Regulatory Toxicology and Pharmacology 80 (2016) 321–334

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Commentary

Regulatory assessment of chemical mixtures: Requirements, current approaches and future perspectives

Aude Kienzler, Stephanie K. Bopp*, Sander van der Linden, Elisabet Berggren, Andrew Worth

European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Systems Toxicology Unit and EURL ECVAM, Via Enrico Fermi 2749, 21027, Ispra, VA, Italy

ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form
13 May 2016
Accepted 16 May 2016
Available online 20 May 2016

Keywords:
Mixture
Combined effect
Risk assessment
Regulation

ABSTRACT

This paper reviews regulatory requirements and recent case studies to illustrate how the risk assessment (RA) of chemical mixtures is conducted, considering both the effects on human health and on the environment. A broad range of chemicals, regulations and RA methodologies are covered, in order to identify mixtures of concern, gaps in the regulatory framework, data needs, and further work to be carried out. Also the current and potential future use of novel tools (Adverse Outcome Pathways, *in silico* tools, toxicokinetic modelling, etc.) in the RA of combined effects were reviewed.

The assumptions made in the RA, predictive model specifications and the choice of toxic reference values can greatly influence the assessment outcome, and should therefore be specifically justified. Novel tools could support mixture RA mainly by providing a better understanding of the underlying mechanisms of combined effects. Nevertheless, their use is currently limited because of a lack of guidance, data, and expertise. More guidance is needed to facilitate their application. As far as the authors are aware, no prospective RA concerning chemicals related to various regulatory sectors has been performed to date, even though numerous chemicals are registered under several regulatory frameworks.

1. Introduction

The number of chemicals and combinations thereof to which humans and the environment are continuously exposed is potentially enormous, ever changing in concentration and identity and to a large extent unknown. This makes it neither realistic nor useful to test every possible combination. However, current human risk assessment (HRA) and environmental risk assessment (ERA) of chemicals mainly focuses on exposure to individual chemicals, mostly considering only a single source.

In 2012, the European Commission published a communication on the combined effects of chemicals (EC, 2012), expressing concerns about the current limitations of assessing compounds individually and proposing a path forward to ensure that risks associated with chemical mixtures are properly understood and assessed. It states that EU laws set strict limits for the amounts of particular chemicals allowed in food, water, air and manufactured products, but that the potential risks of these chemicals in combination are rarely examined.

The hazard and/or risk assessment (RA) requirements for (components of) products on the European market are laid down in specific EU legislations primarily depending on the intended use of the product. These products, e.g. biocides, pesticides, food or feed additives, pharmaceuticals, can consist of an individual compound or of mixtures of several compounds. As the composition of these products is generally known, and the relevant compounds are relatively well assessed individually, the RA is performed prospectively, based on the properties of the individual constituents. Where appropriate, tests can also be carried out on the formulated products. However, when several formulated products are used in combination, i.e. for the application of plant protection products (PPPs) in the field or for the use of personal care products at home, the combined resulting risk is generally not assessed. Similarly, the prospective RA often considers only one route of exposure, e.g. linked to occupational exposure to pesticide, and does not consider potential additional sources of exposure such as the intake via food consumption.

In addition to the regulations that cover the intentional mixtures that are present in specific products, several others focus on

* Corresponding author.
E-mail address: Stephanie.BOPP@ec.europa.eu (S.K. Bopp).

http://dx.doi.org/10.1016/j.yrtph.2016.05.020

Abbreviations

ADI	acceptable daily intake
AOP	adverse outcome pathway
ARfD	acute reference dose
AS	active substance
BPR	biocidal product regulation
CA	concentration addition
DEB	dynamic energy budget modelling
EQS	environmental quality standard
ERA	environmental risk assessment
ILSI-HESI	Health and Environmental Sciences Institute
HI	hazard index
HRA	human risk assessment
IA	independent action
IPCS	International Program on Chemical Safety
MCR	maximum cumulative ratio
MCS	multi constituent substances
MoA	mode of action
MRLs	maximum residue levels
PBPK	physiologically based pharmacokinetic modelling
PPP	plant protection products
PPPR	plant protection product regulation
QSAR	quantitative structure activity relationship
TEFs	toxic equivalency factor
TTC	threshold of toxicological concern
UVCBs	substances of unknown or variable composition, complex reaction products or biological materials
WFD	water framework directive

the exposure to unintentional mixtures. These can be mixtures that are unintentionally formed during the production process or mixtures that are found in the environmental matrix after being emitted (also defined as coincidental mixture, Table 1). Examples of regulations that address these types of mixtures are the Water Framework Directive (WFD), Marine Water Strategy, or Air and Soil related regulation.

Because of their varying composition in space and time, due to both the environmental fate of chemicals and the constant entry of new pollutants, exposure to coincidental mixtures in the environment is never assessed prospectively, and retrospective RAs are scarce, even if this is the most common situation. In special cases, if more information on use would be available, some unintentional mixtures might be assessed prospectively, e.g. PPPs tank mixtures (mixtures of individually assessed formulation that are mixed by the user), or mixtures of chemicals found in the environment after being emitted at the same place and the same time or sequentially (e.g. production plants of specific substances). These cases are currently not covered under the legislation. However several different guidance documents on how to deal with mixtures have been published recently, each focusing on a specific group of compounds or type of assessment. Examples are the guidance on aquatic RA under REACH (Bunke et al., 2013), the assessment of mixture effects of biocides (ECHA, 2014), and for pesticides, how to assess exposure scenarios for RA both using MRL or actual exposures based on monitoring data (EFSA, 2012).

Although methodologies for assessing the combination effects of chemicals are being developed and applied by scientists and regulators in specific circumstances, so far there is no systematic, consistent, comprehensive and integrated approach across different pieces of legislation. As a step forward, a widely accepted framework for the RA of combined exposure to multiple chemicals was developed in a WHO/IPCS workshop (Meek et al., 2011). This framework describes a general approach for RA of combined exposure to multiple chemicals that could be adapted to the needs of specific users. However, its use is often hampered by large data gaps on exposure as well as hazard information.

This review presents an overview of the current regulatory requirements for chemical mixture assessment, with emphasis on the extent to which they address the assessment of intentional and unintentional mixtures. Recent case studies, specifically focusing on mixture RA beyond the current regulatory requirements are summarized to illustrate what lessons can be learned in terms of methodology being used and existing regulatory and data gaps. The lessons learned from the case studies are supplemented by the results from an expert survey. This survey, which was carried out to explore the current use of different approaches for assessing human and environmental health risks from combined effects and the added value of several novel tools that could provide some of the missing information currently hampering the toxicological assessment of mixtures.

2. Mixture terminology and assessment concepts

2.1. Mixture assessment terminology

While the term mixture might seem a clear term at first sight, many similar - but different terms - are used in parallel, to indicate

Table 1
Types of mixtures, characterisation and related regulation.

Type of mixture	Definition	Characterisation	Assessment	Example of related regulation
Intentional	Formulated products marketed as such	Usually of known or well-known composition	Usually prospective based on the properties of the constituents supplemented, where appropriate, by tests carried out on the entire products	Plant Protection Products, Biocides, Pharmaceuticals, Food additives …
Unintentional	Usually from one source; generated by discharge during production, transport, use or disposal of goods	The composition can either be known (effluent) or unknown	If composition unknown, whole-mixture approach.	Water Framework Directive or waste-related regulation.
Coincidental	Originating from various sources	Composition unknown, varying in space and time	Usually not required	a) water/soil/air-related regulation; b) exposure of workers in the workplace, for which a risk assessment is required for all hazardous chemicals, including in combination; c) exposure of humans to multiple chemicals from food and drinking water.

the different types of mixtures and pathways of exposure. Therefore, to avoid future confusion, there is a clear need to define a consistent terminology to identify the different types of mixture scenarios. In this work, we adopted the terminology developed in the context of the WHO, OECD, IPCS, ILSI/HESI initiatives (Meek et al., 2011; OECD, 2011; WHO IPCS, 2009). This means that an exposure to multiple chemicals is defined as a combined exposure, should it be by a single route or by multiple routes (which is sometimes referenced as "cumulative" exposure). Exposure to a single chemical from multiple sources and by multiple pathways and routes is defined as an aggregate exposure (Fig. 1).

In addition, since the terminology sometimes differs between HRA and ERA, we define the following regarding the routes, pathways and sources of exposure. The route of exposure refers to the way a chemical enters the organism, i.e. via dermal exposure, oral exposure or inhalation. The pathway of exposure refers to the medium with which the chemicals are taken up, e.g. with drinking water, air, food. The sources of exposure are the places of release of chemicals such as industrial emissions, waste water treatment plant effluents, etc.

2.2. Mixture assessment models

Two main mathematical models exist to assess the combined toxicological effect of chemicals, either assuming that individual compounds act via a dissimilar mode of action (independent action, IA, or Response Addition, assuming the addition of the response) or by the same mode of action (dose or concentration addition, CA). In CA based models, the total response corresponds to the sum of all the individual concentrations multiplied with their respective potencies. CA models are the most frequently applied, because they generally provide reliable estimates of combined effects, they can more easily be used with existing toxicity data and are considered to be slightly more conservative than IA models. However, the results obtained by both models are usually very similar and the difference between the predictions rarely exceed a factor of five (Backhaus and Faust, 2012; Backhaus et al., 2004; Kortenkamp et al., 2009). Some models are also capable of incorporating interactions (e.g. antagonism or synergism) to some extent. A more extensive overview of mixture assessment models is given in Kienzler et al. (2014), and the most used (i.e Quotient Ratio, Hazard Index, etc …) are presented in Table S1.

Using these models, the overall toxicity of a mixture of known composition can to some extent be determined prospectively. For environmental mixtures, the (eco)toxicity is usually investigated by one of the two following approaches: testing the mixture as a whole (using a simple *in vivo* or *in vitro* test system) or using the (eco)toxicological data on individual components combined with the chemical-analytical concentration data. The later can then feed into a mathematical model to predict the final (eco)toxicity of the mixture. Whole-mixture testing is frequently applied for environmental mixtures, as it allows to assess the (eco)toxicity of mixtures of unknown composition; however, the compounds responsible for the response frequently remain unidentified. The component-based approach is more common, but requires more information regarding identity, concentration and toxicity, including mode of action (MoA) of the individual compounds.

