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REVIEW ARTICLE

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Overview on legislation and scientific approaches for risk assessment of combined exposure to multiple chemicals: the potential EuroMix contribution

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ABSTRACT

This article reviews the current legislative requirements for risk assessment of combined exposure to multiple chemicals via multiple exposure routes, focusing on human health and particularly on food-related chemicals. The aim is to identify regulatory needs and current approaches for this type of risk assessment as well as challenges of the implementation of appropriate and harmonized guidance at international level. It provides an overview of the current legal requirements in the European Union (EU), the United States and Canada. Substantial differences were identified in the legal requirements for risk assessment of combined exposure to multiple chemicals and its implementation between EU and non-EU countries and across several regulatory sectors. Frameworks currently proposed and in use for assessing risks from combined exposure to multiple chemicals via multiple routes and different durations of exposure are summarized. In order to avoid significant discrepancies between regulatory sectors or countries, the approach for assessing risks of combined exposure should be based on similar principles for all types of chemicals. OECD and EFSA identified the development of harmonized methodologies for combined exposure to multiple chemicals as a key priority area. The Horizon 2020 project "EuroMix" aims to contribute to the further development of internationally harmonized approaches for such risk assessments by the development of an integrated test strategy using *in vitro* and *in silico* tests verified for chemical mixtures based on more appropriate data on potential combined effects. These approaches and testing strategies should be integrated in a scientifically based weight of evidence approach to account for complexity and uncertainty, to improve risk assessment.

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1. Introduction

Chemical risk assessments are performed to ensure the protection of human and environmental health. Traditionally, risk assessments are conducted on an individual chemical basis, mostly addressing one source of exposure (single chemical and one route of exposure). However, the human population and the environment are continuously exposed to a multitude of substances from different sources via different routes and duration of exposure, as has been demonstrated in environmental (Malaj et al. 2014; Wu et al. 2014; Maruya et al. 2016) and human monitoring (Woodruff et al. 2011; CDC 2018) studies. Exposure to multiple chemicals may increase health risks, relative to those of individual chemicals, due to potential combined effects, exhibited *via* similar or dissimilar mechanisms. Due to the large number of chemicals present in the environment, risk assessment of chemical combinations is complex and poses a number of challenges for scientists, risk assessors and managers (EFSA 2016). Increasing awareness that in daily-life exposure is to mixtures of chemicals, needing a move beyond chemical-by-chemical assessments, has led to a prioritization of this topic in policy and research. There are a number of different possible scenarios that need to be addressed. These include products containing more than one defined chemical component (intentional mixtures), products comprising a complex mixture of chemicals, not all of which are defined (intentional mixtures of Unknown or Variable composition, Complex reaction products or Biological materials, UVCBs), and exposure to multiple chemicals from different products (incidental or unintentional mixtures). An agreed and sufficiently specific and applicable technical guidance is needed to facilitate a consistent and adequate implementation of a harmonized approach for risk assessment of combined exposure to multiple chemicals via multiple routes (Solecki et al. 2014). Various frameworks on

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this type of risk assessment have been proposed or are actively being developed by European or international organisations, such as the European Food Safety Authority (EFSA), the World Health Organisation (WHO), the International Programme on Chemical Safety (IPCS) and the Organisation for Economic Cooperation and Development (OECD) (reviewed in Kienzler et al. 2016). Several EU research projects focusing on different aspects on combined exposure to multiple chemicals are ongoing or have recently been finished at the date of this publication (Bopp et al. 2018).

Contributing to the further development of internationally harmonized approaches for human health risk assessment of combined exposures to multiple chemicals is the overall goal of the Horizon 2020 project "EuroMix" (European Test and Risk Assessment Strategies for Mixtures; www.euromixproject.eu). More specifically, EuroMix aims to establish efficient and verified testing and tiered assessment strategies for mixtures of chemicals, including the appropriate test methods and tools. In line with existing approaches for risk assessment of combined exposure to multiple chemicals, the strategies being developed within the EuroMix project are mechanism-based. In the EuroMix project, in principle all chemical classes (cumulative) and all routes (aggregate) of combined exposure are considered for their effects on human health. However, this article focuses mainly on food-related chemicals such as plant protection products, biocidal products and their residues, food and feed additives, and contaminants, i.e. chemicals to which humans are potentially exposed *via* the diet.

The aim of this manuscript is to identify regulatory differences and current challenges in the implementation of risk assessment of combined exposure to multiple chemicals and to outline how EuroMix could contribute to the development of new approaches and testing strategies for this type of risk assessment. To that end, the current legislative requirements were reviewed, focusing on human health and particularly on food-related chemicals. An overview is provided of the current legal requirements for assessing the risk to human health from exposure to multiple chemicals in the European Union (EU), the United States (US) and Canada (Section 2). Additionally, this manuscript summarizes general principles, frameworks currently proposed and in-use for risk assessment of combined exposure to multiple chemicals (Section 3). Building on this, the EuroMix contribution to the development of new approaches and testing strategies for such risk assessment is summarized (Section 4). Finally, it outlines challenges in the approaches and testing strategies for risk assessment of combined exposure to multiple chemicals and provides recommendations with regard to international harmonization (Section 5).

It should be noted that in this manuscript the term "risk assessment of combined exposure to multiple chemicals" refers to assessing the combined risk from exposure to multiple chemicals with similar or dissimilar modes of action, via different routes of exposure, with exposure being defined according to the WHO/IPCS workshop on cumulative risk assessment (WHO 2009), i.e. "exposure via all relevant routes and pathways, including concurrent exposures to multiple chemicals or where exposure at different times leads to overlap in the time course of effects as a consequence of their respective toxicokinetics and/or toxicodynamics". The key terms are based on previous initiatives for harmonized terminology of WHO/IPCS (WHO 2009) and EFSA (EFSA 2013a).

2. Legal requirements for risk assessment of mixtures

This section provides an overview of the current legal requirements for risk assessment of combined exposure to multiple chemicals in the EU and describes legal frameworks in the US and Canada. A brief outline on all reviewed legal acts is presented in Table 1.

2.1. European Union

Within the EU, the placing of chemical substances and products on the market is highly regulated in order to ensure a high level of protection for human health. For the registration, evaluation, authorization and restriction of chemicals (REACH) within the scope of Regulation EC (2006), all obligations are related to substances (i.e. products of manufacturing processes) and no separate requirements or guidance for the hazard and risk assessment of combined exposure to multiple chemicals is provided (Kortenkamp et al. 2009; CEFIC 2010). The REACH guidance document addresses aggregate exposure of single substances for consumers (ECHA 2016). In contrast, clear criteria for classification of both substances and intentional mixtures (i.e. products that, as marketed, contain more than one chemical substance) are laid down in the Classification, Labelling and Packaging (CLP) Regulation (EC 2008a), where "*available information on synergistic and antagonistic effects should be taken into account for the classification of mixtures*" (Recital 37). The CLP Regulation provides guidance on the hazard classification of such chemical mixtures and proposes four classification methods, depending on the available data and on the properties of the components of the mixture (ECHA 2017).

Plant Protection Products (PPP) and Biocidal Products (BP) are intentional mixtures, comprising technical formulations with known composition of one or more pesticidal active substance(s) and additional components (e.g. solvents, emulsifiers). They are regulated under different legal acts, based on their intended use. For the authorization of PPPs and BPs, active substances therein need to be approved (EC 2009; EU 2012). The Regulation on BPs (EU 2012) explicitly requires cumulative and synergistic effects (presumably of substances present in the product, but this is not stated explicitly) be taken into account. Furthermore, the BP Regulation even stipulates collaboration between Agencies, Member States and the European Commission to develop and provide guidance on the scientific definitions and methodologies for the assessment of cumulative and synergistic effects. The European Chemicals Agency (ECHA) provides guidance to account for risks exerted by multiple substances within a single BP (ECHA 2015). With regard to the authorization of PPPs, sufficient information to allow for a cumulative risk assessment, considering exposure to more than one chemical substance, is required for pesticidal active substances and co-formulants contained in the product (EU 2013a, 2013b). This

Table 1. Overview of the chemical and mixture assessment requirements for intentional mixtures in the legislation of EU, US and Canada.

