the hazard (or risk) assessment as needed to answer the risk management question defined at the problem formulation stage. The same tier should apply for all substances in the group when moving through the tiers.

The initial hazard tiers of the combined exposures framework are based on the assumption that the substances act by DA/CA, which has the advantage of not requiring extensive (i.e. full dose-response) information on the individual chemicals being considered and is relatively more conservative than other models (WHO, 2009). The tiers range from the incorporation of primarily predictive approaches in the early tiers (i.e. deterministic exposure estimates, use of the most sensitive effect across the substance group, QSAR predictions, potency assumptions), to increasingly more refined data-informed analysis in the later tiers (i.e. refined exposure estimates, refined potency and MOA analysis, physiologically-based pharmacokinetic (PBPK) considerations). Accordingly, later tiers are also more laborious, modelling and data intensive, however, less conservative and are typically associated with a higher degree of certainty than the lower tier approaches.

Different frameworks for hazard characterisation have been developed that follow a tiered approach dependent on data availability (some examples are found in EC, 2010; Meek et al., 2011 (see Figure 1); Backhaus and Faust, 2012; CEFIC, 2012; Backhaus et al., 2013; ECHA, 2013, 2015; Bunke et al., 2014; Kienzler et al., 2014). When the WHO/IPCS framework is applied, the tier at which an assessment can be conducted is influenced by the amount of data available and the need for further refinement (Meek et al., 2011; Meek, 2013). For example, it may not always be necessary to conduct a higher tier assessment,

even though data availability permits, if this level of effort is not appropriate for the magnitude of potential risk outlined at the onset of problem formulation in the determination of the objective and scope of the assessment (e.g. priority setting or risk management).

5.4.1. Tiered approach for considering hazard to human health in the context of assessment of combined exposures to multiple chemicals

For human health assessment, building on the tiered approach outlined in the WHO/IPCS framework (see Figure 1, (Meek et al., 2011)), one can move through different tiers of assessment depending on data availability (see Table 2) to refine the hazard component of a combined assessment of multiple chemicals. The sequence of this tiering approach applies mainly to groups that have been constructed using an endpoint-based approach described in the scoping section (Chapter 4.4) but can also be used to inform the combined hazard assessment of groups constructed from an exposure perspective. If the groups are based on exposure considerations a single assessment group containing all chemicals may be required in tiers 0 and 1. It should be noted that Table 2 includes two concepts that are being refined - the hazard characterisation and the chemicals in assessment groups. These can be independent or dependent factors. Incorporating information that further refines the hazard characterisation might also result in a refinement in identifying chemicals in an assessment group.

Initially in Tier 0, a default approach of DA/CA without refinement can be used and the toxicity of the most potent compound can be applied as a surrogate for all components in the group. In the context of the DA/CA, available Reference Doses (RfDs) or Reference Concentrations (RfCs) can be used as starting point, with understanding that uncertainty factors that had been applied might vary (Benson, 2009) or the use of TTC values could be employed (Meek et al., 2011).

Following to Tier 1, if refinement is required, one could develop PODs for each of the individual components. In many cases, when looking for a specific combined effect such as liver toxicity, the underlying POD data for the components are reported for the effect driving the overall risk of the individual substance, e.g. neurotoxicity, and not specific for the effect under consideration, e.g. for the given common assessment group. Using the lower POD in the DA/CA approach will usually lead to more conservative assessments, which can be sufficient for lower tier and screening level assessments. In higher tier assessments, the specific PODs for the respective effect/target organ chosen to define the common assessment group can be applied.

Table 2. Refinement of hazard characterisation for human health, with increasing consideration of potency.

	Grouping of components in Assessment Groups (AGs)	Data needed	Considering potency of components	Data needed
Tier 0	Based on target organ and assumption of similar AOP/MOA	Minimally need predictive information on each component, but all data types can be considered: *In silico* (QSAR, read across) *In vitro* Phys-chem information In vivo	A Tier 0 is meant to be conservative Default DA/CA for all components without refinement Assume potency of most toxic component in a group for all components in a group Use of available reference doses as starting point, with understanding that uncertainty factors that had been applied might vary Could also apply threshold of toxicological concern (TTC) values	If hazard information available for only some components, an additional uncertainty factor can be added to the unknown component combined with use of the reference value of the most potent known component.
Tier 1	AGs remain as Tier 0	As above	Continue with default DA/CA Refined potency based on individual POD. Correction for the effective doses in the same range across substances Use of benchmark doses	Dose response for at least 1 KE in AOP for each component *In silico, in vitro, in vivo*, depending on availability
Tier 2	Refine AGs based on further information on AOP/MOA for individual components Grouping might be at level of molecular target (e.g. cholinesterase inhibitors)	*In silico, in vitro, in vivo*, can also include omics or other data types in an integrated manner To move to IA, need information to support different AOP/MOA	Consideration of relative potency by using POD on the dose response curve for the common critical effects (e.g. relative potency factors) Or IA approach	Requires similar information across substances from the same test system(s)
Tier 3	Incorporate increasingly refined information on AOP/MOA	Ideally information on several events within an AOP	Incorporate info on TK and toxicodynamic (TD) (PBPK and biologically based dose-response (BBDR) models), probabilistic estimates of hazard	Toxicokinetic-toxicodynamic (TKTD) information, model to translate to internal exposure

For further refinement, relative potencies can be considered in Tier 2, continuing to use DA/CA approach. In the case of DA/CA, the assumption is that the magnitude of the response can be predicted by summing the potency-adjusted doses of substances in the group. In determining which dose-response relationship should be used to compare the substances, one should consider the level of similarity in dose-response curves, information on toxicokinetics and the level of data availability. The adjustment for interspecies differences or route-to-route extrapolations within the database, if it is deemed necessary, is used. In addition, the POD will depend on the level of the available information e.g. selection of a benchmark dose (BMD), no observed adverse effect level/ lowest observed adverse effect level (NOAEL/LOAEL). BMD modelling is considered the preferred approach for deriving PODs, as it takes the entire dose-response curves into consideration (US EPA, 2012; EFSA, 2017; Haber et al., 2018). Often derived no-effect level (DNELs) or NOAELs might be more easily available, even though there are limitations in the comparison (based on different study types, different species and dependent on dose selection). Typically, more extensive information is necessary to carry out the refinement of relative potencies (see Chapter 7).

Further in Tier 2, if there is knowledge that the substances follow different AOPs/MOAs resulting in effects on the same target organ, then RA/IA instead of DA/CA could, in principle, be used as a refinement (EC, 2010; Holmstrup et al., 2010; Altenburger et al., 2013; US EPA, 2016). However, in examining evidence from pesticide combinations EFSA suggested that combined risk assessment methods derived from DA/CA should continue to be used for the assessment of mixtures with dissimilar MOA, provided they produce a common adverse outcome (EFSA, 2013b). The main reasons cited by EFSA are that:

a) Based on the widely used definitions of AOPs/MOAs it is difficult to decide when certain AOP/MOA is sufficiently distinct from another AOP/MOA to permit an assessment using IA;

b) Empirical evidence for the validity of IA as a prediction concept for combined exposure is available in the scientific literature related to bacteria and algae and not to mammals;

c) Under realistic exposure conditions the quantitative differences between predicted mixture effect levels derived from DA/CA and those derived from IA are likely to be small (under one order of magnitude); and

d) No case in the scientific literature demonstrates an example where the IA approach provides a more conservative combined exposure risk prediction than DA/CA (EFSA, 2013b). It was noted that in theory, a more conservative prediction might arise from an IA approach when all components exhibit very shallow dose-response curves.

Also Backhaus and Karlsson (2014) describe how ignoring IA or even using the sum of individual risk quotients as a rough approximation of DA/CA does not have a major impact on the final risk estimate of the examined mixtures. Details of applying the IA approach and approaches for combinations having components that include both similar and dissimilar MOA are further described in the risk characterisation section of the document (Chapter 7).

In Tier 3 assessments, processed information on MOA, including kinetic and dynamic parameters is required. Physiologically-based (PB) models, including PB-toxicokinetic models (PB-TK) and PB-TK-toxicodynamic models (PB-TK-TD) permit probabilistic estimates of hazard and characterisation by incorporating kinetic and dynamic variability and uncertainty. The use of PB-TK/TD models have been proposed in Tier 3 or higher assessments as they can improve the quality of data used by incorporating interspecies differences and human variability, extrapolating experimental data from high-to-low-dose situations and route-to-route and ultimately refining the predicted potency of chemicals (ATSDR, 2004; US EPA, 2007; EFSA, 2008; WHO, 2009; OECD, 2011; Meek et al., 2011). These models are built using anatomical information, physiological information, thermodynamic information and transport information (Fan et al., 2010).

Various PB-TK and PB-TK-TD models have been developed, taking into consideration a number of important metabolic steps, population variability in enzyme induction and metabolic rates, that are applicable for binary mixtures in the field of pesticides (Timchalk and Poet, 2008; Lee et al., 2010) and more complex mixtures such as solvents (Cheng and Bois, 2011; Bois et al., 2010; Fan et al., 2010) and polychlorinated biphenyls (PCBs) (Sasso et al., 2012). So far, these models have not yet been used by regulatory authorities for combined exposure assessment because of a number of limitations such as the lack of detailed knowledge on TK/TD data for multiple chemicals, the fact that they are resource intensive, need specialised expertise and may not always fit the purpose of a specific risk assessment. Since these models based on interactions and effects related to multiple chemicals continue to be evolved and refined, their use will start to increase in the field of regulatory toxicology.

5.4.2. Tiered approach for considering ecological hazards in the context of assessment of combined exposures to multiple chemicals

Similar to approaches for human health, in the ecological context, the two classical concepts in ecotoxicology for the hazard assessment of combined exposures, i.e. DA/CA and RA/IA, can be applied. Between these two approaches, it is generally recommended that the more conservative DA/CA approach should be used as the default where it is assumed that the components have similar MOAs for a species (see Backhaus et al., 2000; Walter et al., 2002; Faust et al., 2003). Table 3 outlines considerations for a tiered approach to refinements of hazard characterisation for environmental risk assessment. This includes increasing consideration of the relative potency of different components.

For risk to the aquatic environment from general industrial chemicals, Backhaus and Faust (2012) recommend applying a DA/CA approach irrespective of the MOA of the components in a first tier using the basic aquatic toxicity data set for algae, crustaceans and fish. In particular, they suggest that summing up ratios of predicted environmental concentrations (PEC) to predicted no effect concentrations (PNEC) (PEC/PNEC ratios) might serve as a justifiable DA/CA-approximation, in order to estimate in a first-tier assessment whether there is a potential risk for an exposed aquatic ecosystem if only base-set data are available. This leverages the results of existing single substance assessments as more demanding combined exposure assessments are requested only if there are first indications of an unacceptable environmental risk. However, when using Reference Values for Ecological/Environmental Risk Assessment (ERA), it needs to be considered that reference values such as the PNEC may be derived from varying species, endpoint and assessment factors. Using PEC/PNEC ratios is comparable to the hazard index (HI), where

PNEC is derived applying assessment factors. The use of PEC/PNEC ratios is less scientifically correct and considered slightly more conservative than the summation of Toxic Units (TU), however is suitable as Tier 1 approach (SCHER, SCCS, SCENIHR (2012)). A more refined approach is to use a summation of TUs, based on a specific (eco)toxicological endpoint.

Chevre et al. (2006) published an easy to calculate and communicate method for defining a Risk Quotient for mixtures (RQm) and an example applying this method for five commonly applied herbicides with a similar MOA. For the calculation of the RQm, consistent and comparable water quality criteria (WQC) are gathered for each single herbicide. The RQm is expressed as the sum of the ratios of the measured environmental concentration taking also into account peaks of different seasonally applied herbicides and the WQC for each herbicide.

The concepts of DA/CA and RA/IA are considered applicable in ERA at the population level. However, different components can have different effects/potencies on the various species and can lead to indirect effects on species and the structure and functioning of communities. While the chemicals act independently, having different MOAs and acting on different trophic levels, they could produce combined effects by both directly affecting invertebrates in the community (insecticide) and reducing the invertebrates' food supply (herbicide). The significance of this combined effect would need to be compared to natural variations in the food supply and the species of interest. So for community level assessments of combined exposures, more ecologically-based approaches are needed instead of toxicologically-based approaches alone.

The use of Species Sensitivity Distributions (SSDs), where data is available, gives an estimate of the potency distribution for entire species assemblages (De Zwart and Posthuma, 2005), thus not addressing indirect effects but at least covering the range of sensitivities across different species. Using SSD-based approaches can also help to overcome differences in data quality for individual species and makes best use of all available data. When using SSDs for the assessment of combined exposures, the best procedure would be, first to apply DA/CA or RA/IA models to each species separately and afterwards combine the results for an SSD for the mixture. This is however, usually not possible because this method is rather data demanding. Gregorio et al. (2013) have noted that when applying DA/CA or RA/IA to SSDs directly may lead to over or underestimation of the mixtures' effects. The size and direction of the error is dependent on a number of factors. In the example of concentration addition, if there are large standard deviations in ecotoxicity data used in constructing the SSD, applying DA/CA on the SSD leads to an underestimation of mixture effects. Therefore, these potential uncertainties should be considered if using this approach.

SCHER, SCCS, SCENIHR (2012) propose a decision tree for the risk assessment of mixtures for both human health and environmental risk assessment. The first step includes the assessment whether significant exposure is likely. For the environment, an exposure driven assessment needs to include at least a preliminary risk characterisation, as any exposure produced by emissions capable to modify the natural background must be considered as significant. Furthermore, in a first tier the comparison of the combined exposure to relevant TTC values is proposed. However, the restriction in applicability to human health assessment is made and it is not currently recommended for ERA. However, in the meantime, an activity on developing the TTC concept also for ERA (ecoTTC) is evolving (Belanger et al., 2015) and should be further explored for ERA of combined exposures once available.

Table 3. Refinements of hazard characterisation for environmental risk assessment, with increasing considerations of potency.

	Grouping of components in Assessment Groups (AGs)	Data needed for grouping	Considering potency of components	Data needed for potency assessment
Tier 0	Start with all chemicals in one group independent of MOA	-	Default DA/CA for all components without refinement In absence of information for individual components, assume potency of most toxic component in a group for all components in a group; Use of available reference doses (PNECs) as starting point, with understanding that uncertainty factors that had been applied might vary Could also apply ecoTTC values in absence of individual data	Acute or chronic toxicity values for e.g. algae, daphnia, fish. The endpoints will be chosen dependent on the scope of the assessment in the problem formulation Use PNEC or Environmental Quality Standards (EQS) from single substance assessments (most sensitive of different trophic levels) as first approximation For compounds without data, use ecoTTC, or PNEC of the most toxic compound or e.g. QSAR predictions
Tier 1	As above	-	Separate assessment for different trophic levels Refined potency based on individual POD (e.g. USA aquatic toxicity benchmark or the relevant endpoint) Instead of PEC/PNECs ratios use TUs	*In silico, in vitro, in vivo*, depending on availability Use specific PNEC per trophic level
Tier 2	Refine AGs based on info on MOA for individual components Refinement could be to divide between narcotic chemicals and specifically acting ones Grouping might be at level of molecular target (e.g. cholinesterase inhibitors)	*In silico, in vitro, in vivo*, also omics	More refined potency Do separate assessment for different trophic levels	WoE for available toxicological information

| Tier 3 | Incorporate increasingly refined information on MOA | Ideally information on several events within an AOP | Incorporate info on TK and TD, using biology based models (e.g. Dynamic Energy Budget models DEB) | TKTD information (e.g. no effect concentration, killing rate, elimination rate), model to translate to other exposure patterns, species and internal exposure* |

Note: *examples: Baas et al., 2010 and Jager et al., 2010.

The combined exposure risk assessment could include a discussion of the probability that different components would adversely affect their respective 'target' organism in a relevant temporal and spatial dimension and that those species would be interdependent to a degree that an ecological effect would result.

5.4.3. Interactions of chemicals and influence of potency

Depending on the type of interactions among chemicals, which can be either greater or less than additive, potency estimates can be influenced positively or negatively. In case of joint action between multiple chemicals that differ from DA/CA or RA/IA and are categorised as less than additive, the terms antagonism, inhibition or masking are used. When the joint action is categorised as greater than additive the synonymous terms synergism or potentiation are applied, depending on the degree of interaction (ATSDR, 2004; US EPA, 2007; EFSA, 2008; EFSA, 2013b).

Besides direct chemical-chemical interactions, hazard assessment and risk characterisation of combined exposure can also be influenced by toxicokinetic and toxicodynamic interactions. Toxicokinetic interactions between chemicals may trigger deviations from additivity by interfering with absorption, distribution, metabolism and/or excretion. For toxicokinetic interactions, it is important to determine whether deviation from additivity is synergistic or antagonistic and when the interaction results in toxification, detoxification or alterations at the expected internal dose that reaches the target organ. Toxicodynamic interactions involve interactions between the biological responses from exposure (internal dose) to the individual substances in the mixture (EFSA, 2013b), e.g. modulation of homeostasis or repair mechanisms. The ratios of the components can also play a role on whether the joint action of the specific components will follow DA/CA, IA or greater than these (synergism) or is less than these (antagonism) as different ratios can lead to different interactions.

Boobis et al. (2011) reviewed 90 studies that reported observing evidence of synergy in mammalian test systems performed at low doses (i.e. close to the POD) for individual chemicals. Only in six of the 90 studies, useful quantitative information on the magnitude of synergy was reported. In those six studies, the difference between observed synergisms and predictions by DA/CA did not deviate by more than a factor of four. Also the available empirical evidence and considerations from various mainly EU committees and panels, suggest that synergisms at dietary exposure levels are rather rare and that synergisms cannot be predicted quantitatively on the basis of the toxicity of components (EFSA, 2013b, ECETOC, 2012). Similarly, for environmental risk assessment, Cedergreen (2014) performed a systematic literature review for binary mixtures within three groups of environmentally relevant chemicals (pesticides, metals, antifouling agents). Synergy was

found in 7%, 3% and 26 % of the 194 binary pesticide, 21 metal and 136 binary pesticide, and metal and antifoulant mixtures, respectively. The extent of synergy was rarely more than a factor of 10. Based on some more in depth analysis Cedergreen concluded that true synergistic interactions between chemicals are rare and often occur at high concentrations. Using standard models as DA/CA is regarded as the most important step in the risk assessment of combined exposures. However, when there is evidence suggesting interactions for the chemical components, these should be considered on a case-by case basis, as confirmed in an expert survey (Bopp et al., 2015).

The concept of interactions in the context of ERA can be widened since, as discussed above, interactions (synergism and antagonism) could occur due to indirect effects in the ecological context. At the community level, the concept of "synergism" is also possible, considering the combined effects of different chemicals on different taxonomic groups and the indirect consequences on the structure and functioning of the community.

Chapter 6. Considerations for Exposure Characterisation to Inform Assessment of Combined Exposures to Multiple Chemicals

	Problem Formulation and Scoping	• Identify regulatory question to be answered and its scope • Determine need to conduct combined exposure assessment
	Hazard and Exposure Characterisation	• Conduct in parallel • Apply tiered approach to both elements progressing until regulatory question can be answered or data limits further refinement
	Risk Characterisation	• Answer regulatory question • Document and communicate uncertainties
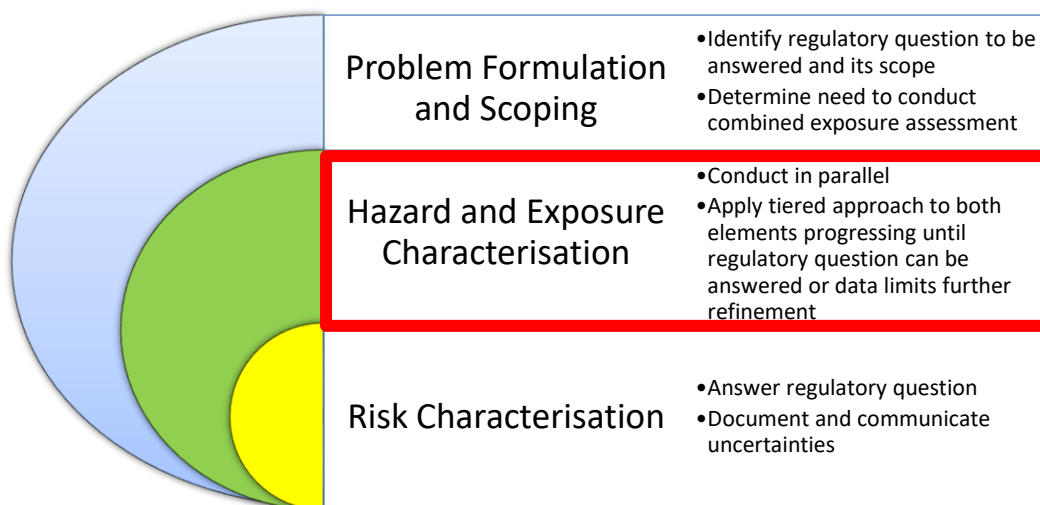
6.1. Introduction

The purpose of this chapter is to examine the many factors to consider when characterising exposure in the context of assessment of combined exposure to multiple chemicals. As highlighted in Chapter 4, information on co-exposure can be used to group chemicals for assessment, or if the group is initially formed from a hazard perspective, then co-exposure information can be used to sub-group chemicals relevant for a combined exposure assessment.