2.3. Mixture assessment tools

In addition to the mathematical models applied to predict the overall toxicity, additional tools are increasingly used to determine

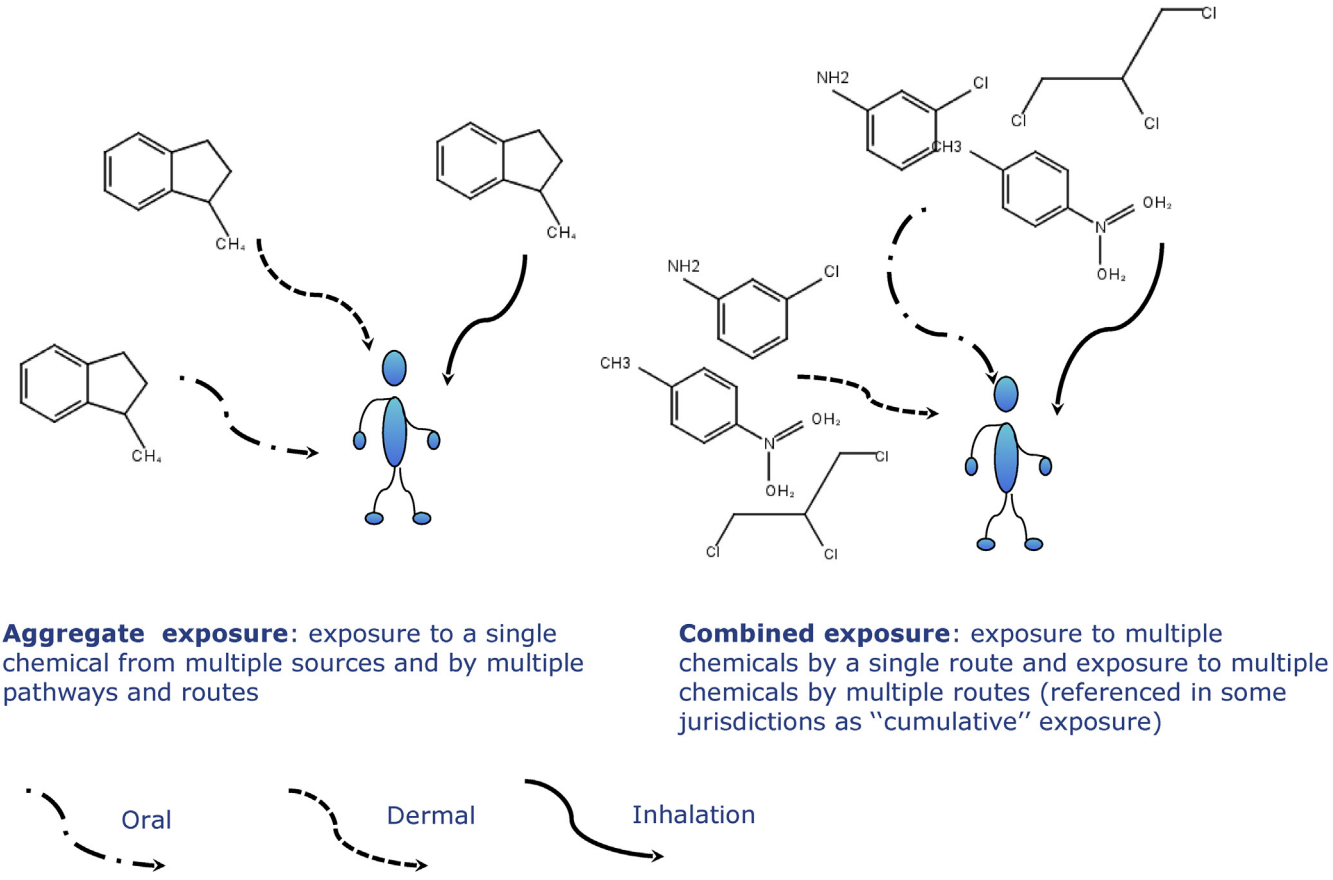

Fig. 1. Aggregate vs combined exposure.

when or whether combined RA is needed at all, e.g. by expressing the individual compound contribution compared to the combined total toxicity of the known compounds in the mixture. As such, these tools do not predict the risk of a mixture *per se* but rather provide a means of investigating data on cumulative exposure to human and ecological receptor and identify when cumulative RA is most needed. An example of such a tool is the Maximum Cumulative Ratio (MCR), which is the ratio between the toxicity of the mixture (based on CA models) and the toxicity of the most contributing chemical in the mixture. The MCR approach is currently applied in various contexts, to determine when cumulative assessments are most required and to discriminate between those mixtures requiring further combined RA and those for which a single-substance assessment is sufficient. Therefore, it helps to decide on the next step of the RA, e.g. undertake a further refinement of mixture RA in case of several main contributors or concentrate on only a few components dominating the effects.

However, for calculating the MCR, at least a screening level mixture RA has to be performed to predict the combined effects to which the effect of the most contributing compound(s) can be related. Moreover, application of the MCR methodology requires knowledge of the concentrations of chemicals in a mixture together with health-based reference values for those chemicals. This tool has also been found to be useful for analysing the pattern of chemical-specific contributions to the total exposure levels of mixtures based on biomonitoring data when Toxic Equivalent Factors (TEFs, see Table S1) or similar approaches are available (i.e. occupational vs. background exposure) (Han and Price, 2013).

Depending on the scope of the assessment and the available data, a tiered approach can be followed. By starting from a screening level with the option for further refinement where needed, resources can focus on the most important factors contributing to risk. However, the criteria used in developing tiers need to be balanced, yet sufficiently conservative so that important factors are not inappropriately screened out. In this context, the utility of the Threshold of Toxicological Concern (TTC) approach as a Tier 0 assessment tool for chemical co-exposures, especially for a data-poor chemical has been demonstrated (Meek et al., 2011): a RA based on monitoring data for 10 chemicals found in water was done using the TTC approach for the substances for which no established chronic health standards or health-based guidance values were available. The resulting HI (see Table S1 for details) of 0.2 suggested that there was no need to further refine the RA.

2.4. Methodological issues and hurdles hampering the risk assessment of chemical mixtures

When having a closer look into RA of chemical mixture case studies, some methodological issues are recurrent. The data sources used are various and the data sets more or less complete, this having a direct impact on the quality of the RA and the related uncertainties. Exposure data are usually modelled, from biomonitoring or published data from surveys on exposure, and exposure data reliability directly depends on the biomonitoring practice (Dewalque et al., 2014; Malaj et al., 2014) and on the quantity of the data. The exposure of persistent and bioaccumulating chemicals is even more challenging as it requires taking into account the kinetics of the chemicals and to consider the body burden as a starting point for the RA, instead of the daily intake, as well as the exposure history, as the exposure patterns might change over time.

Toxicological data are mostly from published databases, but in case of missing data e.g. the TTC approach, or other methods to fill data gaps are used. As a matter of fact, data gaps seem to be the major issue when it comes to deal with RA of chemical mixtures. Those data gaps are numerous, both regarding hazard and exposure data, for pharmaceuticals (Backhaus and Karlsson, 2014), pesticides (Junghans et al., 2006; Kennedy et al., 2015; Nowell et al., 2014), cosmetics, etc. and implies to use extrapolations (i.e. acute to chronic), which increase the uncertainties of the RA. Models to estimate aggregate exposure of consumers in personal care products (PCPs) are being developed (Delmaar et al., 2015), but a sufficiently elaborated data on the frequency of use of those products are still lacking (Gosens et al., 2013) which hamper refinements of the RA if needed.

As a result, RA of chemical mixtures requires a lot of assumptions. Their choice can have a large impact on the outcome and should be carefully documented and justified (Boon et al., 2015; Kennedy et al., 2015). This is also the case for single substance assessments, however, of particular importance for the assessment of mixtures since the uncertainties around single substance assessments are adding up when combined risks are assessed.

Moreover, in the case in which different models are combined and used in the same RA (i.e. for dietary and non-dietary exposure), care must be taken when interpreting the result to recognize possible differences in the degree of conservatism between dietary and non-dietary exposure models. Furthermore, the assessment of combined effects for substances of common effects or common MoA implies that reference values for the specific effect under consideration should be used. Toxicity values reported however are often those driving the single substance risk, i.e. the lowest reference value which might be for a different effect. Using these reference values in lower tiers can be a first conservative estimate, but might lead to large overestimations of the combined effects.

3. Assessment of mixtures under current regulations

As mentioned previously, a general distinction can be made between intentional and unintentional mixtures. Intentional mixtures are generally well addressed by current regulation through a prospective RA prior to the marketing of the product: the existing European regulations dealing with the RA of intentional mixtures have been reviewed in detail and their requirements are presented in Table S2. Nevertheless, this assessment is restricted to a particular use in a given regulatory framework and does not take into account other potential uses related to other regulatory frameworks, i.e. aggregate exposure, although there might be an overlap between the different regulatory frameworks. However, if there is no reason why chemicals allocated to specific regulatory frameworks would have non-overlapping risk profiles, then there is also no reason to expect that mixture RA limited only to chemicals within one regulation can fully capture the risk that may be present to human consumers (Evans et al., 2015).

Overlapping can be illustrated by considering the 428 unique substances registered as pesticides in the EU[1] DG SANCO database. Of these, 38 are also registered as biocides,[2] 55 as industrial chemicals under REACH[3] and six are registered within all these three regulatory frameworks. In addition, one substance (Benzoic acid) is also registered in a fourth framework as a cosmetic ingredient.[4] While this illustrates the potential for aggregate exposure for individual substances, the combined exposure could be even more relevant when considering different chemicals that share the

[1] Source: DG SANCO database, extraction on the 11/05/2015. Except microorganisms.

[2] Source: ECHA database on the 11/05/2015. Except microorganisms.

[3] Source: ECHA database on the 18/09/2014. Except mixtures, reaction products, polymers and petroleum-derivatives.

[4] SCCS, 26/11/2014.

same MoA (Evans et al., 2015).

The assessment of unintentional or coincidental mixtures is generally not required (Table S3), although the assessment of multiple substances from multiple sources is the main issue raised by the European Commission when dealing with the assessment of chemical mixtures (EC, 2012). The requirements regarding mixture assessment of the most important European regulation are reported hereafter.

3.1. Plant protection product (PPPR; Reg 1107/2009), biocidal products regulation (BPR; Reg 528/2012) and maximum residue levels (MRLs) of pesticides (Regulation 396/2005)

The regulations on PPPs and biocidal products both focus on the active substance (AS). AS are assessed by a reporting member state and authorized at the European level, and the preparations made with those AS are registered for specific uses at the national level. Requirements for PPP AS (Reg 283/2013) and formulations (Reg 284/2013) can differ and are addressed by different regulations; in particular, chronic testing is usually only required for the AS and not for the formulation (i.e. mixture). However, the fact that the PPPR requires more data for the RA of AS than for the formulated products (EU, 2013a, 2013b), especially for chronic RA, is often criticized, as formulations are designed to be more effective than the AS itself. Both the PPPR and BPR mention the necessity to take into account interactions between components and require the assessment of cumulative and synergistic effects in the environment. However, for the PPPs this is restricted to the formulation itself. It does not apply to the potential combined effect resulting from the concomitant use of several formulations, as applied in practice, or to the combined effects in the environmental matrix where they end up. Similarly, the potential aggregate exposure to the same AS coming from other sources is currently not addressed for PPPs. Conversely, the BPR requires that for biocidal products that are intended to be used in combination the risks to human health, animal health and the environment arising from these combinations shall be assessed. Moreover, when the evaluating authority considers that there might be some concerns for human health, animal health or the environment because of the cumulative effects from the use of biocidal products containing the same or different active substances, this concern should be documented and included in the conclusions.

