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Review of case studies on the human and environmental risk assessment of chemical mixtures

Identification of priorities, methodologies, data gaps, future needs

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Abstract

Humans and wildlife can be exposed to an infinite number of different combinations of chemicals in mixtures via food, consumer products and the environment, which might impact health. The number of chemicals and composition of chemical mixtures one might be exposed to is often unknown and changing over time. To gain further insight into the current practices and limitations, published peer reviewed literature was searched for case studies showing risk assessments for chemical mixtures. The aim was to find examples of mixture assessments in order to identify chemical mixtures of potential concern, methodologies used, factors hampering mixture risk assessments, data gaps, and future perspectives.

Twenty-one case studies were identified, which included human and environmental risk assessments. Several compound classes and environmental media were covered, i.e. pesticides, phthalates, parabens, polybrominated diphenyl esters (PBDEs), pharmaceuticals, food contact materials, dioxin-like compounds, anti-androgenic chemicals, contaminants in breast milk, mixtures of contaminants in surface water, ground water and drinking water, and indoor air. However, the selection of chemical classes is not necessarily representative as many compound groups have not been covered. The selection of these chemical classes is often based on data availability, recent concerns about certain chemical classes or legislative requirements. Several of the case studies revealed a concern due to combined exposure for certain chemical classes especially when considering specific vulnerable population groups. This is very relevant information, but needs to be interpreted with caution, considering the related assumptions, model parameters and related uncertainties. Several parameters that could lead to an over- or underestimation of risks were identified. However, there is clear evidence that chemicals need to be further addressed not only in single substance risk assessment and that mixtures should be considered also across chemical classes and legislative sectors.

Furthermore, several issues hampering mixture risk assessments were identified. In order to perform a mixture risk assessment, the composition of the mixture in terms of chemical components and their concentrations need to be known, and relevant information on their uptake and toxicity are required. Exposure data are often lacking and need to be estimated based on production and use/consumption information. Moreover, relevant toxicity data are not always available. Toxicity data gaps can be filled e.g. using the Threshold of Toxicological Concern (TTC) approach. Reference values used in single substance risk assessments can be found for several chemical classes, however, they are usually derived based on the lowest endpoint. If a refined toxicity assessment of a mixture for a specific effect/cumulative assessment group is envisaged, this is often hampered by a lack of specific toxicity and mode of action information.

In all case studies, concentration addition based assessments were made, mainly applying the Hazard Index. To further characterise the drivers of the mixture risk, the maximum cumulative ratio was calculated in several case studies. This showed that the scientific methodologies to address mixtures are mostly agreed and lead to reasonable predictions. However, especially for some groups of compounds that are designed as active substances, it cannot be excluded that interactions occur and they should therefore be addressed on a case-by-case basis.

Most of the mixtures addressed in the identified case studies examined specific chemical groups. Only few of them looked at mixtures comprising chemicals regulated under different legislative frameworks. The examples indicated that there is evidence for combined exposure to chemicals regulated under different legislation as well as evidence that such chemicals can elicit similar effects or have a similar mode of action. A mixture risk assessment across regulatory sectors should therefore be further investigated.

1 Introduction

Humans and wildlife can be exposed to chemicals via food, consumer products and the environment, which might impact their health. The number of chemicals and composition of chemical mixtures one might be exposed to is often unknown and changing over time, resulting in an infinite number of different combinations. In 2012, the European Commission published a communication on the combined effect of chemicals (EC, 2012) in which the Commission proposed actions to ensure that risks associated with chemical mixtures are properly understood and assessed. The Communication acknowledges that the current EU legislative framework sets strict limits for the amounts of particular chemicals allowed in food, water, air and manufactured products, but that the potential risks of exposure to these chemicals in combination are rarely examined.

Individual regulations already focus on the assessment of mixtures to some extent, e.g. Regulation (EC) No. 1107/2009, indicates that "interaction between active substance, safeners, synergists, and co-formulants shall be taken into account", and product authorisation for plant protection products and biocidal products requires the assessment of "cumulative and synergistic effects" of the formulations containing more than one active substance and/or "substance of concern". But even though methodologies for assessing/estimating the combination effects of chemicals are being developed and used by scientists and regulators in specific circumstances, so far there is no systematic, comprehensive and integrated approach across different pieces of legislation (Kienzler et al., 2014; Kienzler et al., 2016; EC, 2012).

To gain further insight into the current practices and experiences in performing chemical mixture assessments, the JRC conducted an expert survey to gather information on the current approaches, scientific methodologies, priorities and gaps (Bopp et al., 2015). The survey showed that the main sectors where most experience is already gained in assessing mixtures are in the area of plant protection products and chemicals under REACH. These were also rated highest regarding the priority for performing mixture assessments. However, mixture assessments are also performed in many other areas. Experts had experience with assessing mixtures, both in the context of human health and environmental risk assessment. Mostly concentration addition (CA) based methods are used for predicting mixture effects. In contrast, several experts did not recommend the further use of independent action (IA) based approaches, mainly because of the higher need for input data for IA and considering the small differences in predictions by IA compared to CA. Novel tools (such as *in vitro* methods, omics, Quantitative Structure Activity Relationships (QSARs), read-across, Physiologically Based ToxicoKinetic (PBTK) modelling, Threshold of Toxicological Concern (TTC) approaches, Adverse Outcome Pathways (AOPs), Dynamic Energy Budget (DEB) models, Integrated Approaches to Testing and Assessment (IATA)), are being increasingly used in the hazard assessment of mixtures, but mainly in a research context for the time being, because of a lack of guidance, data and expertise. A general need for clear and harmonised guidance – also between different legislations - for combined exposure assessments can be identified from the survey.

As a next step, published peer reviewed literature was searched for case studies showing risk assessments for chemical mixtures. The aim was to find examples of mixture assessments in order to identify chemical mixtures of potential concern, methodologies used, factors hampering mixture risk assessments, data gaps, and future perspectives. All case studies identified were screened and relevant information extracted. In the following parts of the report, the approach used for the case studies review (section 2), the findings from the case studies (section 3), the discussion of the findings (section 4) and the overall conclusions (section 5) can be found along with the detailed presentation of the individual case studies in Annex I.

2 Methodology

To identify potential case studies, a literature search was performed, taking into account all kinds of chemicals (i.e. not limited to a specific class of chemicals) and all kinds of exposure scenarios (to intentional and unintentional mixtures through all routes and pathways), including human health and environmental risk assessments. Assuming that more recent case studies would include more up to date knowledge and methodology, the review was restricted to the years 2014 until May 2016. Several additional case studies cited from earlier years were included for completion.

The literature was continuously screened using SCOPUS since the beginning of 2014, to find all articles related to search terms such as "combined exposure", "aggregate exposure", "cumulative risk assessment", "cumulative toxicity", "combined toxicity", "mixture toxicity" and "mixture effect". Criteria for the selection of relevant publications were to include only papers showing a mixture risk assessment or testing real samples or realistic artificial samples, in order to assess potential risks. Papers that were exclusively looking at exposure or exclusively reporting testing of combined effects without making the relation between combined exposure and combined effects were not considered as a relevant case study in this review. Several of those excluded papers are however included in the overall discussion. This approach resulted in a more detailed review of 21 relevant case studies.

In order to extract the information in a standardised way and present the case studies in an easily comparable format, a reporting template was developed and compiled for each case study (see Annex 1). For each case study the following was reported:

1. Reference (title, journal, authors and year of publication)
2. Substances assessed
3. Exposure scenario
4. Background and objectives of the case study
5. Problem formulation according to the WHO/IPCS mixture assessment framework
6. Information/data sources used
7. Mixture assessment methodology
8. Overall summary of the outcome and the future perspectives (as provided by the authors in the publications).

WHO/IPCS has published a framework for the risk assessment of combined exposure to multiple chemicals, which describes a general approach for risk assessment of combined exposure to multiple chemicals that can be adapted to the needs of specific users. The framework provides a tiered approach for both the exposure and the hazard assessment. Tier 0 is a screening level assessment using simple semi-quantitative estimates of exposure and default dose addition for all components together. The hazard assessment is based on available toxicity values, TTC values or the value for the most potent component is used for all components as conservative approach. Tier 1 assessments use generic exposure scenarios based on conservative point estimates, while for the hazard assessment individual points of departure (POD) are used. For Tier 2 the exposure assessment is further refined using increasingly measured data, while the hazard assessment is based on refined potency information and groupings based on mode of action is performed. Tier 3 uses probabilistic exposure estimates and ideally leads to probabilistic information on the risk. The Tier 3 hazard assessment includes toxicokinetic aspects in order to consider internal exposure.

An important step in the framework before conceiving of a mixture risk assessment is the related problem formulation. It was therefore decided to include for each case study

the four related questions shown here below from the WHO/IPCS framework (Meek et al., 2011):

1- *What is the nature of exposure? Are the key components known? Are there data available on the hazard of the mixture itself* (i.e., not extrapolated from the hazards presented by the components of the mixture)?

2- *Is exposure likely, taking into account the context?* i.e. for substances that would be used only as industrial intermediates and are not expected to be released in the general environment, the answer would be no.

3- *Is there a likelihood of co-exposure within a relevant timeframe?* To answer this question, the temporal aspects of external exposure, toxicokinetics and toxicodynamics should be taken into consideration. If, based on consideration of those aspects, the likelihood of co-exposure is low, a framework analysis of an assessment group is unnecessary. Biomonitoring data should also be considered as they may indicate co-occurrence of substances in the human body or elimination products.

4- *What is the rationale for considering compounds in an assessment group?* Grouping of chemicals is commonly based on chemical structures, using predictive tools such as (Q)SARs, but may also be based on biological information, e.g. on downstream mechanistic events using *in vitro* methods, based on same outcome, same target organ.

3 Overview of the case study characteristics

Twenty-one case studies were finally selected within the scope of this review and examined further. Relevant information was extracted from the identified papers and was summarised in tables as it was reported by the case study authors. All detailed tables for each individual case study can be found in Annex 1. Table 1 here below shows an overview of the case studies indicating types of mixtures and methodologies included in the assessments.

The case studies were examined regarding the substances that were assessed, the exposure scenario that was considered, the methodology used for assessing hazard, exposure and risk of the mixtures, with the aim to identify potential issues regarding data availability and available methodologies.

Human *vs* environmental risk assessment (RA): Fourteen of the case studies performed a human health risk assessment (HRA), 6 an environmental risk assessment (ERA), and 1 study included both types of assessment for the same samples (exposure to contaminants in surface waters).

Compound classes: Fourteen of the 21 case studies were looking at specific chemical groups (i.e. 6 at pesticides, 2 at phthalates, 1 at parabens, 1 at PBDEs, 1 at pharmaceuticals in general, 1 at antibiotics, 1 at food contact materials including 3 different cases, 1 at dioxin-like compounds). Furthermore, there were 7 case studies focusing on cross-sectorial mixtures, i.e. including chemicals regulated under different regulatory silos, such as human exposure to anti-androgenic chemicals, contaminants in breast milk, 4 studies on mixtures of contaminants in water samples (including surface water, ground water and drinking water) and 1 on indoor air.

Retrospective *vs* prospective RA: Most of the case studies use (bio)monitoring data for the exposure assessment and perform a retrospective RA. However, the same methodology could also be applied for prospective RA. The example of Junghans et al. (2006) shows that predicted environmental concentrations (here for pesticides using FOCUS[1] scenarios), can be used as well.

Types of toxicity and exposure data used: Most of the case studies were using toxicity data extracted from peer reviewed literature or from regulatory authority reports and databases. In some case studies mixture toxicity was also measured. Toxicity test endpoint values such as No observed adverse effect levels (NOAELs) or effective concentrations (EC_{50}) were used as well as reference values including the relevant safety factors used in single substance risk assessments, like acceptable daily intake (ADI) values. Exposure data were mostly based on environmental or human biomonitoring data, but also estimated based on e.g. sales and consumption data or predicted by more sophisticated modelling, e.g. based on food consumption and food residue data.

Mathematical approaches used: All case studies applied concepts based on the Concentration Addition (CA) approach (for general information on the commonly used approaches please refer to e.g. Kienzler et al. (2014). In one case study (Junghans et al., 2006) the CA approach was compared to Independent Action (IA) based predictions. Most of the studies used the Hazard Index (HI) approach. The Sum of Toxic Units (Σ TU) concept was also used frequently mainly in ERA. In one case study (Han and Price, 2013) the Toxic Equivalency (TEQ) concept was applied for dioxin-like compounds. Also the Maximum Cumulative Ratio (MCR) (Price and Han, 2011), which can help to identify the drivers of combined risks, was used in six cases.