Characterisation of combined exposure for humans and the environment is based on the perspective that populations are exposed simultaneously to multiple chemicals via single or multiple exposure routes and pathways. Humans can be exposed indirectly to chemicals through environmental media into which the chemical is released or transported, directly through contact with a consumer product in which it is present, or through the ingestion of contaminated food.

Key sources of exposure of the public include food, drinking water, medicine, tobacco products, soil, dust, air and consumer products (e.g. cosmetics, personal care products, household products, electronics, construction materials, textiles, surface coatings,

adhesives, sealants, disinfectants, automotive care products, toys). Workers are mainly exposed through direct handling of chemicals or through the environment at workplaces.

The main sources of environmental exposure include emissions to the atmosphere, ground water or surface water and soil through a range of processes, such as combustion from industrial sources (e.g. smelters, furnaces, engines), commercial/residential sources (e.g. motorised vehicles, cooking in the home) or deliberate application or discharge (e.g. pesticide or biocide application, cleaning of industrial vessels or surfaces, wastewater treatment system effluents) and sources such as spillage, leakage, runoff or spray-drift. Leaching from products disposed in landfills or through application of bio-solids to agricultural soils could be a source of environmental exposure.

Characterising combined exposure consists of identifying all potential sources of exposure to a chemical or a group of chemicals and identifying those that result in simultaneous exposure. There are multiple factors, which will help determine whether simultaneous exposure to multiple sources or chemicals is a plausible scenario. For both human and environmental exposure, measurements of substances in different environmental media such as air, water, sediment, soil, effluents, or in biological media such as blood or urine, can provide evidence of likely co-exposure. Also co-occurrence in products or likely simultaneous use of products informs co-exposure assessment.

The data to inform combined exposure assessment may be obtained through direct monitoring or measurement or can be modelled information. Additional information gathering may be required to move to higher tiers of assessment.

As with the hazard assessment chapter, this chapter mainly applies to component-based approaches as whole mixture approaches would follow standard assessment practice (e.g. comparing hazard assessment information of the mixture to exposure potential of the mixture). A challenge for exposure assessment for whole mixture approaches is to determine if exposure occurs to the same mixture tested for hazard. As discussed in section 6.2.3 components of mixtures tend to separate in the environment because of differences in physico-chemical properties. Direct exposure assessment of products can be more straight-forward when using a whole mixture approach, but if environmental concentrations are considered (i.e. predicted environmental concentrations of the mixture PEC_{mix}), they may also need to draw on component based considerations to estimate exposure and co-occurrence.

6.2. Factors affecting co-exposure

In characterising combined exposures, the scope of the assessment should be defined first, including: the relevant target population(s), the exposure duration or timeframe, and the sources and routes of exposure along with the chemicals involved (Price et al., 2012a) (see also Chapter 4). The scope of the assessment would identify the sources, uses, the lifecycle of substances, exposure routes, target population(s) and properties of the substance(s) (e.g. physico-chemical properties). This information is incorporated into the conceptual model, which graphically depicts the exposure, and is used, in part, to inform the decision of whether or not to perform a combined exposure assessment.

6.2.1. Sources, use patterns and lifecycle of exposure

All sources and uses of substances(s) identified in the scope of the assessment need to be considered to determine if and how they contribute to the combined exposure. These should be considered for both direct and indirect exposures. The following section presents factors to consider for exposures to chemicals released to environmental media, direct exposures to chemicals that occur as a result of the use of products in the home or workplace and dietary exposures to chemicals that enter the food chain.

For an assessment of human or environmental exposure to substances, collection of information regarding how chemicals are released from sources, and/or how uses lead to contamination, can allow the identification of the major contributors to combined exposure. Points for consideration (Groβ et al., 2011, US EPA, 2000) should include:

- Types and numbers of sources of release to the environment.

- How widespread or disperse the uses or the sources are.

- Location of sources (e.g. proximity to drinking water sources/communities/ecology).

- Waste disposal methods (e.g. releases to landfill/sewage).

- Seasonal release/use patterns (e.g. if release/use coincides with any breeding seasons).

- Concentrations of contaminants at target site (human or ecological).

- If a chemical occurs naturally and the contribution of natural sources to environmental concentrations need to be considered (e.g. certain metals).

When assessing exposure to chemicals from the use of products, collecting information on product use and co-use profiles is essential, especially if one needs to obtain a realistic scenario of combined exposure for a given population, as the assumption that several products are used simultaneously can easily result in overestimation (Bakker et al., 2014). In addition, information on how a product is used is necessary to determining the faction of the chemicals in the product that reach the exposure individual. However, the use of a chemical in a product will also result in exposures other than those that occur from direct use, through the various stages of the lifecycle of a product. This increases the potential for co-exposures with other chemicals.

When considering cosmetic and personal care products for assessment of direct exposure of humans to substance(s), the information gathered should include:

- (Consumer) use pattern information (area of application and/or application duration e.g. leave-on/wash-off, product amount, use frequency).

- Exposure duration.

- Concentration in products and/or dilution during use.

- Products used simultaneously or within a brief time interval (e.g. after brushing teeth using mouthwash).

- How widespread is the use of the product in the target population.

- Route of exposure (inhalation, skin contact, oral via food/drinking water).

Food is an important source of exposure to consider for a large number of chemicals. Therefore, consideration should be given regarding:

- Whether the chemical occurs in food (naturally or via intentionally added substances) or indirectly via migration from food packaging or originated from food processing (e.g. phthalates in the equipment or even the gloves of workers in food processing plants).

- The number of foods that could contain the chemicals of interest.

- Levels of residues in a specific food.

- Dietary patterns of consumption for the affected foods.

The EuroMix project has included the sparse non-negative matrix under approximation (SNMU) method for the identification of pesticide co-exposures (unpublished). This algorithm uses food consumption and pesticide residue data in food and drinking water from national surveys of 9 European countries to identify mixtures of concern and for prioritisation of testing.

Identification of use patterns of substances will lead to the development of exposure scenario(s) for exposure assessment (that define including route, duration and frequency of exposure), data collection or modelling strategies.

Information on use profiles and lifecycle stages of a group of substances, and the lifecycles of products that contain the substances, will help determine whether a combined exposure assessment is required. For example, if two substances of the same assessment group are produced in high volumes and are presented in similar products used by consumers and/or in environmental media and/or food, they might provide a strong case that they could co-occur and contribute to combined exposure. In addition, if more than one industrial use and/or more than one lifecycle stage occur at one industrial site, then a combined exposure assessment may be appropriate. If chemicals are used as alternatives in similar products, their use may also be mutually exclusive.

6.2.2. Pathways and routes of exposure

For both human health and ecological assessments, the exposure pathways and routes that are most relevant to the established potential co-exposure of the substances being considered need be determined. Multiple exposure pathways (e.g. air, water, food, soil, dust) and all routes, including oral, dermal (or via a mucosal membrane) and respiration

need to be considered and the contributions of each exposure pathway and exposure route need to be determined.

Although all the routes of exposure need to be identified for a given substance and a given population, not all exposure scenarios associated with one substance need to be included in the combined exposure assessment. Considerations should be clearly stated and a rationale should be provided; routes through which exposure does occur but does not lead to co-exposure should be identified.

6.2.3. *Physico-chemical and fate properties*

Characteristics of substances, such as physico-chemical properties and/or how they behave once released in the environment (degradation and partitioning) or once they enter an organism, will influence potential exposure. The knowledge of these characteristics informs a better understanding of potential co-exposure. Annex A and B provide examples of physico-chemical properties and how they are relevant to exposure characterisation.

The physico-chemical properties (e.g. water solubility, Kow, Koc, vapour pressure) drive the environmental partitioning of substances. Substances that are released together but partition differently would not necessarily be appropriate candidates for a combined exposure assessment; they may disperse to different media such that the same population of organisms would not be subjected to both. Therefore, in a combined exposure assessment, it is important to understand the fate characteristics of the compounds such as transport, persistence, bioconcentration and bioaccumulation properties in the environment, including biological and abiotic reactions and environmental distribution.

A chemical's transformation in the environment is influenced by biodegradation, photoloysis, hydrolysis and oxidation. The transformation rates for chemical reaction (abiotic and biotic degradation) are expressed in different rates, including reaction rate constants and half-lives. The rates are frequently measured empirically, but can also be predicted based on substances physico-chemical properties (e.g. MacKay level I-III model is used to define distribution and evaluate environmental compartment of concern, based on physical-chemical, fate information and source of emission (RIVM 1996)). Media-specific transformation rates provide a relative measure of the how persistent degradable substances might be in a particular environmental medium.

The roles of physico-chemical properties in transformation and transport are chemical specific. Properties such as Kow are relevant for organics but may not apply to metals and inorganic metal compounds. Other properties such as partition coefficients, suspended particles-water, sediment-water partition coefficients and the cationic exchange capacity may influence the fate of metal ions.

The persistence of a substance in the environment may enhance the likelihood for co-occurrence since releases of chemicals at different times will result in a co-occurrence of chemicals in the same environmental media. In contracts, substances that degrade very quickly are less likely to be reaching the same organism together. Persistence, long-range transport and environmental distribution of substances affect their environmental concentration and are derived from physico-chemical properties.

Biodegradation, the breakdown of organic compounds by microorganisms, is a significant environmental process in all environmental components. Precise estimations of chemical-specific transformation and degradation rates are difficult to calculate and to apply because

they are subject to site-specific physical and biological variables. The concentrations of chemicals in environmental media are also affected by partitioning across media. Certain substances when released to air will adhere to soil and dust particles (e.g. phthalates and naphthalene). This can result in mixtures of chemicals accumulating in dust.

Physical-chemical properties also provide information on potential routes of exposure or co-exposure. If the dermal absorption of substances is expected to be limited because of a large molecular weight then the dermal pathway could be ruled out. If the substance is diluted or degraded in the environment, then the environmentally mediated exposure pathways may be excluded from a combined exposure (Meek et al., 2011).

Physico-chemical properties may also help inform the prediction of the use of chemicals in products. The characteristics of a substance will influence its function (or "role") in a product, which can in turn provide information on whether it is commonly used, and if used, the range of the chemical's weight fractions in products (Isaacs et al., 2016; Phillips et al., 2017). The characteristics of a substance will influence its function (or "role") in a product or mixture, which can provide information on whether it is commonly used. Characteristics of a substance will also determine its potential to be bound to a product matrix, or its propensity to migrate to the surface of that matrix (e.g. plasticisers are not typically bound to the matrix and have a tendency to migrate out of plastic).

Information on the properties of products in which substances can be found is also critical to characterising exposure to that substance and therefore informing the potential for co-exposure. The physical form of a product (powdered, pellets, viscous liquid) or the method by which it is applied (spray and particle size) will determine the extent of potential for exposure and therefore co-exposure.

6.2.4. Magnitude, frequency and duration of exposure

The magnitude, frequency and duration of exposure are important determinants influencing the level of exposure and identifying potential for co-exposure. The magnitude of exposure is driven by the nature of the source, the physico-chemical properties of the substance and the exposure related behaviours of the target population. Frequency of exposure may be expressed as the average number of days in a year in which exposure occurs or it can be the number of exposure events per day/month or year (e.g. number of applications of a cosmetic per day, number of applications of a pesticide per year). The duration of exposure may be the length of time a population has been exposed to a chemical (e.g. a persistent pesticide) or it may be the length of an application event, if further exposure does not occur following application (e.g. 4 hours during paint application, then leaving the application area).

Knowledge of the frequency and duration of exposure to substances helps determine whether exposure is continuous or intermittent. As discussed in Section 5, the hazard assessor need to determine what are the effects that would be expected to occur as a result of short-term exposures and what effects will occur if the exposures are long-term and continuous. The exposure assessor needs to provide the hazard assessor information on peak short-term exposures that occur during exposure events and if the exposures are ongoing, the chronic exposures. These estimates of dose will differ even for the same population.

It is also pertinent to characterise exposures with different frequency and duration patterns in assessing the potential for combined exposures. As discussed previously, sources that

result in continuous long-term exposures are more like to result in co-exposures than sources that result in infrequent short-term exposures because of the greater potential for co-exposure in the same period of time. This can be especially important for the evaluation of acute exposures for sources that occur infrequently. In such cases it is useful to determine if there are any factors that would cause the events to co-occur. For example, application of lawn fertilizer and pesticides are not frequent events but they do tend to co-occur in summers and on weekends.

6.2.5. Specific target populations

Once relevant exposure routes and the major sources/uses contributing to combined exposure have been identified, potential vulnerable sub-populations may be investigated within the population of interest as defined in the problem formulation phase.

For human risk assessment, general population exposure and occupational/worker exposure are commonly considered. However, vulnerable sub-populations may be identified as described in reference documents (US EPA 2011b; US EPA 2002). Considerations for vulnerable human sub-populations are summarised below:

- For children/infants, consider specific locations (e.g. schools, day-care centres and playgroups), behaviour (e.g. chewing toys/objects, drinking pool water, spending more time on the ground), food/water intake (e.g. higher than adults per unit body weight), timeframes/frequency of exposure, etc.

- For pregnant women, consider occupation, timeframes/frequency of exposure, changes to endocrine system/metabolism, etc.

- For the elderly, consider specific locations (e.g. home, hospital and nursing home), medications, reduced ability for organs to detoxify, weakened immune system, etc.

- For workers, consider personal protective equipment, timeframes/frequency of exposure, indirect exposure to family members etc.

For the environmental risk assessment, the target may be a specific species, population, or community of concern in a specific environment, or a set of species representative of the general environment. Relevant pathways and routes of exposure depend upon a combination of environmental compartments they inhabit, the relevant sources of emission and environmental fate of multiple chemicals.

Exposure scenarios should be combined if they target the same population at the same timeframe of exposure. In that case, the combination is realistic and relevant.

6.2.6. Toxicokinetics

Kinetics or toxicokinetics is the study of chemical absorption, distribution, metabolism and excretion (ADME) of a substance from entrance into the organism until the complete excretion of the chemical. Although toxicokinetics is typically used to inform hazard, it can also inform exposure, as information on how a substance enters an organism and how it behaves once it has entered is directly related to its potential to result in exposure at the

target organ. Characteristics of a substance or those of the mixture containing the substance are directly related to its bioavailability (e.g. how easily it can be absorbed through body surface, respiratory organs or digestive tracts). Information on these processes helps to determine the extent of exposure and identify drivers of exposure in a group of co-occurring substances. ADME information can be used in determining when the maximum internal exposure will occur and the internal exposure can be estimated, e.g. the combination of one chemical with a peak internal dose at the first 24 hours while another chemical has a more flat curve over 7 days. Co-exposures for rapidly cleared compounds will typically have to occur at the same time or close together in time. This allows for internal concurrent exposures of the compounds. This is not the case for slowly cleared compounds where an individual's exposures may be separated by years or decades and still result in internal co-exposures (Demond et al., 2006). ADME information may also be used where biomonitoring information data are used as the basis for exposure estimates (Birnbaum and Cohen Hubal, 2006; Christensen et al., 2015) as the information needs to be interpreted to obtain exposure estimates (see Section 6.4.1.).

Substance-specific toxicokinetic properties can also be used to understand which exposure scenarios to pair with which hazard scenarios (e.g. chronic vs acute event) with respect to exposure frequency and effective internal concentrations. In addition, they inform route-to-route or interspecies extrapolations during combined exposure assessment and will facilitate the refinement of default interspecies extrapolation (WHO/IPCS, 2005).

Interspecies differences in toxicokinetics may be substantial among different taxonomic groups (particularly among different classifications, e.g. vertebrates, molluscs, arthropods and green algae) and resulting uncertainty needs to be considered in the practice of interspecies extrapolations. Life stage and size are also important considerations.

6.3. Data for evidence of co-exposure

6.3.1. Data type

The types of data that should be considered to support co-occurrence will depend on the scope and purpose of the exposure assessment and the outcome of the problem formulation. There are two main categories of data - modelled and monitoring data. They can be used together to increase the confidence in an exposure assessment.

The availability of data will vary based on region and location, the timing of the data collected (e.g. sampling dates, age of use pattern information), the target population and the type of analytical measurements. The geographic and temporal relevance of all data should be considered as a factor. The potential for co-exposure is dependent on regional use patterns, local regulations and enforcement, and environmental conditions.

Generally, good quality measured data that represents the exposed population (e.g. workers, consumers) or the environment are preferred over modelled data. As discussed in Section 6.4, monitoring data can be a very useful measurement in determining co-exposures when multiple chemicals are measured at the same time. Such measurements demonstrate the co-occurrence of substances in a sample taken at a specific place and time. Dietary monitoring for multiple substances demonstrates that individuals had oral intakes of specific combinations of substances. Biomonitoring data is especially useful since it is a measurement of the aggregate and cumulative exposures of an individual at a point in time. However, although measurements could provide information on the co-occurrence,

sometimes it is difficult to conduct continuous measurement resulting in limited amounts of data. In contrast, models can generate data taking into account factors that are difficult to measure, for example variable frequency and duration of source of substances. Determination of representative environmental sampling data, in terms of locations and periods covered, is a challenge and risk assessors routinely use their professional judgement to make this determination. Modelling can be used in certain cases to estimate substance concentrations in environmental media (ATSDR 2005). If modelled data are used, then the model should be sufficiently validated, accurate and relevant.

A number of models of inter-individual variation in combined exposure have been developed to inform human health risk assessment. These models not only determine doses for each chemical in an assessment but also model the patterns of co-exposure. The goal of the models is to determine realistic descriptions of inter-individual variation in patterns of co-exposure. Examples of such models include:

- Models of concurrent exposure to dietary residues of pesticides have been developed in Europe (Monte Carlo Risk Assessment Tool (MCRA) https://mcra.rivm.nl/Account/Login?ReturnUrl=%2f) and in the United States, The Cumulative and Aggregate Risk Evaluation System Next Generation (CARES-NG) (http://caresng.org/).

- Models of direct and indirect food additives such as the Flavourings, Additives, and food Contact materials Exposure Tool (FACET) is a software program that models combined exposures to chemicals that migrate from food contact materials or are directly added to foods (Oldring et al., 2014; https://expofacts.jrc.ec.europa.eu/facet/).

- Models of combined exposures to chemicals used in consumer products (Isaacs et al., 2016).

If modelled data are used, then the model should be sufficiently validated, accurate and relevant. In the absence of data or with limited data, the upper bound of deterministic estimates of exposure to the group of chemicals under consideration should be used, and limitations and uncertainties in the assessment should be clearly identified.

Examples of relevant data are provided in Table 4.

6.3.2. Data sources

The most useful data sources and the most useful combination of these data sources will depend upon the scope of the assessment, and relevance to potential co-exposure.

- Information from industry (e.g. through industrial surveys or regulatory submissions) indicating whether substances are part of the same processes at a facility (e.g. could be released together at the industrial stage), or end up in the same products (could be released together at the use stage).

- National programs or national surveys are an important source of information on substance concentrations in exposure pathways and routes (e.g. national programs surveying environmental levels including those in wildlife, food content or food

consumption, or measuring contaminants in human urine or blood (biomonitoring data); see Annex C for examples of such data sources).

- Safety Data Sheets (SDS) or household products databases can be used to identify products in which the substances are co-occurring or could lead to co-exposure.

For worker exposure, epidemiological and industrial hygiene studies can provide information on the various work activities, as well as the duration and nature of exposure (e.g. whether exposure occurring during batch or continuous processing). Epidemiological studies can be also associated with limitations.

Table 4. Examples of key data types to inform evidence of combined exposures

Type of Information	Examples of Type of Data
Substance information	- Physical characteristics (e.g. molecular weight) and chemical structure - Physico-chemical properties - Fate parameters - Toxicokinetics properties
Product information	- Product usage - Composition of products - Product function - Substance function within a product - Market penetration to estimate how broadly used the product is
Use pattern information	- General use patterns/sources (e.g. it is a wide spread/dispersive use or point emission/local source) - Typical co-use of products (e.g. mouthwash typically follows after brushing teeth using paste) - Frequency and amounts (typical products used daily, user survey data); - How and where (indoor/outdoor, open or closed system) - Method of application of a substance (directly applied to the skin, rinsed off, ingested) - Behaviour patterns and variations between seasons, regions, subpopulations and age groups (e.g. food consumption) - Target market for consumer products (age group, gender, etc.) - Basic characteristics of the populations (e.g. definitions on users (professional, industrial, or consumers), demographic information, economic status, health conditions, cultural background, dietary information, living environment) - Environmental compartment of primary exposure
Exposure levels	- Concentration measurements in potential exposure pathways (e.g. air, water, food, soil, dust) - Location of facilities producing or using the substance - Industrial process and lifecycle information such as continuous vs batch, where in an industrial process the substance is produced or added/used, frequency of release (continuous/intermittent) - Type of release (directly into the environment vs released into a sewer) - Wastewater treatment type
Concentration in target population or organisms	Biomonitoring data (e.g. measurement of levels in human biological samples such as urine, blood or breastmilk and in organisms collected in the fields)

6.4. Interpretation of monitoring data

This section provides key considerations to take into account when interpreting monitoring information including biomonitoring data, environmental or other monitoring data to identify co-exposure and to select appropriate methods to estimate exposure. Monitoring data can be used to ascertain co-occurrence and determine relative importance of exposure scenarios in the overall combined exposure assessment. Monitoring data provide information on magnitude of exposure and on pathways that are main contributors to the combined exposure; they are used to derive quantitative estimates of direct or indirect exposure. Typically, the use of monitoring data in an assessment of exposure is considered a refinement and signifies that the assessment is being conducted at a higher tier than Tier 1 (Meek et al., 2011).