Regarding PPP and human health, the RA is not limited to the end-user of the PPP but should cover consumer exposure, operators, workers, residents and bystanders, taking into account, where relevant, the cumulative exposure to more than one AS. It is however not specified if this requirement should be met for AS used in combination in the same PPP only, or also when several PPP are used in combination in the field, in a period that would allow cumulative exposure. When estimating the potential and actual exposure through diet and other sources, the presence of residues arising from other sources (i.e. use as a biocide or veterinary drug) should be taken into account (aggregate exposure), as well as the potential cumulative exposure to more than one AS, where relevant. However it is not specified in detail how to proceed to such an assessment, but reference is made that it should be assessed "where the scientific methods accepted by the authority to assess such effects are available".

Pesticides residues in food and feed are specifically addressed by Reg. 396/2005 on Maximum Residue levels (MRLs), which aims at ensuring that those residues are not present in food and feed products at levels presenting an unacceptable risk to humans and animals. This regulation establishes the maximum quantities of pesticide residues permitted (MRLs) in products of animal or vegetable origin intended for human or animal consumption; MRLs are specific to particular foodstuffs, and for products and/or pesticides for which no specific MRLs are set, a default value of 0.01 mg/kg applies.

Reg. 396/2005 states that MRLs should be set in "view of human exposure to combinations of AS and their cumulative and possible aggregate and synergistic effects" and explicitly addresses the need for carrying out further work to develop methodology and technical guidelines on pesticides residues allowing to take into account aggregate, cumulative and synergistic effects. However, established procedures for safety assessment of MRLs on the basis of ADI (Acceptable Daily Intake) values and food consumption patterns are focused on single substance assessments. The regulation also states that Commission decisions related to MRLs shall take account of the possible presence of pesticides residues arising from sources other than current plant protection uses, without specifying which kind of other sources, and "*their known cumulative and synergistic effects, when the methods to assess such effects are available*". The methodology is currently under development by EFSA see e.g. (EFSA, 2014a).

3.2. Pharmaceuticals (Dir 2001/83/EC and 2001/82/EC)

Regarding pharmaceuticals, both directives on human (Dir 2001/83/EC) and veterinary (Dir 2001/82/EC) pharmaceuticals share some basic features: the RA follows a risk-benefit balance approach, in which the applicant is required to demonstrate that the potential risks are outweighed by the therapeutic efficacy of the product.

Both risk to the patient's health and risk to public health are considered when dealing with human medicines. Wanted and unwanted interactions of substances combined within a medicinal product are addressed, as well as potential interactions of the medicine with other medicinal products, or with alcohol, tobacco, and foodstuffs. Studies on pharmacokinetic and pharmacodynamic interactions are part of the standard dossier requirements (Kortenkamp et al., 2009). Thus, the toxicity of the whole product is taken into account and potential interactions are deeply assessed when it comes to human exposure. An ERA is also required, but does not specifically address any aspect of mixture toxicity (EMEA, 2006).

Regarding veterinary medicines, three types of risks are considered: risks to the target animal, risks to human health (from both exposure to residues of the product in foodstuffs and direct exposure, i.e. during administration), and risks to the environment. Toxicity and ecotoxicity studies and assessments are performed for the product, its active substances and relevant metabolites. Attention is paid to interactions with other medicinal products or feed additives with respect to effects in the target animals, but this point is less deeply assessed than for human medicinal products (Kortenkamp et al., 2009). The ERA of the product is required but does not take into account the toxicity potentially resulting from the joint occurrence of different residues of veterinary products or of other pollutants.

3.3. Food and feed additives (Reg 1333/2008, 1331/2008 and 429/2008)

Neither the terms cumulative, synergistic or potentiating, nor the need for mixture toxicity assessments is mentioned in the food additives regulation (Reg. 1333/2008), although the previous regulation provided a basis for mixture toxicity assessments (Kortenkamp et al., 2009). Besides, established procedures for the safety assessment of food additives on the basis of ADI values for single substances do not specifically consider interactions between additives and food consumption (Groten et al., 2000). However,

Regulation (EC) No 178/2002 laying down the general principles and requirements of food law states that "*regard shall be had* (…) *to the probable cumulative toxic effects*" for human health, without however defining the term "*cumulative toxic effects*", which could either mean a toxic effect resulting from repeated exposure to a single toxicant or a toxic effect resulting from simultaneous or sequential exposure to different toxicants and thus be used as a synonym for *mixture toxicity*. Thus, European Regulation (EC) 1333/2008 neither excludes nor explicitly defines the need for mixture toxicity assessments for food additives (Kortenkamp et al., 2009). Regarding feed additives (Reg 1331/2008), consideration should be given to the cumulative effects in case of additives with multiple components; however, there is no consideration of mixtures assessment from different sources.

3.4. REACH (Reg 1907/2006)

The REACH Regulation aims at ensuring the chemical safety assessment (CSA) of all chemicals unless they are specifically covered by other sectorial regulations (EC, 2006). The REACH registration requirements apply to each of the individual substances in a preparation, but not to the preparation itself. REACH defines a "chemical mixture" as a deliberate combination of two or more individual substances; however, the legal definition of "substance" in REACH can contain up to 20% arbitrary by-products without the need for specific consideration. It also includes Multi-Constituent Substances (MCS) which are substances resulting from a chemical reaction in which several constituents are present at >10%, and UVCB (substances of Unknown or Variable composition, Complex reaction products or Biological materials) which are mixtures that cannot be completely identified by their chemical composition. MCS and UVCB are generally treated as a single substance under REACH, and the testing of hazard and fate properties is therefore made on the mixture itself.

Although there might be multiple sources of exposure to the same substance in real life (i.e. aggregate exposure), a registrant is not obliged to take into account an exposure to the same substance from activities from other producers or importers when doing the exposure assessment (ECHA, 2013), and no specific hazard assessment is required for chemical mixtures, preparations, MCS or UVCBs, unless they have persistent, bioaccumulative and toxic or very persistent/very bioaccumulative (PBT/vPvB) properties; i.e. if they contain more than 80% of a substance with PBT/vPvB properties (ECHA, 2012). Thus, REACH is a typical substance-oriented regulation.

3.5. Cosmetics (Reg 1223/2009)

Cosmetic products are typically a mixture, composed of multiple substances. According to Reg 1223/2009, the assessment of cosmetic products should take into account the anticipated systemic exposure to individual ingredients in a final formulation. It includes a toxicological profile of the substances that should take into account all significant toxicological routes of absorption according to the intended use, as well as possible impacts on the toxicological profile due to interactions of substances.

The safety assessment of substances as individual ingredients should consider the overall exposure to such substances stemming from all sources, which implies the development of a harmonized approach to the use of such overall exposure estimates. However, the regulation does not specify if only sources of exposure linked to cosmetics uses are meant or if other uses (e.g. as pharmaceuticals) are included; although the latest guidance document only mentions cosmetic use (SCCS, 2015). Additionally, the safety of the cosmetic product itself must also be assessed, including possible interactions of the substances contained in the cosmetic product. This regulation does not address potential environmental concerns of cosmetic products specifically, as they are considered to be assessed under REACH, which addresses the assessment of environmental safety of the individual substances in a cross-sectorial manner.

3.6. Water framework directive (WFD, Dir 2000/60/EC) and marine strategy framework directive (Dir 2008/56/EC)

The Water Framework Directive aims to establish the basic principles of sustainable water policy in the European Union, and to assess, maintain or improve the chemical and biological status of European waters (EC, 2000). Thus, this regulation does not address a particular type of chemicals but all of those that could be of concern in surface water. It aims at identifying priority hazardous substances for the aquatic environments on the basis of scientific RA carried out under sectorial regulation (PPP, biocide, pharmaceuticals …), and sets common environmental quality standards (EQS) and emission limit values for chemicals or groups of pollutants (EC, 2011). However, this directive does not mention chemical mixtures or mixture effects, although the EQS guidance document recognises that in some circumstances (i.e. release of known and constant composition mixtures or other mixtures with a partly unknown, reasonably constant composition, that both change after entry into environment) an EQS for mixtures may be preferable to deriving EQSs for the individual constituent substances (EC, 2011). Thus, this guidance document briefly outlines how to estimate EQS for mixtures, using the toxic unit (TU) approach (Table S1) for well-defined mixtures, and the hydrocarbon blocks and the use of non-testing methods such as PETROTOX6 for the derivation of EQS for petrochemical mixtures of unknown or variable composition.

The Marine Strategy Framework Directive adopts an ecosystem-based approach, aiming at a Good Environmental Status (GES) focusing on 11 descriptors related to ecosystem features, human drivers and pressures (Berg et al., 2015). Descriptor 8 is formulated as "concentrations of contaminants are at levels not giving rise to pollution effects", referring to substances or groups of substances that give rise to a level of concern. Where possible, this should also include effects which may be caused by synergistic or cumulative interactions between different contaminants. The list of compounds mentioned is not restrictive, but rather takes the list as indicative. However, it is recognized that the causal relationships between levels of contaminants and observed effects are not well understood, and that there is rarely a direct relationship between tissue levels of contaminants and their effects (Law et al., 2010). The understanding of the effects of mixtures of contaminants and of interactions between contaminants and other environmental stressors is even more limited. Therefore, it was decided that before implementing the Directive, more research was needed *for assessing good environmental status in a coherent and holistic manner to support the ecosystem-based approach* (Commission Decision 2010/477/EU, Article 3). It was still emphasized however, that it is important to consider cumulative and synergistic effects, not only by compounds listed in the WFD and others, but also compounds that may entail significant risks to the marine environment from past and present pollution.

3.7. Drinking water directive (Directive 98/83/EC)

This directive aims at ensuring a good quality of water intended for human consumption, by setting individual parametric values for substances that are of health concern at a level strict enough to ensure human health protection on a life-long basis. Member States are in charge of ensuring that drinking water respects the

minimum requirements of the Directive, of setting values for those parameters which shall not be less stringent than those set out in the Directive, and of setting values for additional parameters not included in this Directive where required by human health protection. They shall also ensure the efficiency of the disinfection treatment applied, while keeping any contamination from disinfection by-products as low as possible and ensure that regular monitoring of the quality of drinking water is carried out. Thus, this regulation requires the monitoring of individual parameters but does not address chemical RA or any mixture issue, although disinfection products or by-products might be of concern for human health (Jeong et al., 2012; Nieuwenhuijsen et al., 2009).

3.8. Conclusions on mixture assessment under current EU legislation

Overall, chemical RA requirement in Europe is most of the time substance-driven and rather sector specific. Clear regulatory requirements for RA of mixtures of chemicals within a given regulatory framework are rare, except for intentional mixtures such as formulated products, and are most of the time prospective. Regulatory requirements for RA of mixtures across various regulatory frameworks is scarce, although for aggregate exposure it is important to acknowledge that numerous chemicals are concerned by more than one regulatory framework. Regarding exposure to multiples substances, it has to be kept in mind that substances regulated under different regulations can elicit similar effects or follow the same mode of action so that combined effects cannot be excluded.