Description of the reviewed legislation		Mixture assessment for human health required?	Guidance document for cumulative risk assessment available?
A) European Union			
Industrial chemical related regulations			
REACH	Regulation (EC) 1907/2006	No[1] mixture assessment, but consideration of cumulative exposure in ECHA (2012)[2]	No
CLP	Regulation (EC) 1272/2008	Yes, defined classification criteria for mixtures.	ECHA (2017)
Pesticide related regulations			
Plant protection products and data requirements	Regulation (EC) 1107/2009 Regulation (EU) 283/2013 Regulation (EU) 284/2013	Yes, for the constituents of the product. No consideration for mixture assessment from different sources.	No
Biocidal products	Regulation (EU) 528/2012	Yes, for the individual components of the product and if the biocidal product is intended to be authorized for use with other biocidal products.	ECHA (2015)
MRLs	Regulation (EC) 396/2005	Yes, for pesticide residues from pesticide uses and other sources.	No
Dietary exposure related regulations			
Food law	Regulation (EC) 178/2002	Yes, cumulative toxic effects for food shall be considered.	No
Food additives	Regulation (EC) 1333/2008	No	No
Additives for use in animal nutrition	Regulation (EC) 1831/2003	No	No
Feed additives	Regulation (EC) 429/2008	No	No
B) United States			
Pesticides	Food Quality Protection Act (FQPA), 1996	Yes	US EPA (2002a, 2016a)
Food additives; New animal drugs; Color additives	Federal Food, Drug, and Cosmetic Act (FFDCA 2018)	No	No
Environmental Pollutants	No legal mandate	Yes	US EPA (2000, 2003b)
C) Canada			
Pesticides	Pest Control Products Act (PCPA 2018)	Yes	[Harmonisation with US EPA]
Environmental Pollutants	Canadian Environmental Protection Act (CEPA 1999)	Yes	Health Canada (2010a, 2010b)

CLP: Classification, Labelling and Packaging of substances; EC: European Commission; ECHA: The European Chemicals Agency; EU: European Union; US EPA: United States – Environmental Protection Agency; REACH: registration, evaluation, authorization and restriction of chemicals; MRL: maximum residue levels.
[1]No mixture assessment of different substances required. The definition of substances under REACH includes impurities and additives necessary for stability.
[2]"The relevant exposure parameter (mean level, peak level, duration, cumulative dose) depends on the health outcome and exposure setting and should be justified. […] In many epidemiological studies, in particular occupational studies, cumulative exposure (cumulative exposure = exposure level * exposure duration, e.g. ppm years) is used as exposure metric."

information is required to evaluate whether PPPs and their residues may have harmful effects on human health, whereby "*known cumulative and synergistic effects where the scientific methods accepted by the Authority to assess such effects are available*", shall be taken into account (EC 2009, Article 4). In the authorization procedure of PPPs, interactions between active substances and other components of the PPP shall be taken into account (EC 2009, Article 29). No harmonized guidance on the implementation of the assessment of combined exposure to multiple PPPs exists, but approaches for operators and consumers were proposed by Stein et al. (2014). Recently, the Group of Chief Scientific Advisors advised the European Commission (EC 2018) that "*The PPP approval and authorisation process must better assess risks associated with PPP mixtures and long-term exposure*". Many activities regarding assessment of combined exposures and combined effects are based on the Regulation defining the setting of limit values for residues in food and feed products (EC 2005), designated as Maximum Residue Levels (MRLs). The MRL regulation clearly requires the development of methodologies for risk assessment of combined exposures to chemicals from multiple products, i.e. incidental mixtures, in order to account for "*[…] pesticide residues arising from sources other than current plant protection uses of active substances, and their known cumulative and synergistic effects, when appropriate methods are available*" (EC 2005, Article 14). Furthermore, the MRL Regulation addresses measures for further development of legislation and technical guidelines on pesticide residues for the assessment of aggregated, cumulative and synergistic effects. This requirement is fundamental in the assessment of potential combined effects, as in many pieces of EU legislation this task is conditional on the availability of appropriate methods. Official guidance would be the first step to enable a sound product and consumer-based risk assessment of combined exposures to multiple chemicals via different routes of exposure.

General principles and requirements for food safety are regulated by the European Food Law (EC 2002), requiring that unsafe food shall not be placed on the market, which includes, amongst others, the determination of whether potential

cumulative exposure might be injurious to health (Article 14). No further explanation for the implementation of this requirement is provided, even though the Food Law provides the legal basis for more specific legislation such as for food and feed additives. No specific legal requirements for evaluating combined exposures and combined effects are laid down in the current Regulation on food additives (EC 2008b) or on feed additives (EC 2003). However, the implementing Regulation on feed additives (EC 2008c) requires a separate assessment of each component and the consideration of the cumulative effect for consumer safety or the assessment of the complete (intentional) mixture. No further guidance on the implementation of combined effect assessments is provided.

Most of the reviewed European Regulations stipulate the need to consider potential combined effects from exposures to multiple chemicals within intentional mixtures of different components within formulated products. As the development of guidance in response to these mandates is still pending, current legal requirements regarding the assessment of risks of combined exposure to multiple chemicals are often conditional to statements like "*where relevant*" or "*known and expected cumulative and synergistic effects shall be considered*". This leads to ambiguity in the consideration of mixture toxicity (i.e. combined effects) that depends on interpretation of the different regulations, expert knowledge and the public availability of toxicity data. Regardless, however, implementation of clear legal mandates in regulations to assess the combined effects of substances in products or of different products, will be difficult as long as harmonized and accepted methods are lacking.

2.2. United States and Canada

In general, legislative mandates give the US Environmental Protection Agency (US EPA) broad authority to protect public health in allowing the use of cumulative risk or chemical mixtures assessment in decision-making. For the risk assessment of pesticides, the Federal Food, Drug, and Cosmetic Act (FFDCA 2018) and the Food Quality Protection Act (FQPA 1996) mandate US EPA to consider, among others, cumulative exposure to different pesticides that have been shown to have common mechanisms of toxicity and aggregate exposure to one pesticide from multiple sources of exposure (food, water, residential and other non-occupational sources). The legislative requirements set by FFDCA (2018) and FQPA (1996) are addressed in several guidance documents (US EPA 1999, 2001, 2002a, 2002b, 2016a). The US EPA has adopted a tiered approach for cumulative risk assessment that begins with a screening level analysis using conservative hazard and exposure assumptions based on the level of scope and refinement needed (US EPA 2016a). Besides pesticides, the FFDCA (2018) also mandates US Food and Drug Administration (FDA) to consider cumulative effects in the process of regulating food additives, new animal drugs and color additives. However, no specific guidance has been developed for these chemical classes. Since this process may be hindered by limited data availability, US EPA (2000) recommends following both a component and a whole mixture based risk assessment approach, giving rise to an integrated summary and uncertainty evaluation.

In Canada, pesticides are regulated by the Pest Management Regulatory Agency (PMRA) of Health Canada, under the Pest Control Products Act (PCPA 2018) and Regulations made under this Act. In the PCPA there is a clear mandate to consider aggregate exposure from diet and residential sources and cumulative effects of pest control and other products that have a common mechanism of toxicity in the process of human health risk assessment. In order to meet this legislative requirement, PMRA is harmonizing with US EPA approaches. As in the US, under the Chemicals Management Plan, Health Canada, Environment Canada and Climate Change Canada have also conducted several risk assessments on incidental mixtures using various approaches including moiety based approaches (metal-containing chemical groups), whole mixture based approaches and component-based approaches (phthalates). Under the Canadian Environmental Protection Act (CEPA 1999), Health Canada has developed guidance documents (Health Canada 2010a, 2010b) on human health risks posed by combined exposures from federal contaminated sites under the Federal Contaminated Sites Action Plan.