6.4.1. Biomonitoring data

Biomonitoring data can be used to quantitatively estimate internal dose or absorbed dose from all exposure routes and can be useful to provide information on co-exposure. Biomonitoring data can be used to evaluate the amount of a chemical in the body and to measure multiple chemicals in the same individual (Birnbaum and Cohen Hubel, 2006; Christiansen et al., 2014; Lober et al., 2015; Qian et al., 2015). The following can be measured:

- Concentration of chemicals and/or chemical metabolites in biological tissues or sera (blood, urine, breath, hair, adipose tissue, etc.);

- Biological effect that occurs as a result of human exposure to the chemical (e.g. alkylated haemoglobin or changes in enzyme induction); and

- Amount of a chemical or its metabolites bound to target molecules.

Limitations to the use of biomonitoring data are analytical approaches, uncertainty and variability associated with the data.

Biomonitoring data provide exposure levels across a study population and provide a means of comparing exposures across population groups by age, sex, ethnicity, or other demographic descriptors (Aylward et al., 2013). Since biomarkers can be measured in humans, uncertainties due to interspecies differences do not apply. If substances are detected together in a significant number of samples from one population, it could be concluded that the probability of co-exposure is high. If a biomonitoring study is large enough and representative of a population, statistics can be applied to the study results to generate probabilities of co-exposure for different combinations of substances, and these probabilities could inform the estimate of combined exposure.

Considerations for selection of biomarkers depend on the purpose of the study. In general, biomarkers should be specific to the chemical or chemicals under the study, relevant to direct estimation of exposure and, ideally, relevant to the internal dose metric of interest in a toxicological context. Selection of biomarkers is also dependent on practical concerns, including invasiveness of sampling (e.g. urine is a more readily obtainable matrix than blood), the physical/chemical characteristics of the chemical (e.g. lipophilicity, volatility, etc.) and the available analytical techniques (Zelenka et al., 2011).

Evaluation of biomonitoring data, in comparison with other monitoring data, such as surveys on product use, environmental concentrations, emissions, etc., can be valuable in examining testing hypotheses about exposure pathways and magnitude of exposure. However, temporal considerations related to relative elimination half-life compared to frequency of exposure and other kinetic and physical/chemical properties will also inform this evaluation.

Biomonitoring data are useful in determining whether the exposures estimated through modelling are within the observed range of exposure and comparable with the integrated exposure of the population, and in identifying the relative contributions of sources of exposure. Population estimates of certain chemicals such as phthalates may differ by age, gender and ethnicity. Hays et al. (2012) compared several methods to estimate overall exposure. Models designed to estimate combined exposure (e.g. Probabilistic Aggregate Consumer Exposure Model (PACEM)) can also be validated by comparing in silico results with biomonitoring data (Dudzina et al., 2015), because it represents total internal burden of a substance in an individual. A comparison of actual versus modelled exposure is only meaningful if the modelled exposure considers all relevant sources and exposure routes, and the database is very good with respect to product information, consumer behaviour patterns and indirect exposure information (e.g. via house dust).

The estimation of actual exposure from biomonitoring data requires an understanding of the compound's toxicokinetics information. In this regard, the time of sampling for biomarkers is critical. It is important to consider how long the biomarker presents after exposure to multiple substances, whether the exposures co-occur, and the specificity and half-life.

Biomonitoring data represent an integration of exposure from all sources and routes, which provide an important perspective on overall exposure. However, without exposure pathways information, it is difficult to relate biomonitoring results to sources and routes of exposure (Albertini et al., 2006), which is a challenge when there is a need to develop effective risk management measures. Biomonitoring data typically cannot provide a direct indication of the source of the monitored chemical or exposure pathway, with a few notable exceptions. Aylward et al. (2011) showed that patterns of concentrations of biomarkers for DEHP versus time since last food consumption were consistent with the hypothesis that exposures were occurring via the food pathway, and Lorber et al. (2015) found associations between type of food consumed and BPA biomarkers. The uncertainty around source determination can be decreased by using survey data (e.g. questionnaire on personal care product use), that can be collected at the time of biomonitoring sample collection, or by comparing the biomonitoring data with relevant environmental or food monitoring data or the result of modelling.

Extrapolating exposure estimates from biomonitoring data at a point in time may not account for the potential continuing increase in the body burden if the similar use patterns continue.

6.4.2. Environmental monitoring data

Detection of several substances in the same environmental sample in a well conducted study in a significant number of samples representative of a region can be very useful evidence of co-occurrence (e.g. phthalates in Canadian dust samples, Health Canada and Environment Canada, 2015). When exposure to organisms (including humans) occurs

through the presence of substances in environmental media, rather than direct exposure to a particular product, the consideration of whether substances originated from the same product may be less important. Similarly, if substances are present at the receptor organism at the same time (co-occurrence), this can be either due to the fact that they were released from the same source or from different sources contributing to the same discharge point or to the same receiving area (e.g. waterbody or airshed). The interest in looking at one or the other or both would have to be determined at the onset of the assessment based on the purpose of the assessment.

Information detailing environmental fate and effects and environmental partitioning is relevant. The number of sources, source strength, timing of release and source locations are also useful. It may be quite difficult when looking at monitoring data to identify the sources of the substances, unless the use patterns are very well known and/or substances are specific in their uses.

Understanding the use patterns of substances, for example whether particular substances can be released through point sources emission from industrial use (local source) and/or diffuse sources by consumers or professionals, can help with interpreting whether monitoring data from different locations and/or timepoints are likely to be representative of levels found in the environment and selecting the best approach for estimating exposure. Widespread and wide dispersive use of a substance may increase the likelihood of co-exposure. As discussed in section 6.2.4, continuous or frequent exposure would be more relevant for co-exposure. If the substances are known to be coming from the same effluent or emission, then monitoring data for this effluent or emission, along with consideration of dilution, dissipation and degradation, may be sufficient for characterising potential exposure levels. If the substances are known to be coming from more than one source, then it would be important to have effluent/emission monitoring data from each of the sources, or alternatively to have monitoring data from the ambient environmental media, which would integrate concentrations from all sources.

The limit of detection and the number of analyses included in the monitoring need to be carefully reviewed based on the problem formulation. In particular, the detection limits should be sufficiently low to support a combined exposure assessment without introducing bias from non-detects (Vallotton and Price 2016).

Monitoring data can also be used to validate or inform models that are designed to quantify combined exposure (e.g. environmental monitoring data were used to validate PACEM, using combined exposure to cyclic siloxane D5 as a case study (Dudzina et al., 2015)). Models are typically used for Tier 1 exposure estimates (Meek et al., 2011). Models might be used preferentially in order to extrapolate to a generic scenario that would be protective of other locations. Alternatively, a first-tier approach might use the highest exposure estimates available for each substance, regardless of whether they come from monitoring or modelling, to be conservative.

In using environmental monitoring data, readers are referred to a previous OECD guidance document "Guidance document for exposure assessment based on environmental monitoring" (OECD, 2013) which describes the basic methodology used to conduct an exposure assessment based on environmental monitoring data. The key issues covered in the document include general considerations when using environmental monitoring data for the purpose of exposure assessment; fundamental properties of monitoring data; selectivity and sensitivity of analytical methods; representativeness of monitoring data in the spatial and temporal distribution of environmental concentrations; consistency of monitoring and modelling approaches, temporal variation of environmental concentrations

and density of sampling points for specific assessment purposes; and statistical data analysis and metric selection.

6.4.3. Other relevant data

Several consumer product models capable of exposure estimates have been developed and are currently available, particularly for cosmetics and personal care products (e.g. CRÈME RIFM, Lifeline, SHEDS, PACEM, Crème Care & Cosmetics) (ECETOC, 2016). A major challenge in estimating exposure in many product categories is obtaining representative information on exposure factors (e.g. habits and practices data, co-use data, chemical occurrence data and presence probability data), as well as potential correlations between these factors.

For the case of several substances in a single product, information on relative bioavailability of each constituent is needed. In the case of co-exposure to substances in different products, likelihood of co- use within the context of metabolic timeframes must be established. It would require the use of population survey data or the creation of a fictive population with use survey information. An estimate of combined exposure should be constructed for a single person considered representative of a given population to ensure that no unrealistic or unrepresentative combinations of exposures are added in the combined exposure estimate (Delmaar and Van Engelen, 2006).

6.5. Data needs, limitations and uncertainties moving through tiers of exposure assessment

The objective of a tiered approach is to ensure that no more resources are invested than are necessary to make a decision, to maximise efficiency in screening and prioritisation. A tiered approach is based on a hierarchical (phased) approach that involves integrated and iterative consideration of exposure and hazard at all phases, with each tier being more refined (i.e. more realistic and less uncertain) than the previous one, but more labor and data intensive (Meek et al., 2011). The analyses of direct and indirect exposure data, and their limitations and requirement for moving through the tiers of the WHO/IPCS framework, are summarised in Tables 5 and Table 6 respectively. The tables also show the limitations and uncertainties associated with the tiers. Consideration of the exposure factors, limitations and uncertainty required for the exposure assessment of multiple chemicals is similar to what is required for the exposure assessment of single chemicals.

The choice of the tier depends on data availability, the purpose of the risk assessment and the resources available. First, at the lower tiers simplistic general scenarios are developed, while more complex representative receptor-orientated scenarios are created at the higher tiers (receptors may be individual organisms or populations in the environment or human individuals, groups, or populations) (EFSA, 2013a, Meek et al., 2011). Exposure assumptions can progress from being deterministic at lower tiers to more complex probabilistic methods (e.g. more robust, more certain exposure estimates although more labour and data intensive) at higher tiers.

In a Tier 1 exposure assessment, generic exposure scenarios are developed. These scenarios would be based on assumptions and modelled data, rather than measured data. Human health scenarios might consider a number of similar uses (e.g. use of cosmetic products)

and make conservative assumptions (e.g. 100% dermal absorption). These types of assessments are very conservative, giving a "worse-case" result that is not necessarily realistic combined exposure estimation (Gosens et al., 2011). Environmental scenarios might consider contaminants with similar environmental transport/fate and include conservative assumptions (e.g. 100% of contaminant not removed by sewage treatment).

At the higher tiers for human risk assessment, a person-orientated approach (Delmaar and Van Engelen 2006) is recommended to avoid overestimations of exposure and therefore scenarios should represent a realistic situation for the individual. A Tier 2 exposure assessment would include better defined and tailored exposure scenarios and thus be more realistic and refined. These scenarios might include chemicals/contaminants with the same mechanism of action and make use of more measured data to make fewer assumptions (e.g. use of measured dermal absorption data). A person-oriented approach, which has been described for assessment of exposures (Price and Chaisson, 2005; Van Raaij, 2009; Bakker et al., 2014; ECETOC, 2016), should be applicable for assessment of combined exposures to multiple chemicals. As suggested, this person may be a hypothetical person that represents an entire subpopulation (e.g. a heavy user simulating a worst-case scenario or an average user of a number of products) or a realistic individual in a population in the case of a probabilistic assessment (Bakker et al. 2014). The method of person-orientation ensures logical consistency in the exposure assessment as it excludes unrealistic combinations of product exposures (e.g. the simultaneous exposure to multiple chemicals used in baby care products and hair dyes) (ECETOC, 2016; Van Raaij, 2009).

Tier 3 exposure assessments may be fully probabilistic with a limited number of assumptions (e.g. based on measured data) (Meek et al., 2011). Consequently more complex exposure scenarios are developed. For example, a high tier assessment of combined exposure to consumer products might consider sub-scenarios for different products, products that are used simultaneously, seasonal use patterns, use patterns for vulnerable sub-groups etc. (Delmaar and Van Engelen, 2006).

Evidence for co-exposure can be based on a number of data types and will need to be more or less refined depending on the level of complexity (Meek et al., 2011) and type of assessment. For a screening/ lower-tiered assessment, reasonable, worst case or upper-bound assumptions can be made and co-exposure can be justified qualitatively (e.g. based on use pattern information or physico-chemical properties). For such assessments, conservative exposure data inputs are used and can be approximate/rough data and may use a number of defined assumptions. If no risk is identified from combined exposures for this scenario, then no further refinement is needed. If it is determined that refinement is needed, then more concrete evidence of co-occurrence may be needed (e.g. biomonitoring or environmental monitoring data).

For the exposure to environment, a similar approach may be applicable. As for physico-chemical properties, emissions and occurrence in the environment, considerations common to the case of single chemical or single source are required. Additionally, geographical distributions of multiple emission sources, different temporal patterns of emissions from these sources, and resulting geographical and temporal distributions of multiple chemicals need to be considered in a more detailed, site-specific manner in higher tier assessments.

Table 5. Relationship between the exposure scenario, approach and limitations in direct exposure data; moving through tiers of the WHO/IPCS framework (Meek et al., 2011; US EPA, 2007; WHO, 2009a)

	Exposure scenario / Data Used	Approach	Limitation
Tier 0	Long term and widespread use across several sectors (e.g. food, pesticides, industrial chemicals, cosmetics and pharmaceuticals) Consideration of sentinel exposure scenarios	Relatively broad assumptions Based on predictive information or hypothetical scenarios Conservative assumptions or safety factors have been incorporated	Analyses of exposure limited to one or two disciplines (e.g. consumer or occupational or environmental exposure) Crude semi-quantitative estimates
Tier 1	Conservative upper bounding estimates of total intake based on simultaneous use of multiple consumer products assuming that a person would use and intake all substances in the products Consideration of use pattern, including similarities and differences in use (e.g. whether products containing the chemicals are used within a relevant time)	Conservative estimates for potentially highest exposed groups Consideration of chemicals in groups, rather than individually or based on combined exposures	Individual differences in exposure, duration, use patterns, inhalation rate, bodyweight, etc. are not taken into account Inconsistency between biological half-life data and predictions based on physico-chemical properties
Tier 2	Consideration of monitoring data for different subgroups (more or less sensitive, more or less at risk than the rest of the population)	Conduct deterministic sensitivity analysis by establishing low, medium, or high values of exposure parameters	More data required
Tier 3	Consideration of differences in absorption and disposition among the substances included in the risk assessment Monitoring data for exposure distribution (who can be more or less exposed)	Use of probabilistic modelling	Extensive data requirements Considerable development work is necessary Considerable representative information on exposure for the scenarios of interest for the relevant populations for different uses

**Table 6. Relationship between the exposure scenario, approach and limitations in
environmental exposure data; moving through tiers of the WHO/IPCS framework (IMM,
2011; Meek et al., 2011; US EPA, 2007; Vermeire, 2009)**

	Exposure Scenario / Data Used	Approach	Limitation
Tier 0	Monitoring data for levels of chemicals, usually only in the environmental medium of the primary concern Generic environmental fate models	Broad conservative exposure estimates Predictive information based on chemical structures and physico-chemical properties	Far from realistic exposure scenarios Rarely a measure of realistic exposure for the entire population
Tier 1	Additional environmental monitoring data including for contaminated sites More detailed environmental fate models Conservative upper bounding estimates of total intake based on maximum levels in air, water, dust, food and feed	Single-medium or multimedia models to estimate environmental exposure Estimated concentration in each intake medium based on generic scenarios	Considers only routes that dominate the total exposure estimation Does not consider consumption rates or variability in types of food consumed
Tier 2	Measured (bio)monitoring data for different subgroups (more or less sensitive, more or less at risk than the rest of the population) Site-specific environmental fate models	More accurate estimate of bioconcentration, biotransfer and bioaccumulation based on representative measured data Refined with information on registered uses, tolerance, use pattern, use information	More data required but uncertainty in exposure estimates can be decreased when reliable and relevant measured data used
Tier 3	Representative population or community of the target species in a spatial scale of interest (e.g. regional or national) Source apportionment as necessary	More accurate spatial estimation of exposure with refined source determination	Extensive data requirements Applying laboratory derived to field situations

Chapter 7. Considerations Regarding Risk Assessment of Combined Exposures and Capturing and Communicating Uncertainties in Findings
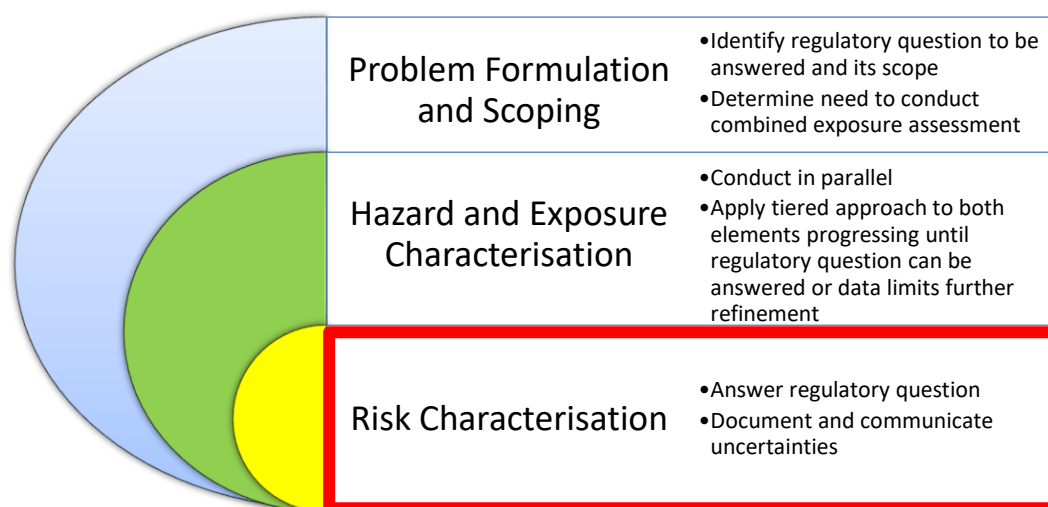
This chapter outlines the principal methodologies and mathematical approaches for the risk characterisation stage of combined exposure to multiple chemicals. It also provides an overview of the major tiered approaches that are described in the open literature in order to optimise resource usage. Afterwards, opportunities for integrating ecological and human health risk assessments of combined exposures are outlined. The chapter ends with a section focusing on identifying and describing uncertainties in the risk characterisation.

7.1. Introduction to risk characterisation of combined exposures using a tiered approach

Characterising the risk from combined exposures to multiple chemicals aims to:

1. Identify whether there are concerns, as determined by the question formulated in the problem formulation and scoping stage.

2. Quantify the magnitude of the risks of the combined exposure and the conditions under which such risks are likely to manifest using a weight of evidence approach that considers multiple lines of evidence.

3. Identify the groups of chemicals that are particularly important risk drivers that should be targeted by risk management activities or controls.

Similar to the assessment of individual chemicals, the process can be mainly prospective in nature, i.e. assessing the consequences of management measures, such as the authorisation of a chemical product with certain uses or the modernisation of a sewage treatment plant, before they are actually implemented. The assessment can also be retrospective, i.e. aiming to characterise the risk of an existing pollution scenario and/or establishing the link between the presence of a complex chemical mixture and an impairment of human health or ecosystem structure and function (see scenarios in Table 1). Given the variety of often-conflicting aims, an appropriate problem formulation, as described in Chapter 4, is essential for any assessment of combined exposures. Additionally, in view of the multitude of possible approaches and the substantial amount of data that is needed (either experimentally determined, modelled, or collected from various literature sources), specific care has to be taken to ensure consistency, transparency and traceability of the assessment process.

The considerations for pairing hazard and exposure data information for the risk characterisation of combined exposures are very similar to those for the risk characterisation of single chemicals. However, there is one important difference. In single substance assessments, the exposure and hazard assessment can be done independently of one-another and their outcomes are then combined in the final risk assessment. In contrast, in the risk assessment of combined exposures, the interdependence between hazard and exposure requires that their assessments be completed in tandem. For instance, before the hazard assessment can begin, some basic exposure information is required showing evidence for potential co-occurrence or co-exposure. Likewise, the hazard assessment provides information on which compounds are drivers of toxicity, and may therefore need more detailed exposure evaluations (while coarse estimates of exposure might suffice for the other, less important chemicals). In addition, the duration of exposures to specific mixtures will dictate if the acute or chronic effects are the relevant endpoints for the assessment.

Tiered approaches are often used in order to refine either hazard, exposure and/or risk estimates in a controlled, stepwise manner. They allow for developing a "fit for purpose" assessment that uses only the resources necessary to answer the risk management question of interest. The tiered approaches for hazard and exposure were described in Chapter 4 and 5 respectively. The most common aim of tiered approaches described in the literature (Table 9) is to assess the risk of combined exposures to either humans or the environment starting with less data and less resources and increasing accuracy when moving through the tiers. Under these conditions, there are only three possible outcomes after the completion of a tier:

1. There are no indications of unacceptable risk, in which case the assessment is considered complete and there is no need for further action.

2. There are clear indications of unacceptable risk, in which case the assessment is also considered complete and risk management measures need to be implemented.

3. There are indications of unacceptable risk and it is unclear whether they are a consequence of the limited, conservative assessment in a given tier.

This last outcome calls for a progression into a higher assessment tier. Often this would require additional data generation and gathering. A shortcoming of the applied tiered

schemes suggested so far is that no clear quantitative guidance is provided for the choice between situations (2) and (3). However, an important question in this context is whether the implementation of the following tier can lead to a different regulatory outcome of the assessment. Depending on the context it may not make sense to invest the resources to implement the next higher tier.

When it is projected that implementation of the following tier may impact the outcome of the assessment and thus appears desirable to conduct, the decision to go ahead may still depend on the legal or practical possibilities to request or generate information, availability of resources and legal timelines. If for such reasons, the tier is not implemented, the uncertainties and their potential impact on the outcome need to be transparently documented.