Regulations on unintentional mixtures in the environment, like the WFD and the MSFD, recognize the importance of mixture effects, but do not provide specific details on how this should be assessed. In these cases, assessment is hampered by a lack of information on pollutants levels, identity and effects, as well as a limited understanding of the causal relationship between level of contaminants and biological effects.

3.9. Mixtures risk assessment in the US

Also in the US; exposure to multiple chemicals is considered in various regulatory frameworks. The CRA of contaminated site specifically requires mixture RA for the evaluation of risks stemming from hazardous waste sites and chemical accidents (USEPA, 1989, 1987). Exposure assessments are made on “reasonably maximally exposed” people, and the toxicity assessment is based on reference toxicological value for each chemical. For carcinogens, it is assumed that there is no dose threshold, and that the dose-response function is essentially linear. For non-carcinogenic chemicals, a HI is calculated using the CA methods. If $HI \leq 1$, it is assumed that there is unlikely to be a risk; if $HI > 1$, further analysis may be performed to determine whether application of dose additivity to all the chemicals simultaneously is justifiable. In 2000, the US EPA published a Supplementary Guidance for Health Risk Assessments for Mixtures, which introduces an Interaction Hazard Index (USEPA, 2000).

Pesticide RA requires the estimation of health risks from combinations of pesticides with a common MOA. In order to do so, the US EPA developed guidelines to determine which pesticides should qualify for inclusion in common mechanism groups (USEPA, 1999), and a guidance document concerning the application of the HI principle to pesticides (USEPA, 2002a), which deals with simultaneous exposures from food, drinking water and residential (non-occupational) use of pesticides for the general population. This RA procedure was used to extensively assess the risk linked to mixtures of organophosphates, carbamates, triazines and chloroacetanilides (USEPA, 2007, 2006a, 2006b, 2002b). It identifies several groups of chemicals that are considered to induce a common toxic effect by a common mechanism, a so-called common mechanism group (CMG). Pesticides that contribute to exposures by minor pathways are excluded, forming a subset called cumulative assessment group (CAG). For each CAG member, dose response analyses are performed to determine its toxic potency for the common effect. The concept of CA is normally used to estimate the combined risks in the CAG, and the relative potencies of the CAG members to one selected index chemical are defined for the standardization of their common toxicity in terms of relative potency factors (RPF). Exposure assessment is made through detailed exposure scenarios, including all relevant pathways, durations and routes where simultaneous exposure may occur, as well as sequential exposures. The output of this analysis is an aggregation of exposures via all routes and pathways, for each chemical, which is then expressed in terms of an equivalent exposure of the index chemical, by using RPFs. The risk contributions from each pathway and route should be evaluated both individually and in combination, in order to identify risk contributors. This risk characterisation step also includes descriptions of variability and major areas of uncertainty and the need for uncertainty and safety factors is determined.

The RA of drinking water (mainly focusing on disinfection by-products) and air pollutants also requires consideration of chemical mixtures, however the approaches developed for such an assessment took minimal considerations of synergistic or antagonistic effects, nor were non-chemical stressors taken into account (Kortenkamp et al., 2009).

In 2003, the US EPA also published a Framework for Cumulative Risk Assessment (USEPA, 2003), which provides starting principles for EPA’s CRA, for the future development of a comprehensive and detailed guidance on methods for evaluating cumulative risk. This report emphasizes chemical risks to human health including the effects from a variety of stressors, including non-chemical stressors. This was further developed in the 2006 publication on the “Considerations for developing alternative health risk assessment approaches for addressing multiple chemicals, exposures and effects” (USEPA, 2006c), which presents concepts that could assist the development of detailed guidance and provides explicit approaches for addressing some of the complicating “multiples” in CRA. These approaches include new methods and the extension of existing methods to address health risk from multiple chemicals and multiple exposure pathways and times.

4. Chemical mixture risk assessment approaches: experiences from case studies and an expert survey

4.1. Case studies focusing on the assessment of mixtures

Case studies on chemical mixture RA are numerous in the literature and help in assessing the applicability of approaches and identifying data gaps and hurdles in the context of mixture RA. Recent case studies on a broad range of chemical classes and exposure scenarios have been selected and reviewed (methodology being used, data gaps identified, outcome) and are reported hereafter.

4.1.1. Chemical class based examples

4.1.1.1. Pesticides and environmental risk assessment. Because pesticides are designed to be biologically active, directly emitted into the environment and used at fairly high volume, there are clear data requirements regarding the toxicity on target and non-target species. Therefore, pesticides are amongst the more data rich chemicals regarding toxicity and they are frequently included in

environmental monitoring programs and case studies on the effects of co-occurring pesticides.

Junghans et al. (2006) showed that pesticides in mixtures are clearly more toxic to algae than any individual component, which clearly highlights the limit of current prospective RA process based on individual chemicals. Overall, CA showed a good predictive quality over the complete range of effects considered, irrespective of the similarity or dissimilarity of their mechanisms of actions. Gregorio and Chèvre (2014) also used the CA model and the risk quotient methodology to retrospectively assess the risk posed by mixtures of chemicals (mainly pesticides) in the Geneva Lake and the Rhone River, and identified the most problematic substances demanding risk reduction. The authors showed that the risk levels associated with mixtures of compounds can rapidly exceed critical aquatic thresholds, and that therefore, it is the sum of the substances that is problematic. However, when the risk quotient is greater than 1, it is often due to only a few chemicals (1–4 in this case).

The pesticide toxicity index methodology has also been used as a screening tool to assess potential aquatic toxicity of complex pesticide mixtures by combining measures of pesticide exposure and acute toxicity in an additive toxic-unit model (Nowell et al., 2014), but this methodology is a relative ranking system that indicates that one sample is likely to be more or less toxic than another sample, without indicating that toxicity will necessarily occur.

Moreover, those methodologies are limited because they do not consider synergistic effects, which are known to be possible with pesticides. As an example, the combination of pyrethroid insecticides and azoles fungicides such as deltamethrin and prochloraz, is known to be much more toxic to bees than the chemicals individually with a ratio ranging from 366 to 1786 fold (Colin and Belzunces, 1992; Sammataro and Yoder, 2011). The proposed mechanism is that those fungicides, by inhibiting ergosterol biosynthesis via the inhibition of cytochromes P450 also involved in detoxification, decrease the capacity of the organisms to detoxify other chemicals. Similar interaction has been found between miticides and pyrethroids, or between miticides (Sammataro and Yoder, 2011). Synergism has also been shown to occur between organophosphates and carbamates pesticides in salmon (Laetz et al., 2009).

Additionally, linking toxic effects to monitoring data can only consider chemical substances that are identified; substances that are not analysed nor detected because they are present at concentrations below the limit of detection are not taken into account, although they might be biologically active. Other methodology could be used, such as a two-step model approach mixing CA for modelling mixture toxicity of individual MoA, and IA to combine the toxicity of different MoA (De Zwart and Posthuma, 2005); or the use of CA or IA on species sensitivity distributions, which can be much more robust, but requires a huge quantity of ecotoxicity data, which are often not available (Gregorio et al., 2013).

Most of the case studies on pesticides are carried out retrospectively, based on monitoring data; however, such types of RAs could also be carried out prospectively, prior to placing a product on the market, and based on calculated Predicted Environmental Concentration (PEC) data, in order to screen and detect the combinations that could be of concern. One way of addressing combined environmental risk from pesticide co-exposure could be to base the selection of co-occurring pesticides on their use patterns in specific crops or based on common tank mixes. Data collections on use patterns have been performed throughout Europe that could serve as a basis (Garthwaite et al., 2015).

Moreover, prospective RA of pesticides could be improved by the development of environmental scenarios for mechanistic effect modelling of pesticides, defined as a combination of abiotic, biotic and agronomic parameters that are thought to represent a realistic worst-case situation for the environmental context in which the model is to be run (EFSA, 2014b).

4.1.1.2. Pesticides and human health. Aggregate exposure assessment combining dietary and non-dietary sources for a single substance allows identifying the relative contributions to exposure, which can differ between particular scenarios and populations. For instance, Kennedy et al. (2015) identified inhalation as the main route of exposure to pesticides for spray users, and dermal exposure for operators. For child bystanders, non-dietary (dermal) exposure is estimated to be small compared to dietary exposure. However, data are lacking on realistic frequency or use of plant protection products by amateurs and more generally on non-dietary exposure, which would be essential for chronic RA.

Regarding combined exposure to multiple pesticides, EFSA published a guidance document on probabilistic modelling of dietary exposure, including an optimistic and a pessimistic model, which were applied to pesticide residue mixtures from the triazole group (Boon et al., 2015). The grouping of the chemicals was based on the toxicological effect. In the optimistic model run, none of the simulated acute nor chronic exposures exceeded the reference toxicological value (i.e. respectively Acute Reference Dose—ARfD- for acute and ADI for chronic); however in the pessimistic model run, which takes into account animal commodities including cattle milk and meat at the level of the MRLs, an exceedance of the ARfD or ADI was frequently observed, and the model was judged to result in unrealistic conclusions regarding the contribution of animal commodities to the dietary exposure. The authors conclude that the pessimistic model runs, besides being laborious, could provide results that are too far from reality, and that the optimistic model runs would likely give results underestimating the real exposure. Some kind of intermediate 'realistic' scenario is therefore needed, which would result in more realistic acute and chronic exposures, conservative enough (precautionary principle) without being over-conservative (Boon et al., 2015).

In the case of retrospective RA using biomonitoring data, all the sources of exposure are by default included in the RA, but when doing a prospective RA, this is not the case, and residues of pesticides from sources other than current plant protection uses of active substances are usually not taken into account; except for biocides and veterinary drug uses in the settings of MRLs. Moreover, there were no case studies identified on Human Health regarding combined exposure to several active substances including both dietary and non-dietary exposure, nor were case studies found considering aggregate exposure to pesticide active substances across regulatory frameworks, which could be of interest.

4.1.1.3. Biocides. The methodology published by ECHA for HRA addressing combined exposure to multiple substances within a single biocidal product (ECHA, 2015) can theoretically be applied to assess aggregate exposure to multiple biocidal product types containing the same AS, by combining the exposure estimates from uses/releases from the different product types, although this would require lots of data. It could also theoretically be applied to combined exposure to multiple substances coming from different sources of release and/or uses, provided that the various exposure scenarios and cumulative effects are taken into account, and that sufficient data are available to do so. However, to our knowledge this has not been put into practice so far.

4.1.2. Groups of compounds or matrix-based examples

4.1.2.1. Environmental risk assessment. Several studies focus on relating effects to the compounds present, including many effects

currently not covered by the respective regulations. E.g. Tang et al. (2014) investigated waste water and recycled water samples, considering 299 chemicals present at concentrations below the regulatory safety limit. Artificial mixtures of those chemicals were found to explain less than 3 and 1% of the observed effluent cytotoxicity and oxidative stress response respectively, showing that the identified compounds do not explain the observed toxic effect. This large proportion of unknown toxicity, which could either be due to other non-monitored chemicals or to mixture effects, calls for effect-based monitoring complementary to chemical monitoring (Ohe et al., 2004; Tang et al., 2014).