Overall, in the US and Canada there are legal mandates for cumulative risk assessment of intentional mixtures and for incidental mixtures occurring in a number of different scenarios. These mandates are addressed in specific approaches described in relevant guidance documents, which are implemented as part of the regulatory processes.

2.3. Regulatory needs

Current legislative frameworks within the EU, the US and Canada highlight the need to assess potential combined effects from exposure to multiple chemicals. However, substantial differences were identified in the legal requirements for risk assessment of combined exposure to multiple chemicals and its implementation between the countries and regulatory sectors (silos) approving different groups and intended uses of the chemicals reviewed. Whereas in the US and Canada, guidance and risk assessments of combined exposure to multiple pesticides have been advanced, in the EU suitable approaches for this are still under development. Although there are legal mandates regarding risk assessment of combined exposure to multiple chemicals, cumulative risk assessment has been considered in various ways across authorities and organizations. Thus, a bottleneck appears to be the lack of available and accepted scientific methods and harmonized approaches to provide guidelines on how to assess and evaluate combined adverse effects from exposure to multiple chemicals. This type of risk assessment conducted in different countries and under different regulatory frameworks should be based on similar principles in order to avoid significant discrepancies between regulatory sectors or countries. Thus, harmonized and clearly structured frameworks, which lead risk assessors through the process of assessing risks of combined exposures to multiple chemicals, are required. This would be facilitated by setting up a network involving European and Non-European authorities and

international organizations such as EFSA, Joint Research Center (JRC), OECD, WHO, and US EPA to generate synergies and combine efforts to develop harmonized approaches.

3. Existing frameworks for health risk assessment of chemical mixtures

As indicated in Section 2, most regulatory authorities and organizations require some assessment of human health risks associated with combined exposure to multiple chemicals, whereas only a few propose and use existing frameworks. Table 2 provides a structured overview and comparison of 14 frameworks identified for review, in terms of the scope, purpose and some general principles applied. These frameworks have been developed by different organizations under different jurisdictions and regulatory settings. Some are meant to be general approaches applicable for different purposes (e.g. EFSA 2018a focused on the food and feed safety areas, but can be broadened to other regulatory areas and across regulatory sectors), while others have been developed for assessment of chemical mixtures from a specific exposure source (e.g. ATSDR 2018) or belonging to a group of chemicals with a specific use and/or regulated under product-specific legislation (e.g. US EPA 2002b; EFSA 2008; Stein et al. 2014; ECHA 2015; US EPA 2016a). Although, the purpose, scope, considerations for problem formulation and principles applied vary to some extent between the existing frameworks, they also show many similarities. EFSA has previously conducted a review to summarize the terminology, methodologies and frameworks developed by national and international agencies and to provide recommendations for future activities at EFSA in this area (EFSA 2013a). Some additional frameworks have been presented by the German Federal Institute for Risk Assessment (Stein et al. 2014), ECHA (2015), the European Chemical Industry Council, CEFIC (Price et al. 2012) and the Health and Environmental Sciences Institute (HESI) (Moretto et al. 2017), as well as an updated approach from the US Agency for Toxic Substances and Disease Registry (ATSDR 2018).

EFSA evaluated available scientific principles and frameworks for the assessment of human health risks associated with combined exposure to multiple chemicals. Based on this, EFSA developed a flexible overarching framework for human, animal and ecological mixture risk assessment, which was recently published as a draft guidance document (EFSA 2018a). Therein, EFSA proposes harmonized risk assessment methodologies for combined exposure to multiple chemicals and describes tiered and stepwise approaches for all tiers of the risk assessment. Peculiarities related to genotoxicity assessment of chemical mixtures are addressed in a specific EFSA statement (EFSA 2018b).

The frameworks reviewed here commonly take the form of decision trees or tiered processes, or a combination of the two. In this context, decision trees provide stepwise guidance for how to proceed through the risk assessment process based on conclusions regarding, for example, the hazard or data availability in previous steps. The US EPA presents an example of such a decision tree (US EPA 2000, 2002b) and the Scientific Steering Committee of the Norwegian Scientific Committee for Food Safety proposes a step-by-step approach for the risk assessment of multiple chemical exposures (VKM 2008). In tiered frameworks, assessment is described as being conducted in phases, with conservative risk assessments based on deterministic worst case scenarios and default estimates of exposure and hazard at the lower (first) tiers and with each consecutive tier being more refined, i.e. less uncertain, than the previous one. The WHO/IPCS tiered framework for the risk assessment of combined exposure to multiple chemicals (Meek et al. 2011) is well recognized and has been applied and adapted by several other organizations for their purposes, for example by the scientific committees of the European Commission (SCHER/SCENIHR/SCCS 2012), CEFIC (Price et al. 2012) and ECHA (2015). The CEFIC framework further provides an example of a combination of a decision tree and tiered process.

Risk assessment of combined exposures to multiple chemicals via different routes of exposure may be based on exposure and hazard data of the whole mixture or, alternatively, on data for the individual components. With the exception of the early guidance from US EPA (2000), which states that a whole mixture approach is generally preferred, a component-based approach, where feasible, seems to be generally accepted as the most appropriate approach for a great number of mixture risk assessments. The US Agency for Toxic Substances and Disease Registry (ATSDR 2018) and EFSA (2018a) recognize that a whole mixture approach may be applied where feasible and depending on the problem formulation of the assessment. While a whole mixture approach may be useful, or indeed necessary, if data are available only on the specific mixture, it is only applicable when exposure is from a single source and for mixtures that do not change significantly in their composition (Price et al. 2012; SCHER/SCENIHR/SCCS 2012). Whole mixture assessments are limited to the endpoints that can be measured in toxicity studies of that mixture. A stepwise application of both approaches might be appropriate for specific assessments.

Structured, tiered frameworks, as well as basic methodologies, have been developed, proposed and thoroughly discussed by several organizations. However, often the lack of agreed and sufficiently specific and applicable technical guidance is the major obstacle for a consistent and adequate implementation of mixture risk assessment (Solecki et al. 2014).

3.1. Deterministic and probabilistic assessment

A common theme in tiered frameworks for assessing risks from combined exposure to multiple chemicals is to apply a deterministic approach to generate conservative estimates of risk at lower tiers, and consequently refine the assessment if needed at higher tiers. As the level of complexity in the assessment is increased, estimates of risk become more realistic but more laborious with higher data requirements. The EFSA (2008), WHO/IPCS (Meek et al. 2011), HESI (Moretto et al. 2017), and ATSDR (2018) frameworks, for example, provide descriptions of data needs and specific approaches at

Table 2. Overview of the scope and strategies of 14 different approaches to risk assessment of chemical mixtures.

Reference (organization)	Scope and purpose, strategy for grouping	Exposure assessment approach	Hazard assessment approach	Dose addition as default assumption?	Recommended RA strategy when assuming dose addition	Recommended RA strategy when assuming independent action	Recommended RA strategy when assuming interaction
US EPA (2000)	A consistent US EPA approach for assessing health risks from exposures to multiple chemicals Grouping based on: 1) Co-exposure 2) Toxicological similarity *Further guidance and examples provided in US EPA (2003, 2007)*	Refers to traditional exposure assessment methods	A whole mixture approach is preferred. Recommended guidance value/PoD: – RfD/RfC – TTD/TTC – BMD	Yes, when the dose for each individual component is at a level at which effects are not expected to occur, be observable, or be of concern	– HI – refined using RPF/TEF-based if possible	Response addition, add individual risks together	Interaction-Based HI/BINWOE-method PBPK models useful to investigate the potential for interactions
US EPA (2002)	A stepwise procedure for performing cumulative risk assessments of pesticides with a common mechanism of toxicity Grouping based on: – Toxicological similarity	Stepwise approach: Identify potential exposure pathways and routes for all compounds in the common mechanism group (CMG). Establish detailed exposure scenarios for all compounds in CMGs and determine the magnitude, frequency, and duration for all pertinent exposure pathway/route combinations. Should reflect "real-world exposure" with the aim to identify the main drivers of toxicity in the CAG.	Stepwise approach: – Characterize and select common endpoints for all compounds in the CMG. – Conduct dose-response analyses, determine relative potencies and points of departure for all compounds in CAGs. Recommended guidance value/PoD: – BMD – NOAEL if BMD is not possible – Refinement using PBPK modeling	Yes	– RPF-based (ideally based on PBPK-data)	Not within the scope of this approach	Refers to US EPA (2000)
VKM (2008)	A step-by-step approach intended to guide VKM panels in health risk assessment of multiple chemicals Grouping based on: Toxicological similarity	Not addressed specifically	Provides stepwise guidance for component-based approach Recommended guidance value/PoD: – ADI – NOAEL/LOAEL	Not clearly stated	– HI – PoD index – MoE/MoS – RPF/TEF-based	Not addressed	Not addressed

(*continued*)

Table 2. Continued.