7.2. Methodologies and Mathematical Approaches Applied for Risk Characterisation

As introduced in Chapter 3 and 4, approaches for risk assessment of combined exposures can be grouped into two fundamentally different classes, whole mixture testing approaches (WMAs) and component based approaches (CBAs). Risk assessment for both approaches is described, and the mathematical concepts outlined, in Table 7.

7.2.1. Whole mixture testing approaches (WMAs)

WMAs, sometimes also called top-down approaches, are used to assess complex mixtures of sometimes unknown chemical composition, such as wastewater effluents, but also chemical products (natural fragrances, fermentation products and formulated pesticides), as if they were a single chemical (Rider and Simmons, 2015; US EPA, 2000). WMAs are for example used for assessing so-called UVCB substances (Substances with Unknown or Variable Composition, or of Biological Origin) under REACH (Fisk, 2014), for Classification and Labelling purposes (CEFIC, 2016), but are also part of the risk assessment procedure of pesticide and biocide products in Europe (EFSA, 2009a, 2013c, 2013d; ECHA, 2014).

The biggest advantage of WMAs is their inherently holistic nature, as all components found in a sample, even if unidentified, contribute to the assessment result, and potential synergistic interactions are considered (Kortenkamp et al., 2009, Backhaus et al., 2010). This approach works well when the source of exposure is a discrete mixture such as a commercial product and there is a direct exposure to the mixture. WMA cannot be used for many assessments of combined exposures when the exposure pathway results in different exposures to different components of the mixture. For example, exposure to gasoline vapours during vehicle refuelling is not the same as exposure to gasoline since the composition of the vapour differs from gasoline (contains more of the highly volatile components). WMA also does not work well when the composition of the source of exposure changes over time. A second limitation of WMA is the need for separate data from different mixtures. This suggests that WMA are most useful when there is an understanding of the chemicals driving the observed toxic effects and/or their interactions that allows the ability to extrapolate across similar whole mixtures (Rider and Simmons, 2015).

Whole mixture testing approaches, e.g. in soil, sediment or surface water, can also be used to identify areas of concern and thus guide combined exposure efforts to the relevant cases. They can also serve to calibrate component-based predictions (as a "real world check").

The Mixture RfD/RfC (e.g. derived from testing of the whole mixture) can be applied for whole mixtures and sufficiently similar mixtures. Application of this approach to a whole mixture uses the same methods/procedures as that of a single substance assessment (Rider and Simmons, 2015). In the absence of data for the whole mixture, data from a surrogate whole mixture, determined to be chemically and (eco)toxicologically sufficiently similar, can be used for estimating the risk of the mixture of interest (US EPA, 2000).

Comparative potency can be used for groups of similar whole mixtures, referring to chemically related classes of whole mixtures that act by a similar mode of action with closely related chemical structures. Available information about the substance and shifts in chemical structure and resulting relative potency ratios of the components can be used to perform the risk assessment. The scaling factor can then be applied across the group of similar substances to estimate the relative toxicity in relation to the reference substance (US EPA, 2000).

Groups of whole mixtures for which environmental processes can affect exposure, as well as the resulting toxicity, can be assessed using Environmental Transformation Approaches. The concept is to adjust the risk assessment based on what is known about the mixture because of environmental transformations (e.g. changes in a mixture's composition in the environment). In this approach, toxicity information available for selected environmental mixtures can be used as a surrogate for the toxicity of similar environmental mixtures (US EPA, 2000).

7.2.2. Component Based Approaches (CBAs)

CBAs are used to estimate the hazard or risk of combined exposures based on information on exposure and hazard for each individual component. They are therefore dependent on the availability of information on the individual toxicity and exposure information for all relevant components. Consequently, CBAs are most appropriate when all of the components are defined. A strength of this approach is that they can be used prospectively, without the need to conduct experimental hazard characterisation on a combination of interest.

Most CBAs can be traced back to only two different fundamental concepts for predicting/calculating combined risks (Boedeker et al., 1992). DA/CA and IA describe a quantitative relationship between single substance toxicities and the toxicity of a combination of these chemicals (Kortenkamp et al., 2009, SCHER, 2012, Bopp et al., 2015). As described in Chapter 3, they are based on opposite assumptions on the respective similarity or dissimilarity of the mode of action of the components within the combined exposure assessment. Both concepts can also be found under various other names (Faust et al., 2001) and both approaches are based on the assumption that the components do not influence each other's toxicity and do not chemically interact.

According to DA/CA, the toxicity of combined exposures of toxicologically similarly acting chemicals can be estimated directly from the sum of the doses/concentrations, scaled for their relative toxicity. DA/CA has become popular in risk assessment of combined exposures, due to its ease of application, comparatively broad empirical foundation and the notion that it often provides a slightly cautious approach to risk estimation of combined

exposures (Kortenkamp et al., 2009; SCHER, 2012). It is implemented in a diverse set of models for predicting and assessing combined exposures (see below and Table 7).

In contrast, IA estimates the effect of combinations of toxicologically independent acting chemicals from the probability of responses to the individual components (Kortenkamp et al., 2009). As outlined in SCHER et al. (2012), the toxicity of a mixture in terms of the probability of an individual being affected can be expressed as:

$$pM = 1 - (1\text{-}p1)\,(1\text{-}p2)\,(1\text{-}p3)\dots(1\text{-}pn)$$

with pM being the response to the mixture and p1, p2, …, pn being the responses due to exposure to the individual components C1, C2, … Cn when present in a specified concentration. This can also be denoted as:

$$E(Cmix) = 1 - \prod_{i=1}^{n}(1 - E(C_i))$$

where E(Cmix) is the combined effect at the mixture concentration (Cmix), and E(Ci) is the effect of the individual mixture component (i) applied at the concentration (ci). Effects are expressed as fractions of a maximum possible effect ($0\% \leq E \leq 100\%$).

It should be emphasized that an incorrect understanding of the underlying modes of action leading to the selection of IA as the concept of choice might lead to an underestimation of the expected toxicity of the combined exposures (Kortenkamp et al., 2009). The application of IA has been approached as response addition (the sum of biological responses or effects addition). However, it should be noted that even where IA is a reasonable choice, the application of a calculation of a simple summation of effects in order to estimate the combined effect of a group of chemicals can lack a sound conceptual basis and the experimental evidence regarding the performance of IA calculations is limited (Backhaus et al., 2010).

Component Based Approaches Using Dose Addition/Concentration Addition

The methodologies that are applicable to DA/CA that are most commonly used in risk assessment of combined exposures include: Hazard Index (HI), Target-organ Toxicity Dose (TTD), Point of Departure Index (PODI), Toxic Unit (TU), Sum of Internal Toxic Units (SITU), Human-Relevant-Potency-Threshold (HRPT), Combined Margin of Exposure (MOET) and Relative Potency Factor (RPF). The selection of a method depends on data availability, the tier of the assessment (as initially identified during the problem formulation) and the level of refinement necessitated in the context of a tiered approach. The mathematical approaches are briefly described below and in Table 7.

The Hazard Index (HI). HI is one of the more straightforward ways to assess combined exposures to multiple chemicals. The HI is equal to the sum of each chemical component's Hazard Quotient (HQ = Exposure ÷ Safe Dose). When selecting the safe dose to derive an HQ, there are several options. In the context of human health risk assessment the DNEL, Acceptable Daily Intake (ADI), TTC, RfD or Benchmark Dose Lower Confidence Limit (BMDL) are often used. In the context of ecotoxicity the PNEC, EQS, or BMD are typically used and the HI is typically referred to as the Risk Quotient (RQ). Uncertainty is already incorporated into the HI and, therefore assessment factors do not need to be applied. However, different critical effects may have driven the derivation of the reference values for the components. Importantly, the type of reference values used within a single HI calculation should be consistent because each value is derived using slightly different assumptions (US EPA, 2011c; Bjarnason, 2004).

Target-Organ Hazard Index (Target-Organ HI). This method is a refinement of the HI approach. In this approach, target-organ toxicity doses (TTDs) are derived as specific reference values for the components, even if this endpoint is not necessarily the critical toxicological effect. These TTDs are then used to derive an organ-specific HI. For example, TTDs could be derived for liver effects across components and a liver specific HI calculated based on these TTDs, whereas the component specific reference values may be driven by different critical effect levels (ATSDR, 2004; EFSA, 2013a; Kienzler et al., 2014).

The Point of Departure Index (PODI). The PODI (also called the Reference Point Index (RPI)) is the sum of the ratio of chemical exposure to the POD for each chemical component in terms of their relative potencies (relative to a particular biological response). A single, group assessment factor is applied (either a default uncertainty factor (UF) or a chemical-specific adjustment factor (CSAF) which can take into consideration both data and policy aspects). The approach has been cited as a more appropriate method of addition to calculate the toxicological potency of the mixture and the contribution of relative components, prior to the application of assessment factors. However, deriving a group assessment factor will need to account for variation between the datasets for different components (Wilkinson et al., 2000; EFSA, 2013a; Kienzler et al., 2014).

The Combined Margin of Exposure (MOET). The MOET approach is the reciprocal sum of the reciprocals of the MOEs (or, the reciprocal of the PODI). Although there are no strictly established criteria to define the magnitude of an acceptable MOE (for single chemicals or combinations), an MOE of 100 (which relates to a 10x10 uncertainty factor for inter- and intra-species variability) is typically considered to be the minimum acceptable margin (Wilkinson et al., 2000; EFSA, 2013a).

The Toxic Unit (TU). The TU model is often used in ecotoxicology to assess the ratio between the concentration of a component in a mixture and the toxicological effect (acute or chronic), which makes it conceptually similar to HI. However, while the HI summarises over different endpoints, the TU is typically done across one common endpoint (making it similar to the PODI). The TU of a mixture (TUm) is the sum of TUs of the individual chemicals. No assessment factors are involved when calculating the sum of toxic units (Kienzler et al., 2014; EFSA, 2012b; SCHER, 2012).

The Sum of Internal Toxic Units (SITU). A SITU (also called the Critical Body Residue, CBR) is produced by summing internal toxic units (based on concentrations in the tissues of the organism), rather than on external exposure concentrations (e.g. in water). An advantage of this approach is that it accounts for the absorption and delivery of a substance to a site of toxic action within the organism (Dyer et al., 2000; Escher et al., 2010). A major limitation is that the internal concentration is often not measured but estimated by multiplying the substance's concentration in the environmental medium by its bioaccumulation factor, which results in additional uncertainties of the final risk estimate, especially if major detoxification mechanisms or other toxicokinetics parameters are not considered.

The Relative Potency Factor (RPF). The RPF (also called the 'scaling factor') approach uses toxicity data for a single index chemical (IC). It is calculated by normalising the potencies of all chemicals in the mixture to the IC (i.e. the RPF is derived using a ratio of a reference dose of the IC to each individual chemical). The RPF approach assumes a similar mode of action and similar toxicological effect for each of the individual chemicals in the group and requires sufficient data to identify a common measure of effect in order to establish relative potency. Considerations in selecting the index chemical include the adequacy of the toxicological database of the IC and how representative the IC is of the other chemicals in the group. In order to limit the propagation of uncertainty, the IC must have a robust database (Wilkinson et al., 2000). Considerations in selecting the index chemical include:

- Adequacy of the toxicological database of the IC.

- Similarity of the IC to the other chemicals considered in the chemical group.

- Representativeness of the IC of the individual chemicals in the group.

Terry et al. (2015) followed a strategy of comparative assessment utilising MOA data, relative potency, hazard characterisation, read-across, predicted exposure and TTC to generate a robust database for sulfoxaflor and its metabolites. This information was then used to develop RPF, compared the metabolites with the parent molecule and assessed the hazard and human risk presented by these metabolites.

The use of toxic equivalent factors (TEFs) in risk assessment is a specific type of RPF and has been historically applied in human health and environmental risk assessment. TEFs that have been formally adopted by international regulatory agencies currently exist for polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like PCBs (EFSA, 2013a; Van den Berg et al., 2006). When relative effect potency estimates (REPs) are derived from heterogeneous data sets using different biological models or endpoints, high uncertainty and variability might characterise the TEFs (Van den Berg et al., 2006; Haws et al., 2006). Using better data can identify the most relevant (the most contributing) compounds and improve the certainty of their corresponding TEF values and relative potencies (Van den Berg et al., 2006; Haws et al., 2006; Rowlands et al., 2013). It should be noted that as TEF approaches were primarily designed to estimate exposure and risks via oral ingestion they may not be directly suitable for other applications (Van den Berg et al., 2006) and internal dosimetry needs to be accounted for when comparing to systemic exposures (van Ede et al., 2016).

The related toxic equivalency quotient (TEQ) approach was used in the Canadian assessment of nonylphenol and its ethoxylates (Health and Environment Canada, 2001). In this case, for the distributional assessment, there was greater confidence in using the nonylphenol toxicity data together with the relative potencies for other metabolites to derive the TEQ than in using toxicity data for the metabolites alone. In addition, for the same purpose the average effluent concentration at each site of different receiving watercourses was used as the estimated exposure value (EEV). For the purpose of determining acceptable levels of toxic substances such as polycyclic aromatic hydrocarbons (PAHs), nonylphenol and its ethoxylates, dioxins and furans (PCDD/Fs), Environment Canada developed environmental quality guidelines that consider the combined effects of these groups of chemicals in water, sediment, or soil by using TEQs (CCME, 2002a,b; 2010).

Additional Approaches

The Maximum Cumulative Ratio (MCR) is defined as the ratio of the cumulative toxicity to the largest toxicity from a single component. As such, it is not a measure of toxicity or risk. MCR is instead a measure of the degree that an individual's hazard from a combined exposure is driven by a single chemical. The larger the value the more the risk is driven by combined exposures. The MCR is a metric that can be determined using many of the methods described in Table 8. For example, the MCR is the ratio between the sum of all HQ values (the HI) and the maximum HQ, where the HI is used to normalise exposures across chemicals.

MCR provides a quantitative measure of the degree to which toxicity is underestimated when a cumulative risk assessment is not performed (Price and Han, 2011); however, it can only be determined retrospectively after a risk assessment of combined exposures is carried out. MCR is also a measure of the ratio of the toxicity of an individual's exposures as measured using the DA/CA and IA models of combined toxicity (Junghans et al., 2006; Price and Han, 2011). MCR values close to 1 indicate that a single substance is driving hazard to an individual and that DA/CA and IA models would produce similar estimates of risk. MCR values have been found in a number of studies of exposed populations to decline with increasing prediction of risk (Price and Han, 2011; Price et al., 2012a; Han and Price, 2011). This observation is based on a small number of case studies, and must therefore be confirmed in larger populations and additional exposure scenarios.

The MCR approach has been applied to mixtures in ground water wells (Han and Price, 2011), to biomonitoring data on dioxin-like chemicals (Han and Price, 2013), phthalates (Reyes and Price, 2018) mixtures in surface waters (Vallotton and Price, 2016) and as a screening tool for the evaluation of mixtures in residential indoor air (Brouwere et al., 2014). It is also noted that the MCR only describes the minimum ratio between the risk of a single mixture component and the complete mixture (Backhaus and Karlsson, 2014).

Considerations of Interactions in the Context of the Hazard Index

Methods have been developed for adjusting the HI approach for addressing interactions (see ATSDR, 2004 for a detailed explanation). These include an interactions-based HI where the HI for the mixture is modified by an uncertainty factor (either a default of 10 or a data-derived uncertainty factor quantifying the magnitude of interaction), which is then combined with a numerical Weight of Evidence (WoE) score (negative for antagonistic interactions and positive for synergism). This score is based on tables developed by the ATSDR and the US EPA and are derived from the nature of the interaction, the quality of the available data, the biological/toxicological plausibility of the interaction at actual exposure conditions and the relevance for human health (US EPA, 2007; ATSDR, 2004; Sarigiannis and Hansen, 2012).

Building on this approach a second method was developed that modifies the individual HQs instead of the HI in order to take into account binary interactions and their WoE (BINWOE). The BINWOEs provide a classification that indicates the expected type of interaction (greater than additive, less than additive, additive, or indeterminate) and score the data qualitatively using an alphanumeric scheme that takes into consideration mechanistic understanding, toxicological significance, and relevance of the exposure duration, sequence, bioassay (in vitro versus in vivo) and route of exposure.

Table 7. A summary of mathematical approaches available for risk assessment of combined exposures using dose addition. [a]

Approach	Formula	Strengths	Limitations	Interpretation
Hazard Index (HI) Risk Quotient (RQ)	$$HQ = \frac{Exposure}{Reference\ Value}$$ $$HI = \sum HQ$$ $$RQ = \sum \frac{PEC}{PNEC}$$	Simple, flexible and can be quickly applied; Provides a transparent index of acceptable risk; Does not require selection of the same endpoints for reference value selection for each chemical in the group; Accommodates varying data of chemical group; Uncertainty factor applied to each chemical in the group, as appropriate; Allows for identification of percent contribution of each substance which can permit identification of target chemicals for risk management.	Potential to be overly conservative; It ignores chemical interactions; PEC/PNEC ratios are considered slightly more conservative than TU summation and suitable as Tier 1 approach [SCHER, 2012]; Toxicokinetic or toxicodynamic differences are not considered; Difficult to rationalise the biology in the absence of mechanistic information; Requires that reference values are available for all chemical group members; these values are often not available.	HI or RQ ≤ 1: combined risk acceptable HI or RQ > 1: potential concern
Target Organ Hazard Index	$$Target\ Organ\ HQ$$ $$= \frac{Exposure}{TTD}$$ $$Target\ Organ\ HI$$ $$= \sum Target\ Organ\ HQ$$	Similar to hazard index, except focuses on a specific target organ which can reduce overestimates of potential risk of specific toxicity if reference values based on various endpoints are used in the HI.	TTD values are required for each component for the target organ of interest.	Target Organ HI ≤ 1: combined risk acceptable Target Organ HI > 1: potential concern

Point of Departure Index (PODI) or Reference Point Index (RPI)	$PODI = (\sum \frac{Exposure}{POD}) x UF$ (UF: chosen group uncertainty factor, usually a default value of 100)	Sums the exposures to the different components in relation to relative potencies; Single group UF or CSAF applied to the chemical group as the last step in the approach which in comparison to the HI allows for calculation of the toxicological potency of the mixture and the contribution of relative components, prior to the application of assessment factors.	Interpretation of the PODI (RPI) depends on the value of the UF or CSAF applied to the group; group assessment factor will need to account for variation between the datasets for different components. Potential overestimation of risk.	If PODI ≤ 1: combined risk acceptable PODI > 1: potential concern
Combined Margin of Exposure (MOE(T))	MOE(T) = 1/[∑ (1/MOE)]	Because the MOET is an extension of an approach that is commonly used for the risk assessment of single chemicals, it might be more user friendly due to the familiarity of the concept of an 'MOE'.	There are no strict criteria to define the magnitude of an acceptable MOE.	If the MOET is greater than 100 or another alternative value specified for the MOE by the risk manager, the combined risk is considered acceptable.
Toxic Unit (TU) Summation	TUm=∑TUs $TU = \frac{exposure\ concentration}{effect\ concentration}$	Like the other approaches TUm can provide an indication of relative contribution of each substance to mixture toxicity, which can permit identification of target chemicals for risk management.	Similar (eco)toxicity data is required for each chemical; i.e. data from the same (eco)toxicological endpoint.	An acute lethal TUm=1 means that the mixture would cause 50% lethality. An acute lethal TUm=10 means that a dilution to 10% of the mixture would produce 50% lethality.

Sum of Internal Toxic Units (SITU)	$ITUm = \sum ITUs$ $ITU = \dfrac{exposure\ concentration \times BAF}{critical\ body\ residue}$ BAF : bioaccumulation factor	Accounts for ability of substances to be taken up by organisms and reach target site of effect.	Requires data on bioaccumulation for each substance; Critical body residues may not be available if the chemicals act through a mode of action other than narcosis; Assumes even distribution of the chemicals in the body of the exposed organism.	An acute lethal ITUm=1 means that the mixture would cause 50% lethality.
Relative Potency Factor (RPF) or Toxic Equivalence Factors (TEF)	$RPF1 = \dfrac{TS1}{TSindex}$ TS1 is the toxicity of the individual substance (S1) and TSindex is the toxicity of the index compound or $RPF1 = \dfrac{Slope\ Factor\,(S1)}{Slope\ factor\,(index)}$ Then, for the mixture: $Mixture\ Dose = \sum(Exposure \times RPF)$ Alternatively, $TEQ = \sum(Exposure \times TEF)$ Where TEF is the relative toxicity of individual substance compared to the index chemical	Transparent, easy to use; Provides sound basis for understanding toxic dose metrics; Separates potency correction (hazard) from exposure.	Increased reliance on data requirements; Selection of index chemical is critical; the used toxicological database should have the lowest uncertainty possible for the index chemical; Parallel dose-response curves assumed.	Potential toxicity of the mixture assessed by reporting the mixture dose on the dose-response curve of the index chemical (IC).