[1] FOCUS: FOrum for Co-ordination of pesticide fate models and their USe. http://esdac.jrc.ec.europa.eu/projects/focus-dg-sante

Table 1: Overview of main characteristics of the reviewed case studies

Study ID	Reference	Chemical sector	HH	ERA	Exposure data	Hazard data	Assessment groups	RA method
1	Junghans et al 2006	Pesticides		X	Modelled exposure (FOCUS scenario R1)	Measured algal toxicity of individual compounds and mixture	No grouping	CA vs IA, Σ TU
2	Nowell et al 2014	Pesticides		X	Literature data	Toxicity data from databases such as USEPA ecotox DB	No grouping	Pesticide toxicity index (based on CA)
3	Kennedy et al 2015	Pesticides	X		Modelled dietary and non-dietary aggregate exposure (MCRA tool), using optimistic and pessimistic scenario	From literature or dossiers	Based on similar toxic effect	RPF within group
4	Boon et al 2015	Pesticides (triazoles)	X		Modelled dietary exposure (MCRA tool) according to EFSA guidance, optimistic and pessimistic scenario	From literature or dossiers	Based on similar toxic effect (hepatotoxicity)	RPF within group
5	Evans et al 2015	Pesticides	X		International Estimated Daily Intakes (IEDI)	ADIs from JMPR	No classical groups, but grouping according to health impact categories in Tier 2	WHO/IPCS framework, HI, Tier 0, Tier 1, Tier 2

Study ID	Reference	Chemical sector	HH	ERA	Exposure data	Hazard data	Assessment groups	RA method
6	Ccanccapa et al 2016	Pesticides		X	Sediment concentrations used to predict pore water concentrations	Collected from pesticide property database	No, all together based on acute toxicity	Σ TU
7	Dewalque et al 2014	Phthalates	X		Human biomonitoring data; Exposure data from food consumption etc. to estimate contribution of dietary and non-dietary sources	EFSA TDI and RV from literature	Structurally similar group with similar toxic profile	HI
8	Hartmann et al 2015	Phthalates from consumer products	X		14 metabolites detected in urine sample from 10 parent phthalates	Reference dose for anti-androgenicity, tolerable daily intake	Dose-addition concept is considered for anti-androgenic phthalates	Cumulative risk assessment
9	Meek et al 2011 Case study A	PBDEs, based on commercial mixtures	X		Semi-quantitative estimates based on volume of production, producing and using companies (Tier 0), use of Canadian intake data (Tier 1); comparison to biomonitoring data	No TDI data, instead LOEL for most toxic congener (Tier 0), critical effect level derived from all data on neurobehavioral effects (Tier 1)	One group of 7 isomers with similar use and common target organ	Tier 0 and Tier 1 of WHO/IPCS framework; sum of risk quotient like approach using the data outlined

Study ID	Reference	Chemical sector	HH	ERA	Exposure data	Hazard data	Assessment groups	RA method
10	Gosens et al 2013	Parabens from personal care products	X		Aggregate exposure of children of 0-3 years 4 most frequent parabens, not considering combined exposure Tier 1 WC deterministic approach; Tier 2: probabilistic based on product use	NOAEL data	n.a., only aggregate exposure considered	n.a.
11	Backhaus and Karlsson 2014	Pharma-ceuticals		X	Literature data on sewage treatment plant effluents	From literature and databases	No grouping, similarly and dissimilarly acting compounds considered together	comparison of sum of PEC/PNEC ratios to ΣTU, use of MCR
12	Marx et al 2015	Antibiotics		X	Predicted PEC from hospital and ambulant antibiotic prescription information	PNECs from literature on bacteria, algae, daphnia	All in one group and division of contribution of different categories of antibiotics to HI	HI and HI_{int}
13	Price et al 2014	food contact materials	X		Literature data	Use of existing ADI data or TTC	No grouping	HI and MCR, CEFIC decision tree
14	Han and Price 2013	Dioxin-like compounds	X		Human biomonitoring data for general population and two	WHO TEF values	All in one group since known for common	TEQ and MCR based on TEQs

Study ID	Reference	Chemical sector	HH	ERA	Exposure data	Hazard data	Assessment groups	RA method
		(DLCs)			groups of workers with relevant occupational exposure		effects/MoA	
15	Kortenkamp and Faust 2010	15 anti-androgens (phthalates, pesticides, cosmetic ingredients)	X		Data from literature and public databases; using median human intake and highly exposed population groups	Reference values (NOAEL, BMD) for anti-androgenicity	All in one group selected for similar action (anti-androgenicity)	HI
16	Price et al 2012a	Organic and inorganic compounds detected in surface water and effluent samples	X	X	Use of large dataset of surface water (sw) and effluent monitoring; for sw direct consumption assumed as WC, for effluents a 10x dilution; 2 options for non-detects compared	RV from literature and databases, if not available TTC	No grouping	HI and MCR, CEFIC decision tree
17	Han and Price 2011	Ground-water (VOCs, PPPs, metals, inorganics)	X		USGS groundwater monitoring data, direct groundwater consumption as WC assumption	Permitted doses from USEPA and ATSDR databases	No grouping	HI and MCR
18	Meek et al 2011 Case study B	10 substances found in surface water monitoring to create	X		Based on surface water monitoring data to create hypothetical case study on human exposure via water consumption	Use of TTC (pretending the case that no toxicity data are available)	All in one group as WC for Tier 0	HI

Study ID	Reference	Chemical sector	HH	ERA	Exposure data	Hazard data	Assessment groups	RA method
		hypothetical mixture (fragrances, PPPs, surfactants, personal care products, solvents, petro-chemicals						
19	Malaj et al 2014	Organic chemicals detected in freshwater		X	Monitoring data of the waterbase; measured concentrations for 223 chemicals from 4000 sites from 91 rivers. Use of C_{mean} and C_{max}.	From toxicity databases. Using experimental, predicted or baseline toxicity data to derive the acute and chronic toxicity threshold using assessment factors.	No grouping	Calculation of a Chemical Risk Index considering 3 organism groups (fish, daphnia, algae), number of sites a chemical occurs and its concentrations related to a river basin, derivation of 5 risk classes, other approaches used to compare sites
20	Evans et al 2016	Cross-sectorial human milk contami-nants	X		From literature data of contaminants in human milk	Reference doses collected from literature and authorities	No grouping	HI

Study ID	Reference	Chemical sector	HH	ERA	Exposure data	Hazard data	Assessment groups	RA method
21	De Brouwere et al 2014	VOCs and NO_2 in indoor air	X		From indoor air monitoring and personal monitoring studies	Reference values from different sources	No grouping	HI and MCR

4 Discussion of case study characteristics and findings

4.1 Case study summaries in the context of other relevant literature

4.1.1 Pesticides

Pesticides were among the most often investigated mixtures, with three studies regarding human health and three environmental assessments. This might have several reasons, like e.g. the advanced work of the European Food Safety Authority (EFSA) to develop a methodology for cumulative risk assessment of pesticides, but also based on the fact that pesticides are generally data rich chemicals, with extensive data requirements for their approval.

Junghans et al. (2006) showed that pesticides in mixtures are clearly more toxic to algae than any individual component, and that CA showed a good predictive quality over the complete range of effects considered, irrespective of the similarity or dissimilarity of their mechanisms of actions.

Nowell et al. (2014) developed the Pesticide Toxicity Index (PTI) as a relative ranking tool regarding pesticide mixture impacts on freshwater organisms. Two approaches were compared: the median-PTI based on median toxicity values within each taxonomic group, and the sensitive-PTI based on the most sensitive endpoint within a taxonomic group. Both were correlated well, with the median-PTI being more robust to outliers. The median-PTI was proposed for sample and site comparison, while the sensitive-PTI was proposed more for screening level as a conservative tool. Also the PTI is based on CA and does not account for potential interactions.

Ccanccapa et al. (2016) looked at pesticides detected in the river Ebro (Spain), and looked at the potential environmental risks from the 42 detected pesticides and some of their degradation products to aquatic organisms, exposed via water or sediment pore water. The sum of toxic units (Σ TU) based on acute EC_{50} values showed no risk in all sites. However, when using risk quotients (RQ=PEC/PNEC) based on chronic NOEC values for the individual pesticides, some exceeded 1 and could be of concern.

Kennedy et al. (2015) assessed aggregate exposure to conazole pesticides, integrating dietary and non-dietary human exposure. Dietary exposure models used food consumption and pesticide residue data. Non-dietary exposure was modelled for operators and bystanders. Optimistic and pessimistic model scenarios were applied. The outcome was presented as relative contributions from various sources to the overall risks, e.g. showing in the hypothetical cases investigated that for a UK arable spray operator inhalation and dermal exposure may be main routes of exposure, while non-dietary exposure played a minor role for child bystanders. Model specifications had a large impact on the outcome and should be duly justified.

Boon et al. (2015) used the EFSA guidance and applied an optimistic and a pessimistic scenario for the cumulative dietary exposure to triazole pesticides using the Monte Carlo Risk Assessment (MCRA) tool. Regarding acute exposures, in the optimistic model run none of the scenarios exceeded the ARfD, while in one of the pessimistic scenarios an exceedance was predicted. In the chronic assessment, again no exceedance of the ADI were found using the optimistic model run, while 6% and 4.3% of the population had a simulated chronic exposure exceeding the ADI in the pessimistic model run in Denmark and Italy, respectively. The model provides also probabilities for exceedances and related uncertainties. Results for the different countries varied substantially and when using MRLs for the exposure calculations, animal commodities (milk and meat) contributed most to the exposures. The authors concluded that the optimistic model runs are rather easy to perform but probably underestimating exposure, while the pessimistic model run is laborious and might produce unrealistically high exposures.

Evans et al. (2015) applied the WHO/IPCS scheme for the risk assessment of combined exposures to the assessment of 67 pesticides, for which individual assessments by the FAO/WHO JMPR were available. A Tier 0 screening level assessment was performed assuming that no hazard data would be available and TTC values for each compound were used. HI values obtained based on TTCs (Tier 0) were in the range 37.5-146, and were up to 16 times greater than HIs based on ADIs. The Tier 1 assessment based on ADI values and 13 WHO food cluster regions showed an HI>1 in all 13 regions, with one region exceeding an HI of 10. The HI was never driven by just one compound and 80% of the HI were contributed by each 9-18 chemicals in the mixtures. The Tier 2 refined assessment was not possible due to a lack of relevant input data. However, an assessment was presented applying a subgrouping based on the Pesticide Property Database (PPDB[2]) nine human health issues as surrogate information for the mode of action. That way in some cases the resulting HI would remain <1.

4.1.2 Phthalates

Phthalates were assessed in two case studies regarding combined human health effects. Many more studies in the literature address combined effects of or combined exposure to phthalates, but these were the only two recent case studies identified performing a complete risk assessment.

Dewalque et al. (2014) investigated the daily intake of 5 phthalate diesters in the Belgian general population, the contribution of dietary exposure to the overall exposure, and the related risks using general reference values (HI_{TDI}) as well as anti-androgenic specific endpoints (HI_{RfDAA}). Exposure was assessed based on human biomonitoring (HBM) data. Few participants of the study showed a HQ>1 for individual phthalates, but 6.2% of the adults and 25% of the children showed a HI_{TDI}>1. HI_{TDI} was 3-4 fold higher than HI_{RfDAA}, showing the influence of the values used for the hazard assessment. For DEHP the main exposure pathway was via food while for all other congeners dietary intake seemed to play a minor role.

Hartmann et al. (2015) assessed the combined risk from exposure to 11 phthalates and 14 metabolites in children in Austria using HBM data as well. Median HIs based on all acceptable levels of exposure are far below 1. HI>1 was however found using TDIs, whereas no exceedances were found using anti-androgenic RfD.

Dewalque et al. (2014) suggest that larger biomonitoring studies including pertinent biomarkers of exposure to other anti-androgenic compounds should be performed. This wide exposure was also shown earlier by Becker et al. (2009), who detected 12 phthalate metabolites in urine samples from 600 German children from 3 to 14 years old. The phthalate concentrations decreased with increasing age and children contamination were 3-5 times higher than in adults. This might be a situation of concern, as anti-androgenic effects are known for phthalates and exposure could occur at all life stages. Furthermore, phthalates are not the only anti-androgenic chemicals to which humans are exposed. This is confirmed by another case study assessing cumulative anti-androgenic effects of 15 chemicals including phthalates, pesticides, and cosmetics, (Kortenkamp and Faust, 2010), which concludes that although the cumulative risk obtained for median exposures can be judged tolerable, it exceeds acceptable levels for people on the upper end of exposure levels.

4.1.3 Polybrominated diphenyl esters

Polybrominated diphenyl esters (PBDEs) were assessed by Meek et al. (2011) applying the WHO/IPCS framework Tier 0 and Tier 1. Assessments were based on three commercial mixtures. The Tier 0 RA used semiquantitative exposure estimates based on production volume, number of manufacturing plants etc. and the LOEL for the most toxic

[2] http://sitem.herts.ac.uk/aeru/ppdb/en/

congener in the mixture. Thus the HI exceeded 1 and a refinement was performed. The resulting Tier 1 assessment used conservative intake estimates based on maximum levels in air, water, dust, food and human breast milk for six age groups in the Canadian population, versus critical effect levels based on neurobehavioural effects. The margin of exposure (MoE) was ca. 300, while MoEs considering HBM data were 10 fold less with however higher uncertainty. Food was considered the major source of exposure, with highest percentages of dietary contribution for breast-fed infants.

4.1.4 Parabens

Parabens used in personal care products were investigated by Gosens et al. (2013). This study considered no real combined exposure to multiple chemicals, but examined the exposure from various sources to individual substances. Aggregate exposure to 4 parabens for children of 0-3 years of age was assessed in two ways: Tier 1 used a worst-case deterministic approach based on the maximum amounts of parabens used in products and default use amounts to predict exposure. Tier 2 used a person-oriented probabilistic approach. Exposure was then compared to NOAEL values based on male reproductive effects. In Tier 1, for methyl and ethyl paraben, the MoE was above the "safe" MoE of 100, while for propyl- and butyl-paraben the MoE was only 8 and 10, respectively. This might be due to the worst case assumptions, like use of all relevant personal care products in parallel. The more realistic probabilistic approach in Tier 2 including an uncertainty analysis allows deriving fractions of the population that might be exposed above a critical level. This refinement results in a much more realistic assessment but is also very data demanding. Further refinement is difficult as detailed data on the use of personal care products is scarce, especially for children, and it is unknown whether extrapolation from adult use by scaling the amount of product used to body surface is appropriate. Furthermore, those chemicals are also used in other types of products such as pharmaceuticals and as food additives and those uses have never been assessed together, as they are regulated under different legal frameworks. More exposure data via these products would therefore be needed to obtain an even more accurate aggregate estimated exposure to parabens.

4.1.5 Pharmaceuticals

Pharmaceuticals entering the aquatic environment via waste water treatment plant (WWTP) effluents were investigated in Backhaus and Karlsson (2014). Exposure to aquatic organisms was estimated from published monitoring data. For the hazard assessment only limited chronic effect data were available so that the assessment was based on acute toxicity data for algae, daphnia and fish, following the REACH guidance for estimating PNECs with an assessment factor (AF) of 1000. Two different approaches based on CA were used, i.e. a risk quotient based on measured concentrations (MEC) and PNEC values ($RQ=\Sigma(MEC/PNEC)_i$) and a ΣTU (with $TU= MEC/EC_{50}$) for each trophic level, multiplied in the end with the appropriate AF. The maximum cumulative ratio (MCR) was also calculated and showed ratios between 1.2-4.2, meaning that up to 4-5 components were the main drivers of mixture toxicity. The RQ regularly exceeded 1, indicating a potential risk depending on the dilution in the recipient stream. The top ten mixture components contributed more than 95% of the overall mixture risk. Algae were found as most sensitive group, fish as least sensitive. The differences between the two approaches remained within a factor of 1.3.

Marx et al. (2015) looked specifically at mixtures of antibiotics and potential synergistic effects to aquatic organisms exposed via WWTP effluents. Exposure for 15 antibiotics was calculated based on known prescriptions for humans, not including veterinary uses that were considered of lower relevance in the study area. PNEC values were derived from the literature as well as information on binary interactions. Common HI as well as HI_{int} considering interactions were compared. HI over a 7 year period had a mean value of 0.37, with 20% of all weeks exceeding 0.5, and one week exceeding HI>1. The share of contribution to the mixture risk changed between different antibiotic classes over

time, but not the overall HI. HI_{int} showed a 50% increase in risk in a worst-case scenario, so that HI>1 would be found in 25 weeks over 7 years. It needs to be kept in mind that most underlying data on interactions were gained from much higher concentrations compared to realistic environmental exposures.