Reference (organization)	Scope and purpose, strategy for grouping	Exposure assessment approach	Hazard assessment approach	Dose addition as default assumption?	Recommended RA strategy when assuming dose addition	Recommended RA strategy when assuming independent action	Recommended RA strategy when assuming interaction
EFSA (2008)	A tiered framework for assessing health risks from pesticide residues for the purpose of setting MRLs Grouping based on: – Toxicological similarity (CAGs) *Further guidance on exposure assessment and grouping provided in EFSA (2012, 2013b, 2013c).*	Tier 1: deterministic, MRL or field data, EFSA consumption model data Tier 2: deterministic, higher and mean residues from monitoring programs, EFSA consumption model data Tier 3: deterministic refined, using processing data Tier 4: probabilistic, distribution of monitoring data, distribution of national Food categorization system (FCS) data Tier 5: probabilistic, assess fraction of population instead of fraction of person-days	Recommended guidance value/PoD: Tier 1: deterministic, ADI or NOAEL/BMD Tier 2: deterministic or probabilistic, use RPF based on NOAEL or BMD Tier 3: RPF, further refinements using PBTK-BBDR	Yes	– HI – RfPI – RPF/TEF-based	Not within the scope of the report	Not within the scope of the report
IGHRC (2009)	A decision tree and tiered approach for addressing mixture issues when assessing health risks of chemicals at levels of exposure that may be encountered by people in their daily activities Grouping based on: 1) Co-exposure 2) Toxicological similarity	The approach taken will depend on the circumstances	A step-wise approach to component-based assessment. Recommended guidance value/PoD: no specific reference point promoted, refers to approaches set up by other agencies, e.g. US EPA and ATSDR	Yes	– HI (TTD-modified if possible) – RPF/TEF-based	Response addition (refers to US EPA)	HI-approach with extra uncertainty factor BINWOE-method PBPK models useful to investigate the potential for interactions
Meek et al. (2011) (also known as WHO/IPCS framework)	A tiered framework to aid risk assessors in identifying priorities for risk-management for a wide range of applications where co-exposures to multiple chemicals are expected. Grouping based on: 1) Co-exposure 2) Toxicological similarity or interactions	Tier 0: simple semiquantitative estimates of summed exposure for the various components Tier 1: "summation of deterministic estimates of exposure for all components of the assessment group based on measured or modeled data" Tier 2: refined deterministic assessment, more data and more realistic but still conservative estimates Tier 3: Probabilistic exposure estimates	Component-based Recommended guidance value/PoD: Tier 0: ADI, TDI, RfD, OEL (for the most toxic component, i.e. all components are assumed to have the same potency as the most toxic component) Tier 1: NOAEL, LOAEL, BMD/C Tier 2-3: RPF, PBPK modeling	Yes	– HI (using ref value for the most toxic component at Tier 0 and individual ref. values at higher tiers) – PoDI – RPF/TEF-based	Not addressed	Not addressed

(*continued*)

Table 2. Continued.

Reference (organization)	Scope and purpose, strategy for grouping	Exposure assessment approach	Hazard assessment approach	Dose addition as default assumption?	Recommended RA strategy when assuming dose addition	Recommended RA strategy when assuming independent action	Recommended RA strategy when assuming interaction
SCHER/ SCENIHR/ SCCS (2012)	A decision tree for the assessment of health and environmental risks from chemical mixtures Grouping based on: – Toxicological similarity	Supports the WHO/IPCS Tiered approach	Supports the WHO/IPCS tiered approach. Includes the use of TTC at the first step Promotes a component-based approach; whole mixture approaches are explicitly not recommended. Does not propose any specific guidance value/PoD PBPK modeling may be useful for a higher-tier assessment	Yes	– HI (based on reference value for the most toxic component at Tier 0 and individual reference values at higher tiers) – PoDi – RPF/TEF-based	Response addition (add probabilistic risks together) Effects addition (add biological responses together)	Case-by-case RA
Price et al. (2012) European Chemical Industry Council (CEFIC)	A decision tree and tiered approach to guide the assessment of health and environmental risks from chemical mixtures Grouping based on: Not addressed	Supports the WHO/IPCS tiered approach	States that a whole mixture approach may be applied when such data is available. Does not propose any specific guidance value/PoD but refers to the WHO/IPCS approach. Uses the concept of MCR (Price and Han 2011)	Yes	– HI/HQ	Not addressed	Not addressed
Stein et al. (2014) German Federal Institute for Risk Assessment (BfR)	A tiered approach taking cumulative aspects into account when assessing health risks from plant protection products and biocides under their respective EU regulations Grouping based on: – Toxicological similarity (EFSA CAGs)	Acute CRA: Use deterministic international estimated short-term intake (IESTI) Chronic CRA: Can be conducted in principle but resource demanding. Should then use supervised trails median residue (STMR) values for the product/mixture considered and background levels for all other pesticide residues in the same CAG, as well as simulations (e.g. Monte Carlo) to estimate exposure.	Promotes a component-based approach. Only provides guidance for acute risk assessment for pesticide residues as chronic risk assessment is very resource demanding and "not expected to reveal risks that have been overlooked in the past". Recommended guidance value/PoD: ARfD	Yes	– HI/HQ – RfPI – RPF-based	Not addressed	Case-by-case

(*continued*)

Table 2. Continued.

Reference (organization)	Scope and purpose, strategy for grouping	Exposure assessment approach	Hazard assessment approach	Dose addition as default assumption?	Recommended RA strategy when assuming dose addition	Recommended RA strategy when assuming independent action	Recommended RA strategy when assuming interaction
ECHA (2015)	A tiered approach as well as a decision tree, intended as guidance for assessing combined exposures under the Biocidal Products Regulation Grouping based on: – Toxicological similarity	Provides guidance for combined exposure to multiple substances – from one source/use – from different sources/uses – Internal exposure levels are used, thus exposure from different sources is taken into account. Refers to the WHO/IPCS Tiered approach	Component-based, tiered approach (same as Stein et al. 2014). The first tier is to verify risk acceptability for single components of the mixture/product. Refers to WHO/IPCS. Recommended guidance value/PoD: AEL or another European validated value, e.g. a DNEL. PBPK modeling may be useful at higher tiers.	Yes, but if independent action is shown to be the appropriate assumption it is already covered in Tier 1 (assessment made substance by substance)	– HI/HQ – PoDI – MoET – RPF/TEF-based	Response addition	Interaction-based HI
US EPA (2016a)	A tiered CRA screening framework for pesticides with increasing levels of refinement Grouping based on: – Toxicological similarity	Stepwise approach: The framework for CRA screening describes three options: Option 1: no common mechanism groups (CMGs), no CRA necessary; Option 2, "candidate CMG", screening for toxicology and exposure following a tiered approach; Option 3: CMG established, CRA in line with US EPA (2002a).	Stepwise approach: For Option 2, a MoA/AOP hypothesis is set and an index chemical with the most robust toxicological database for the common toxic effect *via* the exposure routes of interest, is selected. RPFs are estimated in relation to the MoA/AOP hypothesis and the common toxic effect for the group of pesticides. RPFs and PoDs do not have to be accurately assessed; variation in level of refinement e.g. may be derived from the PoDs from single chemical risk assessments, the NOAELs/LOAELs from specific toxicological studies or possibly BMDs. Refinement using PBPK modeling not required. For Option 3 the US EPA (2002a) CRA guidance is followed (see above).	Yes	– RPF-based	Not within the scope of this framework	Consistent with the principles of dose addition described in US EPA 2000 (see above)

(*continued*)

Table 2. Continued.