Source: [a] Adapted from Kienzler et al., 2014

Table 8. The Maximum Cumulative Ratio Approach

Approach	Formula	Strengths	Limitations	Interpretation
Maximum Cumulative Ratio (MCR)	When the HI approach has been used, MCR=HI/maximum HQ of an individual mixture component $1<MCR<n$ n='number' of chemicals in the mixture A similar ratio can be applied when other methods (e.g. TU) are used to normalise exposures across chemicals	Tool for identifying the significance of combined toxicity compared to single component toxicity. Provides information on the value of further refining a combined exposure assessment or focusing on a single component from a risk management perspective. Can be integrated into tiered screening and assessment approaches. Can be used as an analysis tool when additive models have been applied.	To date there are limited use examples; further validation of this approach is required to identify advantages and limitations.	MCR~1: one chemical completely dominates the potential risk; (under the assumption of CA) MCR ~"n": each chemical contributes equally to the risk estimate (under the assumption of CA)

7.3. Tiered Approaches to Assessment of Combined Exposures to Multiple Chemicals

Chemical risk assessment is an inherently resource-intensive and often time-critical activity. It is therefore often implemented in the form of a tiered approach, starting with simple, fast and conservative approaches and then, if needed, gradually moving to more complex and realistic methods that are substantially more data-, time- and resource-intense. In principle, tiering in the context of combined exposure assessments can take place during the hazard, the exposure and/or the risk assessment stage. Tiered approaches can either be implemented with the aim to (1) quantify total exposure / hazard / risks, (2) to identify drivers of the exposure / hazard / risk or (3) to provide guidance for the safe use of chemical mixtures. In any case, it is essential that lower tiers avoid false negatives, i.e. that the likelihood of underestimating risks / hazards / exposures is minimised.

Tiered approaches can be used in the context of CBAs and WMAs. Tiering in the case of WMAs follows standard chemical-risk assessment practice and is therefore not further considered in the following. However, the recorded toxicity of complex environmental samples can also form the starting point for subsequent efforts to identify which (groups of) chemicals are responsible for the observed toxic effects. Tiered approaches in this context have been most recently suggested by Brack and colleagues (2016). Each tier of the suggested effect-directed analysis methodology provides additional information for the

identification of the "mixture toxicity drivers". Their relevance can finally be validated by experimentally testing a reconstituted mixture. Tiered approaches for the exposure, hazard and/or risk characterisation of combined exposures to multiple chemicals have been suggested by national and international authorities, private sector organisations and academic scientists. These approaches are summarised in Table 9.

Several additional schemes have been published that are not tiered in the sense described in Chapter 7.1, i.e. they do not provide "off-ramps" for finishing the assessment at an early stage. Instead, they provide a decision map in order to systematically and transparently guide decision making within the assessment process. These include:

1. The US EPA's resource document on cumulative health assessments (US EPA, 2007), which provides approaches to systematically identify and characterise the exposure situation relevant for human health in a given area (who, where, when, how much?).

2. The US EPA's guidance on the cumulative risk assessment of pesticides that have a common mode of action (US EPA, 2002). The document describes a step-wise approach on how a decision can be taken whether the considered pesticides co-occur and whether they should be compiled into a common mechanism group (CMG, group of chemicals that include a common toxic effect by a common mechanism of toxicity). Hazard, exposure and risk assessment are then implemented based on relative potencies, points of departure and "detailed exposure scenarios".

3. A series of reports and academic papers describe decision trees to guide the analysis whether whole-mixture approaches or component-based methods are more suitable for a given mixture assessment and, if component-based approaches are recommended, which methods are to be used (US EPA, 2000; Groten et al., 2001; SCHER, SCENIHR & SCCS, 2012, Price et al., 2012a).

4. The scheme for assessing the aquatic toxicity of mixtures published by the European Centre of Ecotoxicology and Toxicology of Chemicals (ECETOC, 2011), which provides an overview of how to identify causes of ecosystem impacts and the role that mixtures play.

In general, the overarching rationale is to reduce uncertainty by increasing the amount of information that is used for the assessment. Therefore, although tiered approaches are usually tailored towards particular regulatory areas and often comprise approaches that are specific for either human health or environmental assessments, the approaches have several elements in common. In particular, the classic assessment concept of Concentration Addition is used in every tiered assessment scheme. In fact, lower tiers are usually designed to provide a first risk estimate using DA/CA as a default approach, without considering the AOP/MOA of the components.

Tiering is then often used to identify which DA/CA-based assessment method is most suitable for the task at hand, i.e. the schemes move from the simple summation of PEC/PNEC ratios or hazard quotients to trophic-level and endpoint-specific assessments. The competing concept of IA is used, if at all, only in the later stages of assessment. In fact, one particular aim of tiered approaches is to come to a decision on whether there is a need to consider IA (NRC, 2008; EFSA, 2008, 2009b, 2013d; Backhaus & Faust, 2012). Taking

this decision in a defendable way is currently a major challenge, given the scarcity of mechanistic (eco)toxicological information.

In general, information on the AOP/MOA of the components is consistently considered valuable to improve the assessment. Especially during the human-health oriented assessment of combined exposures, AOP/MOA information is suggested as a scientific basis to group chemicals into so-called common assessment groups (CAGs) in higher tiers of the assessment (EFSA, 2009b, 2013d). These approaches were further described in Chapter 5.

It is also common amongst all documented tiered approaches that interactions, i.e. synergistic or antagonistic effects, are not systematically considered. Usually the assessment schemes include a request to consider existing knowledge especially on synergistic interactions, but it is not explicitly included in the tiering approach.

Table 9. Tiered approaches for the exposure, hazard and/or risk characterisation of combined exposures to multiple chemicals

Published by	Hazard / Risk Human / Environment	Chemicals Covered	Aim / Scope	Approaches and Starting Points
Danish Veterinary and Food Administration, (2003)	Assessment of hazards to human health	Chemicals found in food and the environment	Guide decision between hazard index approach and toxic equivalency factor.	CBA, based on defined chemical mixtures with known human exposures.
Backhaus & Faust (2012)	Risk to the environment	Industrial chemicals registered or authorised under REACH	Tiered risk assessment with major emphasis on hazard assessment.	CBA, based on defined chemical mixture with known environmental exposures. Basic information on ecotoxicology and/or environmental hazards available for the components.
Backhaus et al. (2013); ECHA (2014); ECHA (2015)	Risk to the environment	Chemicals used in biocidal products on the European market	Tiered risk assessment with major emphasis on hazard assessment.	Combination of CBA and WMA. Formulated biocidal product of known or unknown chemical composition.
Kortenkamp et al. (2012); EFSA (2013b)	Risk to human health	Dissimilarly acting pesticides in food	Tiered risk assessment with the aim of setting maximum residue levels.	Exclusively CBA. Defined pesticide mixtures occurring in food and composed of chemicals with dissimilar modes of action. EFSA (2013a) restricted the analysis on pesticides with a common

				adverse effect on the same organ/system.
Report to EFSA by Nielsen et al. (2012)	Input for human health hazard assessments	Pesticides	Grouping of pesticides into common assessment groups for further consideration in risk assessments.	Pesticides authorised for the European market (listed on Annex I of EU Council Directive 91/414/EEC).
EFSA (2008, 2009b)	Risk to human health	Pesticides	Tiered risk assessment with the aim of setting maximum residue levels.	Exclusively CBA, using independent, tiered approaches for hazard and exposure. Only exposure via food considered.
EFSA (2009a, 2013c, 2013e)	Risk to the environment	Pesticides	Demonstrating safe use for (re)authorisation of pesticide products in Europe. Separate guidelines/opinions for birds and mammals (EFSA, 2009a), edge of field scenarios (EFSA, 2013c) and bees (EFSA, 2013d).	Focus on pesticide products with known chemical composition. Use of CBA and WMA.
German Environmental Agency (Bunke et al., 2014)	Risk to the environment	Industrial chemicals (substances registered or authorised under REACH)	Tiered risk assessment with independent tiers for hazard, exposure and risk assessment.	Focus on defined mixtures of REACH chemicals using CBA. Prioritisation criteria are proposed, also assessment options for coincidental mixtures are discussed as well as options for different stakeholders.
German Federal Environment Agency (Stein et al., 2014)	Risk to human health	Chemicals used in biocidal or plant protection products on the European market	Tiered risk assessment with major emphasis on hazard assessment.	CBA, based on formulated biocidal or plant protection product with known chemical composition.
WHO / IPCS (2009a); Meek et al. (2011)	Risk to human health	Non-carcinogenic chemicals	Tiered risk assessment with independent tiers for hazard and exposure assessment.	Defined chemical mixture of potential relevance for human health, using CBA.

US Department of Health (2004)	Risk to human health	Non-carcinogenic chemicals & carcinogens	Tiered risk assessment scheme with emphasis on tiered hazard assessment (separate approaches for non-carcinogenic chemicals and carcinogens).	Defined chemical mixture of potential relevance for human health, using CBA.
Brack et al. (2016)	Risks to the environment	Chemicals detected or suspected to be present in the (aquatic) environment	Identification of drivers of toxicity.	Approaches are presented for assessing complex environmental samples with unknown chemical contamination and/or chemical monitoring data. Fractionation-based approaches that combine CBA and WMA (effect-direct fractionation, effect-directed assessment, toxicity identification and evaluation).
CEFIC (2009, 2010, 2016)	Risk of mixtures	Chemical registered or authorised under REACH	Identification for conditions of safe use (human health and the environment) as well as adaption of the factor to determine safe daily production volumes (environment) for a defined lead substance.	Identification of a lead substance, only classified (CLP/GHS) substances considered.
Health Canada (2017)	Risk to human health	Pesticides	Tiered risk assessment with independent tiers for hazard and exposure assessment.	CBA, focused on chemicals with common mechanism of action. Exposure via food, drinking water and from residential use considered

7.4. Options for integrating the assessment of risks to human health and to the environment in the context of combined exposure assessment

Integrated Risk Assessment (IRA) has been defined by the WHO/UNEP/ILO International Programme on Chemical Safety as "a science-based approach that combines the processes of risk estimation for humans, biota, and natural resources in one assessment" (WHO-IPCS, 2001). Such integration might take place at all steps of the risk assessment process, i.e. problem formulation, exposure and hazard assessment, and finally risk characterisation and communication. IRA acknowledges the interdependence of risks to human health and the environment, as a consequence of common emission sources, routes of exposure, toxicokinetic and toxicodynamic processes that are conserved across species and the interdependency of the quality of human life and the environment (Vermeire et al., 2007).

IRA requires that the assessment of human health as well as the environment are run in parallel and constantly inform each other (Vermeire et al., 2007; Wilks et al., 2015; EFSA, 2015). Integrating human health and environmental assessments of combined exposures (HRA, human health risk assessment, and ERA, ecological/environmental risk assessment) serves two main purposes. First, combined exposure assessments are inherently complex and therefore often require substantial expertise, time and resources. Integrated approaches can help to optimise the process and to maximise the use of information gathered on both exposure and hazard. This may reduce animal testing and minimise the economic burden for the regulatory system. Second, integrated approaches ensure that management measures taken for the protection of human health, e.g. in the context of occupational safety, do not lead to unacceptable risks for the environment and vice versa.

The following section provides a brief overview of challenges and possibilities for integrating HRA and ERA in the context of assessment of combined exposures to multiple chemicals. In principle, human as well as environmental assessments follow a tiered approach for hazard, exposure and risk assessment, beginning with qualitative or semi-quantitative assessments and then continuing to probabilistic tiers with extensive toxicokinetic and toxicodynamic assessment.

Organisms share fundamental biochemical and physiological pathways of toxicological importance, allowing for the application of predictive modelling approaches during the hazard assessment process. Consequently, a response at a biochemical or physiological level might be useful for setting provisional safe exposure levels until more relevant data on a higher level of biological complexity are at hand. The Toxicity Forecasting programme of the US EPA (Toxcast), for example, makes use of a battery of approximately 700 high-throughput assays that encompass a broad range of cellular assays in order to provide basic information on primary molecular interactions (e.g. binding to proteinogenic receptors or nucleic acids) and early cellular responses (e.g. mutagenicity, production of reactive oxygen) that can inform human and ecological hazard assessments alike. These bottom-up approaches have gained momentum during recent years, mainly with the aim to inform the priority setting of chemicals for further evaluation, hazard identification, classification and labelling, and finally the replacement, refinement and reduction of animal testing (Worth et al., 2014).

As previously described, the two basic concepts of DA/CA and IA play central roles in ERA as well as HRA in order to estimate the hazard based on the individual components. A first task for IRA of combined exposures is therefore the development of appropriate technical guidelines on how to design meaningful lower tier assessments that inform HRA and ERA alike, interpret the outcomes from both perspectives and appropriately document the findings.

In terms of hazard assessment, a critical difference between environmental and human health oriented assessments centres on the question of selecting between DA/CA and IA in higher tier assessments. This can particularly relate to the question on whether effects from combined exposures are expected if the components are present merely at levels assumed to be safe for each individual component, see discussions in SCHER et al. (2012). In selecting between the two concepts in an IRA, not only is mode of action information not available for the majority of chemicals, this information is even more limited for wildlife and non-animal species with the possible exception for herbicides where the MOA for effects on plants and algae is usually well known. In addition, the MOAs potentially change in dependence of the exposed taxa, their life stage at the time of exposure and even the dose of each component.

Another complication is that ERA approaches often use apical endpoints such as growth and reproduction that provide inherently integrated parameters for toxic effects. However, HRA often analyses endpoints that are more specific. Furthermore, ERA is aiming at the protection of whole populations or ecological communities from unacceptable chemical impacts, handling vast uncertainties and knowledge gaps in terms of the species actually present at an exposed site, their largely unknown life cycle and physiology. In comparison to these knowledge gaps, the bias that might result from selecting "the wrong" concept might be considered negligible and these uncertainties can be amplified when considering multiple components. HRA on the other hand is aiming to protect individuals of one well-known species, requiring a substantially higher accuracy of the final risk estimates.

Interactions, on either the toxicokinetic or the toxicodynamic level might lead to a joint toxicity of the combined exposures that exceeds or falls below the prediction by either the DA/CA or IA concept. Interactions involving basic biochemical processes (e.g. changes in biotransformation caused by interferences with cytochrome P450's) might be another area where an integration of HRA and ERA would be beneficial. However, interactions might also take place on an organ, individual, population or even ecological level. Specific, case-by-case approaches might be needed to explore the relevance of such interactions.

Similar to hazard assessments, the potential for integration of HRA and ERA seems highest in the lower tiers of the exposure assessment, where simple, sometimes only semi-quantitative, exposure estimates dominate. Higher tier assessments, including for example the probabilistic methods used to integrate human dietary exposures to multiple chemicals from multiple sources or site-specific environmental assessments might be too specialised for being informative for HRA and ERA at the same time.

A major area for the HRA-ERA integration of exposure assessments concerns the analytical methods, modelling approaches and databases used to quantify chemical concentrations in air, water, sediments and soil. Results from those studies provide the basis for deciding on the co-exposures relevant for environmental organisms and humans alike. Areas of particular overlap concern the uptake of chemicals from food and drinking water, as well as the exposure assessment of terrestrial mammals, birds and humans. Exposure assessments in the context of occupational health assessments might be least amenable to integrated approaches, given the uniqueness of the underlying exposure scenarios.

Uptake, bioavailability and toxicokinetic models can be applied in a consistent manner to translate external concentrations to internal doses. Such models then become increasingly specific for HRA and ERA, considering e.g. different lifestyle factors in HRA or the various lifecycles and migration patterns of wildlife in ERA.

In the end, HRA and ERA work towards different protection goals and therefore consider different (eco)toxicological endpoints and exposure scenarios. An integration of approaches for risk assessment of combined exposures might therefore be most achievable in the lower assessment tiers that are based on general exposure scenarios, basic (eco)toxicological assays and conservative safety factors. Consequently, the Scientific Committees of the EU Commission developed a tiered approach for mixture risk assessment that is supposed to be applicable for ERA as well as HRA (SCHER et al., 2012) and case studies have illustrated that the Mixtures Industry Ad-hoc Team (MIAT) decision-tree is also applicable to both HRA and ERA (Price et al., 2012 a,b).

The EQS that are established under the Water Framework Directive 2000/60/EC (WFD) might currently be the most prominent European example of a regulatory framework for a mixture-aware risk assessment that integrates the consideration of concerns for human

health as well as the environment. EQS values are maximum concentrations of hazardous chemicals and chemical mixtures that are accepted in European freshwaters or coastal bodies of water. They are set to protect not only aquatic ecosystems, but also humans that are exposed via drinking water and fisheries product consumption. EQS values have been established in the daughter Directive 2008/105/EC, as last amended by Directive 2013/39/EU, and in 2011, specific guidance on the calculation of EQS values for substances occurring in mixtures was provided (European Communities, 2011). Currently, EU-wide EQS values are established for 45 priority substances (plus an unknown number of river basin specific pollutants), including a mixture-EQS for 29 dioxins and dioxin-like compounds in biota, six brominated diphenylethers (flame retardants), four cyclodiene pesticides and four DDT isomers.

Consequently, although a lot could potentially be gained by integrating HRA and ERA in the context of combined exposures, it will be necessary to discuss and clarify already during the problem formulation phase of an assessment whether human and ecological assessments indeed will benefit from each other or whether efforts to integrate these processes would lead to a counterproductive increase in complexity, time and costs.

Currently, chemical hazard assessments are conducted mainly by scientists that are specialised either in ecotoxicology or in toxicology, but few have a broader understanding beyond their area of specialisation. There are also limited opportunities to learn from other disciplines, and only limited resources are available for this. Capacity building and the joint implementation of IRA case studies are therefore required.

While it still might take some time before fully integrated assessments become more common, a near future goal would be to increase the harmonisation of HRA and ERA for combined exposures. Harmonisation should, in this context, be understood as improving the consistency of the approaches, including e.g. the rationale behind selecting a particular toxicity concept, the use of safety factors, when and how to account for basic synergistic interactions, the application of emission-based modelling approaches to determine the co-occurrence of chemicals in environmental media, etc.

7.5. Uncertainty in the Context of Assessment of Combined Exposure to Multiple Chemicals

Each stage of a chemical safety assessment (hazard assessment, exposure assessment and risk characterisation) involves the derivation of parameters, values and assumptions about the substance(s) and its/their use(s) (ECHA, 2012). The production of a robust, reliable and protective chemical safety assessment depends on accurately reflecting the degree of uncertainty and variability at each of these stages. Capturing and communicating the uncertainty and variability in chemical risk assessment has been documented elsewhere (see for example, US EPA, 1992; IPCS/WHO, 2008; ECHA, 2012; EFSA, 2014; IPCS/WHO, 2014; EFSA, 2016) and the focus of this section is in regards to the context of combined exposure to multiple chemicals.

The assessment of combined exposure assessment is subject to all of the uncertainties considered during the hazard and exposure assessment of an individual chemical. However, there are additional uncertainties that are specific to the assessment of chemical mixtures (SCHER, 2012), or become compounded when considering multiple components. These include determining the uncertainty surrounding:

- The type of mixture, i.e. a simple mixture (known composition, <10 substances) or a complex mixture (unknown composition, >10 substances). Alternatively, a 'mixture' might be a single substance together with its metabolites, or a co-exposure to substances that react inside the organism to form (a) new, toxic substance(s).

- The accuracy to which the components (and their metabolites, if applicable) have been characterised.

- The level of accuracy with which the exposure has been characterised (e.g. if it is a concurrent or temporally separated exposure).

- The approach (and corresponding assumptions) regarding the combined effect of the co-exposure; i.e. dose addition or independent action/response addition.

- The assumptions about additivity or departure from additivity; i.e. a greater-than-additive response (synergism), or a less-than-additive response (antagonism).

- The assumptions regarding similarity in the shape of the dose response curves (for dose/concentration addition).

- The identification of a single point of departure for the combined exposures.

- Grouping chemicals by 'toxic effect(s)' or 'similar' mode of action and the required criteria for substances to qualify as having 'similar' modes of action.

- Methods used to fill data gaps: read-across data, allometric scaling, PBPK modelling and alternative approaches (HTS data, *in vitro, in silico*).

Due to the complexity of ecosystems, there are additional uncertainties for the ecological assessment of combined exposures:

- The structure of biological communities in the exposed ecosystems, with different species having different sensitivities/vulnerabilities towards individual components;

- Differing modes of action of chemicals in different types of organisms (bacteria, plants, invertebrates, vertebrates).

KEMI (2015) summarised the impact of uncertainties in the consideration of combined exposures, in terms of leading to an over- or under-estimation of risk. This is outlined in Table 10.

A proposed tiered approach for characterising uncertainties in the assessment of risk of chemical mixtures has also been recommended (EFSA, 2013a). EFSA (2013a) notes that uncertainty analysis in the context of a risk assessment of combined exposure to multiple chemicals is used for the identification of the sources and magnitude of uncertainty in a tiered manner (qualitative, semi-quantitative or probabilistic) associated with exposure assessment, hazard assessment and risk characterisation. In addition, it provides the opportunity to identify gaps, strengths and limitations of the assessment (whether further refinements of the assessment are needed) and to make recommendations on future research. If risk is identified at lower tiers using default assumptions, higher tier methodologies that address the specific uncertainties of the mixture should be applied. The

data needs, and associated uncertainties, outlined for moving through tiers of assessments highlighted in the hazard and exposure sections of this document should inform the uncertainty analysis, also in a tiered application. Modifications can be made to the approaches used in order to address the concern of underestimation of risk using dose additivity in lowered-tiered combined exposure assessments. For example, the hazard index approach can be modified by using additional uncertainty factors to accommodate for the possibility of deviations from expected additivity as outlined in Chapter 7.2.2 (ATSDR, 2004). The PODI approach improves upon the HI approach because it avoids the incorporation of (often) arbitrary or irrelevant assessment factors (that are included because of their use in deriving the reference value).