4.1.6 Food contact materials

Food contact materials were investigated by Price et al. (2014). The Cefic MIAT decision tree for the assessment of mixtures (Price et al., 2012b) was applied to investigate three examples. ADI values (and where not available TTC values) were used for calculating the HI and MCR. All examples were classified into Group II, indicating low concern. MCR values were in the range of 1.3-2.4, indicating that few compounds drive the overall risk. MCR values declined with increasing HI, which means that if the risk increases, fewer compounds drive this risk.

4.1.7 Dioxin-like compounds

Dioxin-like compounds (DLCs) were assessed by Han and Price (2013) for three datasets comprising general population exposure (NHANES [3]), and two worker groups with occupational exposure (MI, Michigan dataset, and NZ New Zealand dataset). For all individuals in the datasets the total TEQs were calculated as well as the MCR values. The top five major contributors to total TEQs in the NHANES dataset were 12378-PeCDD, 123678-HxCDD, PCB 126, TCDD, and 23478-PeCDF. On average they accounted for 76% of the total TEQ. Total TEQs were higher in the MI and NZ datasets than in the NHANES dataset (58.96 fg/g for MI, 25.5 fg/g for NZ, 19.72 fg/g for NHANES) explained by the occupational exposure. Part of the difference is however also due to the different age distributions: i.e. for persons >45 years of age, NHANES total TEQs were lower than in the MI dataset but higher than for the NZ dataset. Average MCR values (including 2.5^{th} percentile and 97.5^{th} percentile) were: for NHANES 3.5 (2.2/5.7), for MI 3.6 (1.6/5.1), and for NZ 3.2 (1.4/4.6). This indicates that for all 3 groups a small number of DLCs drives the total TEQ. As also in other studies analysing the MCR, it showed a decreasing trend with increasing total TEQ values. Overall more highly exposed people tend to have lower MCR values for the MI and NZ dataset, but not for the NHANES dataset. The person age and total TEQs are positively correlated. In the NHANES dataset two groups of age > or < 45 years can be distinguished with persons < 45 years showing generally lower DLC levels and higher MCR values. For all three groups, the MCR values were larger than in other investigations of MCR of different mixtures (Han and Price, 2011; Price et al., 2012a), indicating a greater need for CRA for DLCs. A single substance RA based only on the largest contributor would underestimate the total TEQ by a factor of 2-6. In the case of occupational or local sources of exposure, the impact of performing a CRA compared to single substance RA decreases.

4.1.8 Cross-sectorial and environmental mixtures

Apart from case studies addressing specific chemical groups, several case studies address mixtures of compounds that are regulated under different pieces of legislation, i.e. so-called **cross-sectorial mixtures**. These are sometimes related to known co-exposure via certain consumer products or known co-exposures from HBM or environmental monitoring, but some are also based on known common effects (e.g. anti-androgenic effects).

Kortenkamp and Faust (2010) looked at anti-androgenic effects of 15 compounds including phthalates and other chemicals. Risks were compared using median human intake and highly exposed population groups. Acceptable levels were derived using NOAELs or BMDs applying appropriate uncertainty factors, or ADIs derived on anti-androgenic effects were used. For median exposure an HI of 0.38 was calculated,

[3] http://www.cdc.gov/nchs/nhanes/

whereas for highly exposed populations HI of 2.01 was reached. The authors suggest that risk reductions can be achieved by limiting exposures to the plasticiser diethyl hexyl phthalate, the cosmetic ingredients butyl- and propyl paraben, the pesticides vinclozolin, prochloraz and procymidone and bisphenol A. All those results (considering also Dewalque et al., 2014; Hartmann et al., 2015) indicate that combined exposures to anti-androgens might have reached levels of concern. Moreover, it has to be highlighted that these case studies do not take into account synergistic effects, although synergisms have been observed with a mixture of anti-androgens with diverse mode of action (MoA) for particular endpoints (Christiansen et al., 2009). For all these reasons, further work should be performed to know whether this is a phenomenon of concern that should be taken into account in anti-androgenic RA.

Price et al. (2012a) applied the Cefic MIAT decision tree for assessing combined risks to humans and the environment from exposure via surface water and WWTP effluents. Surface water was assumed to be directly consumed without dilution by humans. A 10-fold dilution was assumed for effluents. All detected chemicals (up to 49 were detected from 222 measured substances) were considered to contribute in an additive way without grouping. The human RA followed the WHO/IPCS Tier 0 using available reference values (RVs) or TTCs, for environmental risk assessment (ERA) RVs were available to determine WHO/IPCS Tier 1. Non-detected chemicals were either assumed to be absent or considered at a concentration of the limit of detection divided by $2^{0.5}$. For human health, 98% of the mixtures showed HI<1. For ERA, 68% of the mixtures had HI>1 with one or more substances individually showing an HQ>1, 19% had HI<1 and about 12% had an HI>1 which was not due to individual substances exceedances. The tree enables to identify the MoA of chemicals where refinements would be most useful. Han and Price (2011) applied the same methodology also to groundwater used for drinking water supplies, with the aim to mainly look at the usefulness and applicability of the MCR approach. They found that MCR is negatively correlated with HI, i.e. the risk of mixtures with higher HI is usually driven by fewer compounds. The way how chemicals below the limit of detection are considered can greatly influence the related MCR for mixtures with small HI, but has little impact on MCR for mixtures with HI>1. MCR is positively correlated with the number of chemicals detected, i.e. the higher the number of chemicals the higher also the MCR. The average MCR in all samples was 2.2-3.1, so mixtures were mainly dominated by few chemicals.

Boobis et al. in Meek et al. (2011) took a similar approach assessing a theoretical mixture of potentially detectable surface water contaminants using the WHO/IPCS Tier 0. For the cross-sectorial mixture of 10 substances, using exposure monitoring data and TTC values, the resulting HI was 0.2. In this case a screening assessment would have been sufficient and no further refinement would have been triggered.

Malaj et al. (2014) investigated the risk for freshwater organisms from organic chemicals on a continental scale, using median and maximum annual exposure concentrations from 4000 European monitoring sites covering 91 European rivers. A chemical risk index for each organism group per river was calculated. The mean concentrations were compared to chronic toxicity thresholds, while the maximum annual concentrations were compared to the acute toxicity threshold. For 14% of sites the organic chemicals were likely to exert acute effects and for 42% chronic effects on sensitive fish, invertebrate or algae species. Major contributors to the risk were pesticides, tributyltin, Polycyclic Aromatic Hydrocarbon (PAHs), brominated flame retardants. Pesticides were responsible for 81, 87, and 96% of observed exceedances of the acute risk threshold for fish, invertebrate and algae, respectively. The risk increased with the number of chemicals analysed per site.

Evans et al. (2016) include a case study on breast-fed children exposed via human milk. It was assumed that all detected chemicals would act in an additive way, but also a subdivision in different chemical categories was performed. HI>1 was identified for several chemical groups, i.e. organochlor pesticides and PCBs. The overall HI for the

whole mixture was 66, indicating a potential risk. Mapping the chemicals to the different regulatory framework shows that some of them fall simultaneously under different legislation and all together several regulatory silos become relevant. This underlines that mixtures need to be addressed across regulatory silos.

In the same context of exposure to unintentional mixture, exposure via indoor air becomes relevant. People in modern society spend approximately 90% of their time indoors, in which 2/3 would be spent at home (Le Cann et al., 2011). De Brouwere et al. (2014) applied the MCR tool to evaluate mixtures in residential indoor air. Several data sets across Europe were compared. The average MCR value was 1.8, with a range from 1 to 5.8. MCR was found to be small compared to the number of chemicals in the mixtures, indicating that generally the overall effect was driven by only a few chemicals. The MCR was significantly declining with increasing HI. The large majority of samples from the Flemish school survey were categorised in the "low concern group II", while Flemish home samples were mostly falling into the "concern for combined effects group III", and to the "single substance concern group I". Most of the OQAI dataset were assigned to the "single substance concern group I". Substances identified as biggest contributors were NO_2, trichloroethylene, acrolein, and xylenes. These were however, not consistently measured in all the studies, so comparison of datasets and overall drivers is difficult. The study showed a significant number of cases where combined effects should be considered further and a chemical-by-chemical approach would be insufficient. However, the mixtures showing concern for combined effects were not those with the highest HIs. Highest HI values were observed for samples where single substances were dominating the overall risk. Personal measurements had generally a higher HI than indoor air measurements. The average ratio for HI from personal measurement vs. indoor air monitoring was 1.5 (range 0.15-19), thus the use of indoor air could lead to some underestimation. A problem was the availability of reliable chronic inhalation toxicity data for non-cancer effects. The choice of the RVs had a large impact on the overall results. Using minimum RVs instead of the basic RVs moved most samples to the group of "single substance of concern I".

4.1.9 Need to address mixtures across regulatory sectors

Different chemical classes are often regulated under different pieces of legislation, for instance pesticides, biocides, cosmetic ingredients, industrial chemicals etc. Several studies in the recent literature show that chemicals from different classes are able to elicit similar effects (e.g. Evans et al., 2015; Kortenkamp and Faust, 2010; Maffini and Neltner, 2014) and numerous monitoring studies provide evidence for relevant co-exposure. Several of the case studies presented in section A.8 (De Brouwere et al., 2014; Evans, et al., 2015; Kortenkamp and Faust, 2010; Malaj et al., 2014) show that mixtures of chemicals spanning different regulatory silos can be of concern particularly for vulnerable / highly exposed subgroups. An additional example is shown in Carvalho et al. (2014), who tested two artificial mixtures, designed of 14 and 19 substances selected to cover different classes and modes of action, each present at their individual safety limit concentration imposed by the European legislation for surface water (Water Framework Directive). The toxic effects of the two mixtures were investigated in 35 bioassays based on 11 organisms representing different trophic levels. The mixtures elicited quantifiable and significant toxic effects on some of the test systems, showing the need of precautionary actions on the assessment of chemical mixtures even in cases where individual toxicants are present at seemingly harmless concentrations. As concerns were identified in several cases, further case studies should address also mixture risk assessments across regulatory silos.

4.2 Conclusions related to chemical classes considered

Among the considered case studies, several chemical classes have been addressed, but this does not mean that the overview is representative as many groups of compounds

have not been covered. The selection of these chemical classes is often based on data availability, recent concerns about certain chemical classes or legislative requirements. However, many more chemicals, including emerging substances, could be considered in the future. Wang et al. (2016) for example investigated perfluoroalkyl phosphonic and phosphinic acids (PFPAs and PFPiAs) used as defoamers in pesticide formulations and wetteners in consumer applications, which individually have a low risk. However, they conclude that combined exposure to them could be of concern due to similar MoA with other chemicals and their potential for long-range transport and potential for bioaccumulation in aquatic and mammalian organisms.

Several of the case studies revealed a concern due to combined exposure for certain chemical classes especially when considering specific vulnerable population groups. This is very relevant information, but needs to be interpreted with caution, considering carefully the assumptions, model parameters and related uncertainties. However, there is clear evidence that chemicals need to be further addressed not only in single substance risk assessment and that mixtures should be considered also across chemical classes and legislative sectors.

4.3 Potential for over- and underestimating risks from combined exposure to chemical mixtures

For some case studies described above, a concern for the environment or human health was identified. Each case study however, needs to be interpreted carefully taking into account the related assumptions and uncertainties.

Several factors in these studies might lead to an **overestimation of risks**, such as e.g. conservative assumptions that:

- all chemicals in a mixture contribute to a combined effect,
- exposure takes place to all chemicals simultaneously,
- high-end exposures for all chemicals in parallel, e.g. in lower tiers of the WHO/IPCS scheme (Meek et al., 2011).

The idea in a tiered scheme is however to start on purpose with conservative assumptions to reduce data requirements and allow a simpler screening assessment that is protective. This can be refined based on more realistic assumptions and additional data where a potential concern is identified.

However, many factors could also result in an **underestimation of the overall risk**, such as:

- the limited knowledge of the number and identity of chemicals humans and the environment are really co-exposed to. This is usually based on known exposure data from chemical monitoring, which can only detect the limited set of chemicals we are specifically looking for (e.g. De Brouwere et al., 2014; Kortenkamp & Faust, 2010; Malaj et al., 2014). This could be partly overcome by non-targeted monitoring or effect-based monitoring, potentially followed by effect-directed analysis to identify chemicals driving the risk;
- mixture assessments that consider only a certain compound class, which do not take into account co-exposure to other compounds that might contribute to a combined risk (e.g. leading to the same adverse outcome) (Dewalque et al., 2014);
- neglecting potential bioaccumulation in the organism using only external exposure concentrations (Kortenkamp & Faust, 2010);
- not addressing possible synergistic effects by assuming only concentration addition (Kortenkamp & Faust, 2010; Marx et al., 2015);
- neglecting chemical metabolites that are more toxic than the parent compound, might contribute to the overall risk, since most often only the parent compounds are considered (Malaj et al., 2014);

- in the case of exposure via environmental samples or effluents, the underlying sampling and extraction method can influence the chemicals that can be detected in the chemical (or biological) analyses. In the case of effluents, the assumptions made on their dilution in receiving waters can underestimate concentrations for small streams receiving several discharges (Malaj et al., 2014; Price et al., 2012a);
- in the use of monitoring data, the decision how non-detected chemicals are treated can make a big difference. If they are considered as absent, an underestimation is probable, while if they are assumed to be present at a certain fraction of the limit of detection or quantification, this can lead to overestimations (e.g. De Brouwere et al., 2014; Han and Price, 2011, 2013; Price et al., 2012a).

4.4 Mixture assessment approaches

4.4.1 Prediction models

All case studies performed a mixture risk assessment based on **Concentration Addition** (CA) except for Gosens et al. (2013) which considered only aggregate exposure to individual substances from different sources. One case study (Junghans et al., 2006) applied CA and **Independent Action** (IA) to the same dataset, which slightly underestimated the mixture toxicity. However this underestimation is significant only with increasing effect levels. At the 50% effect level the confidence interval of the EC_{50} predicted according to IA still overlaps with the confidence interval of the EC_{50} derived from the measured concentration-response data. Moreover, the EC_{50} values that can be derived from each prediction only differed by a factor of 1.3. Those results suggest that CA provides a precautious but not overprotective approach to the predictive hazard assessment of pesticide mixtures under realistic exposure scenarios, irrespective of the similarity or dissimilarity of their mechanisms of action. Junghans et al. (2006) identified two circumstances that can challenge the precautionary character of the CA approach, one of them being rather flat concentration-response curves so that IA could predict a higher toxicity than CA. The other could be caused by potential synergistic effects that are not covered by CA or IA approaches. Interactions need to be assessed on a case-by-case basis.

CA based approaches were also the ones most used by the experts participating in the JRC expert survey (Bopp et al., 2015). Experts used mostly the HI, TEQ, and ΣTU, as reflected also in the case studies discussed in this report. Some experts in the survey specifically mentioned IA based calculations as an approach they have abandoned, since the prediction outcome is usually similar to CA based predictions, while IA calculations require a lot more input information (full dose-response curves) to enable such calculations, which is often not available.