Reference (organization)	Scope and purpose, strategy for grouping	Exposure assessment approach	Hazard assessment approach	Dose addition as default assumption?	Recommended RA strategy when assuming dose addition	Recommended RA strategy when assuming independent action	Recommended RA strategy when assuming interaction
Moretto et al. (2017) Health and Environmental Sciences Institute (HESI)	A tiered approach for assessing potential adverse health effects from combined exposure to multiple chemical and non-chemical stressors. Includes consideration of co-exposure to modulating factors. Grouping based on: 1) Co-exposure 2) Toxicological similarity	Different approaches (i.e. deterministic or probabilistic), appropriate for different types of evidence and problem formulations, are briefly discussed.	Component-based. Different approaches (i.e. deterministic or probabilistic), appropriate for different types of evidence and problem formulations, are briefly discussed. Does not promote any specific guidance value/PoD. A single deterministic value, range or distribution can be used. Mixture components considered to not contribute significantly to the health risks of the mixture are identified for exclusion using the Risk21 matrix.	Yes	"Appropriate" method, e.g: – HI – CRI – RfPI – MoET – RPF/TEF-based Note: the RPF/TEF-based approach is directly applicable for the Risk21 matrix, while other values need to be adjusted since they already include assessment factors.	Not addressed	A starting assumption in this approach is that interactions are not likely to occur to a significant extent at doses/exposure levels of individual chemicals at or below their no-effect levels.
ATSDR (2018)	A 3-tiered approach intended to assist ATSDR environmental scientists and toxicologists in determining whether combined exposure to multiple chemicals and other stressors (e.g., noise, radiation) at sites of environmental contamination may impact public health. Grouping based on: 1) Co-exposure 2) Similar toxicity is considered in Tier 3 only.	Based on site-specific exposure information. Tier 1: identify chemicals and exposure pathways of concern. Tier 2: Preliminary analysis of exposure to multiple substances. Tier 3: Refined exposure assessment, e.g. by considering sub-populations, using probabilistic exposure data or models and/or including additional stressors.	Whole mixture or component-based method determined by problem formulation. Separate procedures for non-carcinogens and carcinogens. Tier 1: evaluation of single substances. Substances considered to be of concern are retained for evaluation in Tier 2. Tier 2: Preliminary analysis of multiple substances. Note: no grouping based on similar toxicity in Tier 2. Tier 3: Further refinement and grouping of compounds with similar toxicity. Recommended guidance value/PoD: Health based guidance values, e.g. ATSDR Minimal Risk Levels or US EPA RfD/RfC.	Yes, for non-cancer endpoints. For cancer, response addition (combined cancer risk estimates from carcinogenic agents of concern) is the recommended approach.	– HI	Response addition is applied for carcinogenic substances. For whole mixture-based approaches: combined cancer risk estimate based on the cancer slope factor for the mixture of concern or a sufficiently similar mixture. For component-based approach: the sum of the cancer risk estimate for the individual components.	BINWOE method. Possible greater-than additive or less-than-additive interactions are considered in Tier 3.

(*continued*)

Table 2. Continued.

Reference (organization)	Scope and purpose, strategy for grouping	Exposure assessment approach	Hazard assessment approach	Dose addition as default assumption?	Recommended RA strategy when assuming dose addition	Recommended RA strategy when assuming independent action	Recommended RA strategy when assuming interaction
EFSA (2018a)	A tiered framework for harmonized risk assessment methodologies for human health, animal health and ecological areas based on problem formulation, exposure assessment, hazard identification and characterization, and risk characterization including uncertainty analysis. Grouping based on: 1) Regulatory requirements (common regulatory domain) 2) Co-Exposure (common source or environmental media) 3) Similar toxicity (common: functional group(s), constituents or chemical classes, breakdown products, "critical" target organ(s), MoA or AOP as well as similar toxicokinetics)	The tiering principles are flexible, depend on purpose of the RA, data availability, time and resources and range from qualitative, semi-quantitative to fully probabilistic approaches. Whole mixture approach (WMA): – Step 1 - Characterization of the whole mixture – Step 2 - Assembling the chemical occurrence (concentration) data – Step 3 - Combining occurrence data and consumption data – Step 4 - Report exposure data Component-based approach (CBA): – Step 1 – Components of the assessment group – Step 2 – Assembling chemical occurrence data – Step 3 – Combine occurrence and consumption data – Step 4 – Report exposure data	WMA or CBA method determined by problem formulation. WMA: – Step 1: Hazard data collection – Step 2: Reference points – Step 3: Reference values – Step 4: Report CBA: – Step 1: Confirm chemicals and establish components of the assessment group – Step 2: Collect available hazard information – Step 3: Evidence for combined toxicity – Step 4: Hazard characterization – Step 5: Summarize hazard metrics	Yes, the use of dose (or concentration) addition as a default assumption	– HI – TTD – RfPI/PoD Index	Independent joint action with the associated assessment approach of response addition	Mixture interactions should be selected considering the nature (toxicokinetics, toxicodynamics or both) and the quality of the evidence available on such interactions (*in vitro*, *in vivo*, single dose or full dose response) As more hazard data become available, risk assessors have the option to refine the grouping of chemicals using weight of evidence approaches, dosimetry (toxicokinetic) or mechanistic data (MoA, AOP, etc.)

ADI: acceptable daily intake; AEL: adverse effect level; AOP: adverse outcome pathway; ArfD: acute reference dose; BINWOE: binary weight-of-evidence; BBDR: biologically-based dose-response; BMD: benchmark dose; CAG: common assessment group; CBA: component-based approach; CMG: common mechanism group; CRA: cumulative risk assessment; CRI: cumulative risk index (sum of the reciprocals of the HQs); DNEL: derived no-effect level; HI: hazard index (sum of the individual hazard quotients for all components of the mixture); HQ: hazard quotients (ratio of estimated exposure to the reference value, e.g. an ADI or TDI, for a single chemical); LOAEL: lowest observed adverse effect level; MCR: maximum cumulative ratio; MoA: mode of action; MoE: margin of exposure (ratio of the reference point and exposure); MoET: combined margin of exposure (reciprocal of the sum of the reciprocals of the individual MOEs); MoS: margin of safety; MRL: maximum residue levels; NOAEL: no observed adverse effect level; OEL: occupational exposure limit; PBPK: physiologically-based pharmacokinetic; PoD: point of departure; PoDI: Point of departure index (sum of exposures divided by the point of departure for each of the individual components of an assessment group); RA: risk asssessment; RfC: reference concentration; RfD: reference dose; RfPI: reference point index (sum of the ratios of exposure to each compound expressed as a fraction of its respective reference point for the relevant effect (e.g., the dose that causes a 10% effect, BMD10; or the NOAEL); RPF: relative potency factors; RPF/TEF: relative potency factors/toxicity equivalence factors (identification of an index compound (e.g. 2,3,7,8-TCDD for dioxins) and normalization of all chemicals of the group to the potency of the index compound); TDI: tolerable daily intake; TTC: target organ toxicity concentration; TTD: target organ toxicity dose.

different tiers. For exposure, low tier assessment may be based e.g. on information on sale or use patterns or on MRLs in the case of pesticides to provide worst case conservative exposure estimates for the mixture. At higher tiers, deterministic exposure estimates may be derived by using point estimates of high and mean values from measured data, e.g. from monitoring programs, and modeling data for food consumption. Deterministic exposure assessments may be further refined, e.g. for different groups of the population or by using physiologically based pharmacokinetic (PBPK) modeling to estimate internal exposure levels. The assessment of a realistic exposure of humans and the environment to certain chemicals is a major challenge as different exposure routes have to be considered and the total exposure has to be assessed. However, extensive monitoring data for an exposure are rarely available.