In addition, quantitative modelling of the uncertainty in inter and intra species uncertainty factors has been used to explore the uncertainty in predictions of combined risk (Price et al., 2009). The study indicated that probabilistic descriptions of uncertainty factors could be used to describe uncertainty in the toxicity of discrete mixtures of chemical in surface waters. This approach provides a method of accounting for differences in the sizes of the various chemical-specific uncertainty factors used in component based approaches.

The idea of applying a Mixture Assessment Factor (MAF) has also been proposed (KEMI, 2015; ECHA, 2014). The MAF is targeted towards the additional uncertainties that are encountered while assessing the risk of a chemical mixture and could account for potential synergies, (eco)toxicological data gaps and lack of full composition information. A full analysis of the possibilities and limitations of a MAF was explored by the Swedish Chemicals Agency (KEMI, 2015). The approach could also be used to account for uncertainties with regard to exposure to multiple chemicals that cannot be assessed if they were not measured, as well as for the occurrence of various problematic mixtures in time and space. These "multiple mixtures" will add to an overall effect of chemicals (e.g. multiple applications of different pesticide products over the year), or different pollution patterns in certain watersheds (e.g. agricultural areas, wastewater influenced, mining areas, etc.).

Table 10. Uncertainties leading to over- or under-estimation of risk from combined exposures to multiple chemicals *

Source of Uncertainty / Bias	Consequences	Discussed and described in:
The simultaneous presence of compounds as mixtures is ignored or falsely assumed	Risk underestimation/ overestimation	Kortenkamp (2009) Kortenkamp & Faust (2010) ECETOC (2011a) SCHER, SCENIHR & SCCS (2012) De Brouwere et al. (2014) Malaj et al. (2014)
A mixture not entirely composed of similarly acting substances is assessed by CA	Hazard/risk overestimation, unless the concentration response curves of the individual compounds are exceedingly flat.	Junghans (2006) Kortenkamp (2009) Altenburger (2013)
Antagonistic interactions of the components in a mixture that is assessed by CA	Hazard/risk overestimation	Kortenkamp (2009)

Synergistic interactions of the components in a mixture that is assessed by CA	Hazard/risk underestimation	Kortenkamp (2009) Kortenkamp & Faust (2010) Backhaus (2013) Altenburger (2013) Cedergreen (2014) Marx et al.(2015)
Insufficient (eco)toxicological knowledge on the mixture components to calculate the CA-expected toxicity.	Hazard/risk underestimation if the compounds with insufficient knowledge are simply ignored in the assessment. Otherwise the bias of the mixture toxicity assessment depends on the quality of the (eco)toxicological modelling and data bridging that is applied (e.g. QSAR estimates)	Backhaus (2010) Altenburger (2013)
Not all components included in the CA-based (eco)toxicity assessment of a complex exposure situation	Hazard/risk underestimation	Escher (2013) Dewalque et al. (2014) Tang et al. (2014a,b)
Neglecting potential bioaccumulation in the organism using only external exposure concentrations	Hazard/risk underestimation	Kortenkamp & Faust (2010)
Neglecting chemical metabolites that are more toxic than the parent compound	Hazard/risk underestimation	Malaj et al. (2014)
Sampling methods influencing the chemicals detected in environmental or biological samples; or exposure estimation methods that are then applied (e.g. handling of non-detects)	Exposure/risk underestimation or overestimation	Price et al. (2012a) Malaj et al. (2014)

Source: * adapted and updated from KEMI, 2015 and also drawing from Bopp et al. (2016)

7.5.1. *Data quality*

Quality of data is an important aspect in both human health and environmental risk assessments. Limited data from incomplete reports are not acceptable for use in robust exposure or hazard estimation but may be of value to build a database from past studies or to inform Tier 0 assessment with uncertainties highlighted. Depending on the type of assessment to be conducted, data generated from thoughtfully designed protocols and carefully conducted studies may be required (ATSDR, 2005; ECHA, 2011, 2015). Uncertainties associated with the data should be clearly stated in the assessment and will factor into the ability to refine and/or rely on assessment outcomes.

Adequacy of the data used is important. The adequacy of data can be defined by two basic elements: reliability, covering the inherent quality of the data relating to the methodology and the way that the performance and results are described to give evidence of the clarity and plausibility of the findings; and relevance, covering the extent to which the data is

appropriate for combined exposure assessment. The greatest weight should be attached to the most reliable and relevant data.

Toxicity data obtained using standardised protocols are preferred over data from studies that did not use such protocols. Since newer exposure data are more likely to be performed using such protocols they may be preferred over older studies. Although older data can still be used, professional judgement should be exercised to assess reliability of results, and uncertainties associated with the data should be reported for transparency. Systems for evaluating data quality such as Klimisch (Klimisch et al., 1997) and newer system for reporting and evaluating ecotoxicity data (CRED) (Moermond et al., 2015) can be used.

7.5.2. Documenting Uncertainty

Very few agencies/programs report detailed quantitative information on uncertainties. To address this, several organisations have produced frameworks on transparency and uncertainty analysis (ECHA, 2012; EFSA, 2016) that discuss best practices for reporting, documenting and communicating uncertainty at the different levels of analyses. General guidance has included the following recommendations:

- All uncertainty—including assumptions, data gaps, model limitations and lack of knowledge—should be reported and discussed;
- When possible, sources of uncertainty should be quantified and presented;
- All uncertainties should be addressed individually and then as a whole;
- The possible effects of the sources of uncertainty on the conclusions/outcome of the assessment should be discussed.

A high degree of uncertainty characterisation may not always be feasible and approaches that are more pragmatic may be necessary. The US EPA (1992; 2014; 2016) and EFSA (2013a; 2016) recommend that the level of detail of uncertainty analysis should be appropriate to the context and proportionate to the possible consequences of the decision. As a result, the use of a tiered approach in conducting evaluations of uncertainty is emphasized.

The documentation/communication of uncertainty should always include key limitations, assumptions and any uncertainties associated with any approaches used in the assessment, while still being understandable by decision-makers. At a minimum, the main uncertainties should be captured and communicated. It is important to consider the magnitude and impact of the sources of uncertainty and if reduction of the uncertainty would be likely to lead to a different outcome to inform decision-making. A consensus has evolved that uncertainty analysis should also follow a tiered approach that can be refined as necessary (and as the data allows).

Chapter 8. Conclusions

There are an infinite number of chemical combinations to which humans and the environment can be exposed. Ensuring that the individual chemicals are adequately assessed and managed is a critical component of ensuring protection of human health and the environment. However, it is also important to consider the impacts of combinations of these chemicals. This document has provided elements to consider when assessing combined exposure to multiple chemicals. Given the diversity of possible combinations, and the diversity of ways of prioritising their examination (e.g. based on uses, releases vs similar effect profiles), the elements have not been presented in a strict manner; the application of different approaches and methods will depend on the assessment context and the problem formulation. However, it is clear that a tiered approach should be applied in order to identify where additional resources should be targeted for the refinement of assessment approaches, further data generation or gathering, or the consideration of risk management activities.

As noted throughout the document, there are a number of limitations and uncertainties associated with the various approaches. However, this is also true of single substance assessment methods. As the regulatory application of the approaches has not been extensive to date, the continued gaining of experience from use of the methods and the identification of key gaps and uncertainties during the application will help build experience and refine methodologies moving forward.

Annex A. Examples of physico-chemical properties and how they are relevant to exposure characterisation

Property	Abbreviation	Unit(s)[b]	Description
Water Solubility	NA[a]	g/L; g/m^3	Water solubility is the maximum concentration of a chemical that dissolves in a given amount of pure water. Environmental conditions, such as temperature and pH, can influence a chemical's solubility, which, in turn, also affects a contaminant's volatilisation from water. Solubility provides an important indication of a contaminant's ability to migrate in the environment: highly soluble compounds will tend to move with groundwater and remain in the water column of surface waters rather than partitioning to sediment, and move in soils, while insoluble compounds do not.
Density	d	Mass/volume	Density of liquid is the liquid's mass per volume. For liquids that are insoluble in water (or immiscible with water), liquid density plays a critical role. In groundwater, liquids with a higher density than water may penetrate and preferentially settle to the base of an aquifer, while less dense liquids will float.
Vapour Pressure	NA	Pa; mm Hg	Vapour pressure is a measure of the volatility of a chemical in its pure state. Thus, the vapour pressure largely determines how quickly contaminants will evaporate from surface soils or water bodies into the air, also affects a contaminant's volatilisation from soils or water. Substances with higher vapour pressures will evaporate more readily and may be more likely to be emitted from consumer products and be found in indoor air.
Henrys Law Constant	H	unit less	Henry's Law Constant is a measure of the tendency for a chemical to pass from an aqueous solution to the vapour phase. It can be expressed as the dimensionless ratio between the aqueous-phase concentration of a chemical and its gas-phase concentration. It is a function of molecular weight, solubility and vapour pressure. A high Henry's Law Constant corresponds to a greater tendency for a chemical to volatilise to air.

Organic Carbon Partition Coefficient	K_{oc}	unit less	The organic carbon partition coefficient (Koc) describes the sorption affinity a chemical has for organic carbon and consequently the tendency for compounds to be adsorbed to soil and sediment (based on the organic carbon content of the soil or sediment). This coefficient is often referred to as the adsorption coefficient. A high Koc indicates that organic chemicals bond tightly to organic matter in the soil so less of the chemical is available to move into groundwater or surface water.
Octanol/Water partition Coefficient	K_{ow}	unit less	The octanol/water partition coefficient (Kow) indicates a chemical's potential to accumulate in animal fat by representing how a chemical is distributed at equilibrium between octanol and water. Contaminants with higher Kows are more likely to bioaccumulate and can be more readily absorbed through human skin or lipid membranes of other organisms.
Melting point	mp	°C; °F	The temperature at which a solid becomes a liquid may indicate if a substance is likely to be present. Will provide an indication of the physical state that the substance will likely have at environmentally relevant temperatures.
Boiling point	Bp	°C; °F	The temperature at which a liquid becomes a gas may indicate if a substance is likely to be present. Will provide an indication of the physical state that the substance will likely have at environmentally relevant temperatures.
Molecular weight	MW	g/mol	The sum of the atomic weights of all the atoms in a molecule. Large molecular weight decreases the likelihood of systemic bioavailability.

[a] Not Applicable

[b] Refers to commonly used units. There may be others.

Annex B. Examples of fate parameters and how they are relevant to exposure characterisation

Property	Abbreviation	Unit(s)[b]	Description
Bioconcentration factor	BCF	L/Kg	The bioconcentration factor (BCF) is a measure of the extent of chemical partitioning at equilibrium between a biologic medium, such as fish or plant tissue, and an external medium, such as water. This factor can be qualitatively used to evaluate the potential for exposure of non-target organisms via the food chain. A high BCF represents an increased likelihood for accumulation in living tissue. Information on the potential for substances to bioconcentrate is also critical for characterisation of human exposure, e.g. in the interpretation of biomonitoring data.
Transformation and degradation rates	Could be expressed as half-life in hours, days or years		Transformation and degradation rates in different media take into account physical, chemical and biologic changes in a contaminant over time. It gives an indication of time between emission and exposure, duration of exposure (acute or chronic), and whether there is potential for long-range transport if substance persists for a long time.

Annex C. Examples of Exposure Data Sources to Inform Co-Exposure Potential

- Canada's CMP monitoring and surveillance program (biomonitoring and environmental monitoring)

- Governmental environmental quality monitoring and surveillance databases (e.g. water, sediments and soil)

- Use of cosmetics (COLIPA, European use of cosmetics (Hall et al., 2007, 2011)

- Cleaning products (by AISE) the EPHECT project (EPHECT, 2012); https://sites.vito.be/sites/ephect/Pages/home.aspx

- Development of a consumer product ingredient database for chemical exposure screening and prioritisation (Goldsmith et al., 2014; Dionisio et al., 2015). Data are available at http://comptox-dev.epa.gov/dashboard/

- Survey on indoor use and use patterns of consumer products in EU Member States (Johnson and Lucica, 2012)

- SPIN, database on the use of Substances in Products in the Nordic Countries, based on data from the Product Registries of Norway, Sweden, Denmark and Finland. Spin database: www.spin2000.net

- USA market basket survey: http://www.ars.usda.gov/main/site_main.htm?modecode=80-40-05-30

- Household products: USA National Institute of Health: http://householdproducts.nlm.nih.gov/

- USGS monitoring of pesticides in surface and ground water: http://water.usgs.gov/nawqa/

- USA: Great Lakes monitoring program: https://greatlakesmonitoring.org/

- Japan: Chemicals in the Environment: http://www.env.go.jp/chemi/kurohon/en/index.html

- IPCHEM - the Information Platform for Chemical Monitoring is the European Commission's reference access point chemical occurrence data. IPCHEM is structured into four modules: Environmental monitoring, Human Biomonitoring, Food and Feed, Products and Indoor Air. https://ipchem.jrc.ec.europa.eu/RDSIdiscovery/ipchem/index.html

Glossary of Terms Related to this Document

The terms in this glossary draw from OECD (2017) and references therein.

ADME

An acronym in pharmacokinetics/toxicokinetics and pharmacology/toxicology for absorption, distribution, metabolism and excretion; describes the disposition of a pharmaceutical/chemical compound within an organism. The four processes all influence the drug/chemical levels and kinetics of drug exposure to the tissues and hence influence the performance and pharmacological/toxicological activity of the compound.

Adverse outcome

An Adverse Outcome is a specialised type of key event that is generally accepted as being of regulatory significance on the basis of correspondence to an established protection goal or equivalence to an apical endpoint in an accepted regulatory guideline toxicity test.

Note: Depending on whether the protection goal is for human health or ecological health, the endpoints considered may differ.

Adverse Outcome Pathway (AOP)

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

Chemical category

A group of chemicals whose physico-chemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity (or other similarity characteristic).

Integrated Approaches to Testing and Assessment (IATA)

An Integrated Approach to Testing and Assessment is an approach based on multiple information sources used for hazard identification, hazard characterisation and/or safety assessment of chemicals. An IATA integrates and weights all relevant existing evidence and guides the targeted generation of new data, where required, to inform regulatory decision-making regarding potential hazard and/or risk. Within an IATA, data from various information sources (i.e. physicochemical properties, in silico models, grouping and read-across approaches, in vitro methods, in vivo tests and human data) are evaluated and integrated to draw conclusions on the hazard and/or risk of chemicals. Within this process, the incorporation of data generated with non-animal testing and non-testing methods is expected to contribute considerably to a reduction of testing in animals. The output of an IATA is a conclusion that, along with other considerations, informs regulatory decision-making.

Mechanism of action

Mechanism of action for toxicity is the detailed molecular description of key events in the induction of cancer or other health endpoints. Mechanism of action represents a more detailed understanding and description of events than is meant by mode of action.

Mode of action (MOA)

Mode of action is defined by WHO (2009b) as "A biologically plausible sequence of KEs leading to an observed effect supported by robust experimental observations and mechanistic data. A mode of action describes key cytological and biochemical events – that is, those that are both measurable and necessary to the observed effect – in a logical framework."

Weight of evidence (WoE)

WoE is a comprehensive, integrated, often qualitative judgment of the extent and quality of information supporting a hypothesis for which the approaches and tools vary, depending on the context.

References

Albertini R, Bird M, Doerrer N, Needham L, Robison S, Sheldon L and Zenick H (2006), The use of biomonitoring data in exposure and human health risk assessments. Environ Health Perspect 114(11):1755–1762.

Ankley G, Bennett R, Erickson R, Hoff D, Hornung M, Johnson R, Mount D, Nichols J, Russom C, Schmieder P, Serrano J, Tietge J and Villeneuve D (2010), Adverse Outcome Pathways: A Conceptual Framework to Support Ecotoxicology Research and Risk Assessment. Environ Toxicol Chem 29:730-741.

Agricultural Research Service (2013), Beltsville (USA): Food Surveys Research Group. http://www.ars.usda.gov/main/site_main.htm?modecode=80-40-05-30 [accessed 2016 Feb 29].

Altenburger R, Backhaus T, Boedeker W, Faust M and Scholze M (2013), Simplifying complexity: Mixture toxicity assessment in the last 20 years. Environ Toxicol Chem 32:1685-7.

Altenburger R, Scholz S, Schmitt-Jansen M, Busch W and Escher B (2012), Mixture Toxicity Revisited from a Toxicogenomic Perspective. Environ Sci Technol 46:2508−2522.

Ashauer R, O'Connor I and Escher B (2017), Toxic Mixtures in Time - The Sequence Makes the Poison. Environ Sci and Technol 51:3084–3092.

ATSDR (Agency for Toxic Substances and Disease Registry) (2004 and further revisions), Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures. US Agency for Toxic Substances and Disease Registry. Division of Toxicology. http://www.atsdr.cdc.gov/interactionprofiles/ipga.html.

ATSDR (Agency for Toxic Substances and Disease Registry) (2005), Public Health Guidance Manual (Update). US Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/hac/PHAManual/PDFs/PHAGM_final1-27-05.pdf.

ATSDR (Agency for Toxic Substances and Disease Registry) (2018), Framework for Assessing Health Impacts of Multiple Chemicals and Other Stressors. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. https://www.atsdr.cdc.gov/interactionprofiles/ip-ga/ipga.pdf

Aylward L, Kirman C, Schoeny R, Portier C and Hays S (2013), Evaluation of Biomonitoring Data from the CDC National Exposure Report in a Risk Assessment Context: Perspectives across Chemicals. Environ. Health Perspect 121(3):287-294.

Aylward L, Lorber M and Hays S (2011), Urinary DEHP metabolites and fasting time in NHANES. J Expo Sci Environ Epidemiol 21(6):615-2.

Baas J, Jager T and Kooijman B (2010), Review of DEB theory in assessing toxic effects of mixtures. STOTEN 408:3740-3745.

Backhaus T, Scholze M and Grimme L (2000), The single substance and mixture toxicity of quinolones to the bioluminescent bacterium Vibrio fischeri. Aquatic Toxicology 49(1-2), 49-61.

Backhaus T, Faust M and Blanck H (2010), Hazard and Risk Assessment of Chemical Mixtures under REACH–State of the Art, Gaps and Options for Improvement. Report to the Swedish Chemicals Agency. https://www.kemi.se/global/pm/2010/pm-3-10.pdf.

Backhaus T and Faust M (2012), Predictive environmental risk assessment of chemical mixtures: A conceptual framework. Environ Sci and Technol 46:2564-2573.

Backhaus T, Altenburger R, Faust M, Frein D, Frische T, Johansson P, Kehrer A and Porsbring T (2013), Proposal for environmental mixture risk assessment in the context of the biocidal product authorization in the EU. Environmental Sciences Europe 25:4.

Backhaus T (2014), A note of caution: the threshold of toxicological concern (TTC) is unsuited as a decision rule for deciding whether mixture risk assessment is necessary. PeerJ PrePrints 2:e526v1 https://doi.org/10.7287/peerj.preprints.526v1.

Backhaus T and Karlsson M (2014), Screening level mixture risk assessment of pharmaceuticals in STP effluents. Water Research 49:157-165.

Bakker M, Biesterbos J, Bokkers B, Delmaar C, Dudzina T, Roeleveld N, Scheepers P, van Engelen J and von Goetz N (2014), FINAL REPORT (CEFIC-LRI project ETHZ-B7): Estimation of realistic consumer exposure to substances from multiple sources and approaches to validation of exposure models, 159. Long-Range Research Institute and The European Chemical Industry Council Project. http://cefic-lri.org/projects/b7-ethz-estimation-of-realistic-consumer-exposure-to-substances-from-multiple-sources-and-approaches-to-validation-of-exposure-models/.

Belanger S, Sanderson H, Embry M, Coady K, DeZwart D, Farr B, Gutsell S, Halder M, Sternberg R and Wilson P (2015), It is time to develop ecological thresholds of toxicological concern to assist environmental hazard assessment. Environ Toxicol Chem 34(12):2864-2869.

Benson R (2009), Hazard to the developing male reproductive system from cumulative exposure to phthalate esters-dibutyl phthalate, diisobutyl phthalate, butylbenzyl phthalate, diethylhexyl phthalate, dipentyl phthalate, and diisononyl phthalate. Regul Toxicol Pharmacol 53(2):90-101.

Birnbaum L and Cohen Hubal E (2006), Polybrominated diphenyl ethers: a case study for using biomonitoring data to address risk assessment questions. Environmental Health Perspectives 114(11): 1770.

Bjarnason S (2004), Toxicology of Chemical Mixtures: A Review of Mixtures Assessment DRDC Suffield TM 2004-016.

Bliss CI (1939), The Toxicity of Poisons Applied Jointly. Annals of Applied Biology 26:585–615.

Boedeker W, Altenburger R, Faust M and Grimme L (1992), Synopsis of concepts and models for the quantitative analysis of combination effects in phytopharmacology and ecotoxicology. Archives of Complex Environmental Studies 4:45-53.

Bois F, Jamei M and Clewell H (2010), PBPK modelling of inter-individual variability in the pharmacokinetics of environmental chemicals. Toxicology 278:256-267.

Boobis A, Budinsky R, Collie S, Crofton K, Embry M, Felter, S, Hertzberg R, Kopp D et al. (2011). Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment. Critical Reviews in Toxicology 41(5):369–383.

Boon et al. (2015), Cumulative dietary exposure to a selected group of pesticides of the triazole group in different European countries according to the EFSA guidance on probabilistic modelling. Food and Chemical Toxicology 79:13–31.