There is an ongoing debate about the relevance of **interactions**. In the reviewed case studies, only one included an assessment of synergistic interactions applying the HI_{int} approach to antibiotic mixtures (Marx et al., 2015). Toxicological interactions modulate toxicokinetic and/or toxicodynamic mechanisms of individual chemicals. Toxicokinetic interactions could be e.g. induction of metabolising enzymes, alterations in uptake mechanisms, all processes linked to the influence of individual chemicals on ADME of others. Toxicodynamic interactions can be based on e.g. modulation of homeostasis or repair mechanisms. Boobis et al. (2011) performed a literature review, identifying 90 studies demonstrating synergisms in mammalian test systems performed at low doses (i.e. close to the point of departure, POD) for individual chemicals. Only in 6 of the 90 studies useful quantitative information on the magnitude of synergy was reported. In those six studies, the difference between observed synergisms and predictions by CA did not deviate by more than a factor of 4. Cedergreen (2014) performed a systematic literature review for binary mixtures within three groups of environmentally relevant chemicals (pesticides, metals, antifouling agents). Synergy was defined as a minimum two-fold deviation from CA predictions. Synergy was found in 7%, 3% and 26 % of the

pesticide, metal and antifoulant mixtures, respectively. The extent of synergy was rarely more than a factor of 10. Based on some more in depth analysis, Cedergreen concluded that true synergistic interactions between chemicals are rare and often occur at high concentrations. Using standard models as CA is regarded as the most important step in the RA of chemical mixtures. In the JRC expert survey (Bopp et al., 2015), most of the experts were in favour of addressing interactions on a case-by case basis, considering whether available information (e.g. regarding the chemical structures, MoA) can be used to anticipate possible interactions.

Two international frameworks developed for the assessment of combined exposure to multiple chemicals were applied in some of the case studies: the **WHO/IPCS framework** (Meek et al., 2011) and the **Cefic MIAT decision tree** (Price et al., 2012b). The latter combines the frameworks of the WHO/IPCS with the decision tree developed by the Scientific Committees (SCHER, SCENIHR, SCCS, 2011) and incorporates the Maximum Cumulative Ratio (MCR) (Price and Han, 2011). The MCR can be used as a tool for prioritising mixtures, prioritising relevant refinements in the mixture RA and identifying where a single substance RA might be sufficient. The main characteristic of the WHO/IPCS framework is its tiered approach for both the exposure and the hazard assessment. Screening level assessments (Tier 0) using e.g. simple exposure estimates based on production or sales volumes and TTC values for missing hazard information and without subgrouping of chemicals were presented in several case studies, as well as more refined Tier 1 case studies. However, it was also shown that further refinement (which includes grouping of chemicals based on e.g. common effect or common MoA) is often hampered by a lack of data (e.g. Evans et al., 2015). The Cefic MIAT decision tree was shown to be applicable to several cases and the **MCR** was demonstrated as a valuable tool in six examples. The MCR was in the range of 1.2-4.2 for pharmaceuticals in WWTP effluents (Backhaus and Karlsson, 2014), 1.3-2.4 for the FCM cases (Price et al., 2014), 2.2-5.7 for DLC in the generally exposed population, 1-5.8 for indoor air contaminants (De Brouwere et al., 2014), and 1-2 for groundwater samples with 5-10 detects, and 1-5 for groundwater samples with 15-25 detected contaminants (Han and Price, 2011). This shows that the number of chemicals that drive the mixture risk is usually low. In all the examples, the MCR decreased with increasing HI. This indicates that the higher the predicted risk, the lower the number of chemicals that are substantial contributors. It was also shown to be useful to present the mixture effects by ranking the chemicals according to their individual RQ from highest to lowest, to identify those chemicals that are contributing most and to see which number of chemicals reaches in the sum a certain percentage of the overall risk. It was shown for example in Backhaus and Karlsson (2014) that the top ranking 10 chemicals contributed 95% of the combined risk. In Price et al. (2012a) it was shown that only 2-5 compounds were significant contributors to the overall risk. This can help in further characterising a mixture, deciding when further refined mixture assessment is needed and developing strategies for such targeted refinements. Another important task is also to identify major sources of exposure, which is relevant for the risk management of combined exposures. Dewalque et al. (2014) and Kennedy et al. (2015) showed ways of comparing e.g. dietary *vs* non-dietary contributions for exposure to phthalates and pesticides, respectively.

4.4.2 Grouping of chemicals

In the case studies reviewed here, mostly lower tier assessments were applied based on conservative assumptions. This implies that in most cases no specific grouping was performed, but all mixture components were supposed to act together leading to combined effects. These assessments are usually based on agreed reference values which were derived based on the most sensitive endpoints, i.e. not necessarily based on the same type of effect. This is a valid conservative approach for lower tiers. If no concern is identified considering all mixture components together, no further refinement and grouping will be needed.

There is some consensus in the current frameworks that in refined assessments a grouping is performed based on a common effect, common mode of action, or common target organ. Depending on the choice, the groups will be larger (resulting in more conservative assessments) or smaller (less conservative). One widely acknowledged advanced framework is the development of cumulative assessment groups (CAGs) for pesticides by EFSA (EFSA PPR Panel, 2014). At CAG level 1, chemicals are grouped based on their toxicological target organ. At CAG level 2 grouping is further refined based on common specific phenomenological effects, at level 3 based on common mode of action and at level 4 based on common mechanism of action. With the usually available chemical hazard information, grouping can mostly be performed until level 2. As nowadays more and more mechanistic information is becoming available, further refinement will be possible. Another EFSA opinion (EFSA PPR Panel, 2013) concluded that the best approach for addressing pesticides eliciting a common adverse effect in the same organ by dissimilar MoA is also CA.

Thus the question remains how far further refinement of groupings should go to remain protective. Most scientific publications and international activities on the risk assessment of mixtures conclude that risks from combined effects are relevant for mixtures of substances with similar mode of action or effect (e.g. SCHER, SCENHIR, SCCS, 2011). However, based on the relatively well studied adverse effects of mixtures of pharmaceuticals, Hadrup (2014) suggested that chemicals with dissimilar mechanisms of action could be of bigger concern in the toxicological risk assessment of chemical mixtures than chemicals with a similar mechanism of action. Examples obtained from cancer and HIV treatment studies, show that pharmacological combination therapy targeting different mechanisms of action is more effective than a strategy where only one mechanism is targeted. Another argument is that also in many diseases several organ systems concomitantly contribute to the pathophysiology, implying that a grouping based on common target organs may be inadequate. In further considerations, it should be however considered that in pharmacology usually higher doses are applied, whereas at lower concentrations some specific effects might not occur. Goodson et al. (2015) reviewed actions on key pathways and mechanisms related to carcinogenesis for 85 chemicals ubiquitously occurring in the environment. The aim was to explore the hypothesis that low-dose exposures to mixtures of chemicals in the environment may be combining to contribute to environmental carcinogenesis. The results of their analysis suggest that the combined effects of "individual (non-carcinogenic) chemicals acting on different pathways, and a variety of related systems, organs, tissues and cells could plausibly conspire to produce carcinogenic synergies". Additional research on carcinogenesis and low-dose effects of chemical mixtures needs to be performed to further investigate the hypothesis. The concept of assessing combined effects strictly based on grouping chemicals according to their MoA or common effect(s) might need to be revisited in order not to underestimate cancer-related risks and systemic diseases. Further investigations on the risks from combined exposure to multiple chemicals should consider synergies of chemicals acting via dissimilar processes, acting on different targets and tissues, and consider synergies between certain pathways.

4.5 Current limitations in performing mixture risk assessments

In order to perform mixture risk assessments using component based approaches, it is a prerequisite to have detailed information on the **mixture composition** regarding the chemical identity and concentrations. This is usually known for formulated products/intentional mixtures, but is sometimes problematic for unintentional and environmental mixtures. For example Tang et al. (2014) have shown that although a total of 299 chemicals were screened in wastewater and recycled water samples, all present below the individual regulatory safety limit, the known chemicals in designed mixture toxicity testing explained less than 3% of the observed cytotoxicity and less

than 1% of the oxidative stress response, and were not related to the observed genotoxicity. Neale et al. (2015) examined Danube river samples using large volume water extracts testing in an *in vitro* test battery and tried to match observed effects with the detected organic micropollutants. Most samples showed rather low response in the bioassays, however, depending on the endpoint, the contribution of the detected chemicals explained in the worst case only 0.2 % of the observed effect (for PXR activation), while five chemicals explained 80% of the observed effect for ER activation. The sometimes large proportion of unknown toxicity in environmental samples could be addressed by widening the range of chemicals analysed and complementing the chemical monitoring with biological effect monitoring. De Brouwere et al. (2014) used 4 different indoor air monitoring data sets in their case study and faced difficulties in comparing the results for the different studies. The substances identified as biggest contributors to the potential risk were NO_2, trichloroethylene, acrolein, xylenes. These were however not consistently measured in all the studies, so comparison of datasets and overall drivers is difficult. The combined assessments from the different studies might lead to an underestimation of risk due to the presence of some major contributors that were not included in the chemical analysis.

Another major problem is the availability of relevant exposure and toxicity data, as well as lack of information on the MoA of mixture components (e.g. Evans et al., 2016; 2015). A major **gap** was identified in the information on **human and environmental exposure** and a new platform for monitoring data was therefore created. IPCheM[4], the Information Platform for Chemical Monitoring data, was developed over the last years as one of the follow up actions to the Commission Communication (EC, 2012). It comprises monitoring data in four modules, i.e. human biomonitoring, environmental monitoring, indoor air and consumer products, and food / feed related monitoring data. Thus it offers great potential for the assessment of mixtures.

Exposure is often predicted from e.g. production volumes, but also assessed using small surveys, e.g. for parabens in personal care products (Gosens et al., 2013). The usually limited number of individuals can lead to high uncertainties on the representativeness. Also the limited spectrum of chemicals analysed in monitoring studies can be a problem (e.g. De Brouwere et al. (2014) indicating problems in comparability between data sets), as well as the reporting of monitoring results. For example, in the case of human biomonitoring data, if only aggregated results are made available, it is impossible to trace back the real co-occurrence of chemicals in individual humans. If monitoring data are used, obviously the higher the number of analysed chemicals, the higher will also become the predicted potential risk. Another major uncertainty is related to the impact of chemicals that are analysed but below the limit of detection. Several approaches can then be used to address these chemicals, which can greatly influence the final outcome (Han and Price, 2011). When external exposure data are used (like environmental monitoring data or exposure predicted from emissions or product uses), there is always the question about internal co-exposure at a target site. Tier 3 of the WHO/IPCS scheme therefore includes toxicokinetic modelling for the prediction of internal exposure concentrations. Such Tier 3 refinements of the hazard assessment are however not reported in any case study and hampered by the availability of relevant input parameters required for the modelling (see also EURL ECVAM Toxicokinetic Strategy, Bessems et al. (2015)).

Furthermore, the **lack of toxicity data** is highlighted in many of the reviewed case studies. For screening assessments, the TTC can be used in several cases to replace specific reference values. If however a refinement and specific values are needed, limited availabilities are encountered, e.g. for pharmaceuticals (Backhaus and Karlsson, 2014), pesticides (Junghans et al., 2006; Kennedy et al., 2015; Nowell et al., 2014) cosmetics, etc. Moreover, if relevant reference values are found from the various sources

[4] https://ipchem.jrc.ec.europa.eu/

used (i.e. literature, public databases, authorities' assessment reports etc.), it is sometimes difficult to select the most reliable one. De Brouwere et al. (2014) for example used chronic inhalation toxicity values and identified up to 300 fold differences in the retrieved values. They developed a decision scheme to select the most reliable RV. Apart from the general RVs, it is often even more difficult to find information on specific effects, which is important when chemicals need to be grouped based on a common effect. So what is often missing is information on the detailed MoA and also on the toxicity values related to such a specific effect. Several databases may be useful for obtaining tissue and organ level information as well as reference values. An example is the publicly accessible COSMOS database[5], which currently hosts toxicological data on cosmetic and food relevant chemicals. Missing toxicity data often imply that extrapolations have to be used, such as acute to chronic extrapolations or read-across from similar compounds, which leads to overall increased uncertainties of the predictions.

For both hazard and exposure assessments, additional assumptions have to be made due to limited data availability and parameters sometimes need to be predicted by modelling. The related model specifications can greatly influence the results (Boon et al., 2015; Kennedy et al., 2015). Therefore it is of utmost importance that the scenario parameters and hypotheses underlying each mixture risk assessment are clearly justified and transparently documented to allow a proper interpretation of the results.

[5] https://cosmosdb.eu/cosmosdb.v2

5 Conclusions

As humans and wildlife can be exposed to a virtually infinite number of different combinations of chemicals in mixtures via food, consumer products and the environment, it is impossible to test or assess all these possible combinations. In this review, 21 examples of case studies on specific mixtures have been identified, but to address the risk assessment in a wider range of mixtures, priorities need to be set. The Scientific Committees (SCHER, SCENIHR, SCCS, 2011) have set out relevant criteria to prioritise mixtures for the assessment, e.g. based on relevant exposure close to health based guidance values for several components, chemicals of higher production volumes or produced as commercial mixtures, likelihood of frequent large scale exposure etc. Once a decision is taken to perform a mixture RA, it can then be performed as screening level RA or higher tier RA, depending on the needs and to be resource efficient. Apart from the tiered approach as outlined in the WHO/IPCS framewrok, the MCR can help to further characterise the main issues around a certain mixture and to decide on the next steps for refinement where needed, e.g. concentrate on few substances driving the risk or identify a need to tackle further the whole mixture composition.

Monitoring data are essential in mixture risk assessment as they can give information on identity, magnitude, duration, frequency and/or timing of real exposure, depending on the monitoring scheme, and allow to assess the co-exposure patterns to chemicals (Qian et al., 2015), both for human and environmental risk assessment. Numerous retrospective ERA have been performed with monitoring data; however so far there is no prospective RA concerning chemical substances related to various regulatory sectors and/or uses, and although numerous chemicals fall under several regulatory frameworks (biocides, pesticides, REACH...), the potential for co-exposure is hardly assessed or taken into account in their risk assessment.

The data sources used are various (exposure data from modelling, monitoring or published data from surveys; toxicological data from published databases, TTC approach, data gap filling, etc.) and the data sets are frequently incomplete, which has a direct impact on the possibility to perform a mixture risk assessment, as well as the reliability and accuracy of the risk assessment. Data gaps seem to be the major hurdle when it comes to deal with risk assessment of chemical mixtures, especially when focusing on particular uses or population subgroups (e.g. amateur uses of pesticides, frequency of use of personal care products for children).