Pesticides, followed by pharmaceuticals and personal care products seem to dominate the observed mixture effects on the environment (Tang et al., 2014), and the mixture risk quotient of pharmaceuticals in sewage treatment plant effluents has been shown to regularly exceed 1, which points out the fact that those mixtures can be of concern (Backhaus and Karlsson, 2014).

However, it has to be highlighted that many case studies do not take into account the degradation products and metabolites of the chemicals in the environment; nor the bioconcentration potential of the mixture. In order to assess the risk of complex effluents based on both acute toxicity and the bioconcentration potential of the mixture, a methodology has been developed, combining the estimation of K_{ow} of a mixture by RP-HPLC on one hand, and the extraction, fractionation and ecotoxicity testing of the fraction on the other hand (Effect Directed Analysis) (Gutiérrez et al., 2008). Finally, a relative hazard index (RHI) for any particular mixture is estimated, ranging from 1 to 10, which takes into account the bioconcentration potential. Instead of further analysing the whole sample, the efforts can be focussed on the more toxic fractions to identify relevant toxic compounds, which reduces analysis costs. This method could add value for whole effluent assessment and help to refine PNEC values, however it has not been applied to real effluents so far (Gutiérrez et al., 2008).

4.1.2.2. Human risk assessment. Investigating food contact materials, olefins and saturated hydrocarbons for the Non Intentionally Added Substances and ethyl-4-ethoxybenzoate for water bottles were identified as main contributors to toxicity from multiple substances released to food (Price et al., 2014), although the risk of adverse effects to individuals were found to be low (HI < 1). However, the study did not consider many inorganics, due to a lack of available reference values. Potential ED effects of five phthalates were evaluated based on human urinary biomonitoring data. The HI of the mixture exceeded the "safe" level for 6.2% of adults and 25% of children (Dewalque et al., 2014). This means that even when focusing on a small subset of compounds, safe levels might be exceeded. DEHP was the only phthalate studied for which the main pathway of exposure was the dietary intake; for all other, it seemed to be a minor pathway, highlighting the importance to take into account all pathways of exposure to make a reliable RA. This wide exposure to phthalates is confirmed by Becker et al. (2009), who detected 12 phthalate metabolites in urine samples of German children, with contamination levels 3–5 times higher than in adults (Becker et al., 2009). This might be a situation of concern, as anti-androgenic effects of phthalates on reproductive health could occur at all life stages and because phthalates are not the only anti-androgenic chemicals to which humans are exposed. This was confirmed by Kortenkamp and Faust (2010), which have assessed cumulative anti-androgenic effects of 15 chemicals including phthalates and concluded that the cumulative risk exceeds acceptable levels for people on the upper end of the exposure levels. The results suggest that combined exposure to anti-androgens have reached levels of concern, and that larger human biomonitoring studies including pertinent biomarkers of exposure of anti-androgenic compounds should be performed. Moreover, those case studies do not take into account synergistic effects, although synergism has been observed with a mixture of anti-androgens with diverse MOA (Christiansen et al., 2009). Furthermore, it is often neglected that the effective internal dose of some anti-androgenic chemicals (i.e p,p′-DDE and BDE 99) may be higher than suggested due to their highly lipophilic nature. A different dose metric and tissue concentrations should be used, although the data necessary for such calculations are currently not available (Kortenkamp and Faust, 2010).

Another study has shown that butyl paraben makes up 50% of the HI of the highly exposed population group to anti-androgens, and that the percentile of the population of children from 0 to 3 year old with an exposure probability to propyl- and butylparaben above the assumed "safe" level, was estimated to be 13% and 7%, respectively. Further refinement of the exposure calculations is therefore necessary (Gosens et al., 2013; Kortenkamp and Faust, 2010), although hampered by the scarcity of detailed data on the use of personal care products, especially for children. Furthermore, those chemicals are also used in other types of products such as pharmaceuticals and food additives and those uses have never been assessed together, as they are regulated under different legal frameworks. More exposure data regarding these products would therefore be needed to obtain a more accurate estimate of the aggregate exposure to parabens.

A case study focusing on dioxins using biomonitoring data of 26 dioxin-like compounds based on toxic equivalency factors (TEFs) found similar MCR values in two occupationally exposed groups and in the general public, although the two occupational groups have higher total toxicity equivalence (TEQ) levels. MCR values indicated that only 2–5 of the 26 chemicals make significant contributions to total TEQ values. This was also the case for human exposure to environmental mixtures in surface water usually dominated by a relatively small number of components (Han and Price, 2011).

When looking at human exposure via surface water or effluents from wastewater treatment plants, 2% of the considered mixtures were of concern for human health effects (HI > 1), although those HH effects would have been sufficiently addressed by chemical-by-chemical approaches and showed little need for an assessment of the combined exposure (individual HQ > 1) (Price et al., 2012). However, the assumption made of a 10-fold dilution of the effluents could be wrong for small rivers under low-flow condition; and for rivers receiving multiple discharges the receiving water might already contain some of the compounds from upstream discharges, which would increase the risk.

Another relevant route for human exposure to unintentional mixtures relates to indoor air, since people spend approximately 90% of their time indoors, of which 2/3 would be spent at home (WHO, 2014a). This makes this type of exposure of concern, especially for subpopulations such as young children since their lung structure and immune system is not yet fully developed. Lead is the most studied indoor pollutant, while VOCs (Volatiles Organic Compounds) and SVOCs (Semi Volatiles Organic Compounds) are still of concern and could be correlated with allergic effects and respiratory symptoms in children (Le Cann et al., 2011). For carcinogenic VOCs the estimated carcinogenic risks were up to three orders of magnitude higher than the one proposed as acceptable by risk management bodies (Sarigiannis et al., 2011), whereas conservative exposure limits were not exceeded for non-carcinogenic effects, except for formaldehyde. However, the RA evaluation process faces difficulties, either due to the relative paucity of indoor air quality measurements in many EU countries, or by the lack of sampling consistency in the already existing studies, indicating the need for additional measurements of indoor air quality following a

harmonized sampling and analytical protocol. Some hazardous VOCs are also directly link to the use of flame retardants; and halogenated organophosphates releasing chlorinated degradation products (Salthammer et al., 2003). Organophosphate flame retardants' exposure has been shown to be widespread, with hand-to-mouth contact or dermal absorption being important pathways of exposure (Hoffman and Stapleton, 2015); However, the RA of poly-brominated diphenyl ether in the indoor environment does not seem to be of concern, except in the US (Fromme et al., 2015; Lim et al., 2014), although according to some authors the high exposure to these substances indoor calls for better risk assessments that include mixtures effects (de Boer et al., 2016).

A high variability has been found in the proportion of samples of concern for mixture toxicity in residential indoor air with the MCR methodology, this variability being due both to the variation in indoor air contaminant levels across the studies but also to other factors such as differences in number and type of substances monitored, analytical performance, and choice of RVs (De Brouwere et al., 2014).

4.2. Expert survey on approaches, experiences and future directions in assessing human and environmental health risks from chemical mixtures

In order to gain an overview of current practices and experiences with assessing the effects and risks from combined exposure, an online survey was performed among experts in the field in the period of January to March 2015, addressing both, human health and environmental RA. Fifty-eight experts from 21 countries, different stakeholder groups and sectors of legislation participated in the survey. The main sectors where most experience is already gained in assessing mixtures are in the area of plant protection products and chemicals regulated under REACH. These were also rated highest regarding the priority for performing mixture assessments (Fig. 2), followed by pharmaceuticals, biocides and food or feed contaminants.

Experts mainly used CA based prediction tools and less IA based approaches. Some experts mentioned IA based approaches as a method they had abandoned due to the large amount of data needed for IA based predictions. In order to perform IA assessments, usually the full dose response curve for each mixture component is needed while in CA assessments the reference values are sufficient (Kortenkamp et al., 2009). When experts were asked about the need for addressing interactions in mixture RA, the vast majority (65 answers) agreed that interactions should be addressed on a case-by-case basis if there is specific evidence from which interactions could be expected. Only a small part of the experts (13 answers) thought that interactions do not need to be addressed specifically since they are either covered by CA based conservative approaches, or since they are anyway rare at relevant concentrations. Only 3 experts agreed to the statement that a conservative default safety factor should be applied to cover potential interactions in a non-case-specific way.

Experts were then asked about the use of novel tools in the RA of mixtures, such as *in vitro* methods, omics, (Q)SARs, read-across, toxicokinetic modelling, TTC approaches, Adverse Outcome Pathways (AOPs), or Integrated Approach to Testing and Assessment (IATA). These methodologies were selected based on their potential to contribute to the improved assessment of combined effects and unravelling modes of action (Bopp et al., 2015). Expert opinions were split between those applying them (often in a research context) and those that generally think these tools are valuable but their use is currently limited because of a lack of guidance, lack of data, or lack of expertise. A general need for clear guidance for combined exposure assessments was highlighted by many experts.

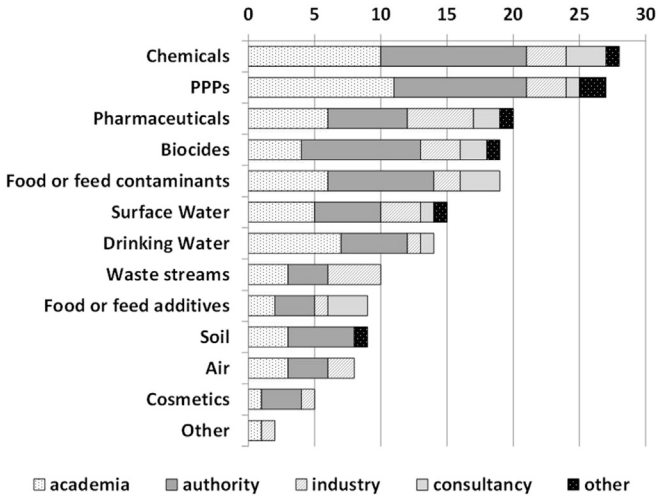

Fig. 2. Replies to the question "Which type of mixture(s) or samples would you identify as highest priority for risk assessment that needs to take mixture effects into account?" divided by stakeholder group. Chemicals were further specified in the survey as "multiconstituent or UVCB substances under REACH". Other mixtures of importance mentioned were those present in human tissues and container systems.

The most used tools in the RA of mixtures were QSARs, Read-across and *in-vitro* tools, both for HRA and ERA (Fig. 3). TTC approach is also often used for HRA of single chemical and chemical mixture; this approach is not frequently used in ERA but an eco-TTC approach is currently being developed to assist ERA (Belanger et al., 2015).