Bopp S et al. (2018), Current EU research activities on combined exposure to multiple chemicals. Environment International 120:544–562.

Bopp S, Kienzler A, van der Linden S, Lamon L, Paini A, Parissis N, Richarz A, 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. EUR 27968 EN, doi:10.2788/272583.

Bopp S, Berggren E, Kienzler A, van der Linden S and Worth A (2015), Scientific methodologies for the combined effects of chemicals – a survey and literature review. EUR 27471 EN; doi:10.2788/093511.

Borgert C, Quill T, McCarty L and Mason AM (2004), Can mode of action predict mixture toxicity for risk assessment? Toxicology and Applied Pharmacology 201:85-96.

Borg D, Lund B, Lindquist N and Håkansson H (2013), Cumulative health risk assessment of 17 perfluoroalkylated and polyfluoroalkylated substances (PFASs) in the Swedish population. Environ Int 59:112-23.

Bradley E, Burden R, Bentayeb K, Driffield M, Harmer N, Mortimer D, Speck D, Ticha J and Castle L (2013), Exposure to phthalic acid, phthalate diesters and phthalate monoesters from foodstuffs: UK total diet study results. Food Additives & Contaminants: Part A 30(4):735-742.

Brack, W et al. (2016), Effect-directed analysis supporting monitoring of aquatic environments - An in-depth overview. Science of the Total Environment. 544:1073–1118.

Brack W et al. (2017), Towards the review of the European Union Water Framework Directive: Recommendations for more efficient assessment and management of chemical contamination in European surface water resources. Science of the Total Environment 576:720-737.

Brouwere K, Cornelis C, Arvanitis A, Brown T, Crump D, Harrison P, Jantunen M, Price P and Torfs R (2014), Application of the maximum cumulative ratio (MCR) as a screening tool for the evaluation of mixtures in residential indoor air. Science of the Total Environment 479–480:267-276.

Bunke D et al. (2014), Mixtures in the Environment – Development of Assessment Strategies for the Regulation of Chemicals under REACH. Environmental Research of the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety. http://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/texte_65_2014_aust _hassold_mixtures_in_the_environment.pdf.

CCME (Canadian Council of Ministers of the Environment) (2002a), Canadian water quality guidelines for the protection of aquatic life: Nonylphenol and its ethoxylates. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg. http://ceqg-rcqe.ccme.ca/download/en/198.

CCME (Canadian Council of Ministers of the Environment) (2002b), Canadian soil quality guidelines for the protection of environmental and human health: Dioxins and Furans. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg. http://ceqg-rcqe.ccme.ca/download/en/275.

CCME (Canadian Council of Ministers of the Environment) (2010), Canadian soil quality guidelines for the protection of environmental and human health: Carcinogenic and Other PAHs. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg. http://ceqg-rcqe.ccme.ca/download/en/320.

CEARO (Canadian Environmental Assessment Review Office) (1994), A reference guide for the Canadian Environmental Assessment Act. Addressing cumulative environmental effects. http://www.ceaa.gc.ca/Content/9/7/4/9742C481-21D8-4D1F-AB14 555211160443/Addressing_Cumulative_Environmental_Effects.pdf.

CPSC (US Consumer Product Safety Commission) (2014), Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives Final Report. http://www.cpsc.gov/PageFiles/169902/CHAP-REPORT-With-Appendices.pdf

Cedergreen N (2014), Quantifying Synergy: a systematic review of mixture toxicity studies within Environmental Toxicology. PLoS ONE 9(5), e96580.

CEFIC (European Chemical Industry Council) (2009), REACH: Exposure scenarios for preparations Methodology for the identification of substances that represent the dominant risks to human health and / or the environment and the drivers for risk management measures.

CEFIC (European Chemical Industry Council) (2010), REACH Practical Guide on Exposure Assessment and Communication in the Supply Chains. Part IV: Mixtures under REACH.

CEFIC (European Chemical Industry Council) (2012), Combination effects of chemicals decision tree. http://www.cefic.org/Documents/PolicyCentre/Identifying_and_assessing_chemical_combinations _of_concern_decision_tree_tool_explained.pdf [accessed 6-8-2015].

CEFIC (European Chemical Industry Council) (2016), REACH Practical Guide on Safe Use Information for Mixtures under REACH - The Lead Component Identification (LCID) Methodology.

Cheng S and Bois F (2011), A mechanistic modeling framework for predicting metabolic interactions in complex mixtures. Environmental Health Perspectives 12:1712-1718.

Chèvre N, Loepfe C, Singer H, Stamm C, Fenner K and Escher BI (2006), Including mixtures in the determination of water quality criteria for herbicides in surface water. Environ Sci Technol 40(2): 426-435.

Christensen K, Makris S and Lorber M (2014), Generation of hazard indices for cumulative exposure to phthalates for use in cumulative risk assessment. Regulatory Toxicology and Pharmacology 69(3): 380-389.

Coors A, Dobrick J, Möder M and Kehrer A (2012), Mixutre Toxicity of Wood Preservative Products in the Fish Embryo Toxicity Test. Environ Toxicol Chem 31(6):1239-48.

Csiszar S, Meyer D, Dionisio K, Egeghy P, Isaacs K, Price P, Scanlon K, Tan Y, Thomas K, Vallero D and Bare J (2016). Conceptual Framework to Extend Life Cycle Assessment Using Near-Field Human Exposure Modeling and High-Throughput Tools for Chemicals. Environ Sci and Technol 50(21):11922-11934.

Danish EPA (Danish Environmental Protection Agency) (2009). Expert workshop on combination effects of chemicals, 28-30 January 2009, Hornbæk, Denmark. http://eng.mst.dk/media/mst/67463/BILAG_2_Expertworkshop.pdf.

Danish Veterinary and Food Administration (2003), Combined Actions and Interactions of Chemicals in Mixtures: The Toxicological Effects of Exposure to Mixtures of Industrial and Environmental Chemicals. Fødevare Rapport 2003:12.

De Brouwere K, Cornelis C, Arvanitis A, Brown T, Crump D, Harrison P, Jantunen M, Price P and Torfs R (2014), Application of the maximum cumulative ratio (MCR) as a screening tool for the evaluation of mixtures in residential indoor air. Science of the Total Environment 479–480:267–276.

Delmaar J and Van Engelen J. (2006), Aggregating human exposure to chemicals: An overview of tools and methodologies. National Institute for Public Health and the Environment (RIVM), The Netherlands. RIVM Report 630700001/2006. https://www.rivm.nl/en/Documents_and_publications/Scientific/Reports/2007/maart/Aggregating_human_exposure_to_chemicals_An_overview_of_tools_and_methodologies.

Demond A, Franzblau A, Garabrant D, Jiang X, Adriaens P, Chen Q, Gillespie B, Hao W, Hong B, Jolliet O and Lepkowski J (2012), Human Exposure from Dioxins in Soil. Environ Sci Technol 46(3):1296-1302.

Dewalque L, Charlier C and Pirard C (2014), Estimated daily intake and cumulative risk assessment of phthalate diesters in a Belgian general population. Toxicology Letters 231(2):161-8.

De Zwart D and Posthuma L (2005), Complex mixture toxicity for single and multiple species: proposed methodologies. Environmental Toxicology and Chemistry 24(10):2665-2676.

Dionisio K, Frame A, Goldsmith M, Wambaugh J, Liddell A, Cathey T, Smith D et al. (2015), Exploring consumer exposure pathways and patterns of use for chemicals in the environment. Toxicology Reports 2:228-237.

Dudzina T, Delmaar CJ, Biesterbos JW, Bakker MI, Bokkers BG, Scheepers PT, van Engelen JG, Hungerbuehler K and von Goetz N. (2015), The probabilistic aggregate consumer exposure model (PACEM): validation and comparison to a lower-tier assessment for the cyclic siloxane D5. Environ Int. 79:8-16.

Dyer SD, White-Hull C and Shephard BK. (2000), Assessments of chemical mixtures via toxicity reference values overpredict hazard to Ohio fish communities. Environ. Sci. Technol. 34: 2518-2524.

Dyer S, Warne MSJ, Meyer JS, Leslie HA and Escher BI. (2010), Tissue residue approach for chemical mixtures. Integrated Environ Assess Man 7(1):99-115.

EC (European Commission) (2005), Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

EC (European Commission) (2009), Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309/1, 24.11.2009, pp. 1–50.

EC (European Commission) (2010), State of the art report on mixture toxicity—final report (Study Contract No 070307/2007/485103/ETU/D.1). Directorate General Environment, European Commission. http://ec.europa.eu/environment/chemicals/effects/pdf/report_mixture_toxicity.pdf [accessed 28-7-2015].

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) (2011a), Development of guidance for assessing the impact of mixtures of chemicals in the aquatic environment. Technical Report No. 111, 75 p.

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) (2011b), Workshop on combined exposure to chemicals. 11-12 July 2011, Berlin. Workshop Report No 22, 39 p.

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) (2012), Effects of chemical co-exposures at doses relevant for human safety assessments. Technical Report 115. http://www.ecetoc.org/publication/tr-115-effects-of-chemical-co-exposures-at-doses-relevant-for-human-safety-assessments/.

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) (2016), Guidance for Effective Use of Human Exposure Data for the Risk Assessment of Chemicals. Proceedings of ECETOC Workshop on Human Exposure Data & Modelling; 2016 26 Jan; Paris (FR).

ECHA (European Chemicals Agency) (2011), Guidance on Information Requirements and Chemical Safety Assessment. Part B- Hazard Assessment, December 2011. http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

ECHA (European Chemicals Agency) (2012), Guidance on Information Requirements and Chemical Safety Assessment. Chapter 19-Uncertainty analysis. http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

ECHA (European Chemicals Agency) (2013), Guidance on risk assessment from combined exposure to multiple biocidal substances within a single biocidal product, on the basis of the tiering principles of refinement as described in the WHO/IPCS.

ECHA (European Chemicals Agency) (2014), Transitional guidance on the biodical products regulation. Transitional guidance on mixture toxicity assessment for biocidal products for the environment. https://echa.europa.eu/documents/10162/23492134/tg_mixture+toxicity_superseded_en.pdf/de8ee de9-45f0-4be3-538e-8e922d21e8dd.

ECHA (European Chemicals Agency) (2015), Guidance on the Biocidal Products Regulation. Volume III Human Health–Part B-Risk Assessment. Version 2, October 2015. http://echa.europa.eu/documents/10162/15623299/biocides_guidance_human_health_ra_iii_partb_en.pdf.

ECHA (European Chemicals Agency) (2017a), Guidance for identification and naming of substances under REACH and CLP. https://echa.europa.eu/guidance-documents/guidance-on-reach.

ECHA (European Chemicals Agency) (2017b), Opinion on an Annex XV dossier proposing restrictions on Four Phthalates (DEHP, BBP, DBP, DIBP). https://echa.europa.eu/previous-consultations-on-restriction-proposals/-/substance-rev/13919/term.

EFSA (European Food Safety Authority) Panel on Plant Protection Products and their Residues (PPR) (2008), Scientific Opinion 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 704:1-85.

EFSA (European Food Safety Authority) (2009a), Guidance of EFSA. Risk assessment for birds and mammals. EFSA Journal 7(12):1438.
http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/1438.pdf.

EFSA (European Food Safety Authority) (2009b), Scientific Opinion on Risk Assessment for a Selected Group of Pesticides from the Triazole Group to Test Possible Methodologies to Assess Cumulative Effects from Exposure through Food from these Pesticides on Human. EFSA Journal 7(9):1–187.

EFSA (European Food Safety Authority) (2012), 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 10(5):2668.
http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2668.pdf.

EFSA (European Food Safety Authority) (2012b), Guidance on the Use of Probabilistic Methodology for Modelling Dietary Exposure to Pesticide Residues.

EFSA (European Food Safety Authority) (2013a), SCIENTIFIC REPORT OF EFSA International Frameworks Dealing with Human Risk Assessment of Combined Exposure to Multiple Chemicals. EFSA Journal 11(7):3313
http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3313.pdf.

EFSA (European Food Safety Authority) (2013b), Scientific opinion on the relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides residues in food. EFSA Journal 11(12):3472.
http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3472.pdf.

EFSA (European Food Safety Authority) (2013c), Scientific opinion on guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 11(7):3290.
http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3290.pdf.

EFSA (European Food Safety Authority) (2013d), Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile. EFSA Journal 11(7):3293.

EFSA (European Food Safety Authority) (2013e), Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 11:268.

EFSA (European Food Safety Authority) Panel on Plant Protection Products and their Residues (PPR) (2014), 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.

EFSA (European Food Safety Authority) (2014a), Scientific report of EFSA on modern methodologies and tools for human hazard assessment of chemicals. EFSA Journal 12(4):3638. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3638.pdf.

EFSA (European Food Safety Authority) (2014b), Scientific opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants. EFSA Journal 12(7):3800. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3800.pdf.

EFSA (European Food Safety Authority) (2014c), Scientific report of EFSA towards an integrated environmental risk assessment of multiple stressors on bees: review of research projects in Europe, knowledge gaps, and recommendations. EFSA Journal 12(3):3594. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3594.pdf.

EFSA (European Food Safety Authority) (2015), Summary report. EFSA Scientific colloquium 21. Harmonisation of human and ecological risk assessment of combined exposure to multiple chemicals. 11-12 September 2014. Edinburgh, UK. http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/784e.pdf.

EFSA (European Food Safety Authority) (2016), Draft Guidance on Uncertainty in EFSA Scientific Assessments. https://www.efsa.europa.eu/sites/default/files/160321DraftGDUncertaintyInScientificAssessment.pdf.

EFSA (European Food Safety Authority) (2017), EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger M, Knutsen K, More S, Mortensen A, Naegeli H, Noteborn H, Ockleford C et al. Update: guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal 15:4658.

EFSA (European Food Safety Authority) (2018a), EFSA Scientific Committee. Draft guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. https://www.efsa.europa.eu/sites/default/files/consultation/consultation/180626-1-ax1.pdf

EFAS (Euorpean Food Safety Authority) (2018b), EFSA Scientific Committee. Statement on genotoxicity assessment of chemical mixtures. https://www.efsa.europa.eu/sites/default/files/consultation/consultation/180626-ax2.pdf

Enoch S, Ellison C, Schultz T and Cronin M (2011), A review of the electrophilic reaction chemistry involved in covalent protein binding relevant to toxicity. Crit Rev Toxicol 41:783-802.

Enoch S and Cronin M (2010), A review of the electrophilic reaction chemistry involved in covalent DNA binding. Crit Rev Toxicol 40:728-748.

Environment Canada and Health Canada (1991), Effluents from Pulp Mills using Bleaching. Priority Substances List Assessment Report. ISBN: 0-662-18734-2 DSS. http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/pulp_mill_effluents_pate_blanchie/index-eng.php.

Environment Canada and Health Canada (1993), Chlorinated Wastewater Effluents. Priority Substances List Assessment Report. ISBN: 0-662-20470-0.
http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/chlorinated_wastewater_effluents_eaux_chlorees/index-eng.php.

Environment Canada and Health Canada (1994), Waste Crankcase Oils. Priority Substances List Assessment Report. ISBN: 0-662-22029-3.
http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/crankcase_oils_huiles_moteur/index-eng.php.

Environment Canada and Health Canada (2001a), Priority Substances List Assessment Report for Textile Mill Effluents. ISBN: 0-662-29341-X http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/textile_effluents/index-eng.php.

Environment Canada and Health Canada (2001b), Priority Substances List Assessment Report for Releases from Primary and Secondary Copper Smelters and Copper Refineries - Releases from Primary and Secondary Zinc Smelters and Zinc Refineries. ISBN: 0-662-29871-3. http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/copper_cuivre_zinc/index-eng.php.

Environment Canada and Health Canada (2013), Draft Screening Assessment. Aromatic Azo and Benzidine-based Substance Grouping. Certain Azo Disperse Dyes.
http://www.chemicalsubstanceschimiques.gc.ca/group/azo_benzidine/dyes-colorants_list-liste-eng.php#azo-disperse.

EPHECT (Emissions, Exposure Patterns and Health Effects of Consumer Products in the EU) [database] (2010-2013), Mol (BE): Vlaamse Instelling voor Technologisch onderzoek. https://sites.vito.be/sites/ephect/Pages/home.aspx.

EPHECT (Emissions, Exposure Patterns and Health Effects of Consumer Products in the EU) (2012), Survey on indoor use and use patterns of consumer products in EU Member States. Mol (BE): EPHECT.
https://esites.vito.be/sites/ephect/Working%20documents/EPHECT%20deliverables%20and%20documents/2.%20Assessing%20EU%20household%20uses%20for%20consumer%20product%20testing%20and%20risk%20assessment%20(WP5)/WP5%20Survey%20Report%20-%20Indoor%20Use%20and%20Use%20Patterns%20of%20Consumer%20Products%20final.pdf.

Escher B, Ashauer R, Dyer S, Hermens J, Lee J, Leslie H, Mayer, Meador J and Warne M (2010), Crucial role of mechanisms and modes of toxic action for understanding tissue residue toxicity and internal effect concentrations of organic chemicals. Publications, Agencies and Staff of the US Department of Commerce. Paper 259.

Escher B, Van Daele C, Dutt M, Tang J and Altenburger R (2013), Most oxidative stress response in water samples comes from unknown chemicals: The need for effect-based water quality trigger values. Environ Sci and Technol 47:7002–11.

European Communities (2011), Guidance Document No. 27: Technical Guidance For Deriving Environmental Quality Standards. doi: 10.2779/43816.

Fan J, Chen S, Chow E and Pang K (2010), PB-PK modeling of intestinal and liver enzymes and transporters in drug absorption and sequential metabolism. Current Drug Metabolism 11:743-61.

Faust M, Altenburger R, Backhaus T, Blanck H, Boedeker W, Gramatica P, Hamer V, Scholze M, Vighi M and Grimme L (2003), Joint algal toxicity of 16 dissimilarly acting chemicals is predictable by the concept of independent action. Aquatic Toxicology 63(1):43-63.

Fisk P (2014), Chemical Risk Assessment: A Manual for REACH. Wiley ISBN: 978-1-119-95368-5

Frische T, Mateszki S and Wogram J (2014), Environmental risk assessment of pesticide mixtures under regulation 1107/2009/EC: a regulatory review by the German Federal Environment Agency (UBA). Journal for Consumer Protection and Food Safety 9: 377-389. DOI 10.1007/s00003-014-0916-6.

Goldsmith M et al. (2014), Development of a consumer product ingredient database for chemical exposure screening and prioritization. Food and Chemical Toxicology 65:269-279.

Gosens I, Delmaar C, Ter Burg W and Schuur G (2011), Aggregate exposure assessment of chemicals in consumer products: Exposure to parabens in cosmetics in children as a case study. National Institute for Public Health and the Environment (RIVM), The Netherlands. RIVM Letter Report 320015005/2011.

Groβ R, Bunke D and Gartiser S (2011), Basic principles for the development of a concept for environmental exposure assessments of single substances released from multiple uses under REACH. Federal Environment Agency (Germany). Project No. (FKZ) 360 01 058.

Gregorio V, Chèvre N and Junghans M (2013), Critical issues in using the common mixture toxicity models concentration addition or response addition on species sensitivity distributions: a theoretical approach. Environmental Toxicology and Chemistry 32(10):2387–2395.

Groten J, Feron V and Suhnel J (2001), Toxicology of simple and complex mixtures. Trends in Pharmacological Sciences, 22(6):316–322.

Haber L, Dourson M, Allen B, Hertzberg R, Parker A, Vincent M, Maier A and Boobis A (2018), Benchmark dose (BMD) modeling: current practice, issues, and challenges. Crit Rev Toxicol 8:1-29.

Hall B, Steiling W, Safford B, Coroama M, Tozer S, Firmani C, McNamara C and Gibney M (2011), European consumer exposure to cosmetic products, a framework for conducting population exposure assessments Part 2. Food and Chemical Toxicology 49(2):408-422.

Hall B, Tozer S, Safford B, Coroama M, Steiling W, Leneveu-Duchemin M, McNamara C and Gibney M (2007), European consumer exposure to cosmetic products, a framework for conducting population exposure assessments. Food and Chemical Toxicology 45(11):2097-2108.

Han X and Price P (2011), Determining the Maximum Cumulative Ratios for Mixtures Observed in Ground Water Wells Used as Drinking Water Supplies in the United States. Int J Environ Res Public Health 8(12):4729-4745.

Han X and Price PS (2013), Applying the maximum cumulative ratio methodology to biomonitoring data on dioxin-like compounds in the general public and two occupationally exposed populations. J Expo Sci Environ Epidemiol 23(4):343-9.

Haws L, Su S, Harris M, Devito M, Walker N, Farland W, Finley B and Birnbaum L (2006), Development of a refined database of mammalian relative potency estimates for dioxin-like compounds. Toxicol Sci 89:4–30.

Hays S, Aylward L, Driver J, Ross J and Kirman C (2012), 2,4-D Exposure and risk assessment: Comparison of external dose and biomonitoring based approaches. Regul Toxicol Pharmacol 64(3):481-489.

Health Canada (2017), Cumulative Risk Assessment Framework. Regulatory Proposal PRO2017-01.

Health Canada and Environment Canada (2001), Priority Substances List Assessment Report for Nonylphenol and its Ethoxylates. ISBN: 0-662-29248-0. Cat. No.: En40-215/57E http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/nonylphenol/index-eng.php#a35 [accessed 18-8-2015].