It has to be taken into account that the list of compounds covered in the reviewed case studies is unlikely to be fully representative of human and environmental exposures. However, based on the identified cases, pesticides followed by pharmaceuticals and personal care products dominate the observed mixture effects in cross-sectorial mixtures. Tributyltin, polycyclic aromatic hydrocarbons, and brominated flame retardants are also major contributors to the environmental chemical risks 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. It is important to be aware of the influence that the choice of model specifications, the parameters and the toxic reference values considered have on the outcome of a mixture RA. There is a need to clearly specify and justify the choices that have been made. Thus, the results should be interpreted carefully in the light of the models used, the underlying hypothesis and the degree of conservatism that has been chosen.

It has to be highlighted that for both environmental and human exposure, there are several factors that might lead to an underestimation or overestimation of the potential risk, e.g. uncertainty in reference values used, incompleteness of monitoring data, etc. A clear potential for underestimation results from neglecting potential synergistic effects, bioaccumulation potential and metabolites. Another potential for underestimation results from the assessment of specific chemical classes or regulated under a specific legislative

framework. Several studies reviewed here indicated a potential concern for mixtures across several regulatory silos.

In order to facilitate mixture risk assessment in the future, it will be relevant to improve data sharing regarding toxicity and exposure information. Relevant platforms such as e.g. IPCheM should be further populated (e.g. by monitoring programmes such as the European Human Biomonitoring Initiative) and made interoperable with other tools.

Future case studies on mixture RA could help to fill the knowledge gaps identified through this review, by:

- Comparison of different populations including vulnerable subgroups;
- Inclusion of substance groups that have not been addressed in mixtures so far, including emerging chemicals;
- Further investigations on the relevance of interactions (particularly synergisms) at relevant low exposure concentrations;
- Developing further criteria to decide under which circumstances and for which mixtures interactions need to be addressed;
- Investigating the impact of different approaches for grouping (based on common effects, common MoA etc.) and related to that, investigating further combined effects of independently acting chemicals considering interactions between pathways, as e.g. for carcinogenesis and systemic diseases;
- Examining further the relevance to address mixtures across different regulatory sectors.

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List of abbreviations and definitions

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism, Excretion
AF	Assessment Factor
Aggregate exposure	exposure to a single substance originating from different sources
AL	Acceptable Level
AOP	Adverse Outcome Pathway
ARfD	Acute Reference Dose
BMD	Benchmark Dose
CA	Concentration Addition The effects of exposure to a mixture of compounds with a similar mode of action are assumed to be the sum of the potency-corrected effects of each component.
CAG	Cumulative Assessment Group
CEA	Cumulative Exposure Assessment
Cefic MIAT	CEFIC (European Chemical Industry Council) Mixture Industry Ad hoc Team
Combined exposure	multiple substances from one or different sources
CR	Concentration-Response
CRA	Cumulative Risk Assessment
DEB	Dynamic Energy Budget modelling
DEHP	diethylhexyl phthalate
DI	Daily Intake
DLC	Dioxin-like compound
EC_{50}	Concentration where 50 % effect was observed/calculated
ERA	Environmental Risk Assessment
HBM	Human Biomonitoring
HH	Human Health

HI	Hazard Index Sum of Hazard Quotients, i.e. ratio between exposure and the reference value for the common toxic effect of each component in a mixture or a CAG.
HI_{int}	Hazard Index considering interactions
HQ	Hazard Quotient
HRA	Human Risk Assessment
IA	Independent Action Occurs where the mode of action and possibly, but not necessarily, the nature and sites of toxic effects differ between the chemicals in a mixture, and one chemicals does not influence the toxicity of another. The effects of exposure to such a mixture are the combination of the effects of each component compounds (also referred to as response-addition).
IATA	Integrated Approach to Testing and Assessment
Intentional mixtures	manufactured products or formulations, including commercial mixtures of industrial substances
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LO(A)EL	Lowest Oserved (Adverse) Effect Level
LOD	Limit Of Detection
MCR	Maximum Cumulative Ratio
MCRA	Monte Carlo Risk Assessment tool
MEC	Measured Environmental Concentration
MoA	Mode of Action
MoE	Margin of Exposure
MRL	Maximum Residue Level
NHANES	National Health and Nutrition Examination Survey
NIAS	Non-Intentionally Added Substances
NOAEL	No Observed Adverse Effect Level
PBDE	PolyBrominated Diphenyl Ether
PBTK	Physiologically Based ToxicoKinetic modelling
PCBs	PolyChlorinated Biphenyls
PEC	Predicted Environmental Concentration

PNEC	Predicted No Effect Concentration
POD	Point Of Departure
PPDB	Pesticide Property Database http://sitem.herts.ac.uk/aeru/ppdb/en/
PPPs	Plant Protection Products
PTI	Pesticide Toxicity Index
QSARs	Quantitative Structure–Activity Relationship
RA	Risk Assessment
RfD	Reference Dose
RPF	Relative Potency Factor
RQ	Risk Quotient
RV	Reference Value
STU	Sum of Toxic Unit (Σ TU)
TDI	Tolerable Daily Intake
TEF	Toxic Equivalency Factors
TEQ	Toxic Equivalency
TTC	Threshold of Toxicological Concern
TU	Toxic Unit
Unintentional mixtures	substances from different sources, deposited separately at a particular site (e.g. in surface water)
US EPA	United States Environmental Protection Agency
VOCs	Volatile Organic Compounds
WC	Worst Case
WHO/IPCS	World Health Organisation / International Programme on Chemical Safety
WWTP	Waste Water Treatment Plant

Annex 1 – Overview of individual case studies

Twenty-two case studies from the literature were selected as described in Section 2. Relevant information from the case studies was extracted and is reported in the tables below. No judgement on the quality/validity of the case studies is included here. **Reported findings and conclusions are those of the case study publications' authors and do not necessarily represent the views of the authors of this report.**

A.1 Pesticides

ID	1
Title	Application and validation of approaches for the predictive hazard assessment of realistic pesticides mixtures (Junghans et al., 2006)
Journal	Aquatic Toxicology, 76, 93-100
Authors	Marion Junghans, Thomas Backhaus, Michael Faust, Martin Scholze, L.H Grimme
Year	2006
Background & Objectives	In freshwater systems located in agricultural areas, organisms are exposed to a multitude of toxicologically and structurally different pesticides. For regulatory purposes it is of major importance whether the combined hazard of these substances can be predictively assessed from the single substance toxicity. This study aimed to analyse whether both concepts of CA or IA may be used to predict the toxicity of environmentally realistic mixtures, including a mixture of chemicals acting by similar and dissimilar MOA. In order to do so, the mixture was studied for its effect on the reproduction of the freshwater algae *Scedenesmus vacuolatus*. The predictability of CA (Σ TU) and IA was then assessed, by comparing the predicted results to the actual measured toxicity.
Substances	A defined mixture of pesticides (25 single substance) reflecting a realistic exposure scenario
Exposure Scenario	Field run-off water leading to exposure of aquatic organisms in edge-of-field surface waters
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Exposure of algae to field run-off water. Key components are known because artificial mixture data available on the hazard of the mixture itself to be compared with theoretical calculated toxicity. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes (for algae) 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP?

	No assessment groups.
Information sources	Exposure: modelled; the physico-chemical characteristics for all active ingredients were collected from registration dossier Toxicity: Single substance and mixture concentration-response relationships were determined experimentally
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	The exposure scenario is based on emission patterns from growing three major crops (cereals, maize, sugar beet), and modelled according to the standard FOCUS scenario "R1", in order to reflect the median load of pesticides in field run-off in central European agricultural areas after pre-emergence treatment in spring. A reasonable worst case (WC) application scenario based on common crop protection strategies has been assumed.
Hazard Assessment	To allow for a comparison of observed mixture toxicity with the prediction according to CA and IA, single substance concentration-response (CR) relationships were determined for all mixture components in a bio-test (24h of exposure). Those data were used to calculate the mixture toxicity according to the CA and IA models. However for 3 out of 25 substances this prerequisite was not or only partially fulfilled: for isoxaflutole algal toxicity, the maximum effect observed at the limit of solubility did not exceed 45%. Therefore, WC estimates of higher effect concentrations were extrapolated from the CR function. For carbosulfan and clopyralid, no CR relationship could be determined within the limits of solubility and within concentration ranges causing no strong acidification of the algal growth medium, respectively. Therefore, they were left out and total mixture toxicity predictions are based on 23 (out of 25) substances only.
RA for Algae	When all components are present at their PEC in run-off water, the growth of the algal population was inhibited by 46%, which is significantly higher than 17% caused alone by the PEC of the most active component atrazine. When comparing this measured mixture toxicity with the predictions, it is slightly lower than predicted according to CA (49%), but higher than predicted according to IA (39%).
Overall summary of outcome	-The resulting mixture proved to be clearly more toxic than any individual component -CA shows a good predictive quality over the complete range of effect. This is consistent with the finding that the toxicity was dominated by a group of similarly acting photosystem II inhibitors (they contribute 0.80 TUs to the total sum of 0.98 TUs), although the mixture included substances with diverse and partly unknown mechanisms of action. -IA underestimates the mixture toxicity slightly; however this underestimation is significant only with increasing effect levels. At the 50% effect level the confidence interval of the EC50 predicted according to IA still overlap with the confidence interval of the EC50 derived from the measured concentration-response data. Moreover, the EC50 values that can be derived from each prediction only differed by a factor of 1.3.

	Those results suggest that CA provides a precautious but not overprotective approach to the predictive hazard assessment of pesticide mixtures under realistic exposure scenarios, irrespective of the similarity or dissimilarity of their mechanisms of action.
Future perspec-tives / Outlook	• Problems: Substance specific degradation and sorption processes are not taken into account. Therefore, a conclusive assessment of the expectable mixture toxicity in receiving water might require a second step of fate and effect modelling. • The major limitation for such modelling exposure is the restricted availability of reliable information on pesticide use. • Two circumstance can challenge the precautionary character of the approach: - If due to rather flat concentration-response curves IA predict a higher toxicity than CA and if the mixture is dominated by dissimilarly acting components, the mixture toxicity can be expected to comply better with IA than with CA. - In case of interaction of the mixtures components, which leads to a mixture toxicity that is higher than predicted by CA. In this case, a more detailed hazard assessment has to be performed.

ID	2
Title	Pesticide Toxicity Index—A tool for assessing potential toxicity of pesticide mixtures to freshwater aquatic organisms (Nowell et al., 2014)
Journal	Science of the Total Environment
Authors	Lisa H. Nowell, Julia E. Norman, Patrick W. Moran, Jeffrey D. Martin, Wesley W. Stone.
Year	2014
Background & Objectives	The Pesticide Toxicity Index (PTI) is 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. This paper addresses exposure to pesticide mixtures and presents the Pesticide Toxicity Index (PTI) as a robust and readily applicable screening tool for interpreting the biological significance of concentration data for pesticide mixtures in hydrologic systems and expands the number of pesticides and degradates included in previous editions of the PTI from 124 to 492 pesticides and degradates, and includes two types of PTI for use in different applications, depending on study objectives.
Substances	Pesticides mixtures (active ingredients and degradates)
Exposure Scenario	Exposure of aquatics organisms
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Exposure of freshwater organisms; key components known; information on the hazards of the sample itself known from literature. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, mixture of pesticides are frequently present in freshwater system 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? No assessment groups
Information sources	Toxicity data: A master list of 484 pesticides were compiled from agricultural pesticide use lists for 1992 to 2011, for which toxicity data has been searched in the USEPA ECOTOX DB, the USEPA registration and RA documents cited in support of the OPP aquatic-life benchmarks, and the Pesticides Properties Database. Exposure data: Published data on concentrations of pesticides in ambient stream water Published data on organism survival in toxicity test conducted in the laboratory with undiluted ambient water were also used to compare with the calculated PTI.
Exposure	No exposure data. Literature data (toxicity of environmental sample to

Assessment	*C.dubia*) were aggregated and used to test how well the PTI approach predicted the incidence of observed toxicity.
Hazard Assessment	The following data were used in order of priority: • Standardized toxicity test data from the ECOTOX database • Toxicity test data from core or supplemental studies underlying OPP aquatic-life benchmarks or summarized in registration documents • Non-standard toxicity test data from the ECOTOX database • Toxicity values compiled from the PPDB Two approaches were used: used of the MTC (median toxicity concentration, calculated as the median of the toxicity value for each compounds toward the taxonomic group) or the STC (sensitive toxicity concentration, either the 5^{th} percentiles if more than 13 data were available, or the minimum toxicity value for each compounds toward the taxonomic group)
RA for aquatic organisms	• The PTI approach is used $$PTI_t = \sum_{i=1}^{n} \left(\frac{E_i}{TC_{i,t}} \right)$$ With t : the taxonomic group, E_i the concentration of the pesticide i, n the number of detected pesticides, TC_{it} the toxicity concentration for the pesticide i for the taxonomic group t. The Median-PTI and the Sensitive-PTI were calculated for each sample.
Overall summary of outcome	• MTC and STC values are significantly correlated with one another within a taxonomic group. • When MTCs (medians) are used, the purpose of the index is to represent the relative toxicity of sites, samples, or individual pesticides - MTC values are relatively robust to outliers. When the STC values are used, the index is better suited for use as a screening level, because it is a more conservative (protective) indicator of the potential for toxicity. • *C. dubia* survival was reduced to ≤50% of controls in 44% of samples with Median-PTI values in the range of 0.1 to 1, and to 0% in 96% of samples with Median-PTI values 1. For the Sensitive-PTI, *C. dubia* survival was reduced to ≤50% of controls in 81% of samples in the range of 0.1 to 1, and in 89% of samples with Sensitive-PTI values >1.
Future perspec-tives / Outlook	Limitation: • The PTI is a relative ranking system that indicates that one sample is likely to be more or less toxic than another sample, but does not indicate that toxicity will necessarily occur. • Toxicity values are based on short-term laboratory data with EC50 or LC50 endpoints and do not reflect long-term/chronic exposure or incorporate sublethal endpoints. • The PTI does not account for environmental factors (dissolved organic carbon, particulates, pH, T°…) which can affect the toxicity and bioavailability of pesticides. • The PTI assumes that pesticide toxicity is additive and there is no chemical interaction which may not be the case for complex mixtures of pesticides from different chemical classes and with different MOAs across

	all taxonomic groups and life stages. • The PTI does not take into account the dose–response curves of either single-chemical or mixtures exposures. • The PTI is limited to pesticides measured in the water column; hydrophobic pesticides may be underrepresented in terms of potential toxicity, especially to benthic organisms. • Uncertainty in the relative toxicity of compounds is high for pesticides with relatively few bioassays available. The 10,837 bioassays in this data set are divided among 440 pesticides and 52 degradates, 559 different species, and three taxonomic groups, making the number in each group relatively small. Although this does not preclude the use of the data as the best available, it demonstrates the sparseness of available data on the toxicity of many currently used pesticides. • The PTI is a relative, but quantitative, indicator of potential toxicity that can be used in study design or to interpret water quality data, relate pesticide exposure to biological condition, and prioritize future assessments IDEAS FOR IMPROVEMENT OF METHODOLOGY: A more rigorous test of the PTI model is needed, but this will require the availability of data for pesticides from multiple classes and MOA, concurrent with data on aquatic toxicity and(or) ecological condition.