Experts were also asked in the survey about their experience with the three most widespread international frameworks developed for addressing combined exposure to chemical mixtures, i.e. the WHO/IPCS framework (Meek et al., 2011), Proposal by the three non-food scientific committees of the European Commission (SCHER et al., 2012), and the proposal by CEFIC MIAT (Price et al., 2012). 73% of the experts were familiar with at least one of those frameworks, mainly with the WHO/IPCS framework, which was rated as an easy and transparent approach. However, it was found to be rather general and lacks criteria when refinement should be stopped. The data available usually allow only to perform Tier 1 and 2 assessments and not to go to higher tiers. The SCHER, SCENIHR, SCCS framework is considered useful for organizing data and deciding how to perform the assessment, but the main limitation mentioned for those two frameworks is that they provide a more conceptual framework and less practical guidance. The CEFIC MIAT framework was judged as useful since it comprises practical tools; however, most input received on this framework was from experts involved in its development.

5. Regulatory challenges and future perspectives

5.1. Legal requirements

As described above, chemical RA and mixture RA is most of the time substance-driven and sector-specific (Kienzler et al., 2014). Clear regulatory requirements for RA of mixtures of chemicals within a given regulatory framework are rare, except for intentional mixtures such as formulated products. Moreover, regulatory requirements for RA of mixtures across various regulatory frameworks is scarce, even though numerous chemicals are subject to the provisions of more than one regulatory framework. Thus, aggregate exposure to one chemical regulated under different legislation as well as combined exposure to different chemicals with similar toxic

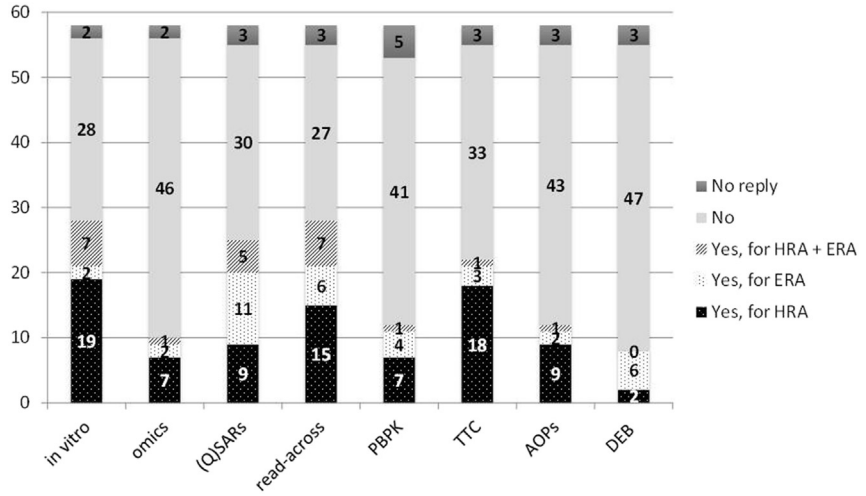

Fig. 3. Replies to the question "Do you apply *in vitro* tools/omics approaches/(quantitative) structure activity relationships ((Q)SARs)/read-across/physiologically based pharmacokinetic (PBPK) modelling/the toxicological threshold of concern (TTC) concept/Adverse Outcome pathways (AOPs)/dynamic energy budget (DEB) models for human health risk assessment (HRA), environmental risk assessment (ERA) or both?".

MoA or similar effects need to be further addressed (Evans et al., 2015).

5.2. Retrospective versus prospective risk assessment of mixtures

The review of case studies identified only retrospective mixture assessments, although several environmental scenarios have been proposed for prospective assessment of pesticides. These scenarios allow characterizing exposure, direct and indirect effects and recovery of aquatic non-target species in order to assess individual, population and/or community-level effects and recovery under realistic worst-case condition. A conceptual framework for the development of such scenario has been developed (EFSA, 2014b; Rico et al., 2015). Although the proposed scenarios still focus on the assessment of single compounds, in principle they can be used for a prospective assessments of mixtures, taking into account the already authorized uses of other chemicals to identify possible situations of concern. These data can be assessed using the recently developed cumulative assessment groups for PPPs, based on compounds with similar effects or MoA (EFSA, 2014a). Comprehensive approaches are needed, however, as the implementation of risk management measures becomes a challenge if risks from combined exposure to multiple chemicals under different legislation are identified.

5.3. Considering interactions

Current evidence in the literature suggests that interactions (synergistic or antagonistic effects) at lower concentration levels such as environmental concentrations are rare and, if observed, lead to relatively small deviations from CA predictions (Boobis et al., 2011; Cedergreen, 2014; Cedergreen et al., 2012). However, interactions are frequently reported for pesticides (Colin and Belzunces, 1992; Laetz et al., 2009; Sammataro and Yoder, 2011) and can also occur between chemicals from different regulatory silos: it has been shown that a pharmaceutical oestrogen and a persistent organochlorine pesticide, both exhibiting low efficacy when studied separately, were leading to synergistic activation by cooperatively binding to the pregnane X receptor. In this case, the binary mixture induces a substantial biological response at doses at which each chemical individually is inactive, each ligand enhancing the binding affinity of the other (Delfosse et al., 2015). This example again highlights the necessity to consider chemicals exposure beyond regulatory framework boundaries.

It has to be stressed that for both environmental and human exposure, none of the case studies reviewed takes synergistic effects or bioaccumulation (except when biomonitoring data are used) into account, which could underestimate the risks. More knowledge could be gained from additional case studies covering different sectors to further analyse this source of uncertainty. In the survey, most experts stated that interactions should be considered if there is specific evidence for interactions and on a case-by-case basis.

To predict and address interactions, toxicokinetic and toxicodynamic modelling are valuable tools. Toxicokinetic and toxicodynamic information to feed into these models, can be gained e.g. from *in vitro* studies or using QSAR models. Adverse Outcome Pathway (AOP) (Ankley et al., 2010), might also help to identify potential nodes between those different pathways which might trigger interaction and potentiation by giving information on the key events of the different toxicity pathways triggered by the different chemicals within a mixture.

Also read-across information from similar mixtures can be used to identify mixtures where interactions could play a role and should be further investigated (Bopp et al., 2015). A way forward, as chemical characterisation of mixtures might be difficult, would be to further investigate read-across based on biological similarity from *in vitro* screening (Reif et al., 2013, 2010).

5.4. Underlying exposure and toxicity data

For exposure assessments, usually exposure concentrations are estimated based on volume of production or use or on product use surveys, can be modelled using appropriate scenarios or can be based on (bio)monitoring data. Monitoring data are essential as they can provide information on magnitude, duration, frequency and/or timing of real exposure, and allow to assess the co-exposure patterns to chemicals (Qian et al., 2015), both for human and environmental RA. Several authors from the selected case studies highlighted the problem of large data gaps regarding the availability of measured exposure concentrations and appropriate modelling approaches. An important aspect for assessing combined effects is also the temporal profile of co-exposure. Ideally, internal or target organ exposure concentrations over time should be

available. Toxicokinetic modelling plays a prominent role here in gaining further insight, although it is currently rarely considered at higher tier assessments.

For hazard assessment mostly toxicological data from published databases are used (based on peer-reviewed literature or substance authorisation dossiers). Data gaps were often identified in the reviewed case studies. Approaches to fill these are e.g. the use of the Threshold of Toxicological Concern (TTC) or *in silico* approaches (QSAR and read-across) (Bopp et al., 2015). Another issue is also the combination of toxicity data based on reference values that are often derived from different endpoints. For the combined effect assessment, usually CA based predictions for groups of chemicals eliciting similar effects or showing a similar MoA are used. The available reference values from substance authorisation dossiers used in single substance RA are often based on the most sensitive endpoint measured. This is however not necessarily the one for the effect under consideration. To use such reference values in lower tier assessments is acceptable; however, more detailed information on specific effects is needed for further refinements and is often not available.

More data on the underlying MoA are also needed to improve grouping approaches for mixture components. The AOP concept has been shown to provide a valuable framework to map available toxicity data for mixture components to key events in AOP networks relevant for grouping as well as to identify data gaps and tailored testing strategies (Ankley et al., 2010).

The limitation in the availability of appropriate exposure and toxicity data has a direct impact on the uncertainty of the RA outcome. As a matter of fact, data gaps are identified as the major issue when it comes to RA of chemical mixtures, especially when dealing with particular uses or population subgroups (i.e. amateur uses of pesticides, frequency of use of cosmetic for children). The integration of data that were originally derived under different scope and that are not always directly comparable, is linked to an increased uncertainty in the assessment of combined effects.

5.5. Most commonly studied compound groups in mixture risk assessment.

Pesticides followed by pharmaceuticals and personal care products dominated the observed mixture effects in the case studies, whereas chemicals regulated under REACH and plant protection products were the areas where most experts participating in the survey had experience. Tributyltin, polycyclic aromatic hydrocarbons, and brominated flame retardants are also major contributors to the environmental chemical risk of the monitored chemicals. Human exposure to parabens, phthalates and more generally anti- androgenic chemicals seems to be of concern, particularly for highly exposed or more sensitive population subgroups.

5.6. Use of novel concepts and tools for the hazard assessment of mixtures.

A high potential in applying novel tools and scientific methodologies (e.g. AOPs, *in vitro* methods, omics, *in silico* approaches, toxicokinetic modelling, TTC approach, IATA) for the assessment of chemical mixtures was identified based on a literature review and the expert survey (Bopp et al., 2015). These tools allow meaningful information to be obtained on individual mixture components or whole mixtures, enabling a better understanding of the underlying mechanisms of mixture effects. Their main strengths lie in their integrated use and ability to put different aspects regarding the hazard from combined exposure to multiple chemicals into context. In order to benefit from these tools in the hazard assessment of mixtures, more guidance on their use is needed to facilitate a more widespread application. In the survey, a lack of guidance, lack of data, and lack of expertise were frequently cited as main reasons hampering the application of novel concepts and tools.

6. Conclusion

Model specifications, exposure and toxic reference values used can greatly influence the outcome of a mixture RA. Therefore, it is crucial to properly document and justify the choices that have been made, and to carefully interpret the results considering the underlying hypothesis, the related uncertainties, and the degree of conservatism that has been chosen. Two guidance documents have recently been published on characterisation of uncertainties in RA (EFSA SC, 2015; WHO, 2014b), however the assessment of uncertainty in the hazard characterisation for mixtures and for cumulative exposure to multiple stressors still need to be further investigated (WHO, 2014b).

Several frameworks for the assessment of chemical mixtures have been developed by international bodies in recent years, i.e. WHO/IPCS (Meek et al., 2011; Price et al., 2012; SCHER et al., 2012). These frameworks provide high-level guidance as well as tiered approaches for screening level assessments and further refinements. A limitation in their application arises however due to the lack of data for performing higher tier assessments. Therefore, there are still many open issues and more detailed guidance is needed, that harmonises approaches used across different legislative sectors. There is now a need to build on all these frameworks to develop a robust and transparent approach not only for conducting, but also reporting a chemical mixture RA.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.yrtph.2016.05.020.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2016.05.020.

References

Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrrano, J.A., Tietge, J.E., Villeneuve, D.L., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ. Toxicol. Chem. 29, 730–741. http://dx.doi.org/10.1002/etc.34.

Backhaus, T., Arrhenius, A., Blanck, H., 2004. Toxicity of dissimilarly acting substances to natural algal communities: predictive power and limitations of IA and CA. Environ. Sci. Technol. 6363–6370.