At the highest tiers, probabilistic exposure assessment may be conducted. In this approach, exposure to chemical mixtures in food is based on distributions of the concentrations of mixture components in different food items, as well as on distributions of the consumption of these foods in the population or in specific population groups. This allows conclusions to be drawn about, for example, the distribution of exposure levels in the population to different mixture components and the proportion of the population that may be at risk of exceeding specific exposure levels. Practical examples of integrated probabilistic cumulative risk assessments for exposures to organophosphorus and anti-androgenic pesticides as well as organophosphorus and carbamate insecticides in the Dutch population are shown in Boon et al. (2008), Bosgra et al. (2009), and Müller et al. (2009). Basic probabilistic methodology for modeling dietary exposure to pesticide residues has been described by the EFSA (2012). Van der Voet et al. (2015) proposed the implementation of the more advanced software system MCRA in the EFSA guidance and gave example calculations on the triazole group. The potential of this model is illustrated in case studies for different population groups in country-specific scenarios (Kennedy et al. 2015). Recently, probabilistic modeling is gaining recognition as an approach to exposure assessment, which is reflected in Europe by discussions between the European Commission and EFSA on the implementation of two probabilistic tiers for assessing risks from combined exposure to multiple chemicals. The degree of refinement of the estimates used at the start of the assessment (i.e. which tier) is determined by a number of factors, such as data availability, resources required, urgency of the assessment, and hence should be part of problem formulation.

Analogously, hazard assessment at the lowest tier may be based on the worst-case assumption that all components in the mixture or assessment group have the same potency for inducing adverse health effects as the most toxic component in a dose additive manner. The lowest health-based guidance value, such as an acceptable daily intake (ADI), even if not based on the common effect, identified among the components can be used as a conservative basis for risk estimations at the lowest tier. Deterministic hazard assessment may be refined at higher tiers by using individual points of departure for the common adverse effect (No Observed Adverse Effect Levels (NOAELs) or Benchmark Doses (BMDs)) for each of the components and relative potency factors (RPF) for this effect. At the highest tier(s), assessment may be further refined by use of dose-response data/models and PBPK modeling to provide exposure estimates and thus probabilistic estimates of hazard. Increasingly detailed information on the mechanisms underlying toxicity (mode of action (MoA), adverse outcome pathway (AOP)), if available, may be used to refine the grouping of chemicals for which a risk assessment for combined exposure to multiple chemicals is conducted.

The use of non-animal data, e.g. *in vitro* and *in silico* data, in risk assessment of chemicals is becoming increasingly more important with new advancements in such technologies and a general movement towards replacing, reducing and refining animal studies in toxicity testing. The use of non-animal data in the context of risk assessment of chemical mixtures, e.g. for strategies for refined grouping or distinguishing between similar and dissimilar MoAs, remains to be further explored.

3.2. Principles for grouping

Prioritisation and grouping have been specifically discussed by EFSA (2013b) and US EPA (2002b, 2016a) and are generally addressed in several of the frameworks reviewed here. There are two main factors influencing grouping: one is exposure-based, i.e. the likelihood that the components may co-occur, whereas the other is toxicity-based and relates to similarity in toxicity, or the potential for interactions, between components. To some extent, the choice of approach will be determined by the regulatory requirement and problem formulation of the assessment.

In early guidance from US EPA (2000), as well as in the frameworks presented by the UK Interdepartmental Group on Health Risks from Chemicals (IGHRC) (2009) and the WHO/IPCS (Meek et al. 2011), on the "silo-less" assessment of chemical mixtures, grouping was based first on co-exposure, then on toxicological similarity. The ATSDR (2018) primarily considers co-exposure, since this approach has been specifically developed for assessment of exposure at sites of environmental contamination. Consideration of similar toxicity is included in the highest tier (Tier 3) in the ATSDR framework.

In general, in low tier assessments, estimations of co-exposure could be based on information of use patterns. For example, in the case of pesticides, if substances are expected to be used on the same crops at the same time, or their residues are present in different commodities expected to be consumed during the same time period. At higher tiers, real data on consumption and pesticide residues from monitoring data are used. Estimations of co-exposure may be refined for different sub-groups and by the use of PBPK-modeling and/or probabilistic approaches. In guidance from other organizations, including EFSA and ECHA, that covers groups of chemicals for specific uses such as pesticides, co-exposure is not specifically discussed as a factor for grouping in the hazard assessment. In those cases, grouping is primarily based on similar target organs and/or mode of action. Well-known examples are EFSA's cumulative assessment groups (CAGs)

(EFSA 2013b). However, it should be noted that formation of a CAG is not in itself a final risk assessment and it is not yet clear whether and to what extent EFSA will take exposure into account for this purpose.

EFSA's approach for grouping pesticides into CAGs was based on identifying pesticides that affect the same organ or physiological system and exhibit similar toxicological properties (adverse outcome) in that organ or system (EFSA 2013b). The methodology consists of four stages:

1. Identification of specific and unambiguous toxic effects that adversely affect an organ or system - known as hazard identification (e.g. effects on the thyroid system);
2. Hazard characterization that describes a specific adverse effect to this organ or system (e.g. changes in hormone levels reflecting effects on thyroid follicular cells);
3. Data collection – gathering data on the indicators (e.g. changes in hormone levels at the dose where the adverse effect occurs) that point to a specific toxic effect (e.g. imbalance of the thyroid system) in an organ/system;
4. Grouping of pesticides that exhibit a similar toxicological effect into CAGs by organ/system and effect (e.g. thyroid follicular cell toxicity).

To date, EFSA's Panel on Plant Protection Products and their Residues (PPR) have applied this methodology to define groups of pesticides which are toxic to the thyroid and central nervous systems. Currently, EFSA is conducting pilot assessments of risks caused by multiple pesticides to consumers, which are expected to be finalized by the end of 2018. A Monte Carlo Risk Assessment (MCRA) tool, developed in close cooperation between EFSA and the Dutch National Institute for Public Health and Environment (RIVM), is used to examine combined effects on human nervous and thyroid systems caused by exposure to pesticides in food (EFSA 2018c).

The new EFSA guidance document (EFSA 2018a) describes a flexible approach to the grouping of chemicals, depending on problem formulation, based on

- Common regulatory domain
- Common source, environmental media
- Common functional group(s)
- Common constituents or chemical classes
- Common breakdown products
- Common "critical" target organ(s)
- Common MoA or AOP, similar toxicokinetics.

As more hazard data become available, risk assessors have the option to refine the grouping of chemicals using weight of evidence approaches, dosimetry (toxicokinetics) or mechanistic data such as MoA, AOP etc (EFSA 2018a).

US EPA considers also exposure data to group chemicals into common mechanism groups (CMGs), on the basis that not all chemicals, pathways of exposure (e.g. residential, food, drinking water), or uses are risk contributors requiring risk mitigation measures.

The methodology applied most often for specific pesticide chemical groups (US EPA 2002b), involves:

1. Identification of CMGs, i.e. substances that cause a common toxic effect via similar sequence of major biochemical events following the initial chemical interaction, essentially the same MoA (US EPA 1999);
2. Performance of aggregate risk assessment for each chemical in the CMG based on exposure data (US EPA 2001);
3. Consideration of exposure data for refinement of the CMGs to common assessment groups (US EPA 2002b).