ID	3
Title	A European model and case studies for aggregate exposure assessment of pesticides (Kennedy et al., 2015)
Journal	Food and Chemical Toxicology
Authors	Marc C. Kennedy, C. Richard Glass, Bas Bokkers, Andy D.M. Hart, Paul Y. Hamey, Johannes W. Kruisselbrink, Waldo J. de Boer, Hilko van der Voet, David G. Garthwaite, Jacob D. van Klaveren
Year	2015
Background & Objectives	To assess aggregated exposure and risk to pesticides a new aggregate model/general framework is described, which allows individual users to include as much, or as little, information as is available or relevant for their particular scenario. Depending on the inputs provided, the outputs can range from simple deterministic values through to probabilistic analyses including characterizations of variability and uncertainty. Exposures can be calculated for multiple compounds, routes and sources of exposure, and this aggregate model links to the cumulative dietary exposure model developed in parallel. It is implemented in the web-based software tool MCRA. This work presents case studies to illustrate the potential of this model, with inputs drawn from existing European data sources and models.
Substances	Pesticides mixtures (active ingredients and degradates) from the conazole group
Exposure Scenario	Human exposure: exposure to UK arable spray operators, Italian vineyard spray operators, Netherlands users of a consumer spray and UK bystanders/residents, and finally a hypothetical population performing a combination of these activities.
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Human exposure to pesticides within different scenarios: aggregate exposure combines dietary and non-dietary sources and example of exposure are occupational farming activities, use of amateur or consumer products, or incidental exposures experienced by residents or bystanders; key components known; no data available on the hazard of the mixture itself. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, humans are exposed to pesticides via dietary and non-dietary routes, being consumer, operator, worker or bystander. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? Compounds are grouped into a cumulative assessment group (CAGs)

	if they have a similar toxicological effect.
Information sources	Toxicity data: from the literature or pesticides registration data Exposure: Already existing models, databases and Software have been used (Operator activities: EUROPOEM Databases; Worker activities: BEAT Models and ART; exposure from non-professional uses: ConsExpo Software; Bystander and resident activities: BREAM models).
MIXTURE ASSESSMENT/METHODOLOGY	
HRA	**Exposure assessment:** The model implies several step: • To define an exposure question (selection of an appropriate population, health effects and relevant compounds) • The estimation of non-dietary exposure from one or more activities • Matching non-dietary exposures with dietary exposures at the individual level • Aggregation of those exposures to an appropriate common unit. Each compound is aggregated separately, before a suitable weighted sum is derived to give a total exposure. The weights are derived based on relative potency factors (RPFs, toxic potencies expressed relative to a selected index compound) • If a chronic assessment is required, average daily exposure is calculated per individual. In the acute case, exposure values per individual day should be calculated. Simple absorption factors are used rather than more detailed dosimetry/toxicokinetic modelling, as they are more compatible with the available data in EU.
Overall summary of outcome	• The outputs available from the aggregate model provide estimates of the relative exposure from various sources, which may be more effective for communication. A comparison of risks is easier to process than an individual exposure or probability value in isolation • Those case studies demonstrate how the relative contributions to exposure can be shown to differ between particular scenarios and populations. For example, based on those hypothetical scenarios the main routes of exposure are seen to be inhalation for the spray user, and dermal for the UK operator; for child bystanders, exposure through non-dietary dermal exposure is estimated to be small compared with dietary exposure • Alterative model specifications can greatly influence the results • When interpreting the results, care must be taken to recognize possible differences in the degree of conservatism between dietary and non-dietary exposure models
Future perspec-tives / Outlook	• Data gap: A useful extension of this case study would be to obtain information about realistic frequency of use of this type of products by amateur, and this would be essential for chronic assessments • To reliably assess exposure related to some activities, survey of non-dietary activities would be required • The model could also be adapted to handle non-PPP compounds, if they can be weighted relative to some reference compounds • A significant challenge in this area is the communication of risks and probabilities • In future assessment, the selected scenario parameters and distributions would require specific and detailed justification,

	regarding their impact on the results obtained. • Further refinements will be made based on feedback from stakeholder groups testing and using the model in practice. Particular computation issues may arise as larger CAGs become available and are included

ID	4
Title	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 (Boon et al., 2015).
Journal	Food and Chemical Toxicology
Authors	Polly E. Boon, Gerda van Donkersgoed, Despo Christodoulou, Amélie Crépet, Laura D'Addezio, Virginie Desvignes, Bengt-Göran Ericsson, Francesco Galimberti, Eleni Ioannou-Kakouri, Bodil Hamborg Jensen, Irena Rehurkova, Josselin Rety, Jiri Ruprich, Salomon Sand, Claire Stephenson, Anita Strömberg, Aida Turrini, Hilko van der Voet, Popi Ziegler, Paul Hamey, Jacob D. van Klaveren
Year	2014
Background & Objectives	A cumulative dietary exposure assessment according to the requirements of the EFSA guidance (EFSA Panel on Plant Protection Products and their residues (PPR), 2012) on probabilistic modelling was performed in order to assess the practicality of the guidance.
Substances	Pesticides residues mixture from the triazole group
Exposure Scenario	Human exposure *via* food consumption
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Human exposure to pesticides via food consumption; key components known; no hazard data available on the mixture itself. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, human are frequently exposed to mixture of pesticides via food consumption 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? Compounds are grouped into a cumulative assessment group (CAGs) if they have a similar toxicological effect.
Information sources	Exposure: The acute and chronic cumulative exposure to triazole pesticides was estimated using national food consumption data part of the Comprehensive database of EFSA, and monitoring data on pesticide residue of eight European countries in the period 2007-2010 (acute exposure) and 2 countries for chronic exposure.
MIXTURE ASSESSMENT/METHODOLOGY	
HRA	**Exposure assessment:** • Both the acute and chronic cumulative dietary exposures were

	calculated according to two model runs (optimistic and pessimistic) as recommended in the EFSA guidance: national food consumption data were combined with national monitoring data, including available information on the effect of processing on pesticide residues if appropriate (those data were coming from the German Database developed by the federal Institute for Risk Assessment). • Information on unit variability was included in the pessimistic model run. Additionally, for two countries the chronic cumulative exposure was calculated for the group of triazole pesticides of the chronic CAG (chronic effect: hepatoxicity) according to both model runs. • Calculations were performed with the Monte Carlo Risk Assessment (MCRA) software, developed to facilitate cumulative exposure assessments. Those calculations followed the EFSA guidance procedure for performing an acute or chronic cumulative assessment, and consist in the conversion of single compound concentration databases to cumulative concentration database containing cumulative residues levels per sample, by using Relative Potency Factors.
Overall summary of outcome	• Acute exposure: In the optimistic model run, none of the simulated exposures per country and age class exceeded the ARfD (acute reference dose), whereas in the pessimistic model run person-days with simulated exposures exceeding the ARfD were observed for IT: 10 person-days per million in adults and 20 in adolescents • Chronic exposure: In the optimistic model run no exposures were simulated exceeding the ADI. In the pessimistic model run, 6,% and 4,3% of the population had a simulated chronic exposure that exceeded the ADI in Denmark and Italy respectively, with a 97.5% upper confidence limit of 71,900/48,900. The P99.9 of chronic exposure exceeded in both countries the ADI in the pessimistic model run. • The exposures obtained with these model runs differed substantially for all countries, with the highest exposures obtained with the pessimistic model run. In this model, animal commodities including cattle milk and different meat types (entered in the exposure calculations at the level of the maximum residue limit, MRL), contributed most to the exposure. • In this study the uncertainties due to sampling uncertainty of the food consumption and residue concentration data were quantified in both models run via central 95% confidence intervals around the number of person-days or persons exceeding a toxicological reference value and around the P99.9 of exposure. However, exposure assessments are affected by many other uncertainties (e.g. food conversion factors, monitoring data…) which should also be evaluated. This evaluation, based on the experience of the authors, was therefore subjective. • The authors conclude that application of the optimistic model run on a routine basis for cumulative assessments is feasible; however, the resulting exposure estimates are very likely underestimates of the real exposure. • The pessimistic model run is laborious and the exposure results could be too far from reality.
Future perspec-tives / Outlook	• Differences in exposures results between models and countries are very likely due to the dissimilarities in the approaches/models taken • The link with processing information should be improved to further optimize the application of the optimistic model run. • More experience with the pessimistic model run is needed to

	stimulate the discussion of the feasibility of all the requirements, especially the inclusion of MRLs of animal commodities which seem to result in unrealistic conclusions regarding their contribution to the dietary exposure. Furthermore, tools are needed to standardised pessimistic residue database. • A database with authorised uses of pesticides worldwide that will be updated and maintained over the years would be needed to make it feasible to perform CA according to the pessimistic model on a routine basis. • The use of common effects in CRA with much higher reference values than the most sensitive effect of the index compound may result in conclusions that are contrary to past conclusions based on single compound assessments. Risk assessors and managers should keep this in mind when evaluating the outcomes of cumulative exposure assessments. • More experience is needed with some kind of intermediate 'realistic' scenario combining the optimistic and pessimistic model run in such a way that it results in more realistic acute and chronic exposures which would be conservative enough (precautionary principle) but not over-conservative such as the pessimistic model run.

ID	5
Title	Examining the feasibility of mixture risk assessment: A case study using a tiered approach with data of 67 pesticides from the joint FAO/WHO meeting on pesticide residues (JMPR)
Journal	Food and Chemical Toxicology
Authors	Evans RM, Scholze M, Kortenkamp A.
Year	2015
Background & Objectives	• Case study to illustrate the application of the WHO/ICPS framework for MRA • Applied to a mixture of 67 pesticides, going through the tiered approach • Illustrate data needs and gaps for refinements at the different tiers
Substances	67 pesticides
Exposure Scenario	Exposure to pesticides via food residues
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Dietary exposure to pesticides, based on likely exposure to individual pesticides. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? International estimated daily intakes were used for the individual pesticides, 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes, co-exposure via multiple food residues is possible, even if only theoretically assumed in this case. 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? No classical grouping based on common effects/MoA was applied, but a surrogate based on PPDB health issue categories was performed.
Information sources	• JMPR reports reporting Acceptable Daily intakes (ADIs) and International estimated daily intakes (IEDIs) • Analysis also done for 13 WHO food cluster diet regions
MIXTURE ASSESSMENT/METHODOLOGY	
HRA	• HI approach according to WHO/ICPS tiered scheme • For Tier 0, all pesticides were classified as Cramer Class III, with a TTC of 90 µg/person per day. • For Tier 1, HI calculated using ADI values • Tier 2 calculation based on specific endpoints not feasible due to limitation in relevant data availability. However, a Tier 2 like refinement with a surrogate data set was performed.
Overall summary of	• A low-tier assessment identified a potential risk. For the 67 pesticides HI>1 was calculated for all 13 food cluster diet regions and exceeded 10 in one region (range for all 13 regions was 2.8-11).

outcome	The HI was never driven by just 1 chemical. 80% of the HI are contributed by each 9-18 chemicals in the mixture. • A tier 0 assessment was performed even if not needed due to the availability of ADI values, to investigate the differences in the resulting HI. HI values based completely on TTC ranged 37.5-146 and were up to 16 times greater than ADI-based HI calculations. • Tier 2 refinement was not possible due to a lack of relevant input data for the refinement, however, a surrogate refinement based on human health issues categories of the Pesticide Property Database (PPDB) was performed.
Future perspec-tives / Outlook	• In lower tiers, investigating further the individual HQs allows to identify the drivers of the mixture risk (chemicals contributing most to combined effect) • Data requirements in higher tiers are high and relevant input often unavailable, which represents a major obstacle in MRA • In this case study, an HI>1 would be reached if depending on the food cluster diet region co-exposure would occur to a minimum of 6-24 compounds assuming for each the average HQ individually. • In such a mixture, not all chemicals will have a common effect and contribute to a combined effect; it is however not implausible that 6-7 compounds in a mixture of 67 compounds might have a common effect.

ID	**6**
Title	**Pesticides in the Ebro River basin: Occurrence and risk assessment**
Journal	Environmental Pollution 211:414-424
Authors	Alexander Ccanccapa, Ana Masiá, Alícia Navarro-Ortega, Yolanda Picó, Damià Barceló
Year	2016
Background & Objectives	Previous studies performed in the Ebro River linking occurrence of pollutants, concentrations and toxicity, but most of them have focused on a single chemical family or select one environmental matrix (water, soils, sediments or biota). The objective of this study was to establish pesticide's occurrence, spatial distribution and transport and to evaluate the ecotoxicological risk in three trophic levels (Algae, daphnia and fish), using RQs for each pesticide and sumTUs for each sampling site.
Substances	Pesticides: 42 and some of their degradation products **Azol** (Imazalil, Prochloraz), **Benzimidazole** (Carbendazim, Thiabendazole), **Carbamates** (3-hydroxycarbofuran, Methiocarb), **Chloroacetanilide** (Metoalachlor), **Juvenile Hormone Mimics** (Pyriproxyphen), **Neonicotinoid** (Imidacloprid**), Organophosphorus** (Azinphos Methyl, Chlorfenvinphos, Chlorpyrifos, Diazinon, Diclofenthion, Dimethoate, Fenitrothion, Fenoxon, Fenoxon Sulfone, Fenoxon Sulfoxide, Fenthion, Fenthion Sulfone, Fenthion Sulfoxide, Malathion, Omethoate, Parathion-Ethyl, Parathion-Methyl, Tolclophos-Methyl), **Other Pesticides** (Buprofezin, Hexythiazox), **Triazines** (Atrazine, Deisopropylatrazine, Deethylatrazine, Propazine, Simazine, Terbumeton, Terbumeton-Deethyl, Terbuthylazine Terbuthylazine Deethyl, Terbuthylazine-2 Hydroxy, Terbutryn), **Triazole** (Tebuconazole), **Ureas** (Diuron, Isoproturon)
Exposure Scenario	Exposure (to biota: fish, algae and Daphnia) via water. Sediment concentrations are used to predict pore water concentrations.
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Chemical exposure is estimated based on chemical analysis of the matrices water and sediment. Other components might be present. Based on the compounds analysed, the sumTU was calculated, based on the acute toxicity values. If possible, also the Risk Quotients was calculated 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, because the analysis is referring to water, and pore water in the sediment. Exposure is very likely for fish, algae and Daphnids. Chemicals analysis in fish also show the relevance of exposure. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes. All compounds are analysed in the environment, and exposure

	at the same time is very likely. 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? All compounds were taken together, based on acute toxicity. Although in the presentation of the compounds they were grouped according to family or mode of action, this is not taking into account in the summation of the effect.
Information sources	• Concentrations in water and sediment were measured. • EC50 values collected from the PPDB database http://sitem.herts.ac.uk/aeru/ppdb/en/atoz.htm
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	Exposure is assumed to be via water. Water concentrations are measured directly, while concentrations in the sediment are used to calculate the pore water concentration based on the partitioning coefficient Kd (Cpw = Cs/Kd) where Kd = Koc x foc
Hazard Assessment	Acute 48 h EC50 for D. magna, 72 h EC50 for algae and 96 h LC50 for fish, as well as Chronic 96 h NOEC data for algae and 21 days NOEC for fish and D. magna of each chemical was collected from Hazard is based on acute toxicity values.
ERA	The calculated sumTU is the sum of all the individual TUs which are calculated by TU = ci/EC50i. To evaluate the impact of the pesticides on the Ebro River basin ecosystems, the risk quotient (RQ) method was used employing, whenever possible, the NOEC values obtained from chronic toxicity tests for producing the corresponding PNECs. (RQ = EC/PNEC). ERA was performed for fish, algae and Daphnia.
Overall summary of outcome	The obtained Sum TUs for water and sediment were <1 in all sites, evidencing that there is no acute risk associated with pollution either in water or sediments. The Toxic Unit for water and sediments showed the daphnia was the most sensitive (in 2010). Several pesticides showed a RQ > 1 indicating that pesticide risk to the aquatic communities needs further study.
Future perspec-tives / Outlook	A long-term chronic study on assessment of these mixtures is required. Not all chronic effects could be calculated due to missing information (NOECs)