Backhaus, T., Faust, M., 2012. Predictive environmental assessment of chemical mixtures: a conceptual framework. Environ. Sci. Technol. 2564–2573.

Backhaus, T., Karlsson, M., 2014. Screening level mixture risk assessment of pharmaceuticals in STP effluents. Water Res. 49, 157–165. http://dx.doi.org/10.1016/j.watres.2013.11.005.

Becker, K., Göen, T., Seiwert, M., Conrad, A., Pick-Fuss, H., Müller, J., Wittassek, M., Schulz, C., Kolossa-Gehring, M., 2009. GerES IV: phthalate metabolites and bisphenol A in urine of German children. Int. J. Hyg. Environ. Health 212, 685–692. http://dx.doi.org/10.1016/j.ijheh.2009.08.002.

Belanger, S.E., Sanderson, H., Embry, M.R., Coady, K., DeZwart, D., Farr, B.A., Gutsell, S., Halder, M., Sternberg, R., Wilson, P., 2015. It is time to develop ecological thresholds of toxicological concern to assist environmental hazard assessment. Environ. Toxicol. Chem. 34, 2864–2869. http://dx.doi.org/10.1002/etc.3132.

Berg, T., Fürhaupter, K., Teixeira, H., Uusitalo, L., Zampoukas, N., 2015. The marine strategy framework directive and the ecosystem-based approach – pitfalls and solutions. Mar. Pollut. Bull. 96, 18–28. http://dx.doi.org/10.1016/j.marpolbul.2015.04.050.

Boobis, A., Budinsky, R., Collie, S., Crofton, K., Embry, M., Felter, S., Hertzberg, R.,

Kopp, D., Mihlan, G., Mumtaz, M., Price, P., Solomon, K., Teuschler, L., Yang, R., Zaleski, R., 2011. Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment. Crit. Rev. Toxicol. 41, 369–383. http://dx.doi.org/10.3109/10408444.2010.543655.

Boon, P.E., van Donkersgoed, G., Christodoulou, D., Crépet, A., D'Addezio, L., Desvignes, V., Ericsson, B.-G., Galimberti, F., Ioannou-Kakouri, E., Jensen, B.H., Rehurkova, I., Rety, J., Ruprich, J., Sand, S., Stephenson, C., Strömberg, A., Turrini, A., van der Voet, H., Ziegler, P., Hamey, P., van Klaveren, J.D., 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 Chem. Toxicol. 79, 13–31. http://dx.doi.org/10.1016/j.fct.2014.08.004.

Bopp, S., Berggren, E., Kienzler, A., van der Linden, S., Worth, A., 2015. Scientific Methodologies for the Assessment of Combined Effects of Chemicals – a Survey and Literature Review.

Bunke, D., Groß, R., Kalberlah, F., Oltmanns, J., Schwarz, M., Reihlen, A., Reineke, N., 2013. 4M: Mixtures under REACH. Concepts and Options to Act. Mixtures in the Environment – Development of Assessment Strategies for the Regulation of Chemicals under REACH.

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

Cedergreen, N., Sørensen, H., Svendsen, C., 2012. Can the joint effect of ternary mixtures be predicted from binary mixture toxicity results? Sci. Total Environ. 427–428, 229–237. http://dx.doi.org/10.1016/j.scitotenv.2012.03.086.

Christiansen, S., Scholze, M., Dalgaard, M., Vinggaard, A.M., Axelstad, M., Kortenkamp, A., Hass, U., 2009. Synergistic disruption of external male sex organ development by a mixture of four antiandrogens. Environ. Health Perspect. 117, 1839–1846. http://dx.doi.org/10.1289/ehp.0900689.

Colin, M.-E., Belzunces, L.P., 1992. Evidence of synergy between prochloraz and deltamethrin in apis mellifera L.: a convenient biological approach. Pest Manag. Sci. 36, 115–119. http://dx.doi.org/10.1002/ps.2780360206.

de Boer, J., Ballesteros-Gómez, A., Leslie, H.A., Brandsma, S.H., Leonards, P.E.G., 2016. Flame retardants: dust – and not food – might be the risk. Chemosphere 2014, 10–13. http://dx.doi.org/10.1016/j.chemosphere.2015.12.124.

De Brouwere, K., Cornelis, C., Arvanitis, A., Brown, T., Crump, D., Harrison, P., Jantunen, M., Price, P., Torfs, R., 2014. Application of the maximum cumulative ratio (MCR) as a screening tool for the evaluation of mixtures in residential indoor air. Sci. Total Environ. 479–480, 267–276. http://dx.doi.org/10.1016/j.scitotenv.2014.01.083.

De Zwart, D., Posthuma, L., 2005. Complex mixture toxicity for single and multiple species: proposed methodologies. Environ. Toxicol. Chem. 24, 2665–2676. http://dx.doi.org/10.1897/04-639r.1.

Delfosse, V., Dendele, B., Huet, T., Grimaldi, M., Boulahtouf, A., Gerbal-Chaloin, S., Beucher, B., Roecklin, D., Muller, C., Rahmani, R., Cavaillès, V., Daujat-Chavanieu, M., Vivat, V., Pascussi, J.-M., Balaguer, P., Bourguet, W., 2015. Synergistic activation of human pregnane X receptor by binary cocktails of pharmaceutical and environmental compounds. Nat. Commun. 6, 8089. http://dx.doi.org/10.1038/ncomms9089.

Delmaar, C., Bokkers, B., Ter Burg, W., Schuur, G., 2015. Validation of an aggregate exposure model for substances in consumer products: a case study of diethyl phthalate in personal care products. J. Expo. Sci. Environ. Epidemiol. 25, 317–323. http://dx.doi.org/10.1038/jes.2014.68.

Dewalque, L., Charlier, C., Pirard, C., 2014. Estimated daily intake and cumulative risk assessment of phthalate diesters in a Belgian general population. Toxicol. Lett. 231, 161–168. http://dx.doi.org/10.1016/j.toxlet.2014.06.028.

EC, 2012. Communication from the Commission to the Council - the combination effects of chemicals. Chem. Mix. Com. 252 (final. Brussels).

EC, 2011. Common Implementation Strategy for the Water Framework Directive (2000/60/EC) Guidance Document No.27-Technical Guidance for Deriving Environmental Quality Standards. http://dx.doi.org/10.2779/43816.

EC, 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L396.

EC, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. http://dx.doi.org/10.1039/ap9842100196. OJ L327.

ECHA, 2015. Guidance on the Biocidal Products Regulation - Volume III Human Health - Part B Risk Assessment. European Chemicals Agency, Helsinki, Finland.

ECHA, 2014. Transitional Guidance on the Biocidal Products Regulation - Transitional Guidance on Mixture Toxicity Assess Emtn for Biocidal Products for the Environment. European Chemicals Agency, Helsinki, Finland.

ECHA, 2013. Guidance for the Implementation of REACH - Guidance on Information Requirements and Chemical Safety Assessment - Chapter R. 20: Table of Terms and Abbreviations. European Chemicals Agency, Helsinki, Finland.

ECHA, 2012. Guidance on Information Requirements and Chemical Safety Assessment Chapter R. 11: PBT Assessment. European Chemicals Agency, Helsinki, Finland.

EFSA, 2014a. Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile (2014 update). EFSA J. Eur. Food Saf. Auth. Sci. Opin. Parma, Italy. http://dx.doi.org/10.2903/j.efsa.2013.3293.

EFSA, 2014b. Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products. EFSA J. Eur. Food Saf. Auth. Parma, Italy. http://dx.doi.org/10.2903/j.efsa.2014.3589.

EFSA, 2012. Guidance on the Use of Probabilistic Methodology for Modelling Dietary Exposure to Pesticide Residues. European Food Safety Authority, Parma, Italy. http://dx.doi.org/10.2903/j.efsa.2012.2839.

EFSA SC, 2015. Guidance on Uncertainty in EFSA Scientific Assessment. European Food and Safety Authority. Scientific Opinion, Parma, Italy. http://dx.doi.org/10.2903/j.efsa.20YY.NNNN.

EMEA, 2006. Guideline on the Environmental Risk Assessment of Medicinal. European Medicines Agency, London, UK.

EU, 2013a. Regulation (EU) No 283/2013 Setting Out the Data Requirements for Active Substances, in Accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council Concerning the Placing of Plant Protection Products on the Market. OJ L93.

EU, 2013b. Regulation (EU) No 284/2013 Setting Out the Data Requirements for Plant Protection Products, in Accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council Concerning the Placing of Plant Protection Products on the Market. OJ L93.

Evans, R.M., Martin, O.V., Faust, M., Kortenkamp, A., 2015. Should the scope of human mixture risk assessment span legislative/regulatory silos for chemicals? Sci. Total Environ. http://dx.doi.org/10.1016/j.scitotenv.2015.10.162.

Fromme, H., Becher, G., Hilger, B., Völkel, W., 2015. Brominated flame retardants – exposure and risk assessment for the general population. Int. J. Hyg. Environ. Health 219, 1–23. http://dx.doi.org/10.1016/j.ijheh.2015.08.004.

Garthwaite, D., Sinclair, C., Glass, R., Pote, A., Trevisan, M., Sacchettini, G., Spanoghe, P., Ngoc, K.D., Fevery, D., Charistou, A., Nikolopoulou, D., Arapaki, N., Gerritsen-ebben, R., Spaan, S., González, F.E., Stobiecki, S., Śliwiński, W., Stobiecki, T., Hakaite, P., 2015. Collection of Pesticide Application Data in View of Performing Environmental Risk Assessments for Pesticides. EFSA supporting publication, 2015:EN-846.

Gosens, I., Delmaar, C.J.E., Burg, W., Heer, C., De Schuur, A.G., 2013. Aggregate exposure approaches for parabens in personal care products;: a case assessment for children between 0 and 3 years old, 24, 208–214. http://dx.doi.org/10.1038/jes.2013.33.

Gregorio, V., Chèvre, N., 2014. Assessing the risks posed by mixtures of chemicals in freshwater environments: case study of Lake Geneva. Switz. Wiley Interdiscip. Rev. Water 1, 229–247. http://dx.doi.org/10.1002/wat2.1018.

Gregorio, V., Chèvre, N., Junghans, M., 2013. Critical issues in using the common mixture toxicity models concentration addition or response addition on species sensitivity distributions: a theoretical approach. Environ. Toxicol. Chem. 32, 2387–2395. http://dx.doi.org/10.1002/etc.2320.

Groten, J.P., Butler, W., Feron, V.J., Kozianowski, G., Renwick, a G., Walker, R., 2000. An analysis of the possibility for health implications of joint actions and interactions between food additives. Regul. Toxicol. Pharmacol. 31, 77–91. http://dx.doi.org/10.1006/rtph.1999.1356.

Gutiérrez, S., Fernández, C., Escher, B.I., Tarazona, J.V., 2008. A new hazard index of complex mixtures integrates bioconcentration and toxicity to refine the environmental risk assessment of effluents. Environ. Int. 34, 773–781. http://dx.doi.org/10.1016/j.envint.2008.01.002.