Health Canada and Environment Canada (2015), Proposed approach for cumulative risk assessment of certain phthalates under the Chemicals Management Plan. http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=723C9007-1.

Holmstrup M, Bindesbøl A, Oostingh G, Duschl A, Scheil V, Köhler H, Loureiro S, Soares A, Ferreira A, Kienle C, Gerhardt A, Laskowski R, Kramarz P, Bayley M, Svendsen C and Spurgeon D (2010), Interactions between effects of environmental chemicals and natural stressors: A review. Science Tot Environ 408:3746–3762.

Household Products Database [database] (1993- present), Bethesda (MD): US National Library of Medicine. http://www.householdproducts.nlm.nih.gov/.

Hynes H and Lopez R (2007), Boston University School of Public Health. Cumulative Risk and a Call for Action in Environmental Justice Communities. Journal of Health Disparities Research and Practice 1(2):29-57.

IMM (The Institute of Environmental Medicine) (2011), Human health Risk Assessment of Combined Exposures to Chemicals: Challenges and Research Needs. IMM-Rapport nr/2011, Karolinska Institutet, Sweden.

IPCS/WHO (International Programme on Chemical Safety/World Health Organisation) (2008), Guidance Document on Characterizing and Communicating Uncertainty in Exposure Assessment Harmonization Project Document No. 6.

IPCS/WHO (International Programme on Chemical Safety/World Health Organisation) (2014), Guidance Document on Evaluating and Expressing Uncertainty in Hazard Characterization Harmonization Project Document 11.

Isaacs K, Goldsmith M, Egeghy P, Phillips K, Brooks R, Hong T and Wambaugh J (2016), Characterization and prediction of chemical functions and weight fractions in consumer products. Toxicology Reports 3:723-732.

Jager T, Vandenbrouck T, Baas J, De Coen W and Kooijman S (2010), A biology-based approach for mixture toxicity of multiple endpoints over the life-cycle. Ecotoxicology 19(2):351-361.

Junghans M, Backhaus T, Faust M, Scholze M and Grimme L (2006), Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. Aquatic Toxicology 76:93-110.

KEMI (Swedish Chemicals Agency) (2015), An additional assessment factor (MAF) – A suitable approach for improving the regulatory risk assessment of chemical mixtures? Report 5-15. https://www.kemi.se/global/rapporter/2015/rapport-5-15.pdf.

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. EUR 26675 EN; doi:10.2788/84264.

Kienzler A, Halder M and Worth A (2016a), Scientific options for avoiding chronic fish testing on the basis of existing data and extrapolation approaches. EUR 27907; doi:10.2788/546221.

Kienzler A, Bopp S, Van Der Linden S, Berggren E and Worth A (2016b), Regulatory assessment of chemical mixtures: Requirements, current approaches and future perspectives. Regulatory Toxicology and Pharmacology 80:321-334.

Klimisch H (1997), A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regulatory Toxicology and Pharmacology 25:1-5.

Kortenkamp A (2007), Ten years of mixing cocktails: a review of comnìbination effects of endocrine-disrupting chemicals. Environmnetal Health Perspectives 115(Suppl. 1): 98-105.

Kortenkamp A, Backhaus T and Faust M (2009), State of the art report on mixture toxicity. Final report. 070307/2007/485103/ETU/D.1, 391 p.

Kortenkamp A and Faust M (2010), Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment. International Journal of Andrology 33:463-474.

Loewe S and Muischnek H (1926), Über Kombinationswirkungen. 1. Mitteilung: Hilfsmittel der Fragestellung. Nanyn-Schmiedebergs Arch Exp Pathol Pharmakol (114):313-326.

Lorber M, Schecter A, Paepke O, Shropshire W, Christensen K and Birnbaum L (2015), Exposure assessment of adult intake of bisphenol A (BPA) with emphasis on canned food dietary exposures. Environment International. 77:55-62.

Malaj E, von der Ohe P, Grote M, Kühne R, Mondy C, Usseglio-Polatera P, Brack W and Schäfer R (2014), Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. PNAS 111(26):9549-9554.

Marx C, Mühlbauer V, Krebs P and Kuehn V (2015), Environmental risk assessment of antibiotics including synergistic and antagonistic combination effects. Science of the Total Environment 524-525:269-279.

Meek M, Boobis A, Crofton K, Heinemeyer G, Raaij M and Vickers C (2011), Risk assessment of combined exposure to multiple chemicals. A WHO/IPCS framework. Regulatory Toxicology and Pharmacology 60(Supp 2):S1-S14.

Meek M (2013), International experience in addressing combined exposures: Increasing the efficient of assessment. Toxicology 313:185-189.

Moermond C, Kase R, Korkaric M and Ågerstrand M (2015), CRED: Criteria for reporting and evaluating ecotoxicity data. Environmental Toxicology and Chemistry 35(5):1297-1309.

Moretto A, Bachman A, Boobis A, Solomon K, Pastoor T, Wilks M and Embry M (2016), A framework for cumulative risk assessment in the 21st century. Critical Reviews in Toxicology 47(2):85-97.

Moretto et al. (2015), The use of in vitro testing to refine cumulative assessment groups of pesticides: The example of teratogenic conazoles. Food Chem Toxicol 79:65-9.

NAS (National Academy of Science) (2008), Phthalates and Cumulative Risk Assessment the Task Ahead. Committee on Health Risks of Phthalates. Washington, DC: The National Academies Press. http://www.nap.edu/openbook.php?record_id=12528.

NAS (National Academy of Science) (2017), Using 21st Century Science to Improve Risk-Related Evaluations. https://download.nap.edu/cart/download.cgi?record_id=24635.

Nielsen E et al. (2012), External scientific report submitted to EFSA: Identification of Cumulative Assessment Groups of Pesticides.

NEJAC (National Environmental Justice Advisory Council) (2004), Ensuring Risk Reduction in Communities with Multiple Stressors: Environmental Justice and Cumulative Risks/Impacts (Cumulative Risks/Impacts Workgroup Report). https://www.epa.gov/sites/production/files/2015-04/documents/ensuringriskreducationnejac.pdf.

NRC (National Research Council) (2008), Phthalates and Cumulative Risk Assessment: The Task Ahead. Washington, DC: The National Academies Press.

NRC (National Research Council) (2009), Science and Decisions: Advancing Risk Assessment. Washington, DC: The National Academies Press. https://www.nap.edu/catalog/12209/science-and-decisions-advancing-risk-assessment.

OECD (Organisation for Economic Cooperation and Development) (2011), WHO OECD ILSI/HESI International Workshop on Risk Assessment of Combined Exposures to Multiple Chemicals. Paris, France, OECD Environment Directorate. OECD Environment, Health and Safety Publications. Series on Testing and Assessment No. 140.

OECD (Organisation for Economic Cooperation and Development) (2013), Guidance document for exposure assessment based on environmental monitoring. OECD Environment Directorate, OECD Environment, Health and Safety Publications Series on Testing & Assessment No. 185

OECD (Organisation for Economic Cooperation and Development) (2014), Guidance on Grouping of Chemicals, Second Edition, OECD Environment Directorate, OECD Environment, Health and Safety Publications Series on Testing & Assessment No. 194.

OECD (Organisation for Economic Cooperation and Development) (2016a), Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA). OECD Environment Directorate, OECD Environment, Health and Safety Publications Series on Testing & Assessment No. 260.

OECD (Organisation for Economic Cooperation and Development) (2016b), Guidance document on the Reporting of Defined Approaches to be Used within Integrated Approaches to Testing and

Assessment. OECD Environment Directorate, OECD Environment, Health and Safety Publications Series on Testing & Assessment No. 255.

OECD (Organisation for Economic Cooperation and Development) (2017), Guidance Document on Developing and Assessing Adverse Outcome Pathways, Series on Testing and Assessment No. 184.

Phillips K, Wambaugh J, Grulke C, Dionisio K and Isaacs K (2017), High-throughput screening of chemicals as functional substitutes using structure-based classification models. Green Chemistry 19(4): 1063-1074.

Price P and Chaisson C (2005), A conceptual framework for modelling aggregate and cumulative exposures to chemicals. Journal of Exposure Science and Environmental Epidemiology 15(6):473.

Price P, Hollnagel H and Zabik J (2009), Characterizing the Noncancer Toxicity of Mixtures using Concepts from the TTC and Quantitative Models of Uncertainty in Mixture Toxicity. Risk Analysis 29(11):1534-1548.

Price P and Han X (2011), Maximum Cumulative Ratio (MCR) as a Tool for Assessing the Value of Performing a Cumulative Risk Assessment. Int. J. Environ. Res. Public Health. 8:2212-2225.

Price P et al. (2012a), A decision tree for assessing effects from exposures to multiple substances. Environmental Sciences Europe, 24:26.

Price P, Han X, Junghans M, Kunz P, Watts C and Leverett D (2012b), An application of a decision tree for assessing effects from exposures to multiple substances to the assessment of human and ecological effects from combined exposures to chemicals observed in surface waters and waste water effluents. Environmental Sciences Europe 24:34.

Reyes J and Price P (2018), An analysis of cumulative risks based on biomonitoring data for six phthalates using the Maximum Cumulative Ratio. Environment international 112: 77-84.

Rider C and Simmons J (2015), Toxicological Risk Assessment for Beginners. In: Torres JA, Bobst S, editors. Risk Assessment Strategies and Techniques for Combined Exposures: Spinger. p 111-111-134.

Rieke S, Heise T, Schmidt F, Haider W, Bednarz H, Niehaus K, Mentz A, Kalinowski J, Hirsch-Ernst KI, Steinberg P, Niemann L et al. (2017), Mixture effects of azole fungicides on the adrenal gland in a broad dose range. Toxicology 385:28-37.

RIVM (Rijksinstituut voor Volksgezondheid en Milieu [National Institute for Public Health and the Environment (NL)]) (1996), SimpleTreat 3.0: a model to predict the distribution and elimination of chemicals by sewage treatment plants. RIVM Report:719101025/1996. https://www.rivm.nl/dsresource?objectid=0f348f0d-3cfe-4ece-bebd-178072b56170&type=org&disposition=inline.

Rowlands C et al. (2013), A Genomics-Based Analysis of Relative Potencies of Dioxin-Like Compounds in Primary Rat Hepatocytes. Toxicological Sciences 136(2):595–604.

Sasso A, Georgopoulos P, Isukapalli S and Krishnan K (2012), Bayesian Analysis of a Lipid-Based Physiologically Based Toxicokinetic Model for a Mixture of PCBs in Rats. Journal of Toxicology 2012:895391.

Sarigiannis D and Hansen U (2012), Considering the cumulative risk of mixtures of chemicals - a challenge for policy makers. Environ Health 11(Suppl 1):S18.

SCHER, SCCS, SCENIHR (2012), Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), and the Scientific Committee on Consumer Safety (SCCS). Toxicity and assessment of chemical mixtures. http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf

Schmidt F, Marx-Stoelting P, Haider W, Heise T, Kneuer C, Ladwig M, Banneke S, Rieke S and Niemann L (2016), Combination effects of azole fungicides in male rats in a broad dose range. Toxicology 355-356:54-63.

Schwöbel J, Koleva Y, Enoch S, Bajot F, Hewitt M, Madden J, Roberts D, Schultz T and Cronin M (2011), Measurement and Estimation of Electrophilic Reactivity for Predictive Toxicology. Chem Rev 111(4):2562–2596.

Sexton K (2012), Cumulative risk assessment: An overview of methodological approaches for evaluating combined health effects from exposure to multiple environmental stressors. Int J Environ Res Public Health 9:370-390.

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 for Consumer Protection and Food Safety 9:367-376.

Solecki R, Stein R, Frische T, Matezki S, Wogram J and Streloke M (2014), Paradigm shift in the risk assessment of cumulative effects of pesticide mixtures and multiple residues to humans and wildlife: German proposal for a new approach. Journal for Consumer Protection and Food Safety 9:329-331.

Solomon K, Wilks M, Bachman A, Boobis A, Moretto A, Pastoor T, Phillips R and Embry M (2016), Problem formulation for risk assessment of combined exposures to chemicals and other stressors in humans. Critical Reviews in Toxicology 46(10):835-844.

Tang J and Escher B (2014a), Realistic environmental mixtures of micropollutants in surface, drinking, and recycled water: herbicides dominate the mixture toxicity toward algae. Environmental Toxicology and Chemistry 33(6):1427–36.

Tang J, Busetti F, Charrois J and Escher B (2014b), Which chemicals drive biological effects in wastewater and recycled water? Water Res 60:289-299.

Terry C, Rasoulpour R, Knowles S and Billington R (2015), Utilizing relative potency factors (RPF) and threshold of toxicological concern (TTC) concepts to assess hazard and human risk assessment profiles of environmental metabolites: a case study. Regul Toxicol Pharmacol 71:301-17.

Teuschler et al. (2004), A Feasibility Study of Cumulative Risk Assessment Methods for Drinking Water Disinfection By-Product Mixtures. Journal of Toxicology and Environmental Health, Part A: Current Issues 67:8-10.

Timchalk C and Poet T (2008), Development of a physiologically based pharmacokinetic and pharmacodynamic model to determine dosimetry and cholinesterase inhibition for a binary mixture of chlorpyrifos and diazinon in the rat. Neurotoxicolog, 29(3):428-443.

Tollefsen K, Scholz S, Cronin M, Edwards S, de Knecht J, Crofton K, Garcia-Reyero N, Hartung T, Worth A and Patlewicz G (2014), Applying Adverse Outcome Pathways (AOPs) to support Integrated Approaches to Testing and Assessment (IATA). Regulatory Toxicology and Pharmacology, 70(3):629-640.

Qian H, Chen M, Kransler K and Zaleski R (2015), Assessment of chemical coexposure patterns based upon phthalate biomonitoring data within the 2007/2008 National Health and Nutrition Examination Survey. J Expo Sci Environ Epidemiol 25(3):249-55.

UN (United Nations) (2011), Globally Harmonised System of Classification and Labelling of Chemicals (GHS). Fourth Revised Edition.

UNEP (United Nations Environment Programme) and WHO (World Health Organisation) (2012), State of the science of endocrine disrupting chemicals. http://www.who.int/ceh/publications/endocrine/en.

US Department of Health (2004), Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures.

US EPA (United States Environmental Protection Agency) (1992), Guidelines for Exposure Assessment. Risk Assessment Forum EPA/600/Z-92/001.

US EPA (United States Environmental Protection Agency) (2000), Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum EPA/630/R-00/002. https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=20533.

US EPA (United States Environmental Protection Agency) (2002), Guidance on Cumulative Risk Assessment of Pesticides Chemicals That Have a Common Mechanism of Toxicity. http://www.epa.gov/sites/production/files/2015-07/documents/guidance_on_common_mechanism.pdf.

US EPA (Environmental Protection Agency) (2003), Framework for Cumulative Risk Assessment. EPA/630/P-02/001F http://www2.epa.gov/sites/production/files/2014-11/documents/frmwrk_cum_risk_assmnt.pdf.

US EPA (United States Environmental Protection Agency) (2006a), Organophosphate Cumulative Risk Assessment. EPA-HQ-OPP-2006-0618 https://www.regulations.gov/docket?D=EPA-HQ-OPP-2006-0618.

US EPA (United States Environmental Protection Agency) (2006b), Triazine Cumulative Risk Assessment. EPA-HQ-OPP-2005-0481 https://www.regulations.gov/document?D=EPA-HQ-OPP-2005-0481-0001.

US EPA (United States Environmental Protection Agency) (2007), Concepts, Methods, and Data sources for Cumulative Health Risk Assessment of Multiple Chemicals, Exposures and Effects: A Resource Document. EPA/600/R-06/013F https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=190187.

US EPA (United States Environmental Protection Agency) (2011a), Pyrethrins/Pyrethroid Cumulative Risk Assessment. EPA-HQ-OPP-2011-0746-0003 https://www.regulations.gov/docket?D=EPA-HQ-OPP-2011-0746.

US EPA (United States Environmental Protection Agency) (2011b), Exposure Factors Handbook: 2011 Edition. EPA/600/R-09/052F https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252.

US EPA (United States Environmental Protection Agency) (2011c), National Air Toxics Assessment. Glossary of Terms. https://www.epa.gov/national-air-toxics-assessment/nata-glossary-terms.

US EPA (United States Environmental Protection Agency) (2012), Benchmark dose technical guidance. Risk Assessment Forum EPA/100/R-12/001. https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf.

US EPA (Environmental Protection Agency) (2014), Framework for Human Health Risk Assessment to Inform Decision Making. EPA/100/R-14/001. http://www.epa.gov/sites/production/files/2014-12/documents/hhra-framework-final-2014.pdf.

US EPA (United States Environmental Protection Agency) (2015), Human health risk assessment. Strategic research action plan 2016-2019. EPA 601/K-15/002. http://www.epa.gov/sites/production/files/2015-10/documents/strap_2016_hhra_508.pdf.

US EPA (United States Environmental Protection Agency) (2016), Pesticide Cumulative Risk Assessment: Framework for Screening Analysis Purpose. This document is contained in EPA-HQ-OPP-2015-0422. https://www.regulations.gov/document?D=EPA-HQ-OPP-2015-0422-0019.

Vallotton N and Price P (2016), Use of the Maximum Cumulative Ratio As an Approach for Prioritizing Aquatic Coexposure to Plant Protection Products: A Case Study of a Large Surface Water Monitoring Database. Environ Sci Technol. 50(10): 5286-93.

van den Berg M et al. (2006), The 2005 World Health Organization reevaluation Dioxins and DL-PCBs in foods for infants and young children of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicological Sciences, 93 223-241.

van der Voet H, de Boer WJ, Kruisselbrink JW, van Donkersgoed G and JD van Klaveren (2016), MCRA made scalable for large cumulative assessment groups. EFSA supporting publication 2016:EN-910. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/sp.efsa.2016.EN-910.

van Ede K, van Duursen M and M van den Berg (2016), Evaluation of relative effect potencies (REPs) for dioxin-like compounds to derive systemic or human-specific TEFs to improve human risk assessment. Archives of Toxicology 90(6):1293–1305.

van Raaij M (2009), Principles of aggregate exposure assessment. Page 23-25. Cited in Assessment of Combined Exposures to Multiple Chemicals: Report of a WHO/IPCS International Workshop on Aggregate/Cumulative Risk Assessment. http://www.who.int/ipcs/methods/harmonization/areas/workshopreportdocument7.pdf.

Vermeire T (2009), Aggregate Exposure to Industrial Chemicals and other Contaminants: Assessment of Environmental Exposure. Pages 26-30. Cited in Assessment of Combined Exposures to Multiple Chemicals: Report of a WHO/IPCS International Workshop on Aggregate/Cumulative Risk Assessment. http://www.who.int/ipcs/methods/harmonization/areas/workshopreportdocument7.pdf.

Walter H, Consolaro F, Gramatica P, Scholze M and Altenburger R (2002), Mixture toxicity of priority pollutants at no observed effect concentrations (NOECs). Ecotoxicology 11(5):299-310.

WHO (World Health Organisation) (2001), Integrated Risk Assessment. Report WHO/IPCS/IRA/01/12.

WHO (World Health Organization) (2009a), Assessment of Combined Exposures to Multiple Chemicals, Report of a WHO/IPCS International Workshop on Aggregate/Cumulative Risk Assessment. http://www.who.int/ipcs/methods/harmonization/areas/workshopreportdocument7.pdf.

WHO (World Health Organisation) (2009b), Environmental Health Criteria 240: Principles and Methods for the Risk Assessment of Chemicals in Food (Definitions page A-25). http://www.who.int/foodsafety/publications/chemical-food/en/.

WHO (World Health Organization) (2017), Chemical Mixtures in Source-water and Drinking-water. http://apps.who.int/iris/bitstream/10665/255543/1/9789241512374-eng.pdf?ua=1.

WHO/IPCS (World Health Organisation/International Programme on Chemical Safety) (2005), Chemical-specific adjustment factors for interspecies differences and human variability: Guidance document for use of data in dose/concentration-response assessment Harmonization Project Document No. 2.

Vermeire T, Munns W, Sekizawa J, Suter G and Van der Kraak G (2007), An Assessment of Integrated Risk Assessment. Human and Ecological Risk Assessment: An International Journal 13(2):339–354.

Wilks MF et al. (2015), 'White paper on the promotion of an integrated risk assessment concept in European regulatory frameworks for chemicals', Science of the Total Environment 521–522(1):211–218.

Wilkinson C, Christoph G, Julien E, Kelley J, 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? Regul Toxicol Pharmacol 31:30-43.

Worth A et al. (2014), Alternative methods for regulatory toxicology – a state-of-the-art review, Science and Policy Report by the Joint Research Centre. doi: 10.2788/11111.

Yang C et al. (2015), New Publicly Available Chemical Query Language, CSRML, To Support Chemotype Representations for Application to Data Mining and Modeling. Inf Model 55(3):510–528.

Zelenka M, Barr D, Nicolich M, Lewis R, Bird M, Letinski D, Metcalf S and O'Lone R (2011), A weight of evidence approach for selecting exposure biomarkers for biomonitoring. Biomarkers 16(1):65-73.

The report outlines the technical aspects of the various approaches and methodologies available with respect to the assessment of risks from combined exposures to multiple chemicals. The document draws from approaches applied and experience gained in the regulatory context and will therefore be most relevant to the regulatory authorities addressing chemicals, the regulated community and other interested stakeholders. The considerations are not presented as strict guidance but rather elements to recognise in assessing combined exposures to multiple chemicals.

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