US EPA has grouped a number of pesticides for the assessment of risks from combined exposure. Potential membership of a CMG is based on similarities in chemical structure and pesticidal mode of action. To date, CMGs have been created for: organophosphates, N-methyl carbamates, chloracetanilides, triazines, naturally occurring pyrethrins and synthetic pyrethroids. US EPA also examined thiocarbamates and dithiocarbamates as potential CMGs, but determined the available evidence did not support inclusion in a common mechanism group.

The US EPA developed a risk-based screening approach for pesticides to supplement its existing guidance for establishing CMGs and conducting cumulative risk assessments (US EPA 2016a). In case a common mechanism is not identified (Option 1), cumulative risk assessment is not warranted (US EPA 2016a). Where there is limited evidence of common toxicological profile a candidate CMG is identified (Option 2), screening level toxicology and exposure analysis is applied, where further data might be required on a case-by-case basis (US EPA 2016a). An example of such a candidate CMG is the avermectins (US EPA 2015, 2016b). A CMG can be established, provided that sufficient evidence for a common toxicological profile (based on detailed knowledge on toxicity and exposure) is available (Option 3). Then problem formulations determine the degree of scope and refinement needed.

It is important to note that assumptions used for grouping contribute to the uncertainties in the assessment of chemical mixtures, which have to be carefully considered and handled. For example, uncertainties associated with:

1. Identification of relevant mixture components and the assumptions made regarding whether MoA is similar or dissimilar. In this regard, information on secondary effects may often be especially inadequate;
2. Omissions from assessment groups due to lack of exposure data (US EPA 2007) or lack of information about chemicals, such as pesticides, present on the worldwide market (EFSA 2013b);
3. The level of accuracy with which (co-)exposure to mixtures has been characterized, keeping in mind that exposure estimates may be based on several sources/uses and a number of events at several locations over broad and varied time periods.

The fundamental problem for grouping is that it is not possible to identify and assess all potential chemical

mixtures. This makes the prioritization of chemicals for mixture risk assessment, and the organization into relevant assessment groups, central to the development and refinement of methodologies in this area. The potential for exposure and similar effects/MoA are key factors that should drive prioritization, as well as grouping. However, there is a strong need for development of approaches to further refine groupings, such as the EFSA CAGs, into narrower/smaller groups.

3.3. Dose addition as default assumption

There is general agreement between current frameworks that dose addition should be used as the default assumption for assessing risks from combined exposure to chemical components with the same adverse outcome. Strictly speaking, components should have a similar MoA for dose addition to be applicable but, since mechanistic data commonly is limited, evidence of common target organ and effect is often considered sufficient (US EPA 2000). It has also been shown that dose addition may be appropriate in some cases where mixture components act via different MoAs (discussed below). Dose addition entails estimating the effect and cumulative risks of the mixture from the sum of the doses/concentrations, scaled for the relative potency of the mixture components. The most common approach for applying dose addition is use of the hazard index (HI) methodology. Refinements, using outcome- and exposure route specific RPFs as well as PBPK-modeling are also discussed in many of the current frameworks.

It is generally argued that if there is sufficient evidence to show that components act independently, i.e. lead to the same adverse outcome via completely dissimilar MoAs, response or effect addition (concept of independent action) is more appropriate than dose addition (SCHER/SCENIHR/SCCS 2012). The EFSA PPR panel assessed the relevance of dissimilar MoAs for risk assessment of combined exposure to substances that produce a common adverse effect on the same organ/system (EFSA 2013c). The panel concluded that there was no case documented in the scientific literature where independent action provided predictions that were more conservative than dose addition for the effects of a mixture of chemicals producing the same adverse outcome and hence dose addition would be an appropriately conservative default assumption. This approach is supported by conclusions from other organizations (US EPA 2002b; EFSA 2008; IGHRC 2009; Meek et al. 2011; SCHER/SCENIHR/SCCS 2012; ATSDR 2018). EFSA's PPR panel therefore recommended also using dose addition for risk assessments of combined exposure to mixtures of pesticides with dissimilar MoAs, provided they produce a common adverse outcome. EFSA recognized that this is a conservative assumption and that refinements may be possible if future research shows that it can be justified. That means that clear criteria for the deviation from the assumption of similar MoA and application of the principle of independent action and effect/response addition are needed. However, such criteria for determining when MoAs of different chemicals are sufficiently dissimilar to indicate independent action are currently lacking.

A third scenario is that interactions between mixture components cause synergistic or antagonistic effects. Some of the available guidance discusses methods for risk assessment of mixtures with interactions between components (US EPA 2000; VKM 2008; IGHRC 2009; ECHA 2015; ATSDR 2018; EFSA 2018a). These are often based on modifications of the HI methodology by weight-of-evidence evaluation of binary interaction data (BINWOE method) or by applying an extra uncertainty factor for possible interactions. The BINWOE method categorizes, based on qualitative analyses on empirical observations and mechanistic considerations, the most plausible causes of potential influences of one compound to the toxicity of another in a mixture, for a given exposure scenario (Mumtaz and Durkin 1992).

PBPK models may also be useful to examine alterations in kinetic processes, which may be a mechanism for toxicological interactions between mixture components. However, several authorities and organizations have concluded that there is currently little empirical evidence of interactions occurring between mixture components at (low) dose levels relevant for dietary exposure scenarios in the general population, particularly for regulated chemicals, which are managed so that individual exposure is below the respective health based guidance value (EFSA 2008, 2013c, 2015; SCHER/SCENIHR/SCCS 2012; Moretto et al. 2017). Focus has therefore been on approaches and methods assuming non-interaction, i.e. dose addition or response/effect addition.

4. EuroMix contribution to international risk assessment and testing of mixtures

4.1. Aim of the EuroMix project

EuroMix aims to develop a pragmatic approach for risk assessment of combined exposure to multiple chemicals derived from multiple sources. The approach developed will be assessed in proof-of-principle studies in which the use of *in silico* tools and *in vitro* methods for this purpose will be verified *in vivo* for three adverse outcomes (i.e. liver steatosis, adverse effects on reproduction due to endocrine disruption, and skeletal malformation/cleft palate), as examples. The EuroMix project will deliver an innovative platform of tools and test methods for mixture testing and refined grouping of chemicals into CAGs, for both data rich and data poor chemicals. Hazard and exposure models will be embedded in a model toolbox, made available to stakeholders through an openly accessible web-based platform. Criteria will be set and guidance will be produced on how to use and implement the tiered testing and assessment strategy. Dissemination and harmonization of the approach within EU and more broadly within the international community, by involving, among others, WHO, Codex Alimentarius, OECD and US EPA in the project and by the participation of experts from such organizations will play a key role in helping establish international food safety policies.

4.2. EuroMix contributions to harmonization

During the course of the EuroMix project a series of international harmonization workshops, with participants from several continents and authorities (e.g. Australian Pesticides and Veterinary Medicines Authority (APVMA), Codex Alimentarius, EFSA, Food and Agriculture Organization (FAO), JRC, OECD, US EPA, US FDA, WHO, RIVM, German Federal Institute for Risk Assessment (BfR)) are being organized. In the first two workshops already held, the extent to which risk assessment of combined exposure to multiple chemicals derived from multiple sources is harmonized internationally and across regulatory sectors and the remaining hurdles that need to be overcome to achieve further harmonization were identified (EuroMix 2017). The first workshop was held in London in October 2016. Experts in risk assessment from different regulatory sectors and different regions reviewed approaches being used to assess risks from combined exposure to multiple chemicals. Whilst there were broad similarities in the approaches used, a number of key issues were identified where there is clearly a lack of harmonization. These include transparency of problem formulation, the scope of cumulative risk assessments (which regulatory sector(s) should be covered), the basis for grouping chemicals into assessment groups and how information on MoA/AOPs should be taken into account in such assessments. The second workshop was held in Brussels, May 2017. Experts in risk assessment met with a range of risk managers primarily from Europe and North America, as well as international organizations, to discuss regulatory needs in assessing the risks from combined exposure to multiple chemicals. It was apparent that there is currently no overarching approach to such assessments, either within the EU (across regulatory sectors) or internationally. Approaches to assessing the risks from combined exposure to multiple chemicals vary across regulatory sectors and geographies, sometimes markedly. In some sectors, assessing the risks from combined exposure to multiple chemicals is currently not a significant consideration, whereas in others there is appreciable concern. However, even in the latter case, approaches utilized in different regions show substantial differences. The most common approach to date for developing cumulative assessment groups is use of common structure and/or co-occurrence and/or designed function (e.g. pesticidal mode of action). Work is underway both within and beyond the EU to explore harmonization of approaches to assessing the risks from combined exposure to multiple chemicals within and across regulatory sectors. Future workshops will consider how greater harmonization can be achieved, using in part the results of the EuroMix project. Whilst it is likely that the approaches used will vary, depending on problem formulation, it should be possible to harmonize the scientific principles used and to establish guidance to ensure greater transparency in reporting the approaches adopted.