Han, X., Price, P.S., 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, 343–349. http://dx.doi.org/10.1038/jes.2012.74.

Han, X., Price, P.S., 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, 4729–4745. http://dx.doi.org/10.3390/ijerph8124729.

Hoffman, K., Stapleton, H., 2015. Exposure to organophosphate flame retardants: hand wipes and house dust. Environ. Health Perspect. 123, 160–165. http://dx.doi.org/10.1289/ehp.1408669.

Jeong, C.H., Wagner, E.D., Siebert, V.R., Anduri, S., Richardson, S.D., Daiber, E.J., Mckague, A.B., Kogevinas, M., Villanueva, C.M., Goslan, E.H., Luo, W., Isabelle, L.M., Pankow, J.F., Grazuleviciene, R., Cordier, S., Edwards, S.C., Righi, E., Nieuwenhuijsen, M.J., Plewa, M.J., 2012. Occurrence and toxicity of disinfection byproducts in european drinking waters in relation with the HIWATE epidemiology study. Environ. Sci. Technol. 46, 12120–12128.

Junghans, M., Backhaus, T., Faust, M., Scholze, M., Grimme, L.H., 2006. Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. Aquat. Toxicol. 76, 93–110. http://dx.doi.org/10.1016/j.aquatox.2005.10.001.

Kennedy, M.C., Glass, C.R., Bokkers, B., Hart, A.D.M., Hamey, P.Y., Kruisselbrink, J.W., de Boer, W.J., van der Voet, H., Garthwaite, D.G., van Klaveren, J.D., 2015. A European model and case studies for aggregate exposure assessment of pesticides. Food Chem. Toxicol. 79, 32–44. http://dx.doi.org/10.1016/j.fct.2014.09.009.

Kienzler, A., Berggren, E., Bessems, J., Bopp, S., Van Der Linden, S., Worth, A., 2014. Assessment of Mixtures - Review of Regulatory Requirements and Guidance. JRC Science and Policy Report. European Commission, Joint Research Center, Ispra, Italy. http://dx.doi.org/10.2788/84264.

Kortenkamp, A., Backhaus, T., Faust, M., 2009. State of the Art Report on Mixture Toxicity - Final Report, 391 pp.

Kortenkamp, A., Faust, M., 2010. Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment. Int. J. Androl. 33, 463–474. http://dx.doi.org/10.1111/j.1365-2605.2009.01047.x.

Laetz, C.A., Baldwin, D.H., Collier, T.K., Hebert, V., Stark, J.D., Scholz, N.L., 2009. The synergistic toxicity of pesticide mixtures: implications for risk assessment and

the conservation of endangered Pacific Salmon. Environ. Health Perspect. 117, 348–353. http://dx.doi.org/10.1289/ehp.0800096.

Law, R., Hanke, G., Angelidis, M., Batty, J., Bignert, A., Dachs, J., Davies, I., Denga, Y., Duffek, A., Herut, B., Hylland, K., Lepom, P., Leonards, P., Mehtonen, J., Piha, H., Roose, P., Tronczynski, J., Velikova, V., Vethaak, D., 2010. JRC Scientific and Technical Reports - Marine Strategy Framework Directive. Task Group 8 Report Contaminants and Pollution Effects. Joint Research Centre, Office for Official Publications of the European Communities. http://dx.doi.org/10.2788/85887.

Le Cann, P., Bonvallot, N., Glorennec, P., Deguen, S., Goeury, C., Le Bot, B., 2011. Indoor environment and children's health: recent developments in chemical, biological, physical and social aspects. Int. J. Hyg. Environ. Health 215, 1–18. http://dx.doi.org/10.1016/j.ijheh.2011.07.008.

Lim, Y.-W., Kim, H.-H., Lee, C.-S., Shin, D.-C., Chang, Y.-S., Yang, J.-Y., 2014. Exposure assessment and health risk of poly-brominated diphenyl ether (PBDE) flame retardants in the indoor environment of elementary school students in Korea. Sci. Total Environ. 470–471, 1376–1389. http://dx.doi.org/10.1016/j.scitotenv.2013.09.013.

Malaj, E., von der Ohe, P.C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. Proc. Natl. Acad. Sci. U. S. A. 111, 9549–9554. http://dx.doi.org/10.1073/pnas.1321082111.

Meek, M.E.B., Boobis, A.R., Crofton, K.M., Heinemeyer, G., Raaij, M. Van, Vickers, C., 2011. Risk assessment of combined exposure to multiple chemicals: a WHO/IPCS framework. Regul. Toxicol. Pharmacol. 60, S1–S14. http://dx.doi.org/10.1016/j.yrtph.2011.03.010.

Nieuwenhuijsen, M., Smith, R., Golfinopoulos, S., Best, N., Bennett, J., Aggazzotti, G., Righi, E., Fantuzzi, G., Bucchini, L., Cordier, S., Villanueva, C., Moreno, V., La Vecchia, C., Bosetti, C., Vartiainen, T., Rautiu, R., Toledano, M., Iszatt, N., Grazuleviciene, R., Kogevinas, M., 2009. Health impacts of long-term exposure to disinfection by-products in drinking water in Europe: HIWATE. J. Water Heal 7, 185–207.

Nowell, L.H., Norman, J.E., Moran, P.W., Martin, J.D., Stone, W.W., 2014. Pesticide Toxicity Index-a tool for assessing potential toxicity of pesticide mixtures to freshwater aquatic organisms. Sci. Total Environ. 476–477, 144–157. http://dx.doi.org/10.1016/j.scitotenv.2013.12.088.

OECD, 2011. In: WHO OECD ILSI/HESI International Workshop on Risk Assessment of Combined Exposures to Multiple Chemicals. Series on Testing and Assessment No 140. Organisation for Economic Co-operation and Development. 77pp.

Ohe, T., Watanabe, T., Wakabayashi, K., 2004. Mutagens in surface waters: a review. Mutat. Res. Rev. Mutat. Res. 567, 109–149.

Price, P., Han, X., Junghans, M., Kunz, P., Watts, C., Leverett, D., 2012. 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. Environ. Sci. Eur. 24, 34. http://dx.doi.org/10.1186/2190-4715-24-34.

Price, P., Zaleski, R., Hollnagel, H., Ketelslegers, H., Han, X., 2014. Assessing the safety of co-exposure to food packaging migrants in food and water using the maximum cumulative ratio and an established decision tree. Food Addit. Contam. Part A. Chem. Anal. control. Expo. Risk Assess. 31, 414–421. http://dx.doi.org/10.1080/19440049.2013.865145.

Qian, H., Chen, M., Kransler, K.M., Zaleski, R.T., 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, 249–255. http://dx.doi.org/10.1038/jes.2014.24.

Reif, D.M., Martin, M.T., Tan, S.W., Houck, K. a., Judson, R.S., Richard, A.M., Knudsen, T.B., Dix, D.J., Kavlock, R.J., 2010. Endocrine profling and prioritization of environmental chemicals using toxcast data. Environ. Health Perspect. 118, 1714–1720. http://dx.doi.org/10.1289/ehp.1002180.

Reif, D.M., Sypa, M., Lock, E.F., Wright, F.A., Wilson, A., Cathey, T., Judson, R.R., Rusyn, I., 2013. ToxPi GUI: an interactive visualization tool for transparent integration of data from diverse sources of evidence. Bioinformatics 29, 402–403. http://dx.doi.org/10.1093/bioinformatics/bts686.

Rico, A., Van den Brink, P.J., Gylstra, R., Focks, A., Brock, T.C.M., 2015. Developing ecological scenarios for the prospective aquatic risk assessment of pesticides. Integr. Environ. Assess. Manag. http://dx.doi.org/10.1002/ieam.1718 n/a–n/a.

Salthammer, T., Fuhrmann, F., Uhde, E., 2003. Flame retardants in the indoor environment - Part II: release of VOCs (triethylphosphate and halogenated degradation products) from polyurethane. Indoor Air 13, 49–52. http://dx.doi.org/10.1034/j.1600-0668.2003.01150.x.

Sammataro, D., Yoder, J.A., 2011. Honey Bee Colony Health: Challenges and Sustainable Solutions. CRC Press, 320 pp.

Sarigiannis, D.A., Karakitsios, S.P., Gotti, A., Liakos, I.L., Katsoyiannis, A., 2011. Exposure to major volatile organic compounds and carbonyls in European indoor environments and associated health risk. Environ. Int. 37, 743–765. http://dx.doi.org/10.1016/j.envint.2011.01.005.

SCCS, 2015. SCCS Notes of Guidance for the Testing of Cosmetic Ingredients, 9th revision. SCCS/1564/15. SCCS/1564/15.

SCHER, SCCS, SCENIHR, 2012. SCHER, SCCS, SCENIHR, Opinion on the Toxicity and Assessment of Chemical Mixtures. European Commission, Brussels.

Tang, J.Y.M., Busetti, F., Charrois, J.W.A., Escher, B.I., 2014. Which chemicals drive biological effects in wastewater and recycled water? Water Res. 60, 289–299. http://dx.doi.org/10.1016/j.watres.2014.04.043.

USEPA, 2007. Revised N -Methyl Carbamate Cumulative Risk Assessment (Washington DC).

USEPA, 2006a. Cumulative Risk from Chloroacetanilides Pesticides (Washington DC).

USEPA, 2006b. Triazine Cumulative Risk Assessment (Washington DC).

USEPA, 2006c. Considerations for Developing Alternative Health Risk Assessment Approaches for Addressing Multiple Chemicals, Exposures and Effects - EPA/600/R-06/013A (Washington DC).

USEPA, 2003. Framework for Cumulative Risk Assessment - EPA/630/P-2/001F (Washington DC).

USEPA, 2002a. Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity (Washington DC).

USEPA, 2002b. Organophosphate Pesticides: Revised Cumulative Risk Assessment (Washington DC).

USEPA, 2000. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures (Washington DC).

USEPA, 1999. Guidance for Identifying Pesticide Chemicals and Other Substances that Have a Common Mechanism of Toxicity (Washington DC).

USEPA, 1989. Risk Assessment Guidance for Superfund Volume I Human Health Evaluation Manual-part A-EPA/540/1-89/002 (Washington, DC).

USEPA, 1987. The Risk Assessment Guidelines of 1986-EPA/600/8-87/045 (Washington, DC).

WHO, 2014a. Combined or Multiple Exposure to Health Stressors in Indoor Built Environments - An Evidence-based Review Prepared for the WHO Training Workshop "Multiple Environmental Exposures and risks.". World Health Organization, 82 pp.

WHO, 2014b. Guidance Document on Evaluating and Expressing Uncertainty in Hazard Characterization. World Health Organization. IPCS Harmonization project document no 11. 181 pp.

WHO IPCS, 2009. Assessment of Combined Exposures to Multiple Chemicals: Report of a WHO/IPCS International Workshop on Aggregate/cumulative Risk. World Health Organization. IPCS harmonization project document n° 7. 83 pp.