A.2 Phthalates

ID	7
Title	Estimated daily intake and cumulative RA of phthalate diesters in a Belgian general population (Dewalque et al., 2014).
Journal	Toxicology Letters
Authors	Lucas Dewalque, Corinne Charlier, Catherine Pirard
Year	2014
Background & Objectives	The 5 phthalate diesters taken into consideration in this work are known to exhibit ED properties, especially anti-androgenic effects. The aims of this study were (1) to estimate, in a Belgian general population, the daily intake (DI) of those phthalates based on their urinary measurement, (2) to investigate the diet contribution to the total exposure, (3) to assess the risk of exposure to phthalates by comparing their intake to well-recognized reference values, (4) to assess the risk of cumulative exposure based on anti-androgenic endpoints to several phthalate compounds and (5) finally to compare the risk assessment results in adults and children.
Substances	Phthalates diesters: diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), di-iso-butyl phthalate (DiBP), butylbenzyl phthalate (BBzP) and di-2-ethylhexyl phthalate (DEHP)
Exposure Scenario	Human exposure to phthalates from food consumption and other sources
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Exposure of human to phthalate via food consumption and other sources. The key components are known. No data on the hazard of the mixture itself. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, exposure data comes from biomonitoring data. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? Phthalates are a structurally similar group of chemicals that have been shown to exhibit similar toxicological action, thus additive effect should be expected when considering this assessment group.
Information sources	Exposure data: measurement from biomonitoring study Toxicity value: EFSA TDI and RVs from literature
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure	Daily intake (DI) was based on the urinary measurements of the

Assessment	corresponding metabolites, and estimated using the volumetric model developed by Knoch et al (2003)
Hazard Assessment	Reference value chosen were tolerable daily intakes (TDI) for phthalates established by EFSA or a reference dose for anti-androgenicity (RfD AA) recently developed
HRA	The HI approaches was used: -HQ was calculated (HQ=DI/TDI) -HI=ΣHQ
Overall summary of outcome	• Although very few participants exceeded the threshold of 1 considered as safe for individual HQ, 6.2% of the adults and 25% of the children showed a HI_{TDI} higher than 1. These high HI values warranted further investigations since several studies suggested that anti-androgenic effects of phthalate exposure on reproductive health could occur at all life stages and because phthalates are not the only anti-androgenic chemicals to which humans are exposed. • The HI_{TDI} was 3-4 fold higher than HI_{RfDAA} showing that CEA results are very dependent of the reference value taken into account
Future perspec-tives / Outlook	• This biomonitoring approach has relevant advantages, such as integrating all routes and sources of exposure, and avoiding the external contamination due to the widespread presence of the phthalate diesters in the lab environment. However, it does not provide detailed information concerning exposure pathways. • DEHP would be the only phthalate congener studied for which the main contributor to the daily exposure would be the ingestion of food. For all other congeners, dietary intake seemed to be a minor pathway of exposure, suggesting that other routes should occur. • The TDI and RfD AA determination were based on animals exposed by gavage and therefore did not take into account other route of exposure. The estimation of DI was based on urinary levels measured in spot urine samples and extrapolated to a daily excretion with an estimated urine volume excreted daily. This also implies that these spot samples were considered as representative in terms of daily phosphate levels excreted while more and more studies highlighted the within-person variability of the urinary levels for these compounds. • Larger biomonitoring studies including pertinent biomarkers of exposure of other anti-androgenic compounds should be performed.

ID	**8**
Title	**Human biomonitoring of phthalate exposure in Austrian children and adults and cumulative risk assessment**
Journal	International Journal of Hygiene and Environmental Health
Authors	Christina Hartmann, Maria Uhl, Stefan Weiss, Holger M. Koch, Sigrid Scharf, Jürgen König
Year	2015
Background & Objectives	Assessment of population exposure to phthalates used in consumer products through a biomonitoring campaign, estimation of daily intake, estimation of cumulative risk assessment.
Substances	14 metabolites of 11 parent phthalate compounds
Exposure Scenario	Exposure through consumer products (and home environment) is assumed. Daily intakes are calculated from measured metabolites concentration in urine.
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Exposure occurs mainly though consumer products and house dust. Differences in urine levels show that environmental exposure matters as well as a difference in phthalates metabolites concentrations is observed between samples collected from (sub)urban and rural areas. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes. It occurs through different consumer products (e.g. toys, school supplies, plastic gloves, or paints, as well as food and cosmetic products) 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes. Metabolites of different parent compounds were detected in the same population group, showing that consumer products imply exposure to a mixture of phthalates. 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? Antiandrogenic activity
Case Specific Information sources	• Individual phthalates daily intakes are estimated from metabolites detected in urine samples and compared with acceptable exposure levels • TDI and reference dose for anti-androgenic activity are used for te calculation of the HI for each population class (adults, children, elderly)
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	Total daily intake is calculated depending on metabolites concentrations detected in urine

Hazard Assessment	Dose-addition by using the hazard index for the anti-androgenic phthalates related to the Reference Dose for Anti-Androgenicity or related to the Tolerable Daily Intake.
HRA	Cumulative risk assessment is calculated through the HI for anti-androgenic phthalates.
Overall summary of outcome	Median HIs based on all acceptable levels of exposure used are far below the value of 1. The highest values were identified among children, Exceedances of the HI of 1 existed among all age groups for tolerable daily intake based values, whereas no exceedance was identified for the reference dose for anti-androgenicity (reference doses for anti-androgenicity are higher than tolerable daily intake). Authors report that assuming other exposure to androgenic chemicals (e.g. pesticides residues and cosmetic products) there is potential indication of cause of concern.
Future perspec-tives / Outlook	Inclusion of a larger set of phthalates secondary metabolites.

A.3 PBDEs

ID	9
Title	Example Case study A: PBDEs - Annex A (Meek et al., 2011)
Journal	Regulatory Toxicology and Pharmacology 60 S1-S14
Authors	Bette Meek
Year	2011
Background & Objectives	A screening level RA of PBDEs was conducted under the Canadian Environmental Protection Act and slightly modified to illustrate the WHO/IPCS framework for combined exposure to multiple chemicals (Tier 0 and Tier 1).
Substances	Polybrominated diphenyl ethers (PBDEs) used as flame retardants in a wide variety of consumer products; three main commercial mixtures containing seven isomers were subject of assessment: pentabromodiphenyl ether (PeBDE), or ComPeBDE (usually containing a mixture of PBDEs with 4–6 bromines); commercial octabromodiphenyl ether, (OcBDE), or ComOcBDE (usually containing a mixture of PBDEs with 6–9 bromines); and commercial decabromodiphenyl ether (DeBDE), or ComDeBDE (usually containing PBDEs with 9–10 bromines)
Exposure Scenario	Exposure of general population through consumer products and via the environment
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Focus of the case study on exposure of the population in the general environment including through consumer products. The majority of data relevant of human health risk relate to commercial mixtures with much less information on individual congeners. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes. Direct contact via consumer products containing PBDEs is possible, also via the environment through the use and disposal of PBDEs. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes. There is overlap in congeners within the commercial mixtures and reason to believe that their kinetics will be similar based on similarity in physicochemical properties. 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? The assessment group contains seven isomers with identical base structure, overlap in congeners within the commercial mixtures, similarities in uses and common target organs. Trends in physic-chemical properties and toxicity vary consistently with increasing

	degree of bromination.	
Information sources	• Exposure data available from the assessment under the Canadian Environmental Protection Act (Tier 0); for Tier 1 estimated from available data • Hazard data no tolerable intakes or concentrations were available (Tier 0); for Tier 1 from literature.	
MIXTURE ASSESSMENT/METHODOLOGY		
	Tier 0	**Tier 1**
Exposure Assessment	Semiquantitative measure available from Canadian Assessment; determined based on volume of production, numbers of producing and using companies, and the sum of "expert ranked uses" (based on potential to create exposure for each use)	• Limited data were available, therefore a conservative estimate was based on maximum levels in air, water, dust, food, human breast milk • Standard intake values for six age groups in the Canadian population. • Thus upper-bound estimates of daily-intake of total PBDEs were estimated. Estimates considered conservative since they were based on summed estimates of all congeners for which data were available and highest measured concentrations for many media.
Hazard Assessment	No reference tolerable intakes or concentrations for relevant congeners were available, thus a hazard index could not be developed; as a conservative measure, the LOEL for the most toxic congener was considered.	• Most toxicity data found were for commercial mixtures, less for the individual congeners; • From all data the critical effect level was selected at 0.8,g/kg body weight (PeBDE) based on neurobehavioural effects
HRA	The sum of semiquantitative estimates of exposure exceeded the LOEL of the most toxic congener, so additional assessment was considered necessary→Tier1	• Comparison of critical effect level with upper-bound estimate of exposure to total PBDEs for the potentially most exposed subgroup. • Resulted in Margin of Exposure of approximately 300. • Margins based on available biomonitoring data were approx. 10-fold less, but less confident due to uncertainty in back-calculation of exposure from biomonitoring data
Overall summary of outcome	• Food represented the principal source of exposure for the majority of age groups, highest for breastfed infants with breast milk accounting for 92% of the exposure. Estimates of intake from dermal contact with dust or oral contact with household products were negligible in comparison to uptake via food. • Uncertainties: Degree of conservatism in the derived margin is relevant to its interpretation. One critical aspect is the large interindividual variability in levels of PBDEs in breast milk (mean and median levels observed in the general population were 400 and 200 fold less, respectively, than the maximum levels on which the exposure estimate was based. The hazard was based on the most	

	sensitive effect for the most toxic congener. In other studies the effect levels were 100 times higher than the one used in this assessment. However, continuing increase in body burden was not considered due to limited information availability.
Future perspec-tives / Outlook	• In view of the smaller margin between the most conservative estimated critical values for exposure and effects on the environment in comparison with that for human health and resulting recommended action to protect the environment, in-depth evaluation of PBDEs from a human health perspective was considered a low priority at this time.

A.4 Parabens

ID	10
Title	Aggregate exposure approaches for parabens in personal care products: a case assessment for children between 0 and 3 years old (Gosens et al., 2013).
Journal	Journal of Exposure Science and Environmental Epidemiology
Authors	Ilse Gosens, Christiaan J.E. Delmaar, Wouter ter Burg, Cees de Heer and A. Gerlienke Schuur
Year	2013
Background & Objectives	Many chemical substances in consumer products are used in multiple product categories, leading to multiple source of exposure, but in risk assessment, aggregation of exposures from different sources is not common practice, especially when these sources are regulated under different legal frameworks. Objective is to assess aggregate exposure (exposure to a substance from different sources via different pathways) to the four most common parabens in personal care products for children between 0 and 3 years old. A deterministic approach with conservative assumptions (tiers 1) followed by a person-oriented probabilistic (tier 2) approach for exposure assessment was applied, to gain more insight into the feasibility and necessity of refining an aggregate exposure approach. Parabens are used in a wide variety of products: personal care products for adults and children, in consumer products such as dog shampoo, in pharmaceutical products such as antibiotics and they are used as food additives. Given the estrogenic effects of parabens and the potential severity of the effects during early human child development, the aggregate exposure for children between 0 and 3 years of age was assessed.
Substances	Methyl-, ethyl-, propyl- and butylparaben.
Exposure Scenario	Human exposure to personal care product
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Human exposure, oral and dermal absorption. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes. The parabens considered are the 4 most frequent paraben in personal care product from children from 0 to 3 years old. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? This study considers aggregate exposure (several sources) of one compound and therefore does not include co-exposure.