4.3. EuroMix contributions to cumulative risk assessment

EuroMix aims for a systematic approach to risk assessment of combined exposure to multiple chemicals by identifying the appropriate tools for the various tiers. Referring to the issues of the frameworks discussed in section 3, the outcome of the EuroMix project will facilitate:

1. Comparison of deterministic (conservative) and probabilistic elements in tiered approaches to exposure, hazard and risk assessment. These tiered approaches will be made available as part of the web-based data and model platform MCRA, that will also be used to organize consumption data, residue data and hazard data.
2. Development of a refinement of the current approach for grouping pesticides into CAGs enabling better use of MoA/AOP information organized in AOP networks. This will include methods for an assessment of the likelihood of independent action as relevant for the studied adverse outcomes, and the likelihood of synergistic or antagonistic effects.
3. Reconciliation of potential international differences in the use of inclusion and exclusion approaches to the creation of CAGs. Methods for assessing the uncertainties associated with use of an exclusion approach and an inclusion approach will be developed and means of addressing these proposed, for example by using uncertainty factors.
4. Refinement of methods for assessing combined exposure to chemicals. In considering co-exposure, exposure to different chemicals may occur simultaneously in time and source (e.g. pre-formed mixtures), separated by time (e.g. foods on different days), separated by source (different foods or oral vs. dermal exposure) or separated by both. This will require consideration not only of toxicokinetics but also of the persistence and reversibility of the toxicodynamic response.
5. An overarching approach to risk assessment of combined exposure to multiple chemicals via different routes of exposure. EFSA is developing a guidance document for human and ecological risk assessment of combined exposure to multiple chemicals using existing frameworks as a starting point and tiered approaches for each step (problem formulation, hazard identification, hazard characterization, exposure assessment, risk characterization) (EFSA 2018a). EuroMix will determine how its research outputs can be best integrated into this framework.

5. Conclusions

5.1. Implementation of risk assessment of combined exposure to multiple chemicals

Whilst assessment of the risk from combined exposure to intentional mixtures is mandated by different legislation in several regulatory sectors in Europe, risk assessment of unintentional mixtures is much less widely required and guidance has yet to be finalized on how this should be performed. However, the risk to human health is a consequence of the totality of exposure and it has been recognized that this will require cross-sectorial assessments (Evans et al. 2016).

Even where there is requirement that unintentional mixtures should be assessed, such as for pesticide residues, there are major regional differences in how this is undertaken (e.g. US EPA compared to EFSA). This may, for example, result in different acceptability of standards for pesticide residues in food commodities (e.g. MRLs), with attendant implications related to different levels of consumer protection, risk communication, and international trade.

From a scientific perspective, the methodology for the establishment of CAGs should have the flexibility to include all relevant chemicals to which humans are co-exposed, regardless of regulatory sector and geographical region. However, the introduction of a comprehensive and systematic approach to risk assessment of combined exposure to multiple chemicals will be complex and has many practical and regulatory implications. Hence, a step-wise approach would be more pragmatic and feasible. There is agreement that detailed information on MoAs/AOPs should be utilized in creation and/or refinement of CAGs. However, clarity is needed on how such information will be obtained and utilized. The default assumption for members of a CAG is dose addition.

While the current scientific approaches summarized here represent a broad application of knowledge and integrated methods to address the challenges of risk assessment of combined exposure to multiple chemicals, additional guidance and harmonized method development will increase transparency and structure, as well as improve confidence in risk assessment conclusions. A review of existing legislation and discussions with risk assessors and risk managers has clearly established that no single approach will be applicable in the short to medium term. The approach adopted is as much a policy decision as a scientific one, and should be clearly reflected in problem formulation. Hence, EuroMix is developing an integrated testing and assessment strategy using a range of *in vitro* and *in silico* tests, appropriate to the tier of the assessment, together with more appropriate data on potential cumulative effects which can be combined in a scientifically-based weight of evidence approach to account for complexity, address uncertainty and improve risk assessment.

5.2. Further research and development needs

The current review highlighted the following needs in research and development:

- Development of structured and flexible approaches and harmonized guidance for grouping chemicals into refined and relevant assessment groups;
- Establishing criteria for determining when substances have dissimilar MoAs/AOPs and deviate from the default assumption of dose addition;
- Development of guidance on how to apply an integrated testing and assessment strategy using alternative methodology and non-animal data, e.g. for strategies for refined grouping and for determining RPFs;
- Development of structured approaches for collecting data from exposure to multiple substances, either simultaneously or in sequence, for use in risk assessment of combined exposure to multiple chemicals;
- Development of guidance for analyzing uncertainties associated with risk assessment of combined exposure to multiple chemicals, e.g. uncertainties associated with the grouping of chemicals into assessment groups and assumptions concerning similar MoAs/AOPs and application of dose addition.

In addition, practical guidance for problem formulation in cumulative risk assessment is needed (Solomon et al. 2016). The problem formulation step is critical for structuring the risk assessment by specifying the purpose and scope of the assessment, including e.g. prioritization of chemicals, exposure pathways and endpoints, as well as the approach to be taken, analytical needs, available resources and the level of complexity of the assessment.

The OECD and EFSA have identified the development of harmonized methodologies for combined exposure to multiple chemicals as a key priority area and initiated a number of activities. EFSA is currently working on a guidance document addressing a harmonized framework for risk assessment of combined exposure to multiple chemicals, which is based on the stepwise approaches for problem formulation, exposure assessment, hazard assessment, risk characterization and uncertainty analysis combined with the principle of tiering (EFSA 2018a). Recently, the representatives of several EC funded research projects (EDC-MixRisk, EuroMix, EU-ToxRisk, HBM4EU, SOLUTIONS), have sent a position paper to the EU Commission, proposing 12 key actions and recommendations to help better address combined effects and overcome remaining gaps in chemical mixture research (Altenburger et al. 2018). Contributing to the further development of internationally harmonized approaches for risk assessment of combined exposures to multiple chemicals is also the overall goal of the Horizon 2020 project "EuroMix".

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Declaration of interest

This research is sponsored by European Commission within the EuroMix project. We have disclosed those interests fully to the European Commission and have in place an approved plan for managing any potential conflicts arising from this arrangement. ARB was employed by Imperial College London, where he was involved in academic research and teaching in the fields of biomedicine and toxicology until he retired in June 2017. He is currently employed 4 h per week by the College to work on the EU Horizon 2020 project (EuroMix). He is or has collaborated on a number of activities on the risk assessment of combined exposures to multiple chemicals through the European Commission, FAO/WHO JECFA, FAO/WHO JMPR, WHO-IPCS, EFSA, ILSI HESI, ILSI Europe, and United Kingdom COT and COMEAP. None of these collaborative activities was remunerated and no research funding was received. He consulted for Coca-Cola and for Red Bull on the safety of certain food additives, from 2012 to 2014. This work did not involve risk assessment of

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Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the official views, decisions or policies of the author institutions.

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