	4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? No assessment group
Information sources	• Exposure, Tier 1: Product composition and ConsExpo default value • Exposure, Tier 2: more detailed information on product use has been obtained from a small survey on product use of consumers. • Toxicity: NOAEL
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	• Tier 1: worst case, deterministic approach Parameters used for the exposure calculations: 1) maximum amount of paraben found in a product, 2) default use amounts of PCP as reasonable worst case estimates from ConsExpo 3) ConsExpo defaults of frequency of use as reasonable worst-case estimate. When application on body surface area was involved, the default value was extrapolated to children using a correction factor that account for the smaller total body surface area of children. • Tier 2: Person-oriented probabilistic approach is performed to estimate the variability and uncertainty of exposure in a population. Raw data on weight fraction measurements in 12 product types by the Dutch Food and Product Safety Authority and information from a pilot survey have been used to estimate exposure. The aggregate exposure per day is determined by adding all exposure on the same day for one person and subsequently averaging the daily aggregate exposure for each individual. The result is a distribution of the daily average aggregate exposure for all persons in the population.
Hazard Assessment	NOAEL
HRA	Percentiles of the distribution of exposure can be compared against the NOAEL. It gives an indication on the fraction of the population with average exposures above a certain Margin of Exposure (MoE).
Overall summary of outcome	• The internal exposure for each paraben calculated in Tiers 2 is below the level determined in Tier 1. However, for propyl- and butylparaben, the percentile of the population with an exposure probability above the assumed ''safe'' MoE of 100, is 13% and 7%, respectively (MoE of 8 and 10 respectively) indicating that further evaluation of the exposure calculations is necessary. • In conclusion, a Tier 1 approach can be performed using simple equations and default point estimates, and serves as a starting point for exposure and risk assessment. If refinement is required, the more data demanding person-oriented probabilistic approach should be used. This probabilistic approach results in a more realistic exposure estimate, including the uncertainty, and allows determining the main drivers of exposure. Furthermore, it allows to estimate the percentage of the population for which exposure is likely to be above a specific value.
Future perspec-tives / Outlook	• Refinement is difficult as detailed data on the use of PCP is scarce, and it is unknown whether extrapolation from adult use by scaling the amount of product used to body surface is appropriate. • Steps need to be taken before aggregate exposure can be assessed routinely: it would be useful to perform an extended personal care

	product use survey for children • Uncertainty in the exposure assessment for propyl- and butylparaben could be reduced by collecting more suitable data. • Pharmaceutical products contributed as the second largest product group toward the total paraben exposure. More exposure data via these products would be needed to obtain an even more accurate aggregate estimate

A.5 Pharmaceuticals

ID	11
Title	Screening level mixture RA of pharmaceuticals in STP effluents (Backhaus & Karlsson, 2014a).
Journal	Water Research
Authors	Thomas Backhaus, Maja Karlsson
Year	2014
Background & Objectives	Pharmaceuticals do not occur as isolated, pure substances in an environmental compartment. A broad range of different substances is used simultaneously in human and veterinary medicine, hence pharmaceuticals often occur in the environment as multi-component mixtures. The joint ecotoxicity of such chemical cocktails is typically higher than the toxicity of each individual compound, and even if the compounds of a mixture are present only below their respective toxicity threshold, a joint toxic effect cannot be ruled out *a priori*. Both approaches of the mixture quotient and of the STUs were used for providing a screening level assessment of the environmental risks of pharmaceutical mixtures previously determined in European sewage treatment plant effluents. The aim was to determine whether the detected pharmaceutical cocktails might pose a risk to aquatic organisms, how this relates to the toxicities of the individual pharmaceuticals, which group of organisms (trophic levels) is most sensitive and which are the ecotoxicologically most important compounds. Standard regulatory environmental risk assessment approaches for individual pharmaceuticals were followed as closely as possible.
Substances	Pharmaceuticals
Exposure Scenario	Exposure of aquatics organisms from sewage treatment plant (STP) effluents
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Exposure of freshwater organisms; key components known from previous published data; no information on the hazards of the mixture itself. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, mixture of pharmaceuticals are frequent in freshwater system 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? No assessment groups, mixture contains compounds with similar and dissimilar mode of action

Information sources	Exposure data are based on a comparative exposure assessment of a range of pharmaceuticals previously published in the literature. Hazard data were compiled in the published literature and/or database.
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	Exposure is based on previously published data on pharmaceuticals mixtures: data analysis of 26 pharmaceuticals present in 7 STP effluents was used as a basis.
Hazard Assessment	Toxicity data for chemicals were compiled from reviews, electronic databases, and if needed, primary literature. The European Medicines Agency guideline for the ERA of human pharmaceuticals (EMA, 2006) stated that environmental hazard assessments of pharmaceuticals should be based on chronic data, using an AF of 100 or lower. However, such chronic data are only available for a minority of the pharmaceuticals used in this work; the assessment was then based on acute data for algae, daphnids and fish, following the approach outlined in the REACH guidance document to estimate a PNEC on the basis of acute data, using an AF of 1000 (ECHA, 2008). If more than one EC_{50} was available for a given compound, the lowest value found for that particular species group was used. If no experimental toxicity data were found for a given trophic level, QSAR estimates were used for the EC_{50} values, assuming a common MOA of compounds from a similar chemical class. Differences in toxicity between members of a chemical class are then assumed to be caused by differences in lipophilicity-driven uptake rates.
RA for aquatic organisms	The concept of CA has been used *via* two approaches: 1) Estimation of the risk quotient of a mixture: $RQ=\Sigma\ (MECs/PNEC)_i$ MEC: measured environmental concentration 2) Calculation of the sum of toxic units (STU, with a toxic unit being $TU = MEC/EC_{50}$) in a first step for each of the main trophic levels (usually algae, invertebrates, fish). The final risk quotient (RQ_{STU}) for the mixture equals the sum of toxic units of the most sensitive trophic level multiplied with the corresponding AF, which is set to 1000 if data represent EC_{50} values from short-term toxicity studies with algae, invertebrates and fish (ECHA, 2008).
Overall summary of outcome	• The risk quotient of a single, randomly selected pharmaceutical is often more than a factor of 1000 lower than the mixture risk, clearly indicating that a mixture risk assessment is indispensable for an environmentally realistic risk assessment when it comes to pharmaceuticals. The MCR varies between 1.2 and 4.2, depending on the actual scenario and species group under consideration. • The mixture risk quotients, based on acute data and an assessment factor of 1000, regularly exceed 1, indicating a potential risk for the environment, depending on the dilution in the recipient stream. • The top 10 mixture components explain more than 95% of the

	mixture risk in all cases. However, the ranking profile strongly depends on the considered group of organisms. • Regarding the relative sensitivity of the three trophic level, algae are the most sensitive group, followed by invertebrate, fish being always least sensitive. • The ratio between the $RQ_{MEC/PNEC}$ and RQ_{STU} never exceeds 1.3 for the 7 effluents, if identical assessment factors are used.
Future perspec-tives / Outlook	• Ignoring Independent Action or using the sum of individual risk quotients as a rough approximation of Concentration Addition does not have a major impact on the final risk estimate • The lack of data on the chronic toxicity of most pharmaceuticals as well as the very few data available for *in vivo* fish toxicity has to be regarded as a major knowledge gap in this context

ID	12
Title	Environmental risk assessment of antibiotics including synergistic and antagonistic combination effects
Journal	Science of the Total Environment
Authors	Conrad Marx, Viktoria Mühlbauer, Peter Krebs, Volker Kuehn
Year	2015
Background & Objectives	• Aim of this study is to make a solid estimate on the possible synergistic potential of combined antibiotics • To quantify the subsequent effect for the case of the receiving river Elbe, Germany.
Substances	Antibiotics
Exposure Scenario	Exposure of aquatic organisms in receiving waters of waste water treatment plants (WWTP). Exposures calculated based on 15 most prescribed antibiotics in the investigated catchment area.
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Exposure of aquatic organisms via receiving waters of WWTPs. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, exposure estimates based on antibiotic prescription information and known rates of degradation in WWTP. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes, co-occurrence in Elbe river. 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? Share of different categories of antibiotics in the overall HI was assessed.
Information sources	• Exposure based on available information on antibiotic prescriptions and literature data. • Predicted no effect concentrations (PNEC) from own studies and literature. Information on binary interactions from literature.
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	• Exposure was predicted based on ambulant and hospital prescription data in the study area, excretion ratio, WWTP outflow, daily flow of receiving river Elbe, elimination rates in WWTP. Veterinary uses of antibiotics play minor role in the study area and were therefore neglected.
Hazard Assessment	• Predicted no effect concentrations (PNEC) from own studies and literature. Information on binary interactions from literature for bacteria, algae and daphnia, aggregated for the different antibiotic classes.
RA for aquatic organisms	• HI_{add} (based on concentration addition only) and HI_{int} (including interactions) were calculated.

Overall summary of outcome	• HI_{add} was calculated over a 7 years period with a mean value of 0.37. 20% of all weeks exceeded HI_{add} of 0.5, HI_{add}>1 was calculated only for 1 week in the 7 years period. • The hazard share of different classes of antibiotics changed over time (for some doubled), while the overall HI_{add} did not noticeably change over time. • Considering HI_{int} showing in a worst-case scenario a 50% risk increase, the threshold of HI>1 would is exceeded in 25 weeks over 7 years.
Future perspec-tives / Outlook	• Most underlying data on binary interactions were gained using much higher than environmentally relevant concentrations and using a variety of organisms (algae, daphnia, bacteria). • Some underlying data show that the probability for synergistic interactions increases at lower antibiotic concentration in contrast to many other studies. In summary, since the concentration influence on synergisms depends on the target organism and the combination of substances, no general statement on concentration dependency can be made for antibiotic mixtures. • Different scenarios applied in the HI_{int} lead to the conclusion that antibiotic mixtures tend to exhibit an overall synergistic effect. The resulting increase was between 20-50%.

A.6 Food Contact Materials

ID	13
Title	Assessing the safety of co-exposure to food packaging migrants in food and water using the maximum cumulative ratio and an established decision tree (Paul Price et al., 2014)
Journal	Food additives and contaminants: Part A
Authors	Paul Price, Rosemary Zaleski, Hans Hollnagel, Hans Ketelslegers & Xianglu Han
Year	2014
Background & Objectives	Food contact materials (FCM) can release low levels of multiple chemicals (migrants) into foods and beverages, to which individuals can be exposed through food consumption. This paper investigates the potential for non-carcinogenic effects from exposure to multiple migrants using the Cefic Mixtures Ad hoc Team (MIAT) decision tree. This assessment aims to demonstrate how the decision tree can be applied to concurrent exposures to multiple migrants using either hazard or structural data on the specific components, i.e. based on the acceptable daily intake (ADI) or the threshold of toxicological concern (TTC).
Substances	Food packaging migrants
Exposure Scenario	Human exposure via food and water consumption
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Human exposure via food and human consumption; key components known from previous published data. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? No assessment groups
Information sources	Exposure: Data on co-exposure to migrants were previously reported in a study on non-intentionally added substances (NIAS) eluting from food contact-grade plastic and two studies of water bottles (one on organic compounds and the other on ionic forms of various elements). Toxicity data: Existing ADI value or TTC approach.
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure	Exposure is based on 3 examples previously published of NIAS eluting

Assessment	from food contact material (food and water bottle)
Hazard Assessment	Reference values were based either on existing ADI or on a TTC approach, according to the Cramer class of the chemical, for organics chemicals. Inorganics without RVs were not considered in the analysis.
HRA	Determination of the HQ of each compounds, of the HI and MCR of the mixture
Overall summary of outcome	• The two first examples were assigned to the group II (low concern) by the decision tree. • The cumulative olefins and saturated hydrocarbons for the NIAS study and ethyl-4-ethoxybenzoate for the water bottles study provided the largest toxicity of any of the migrants. • The MCR value in example 1 is greater than 2, but this is impacted by the fact that the driving components are not single compounds but each represent a group of compounds falling into a structure-based chemical class. HIs are not affected by this grouping since the same RV is applied to all compounds. • In example 2, a single compound dominates the toxicity of the mixture (MCR<2). • The co-exposure assessment indicated that while multiple substance were extracted from FCM samples, the risk of adverse effects to individuals was very low (HI<1).
Future perspec-tives / Outlook	Outcomes : • Those three example show that sufficient data are available for the safety evaluation of many co-exposure to migrants that occurs in food and water, except for the inorganics for which 30 RVs were missing on the 57 chemicals. • When RVs are not available for organic compounds, the Cramer class predictions might be used, since the levels of exposure of migrants are low and often fall below the conservative estimates of RVs produced by the Cramer class approach. • The decision tree demonstrated that given the available screening toxicity data, exposures to the reported migrants both separately and in combination were unlikely to cause adverse health effects. • Future work on combined exposure to ionic forms would benefit from additional toxicity information.

A.7 Dioxin-like compounds (DLCs) including dioxins, furans and PCBs

ID	14
Title	Applying the maximum cumulative ratio methodology to biomonitoring data on dioxin-like compounds in the general public and two occupationally exposed populations
Journal	Journal of Exposure Science and Environmental Epidemiology
Authors	Xianglu Han and Paul S. Price
Year	2013
Background & Objectives	MCR values were calculated for three groups of individuals based on monitoring data and the WHO toxic equivalency factors (TEFs) for dioxin like compounds (DLCs)
Substances	Dioxin-like compounds (DLCs) including polyhalogenated dioxins, furans, and polychlorinated biphenyls (PCBs)
Exposure Scenario	2 occupationally exposed groups and one group of general population based on human biomonitoring data
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? 3 biomonitoring study groups, 2 with relevant occupational exposure, 1 with general exposure 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, as this is based on biomonitoring data. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes, as this is based on biomonitoring data. 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? Selection of included chemicals based on common dioxin-like characteristics
Information sources	• NHANES biomonitoring data plus 2 biomonitoring studies on occupational worker exposure • WHO TEF values
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	Lipid-adjusted concentrations of DLCs in serum were used from human biomonitoring studies. NHANES was used for one group of individuals reflecting current and historical DLC exposure in the general population. Further 2 groups of workers occupationally exposed to dioxins were included (trichlorophenol workers in Michigan (MI dataset) and New Zealand (NZ dataset)).
Hazard	35 DLCs were analysed in the 2 worker groups, but not for all of them

Assessment	TEFs are available, thus only 26 DLCs were used in the analysis and calculation of overall TEQs. This was done by multiplying the serum levels with the respective TEFs for each individual person.
HRA	Since the investigated mixture components share the same MoA, the toxic equivalency (TEQ) approach is preferred over the HI approach. TEFs are used to convert doses of each component into an equivalent dose of the index chemical 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). These equivalent doses are then summed up resulting in a toxicologically equivalent exposure to TCDD. The MCR is then calculated as the ratio of the person's cumulative toxicologically equivalent exposures for the mixture divided by the person's maximum chemical-specific equivalent. Total TEQ values were calculated for each individual in the studies. Within each of the 3 study groups, the mean TEQ was calculated for each DLC and ranked from high to low. Subjects with one or more missing values for the top 5 chemicals were excluded from the dataset. Non-detects (NDs) were assumed to be present at the limit of detection/$2^{0.5}$. Persons where NDs would have contributed >50% to the MCR or where the primary chemical was a non-detect, were excluded from the dataset.
Overall summary of outcome	• The top five major contributors to total TEQs in the NHANES dataset were 12378-PeCDD, 123678-HxCDD, PCB 126, TCDD, and 23478-PeCDF. On average they accounted for 76% of the total TEQ. • Total TEQs were higher in the MI and NZ datasets than in the NHANES dataset (58,96 fg/g for MI, 25.5 fg/g for NZ, 19,72 fg7/g for NHANES). Part of the difference is however also explained by the different age distributions, i.e. for persons >45 of age, NHANES total TEQs were lower than in the MI dataset but higher than for the NZ dataset. • Average MCR values (including 2.5th percentile and 97.5th percentile) were: for NHANES 3.5 (2.2/5.7), for MI 3.6 (1.6/5.1), and for NZ 3.2 (1.4/4.6). This indicates that for all 3 groups a small number of DLCs drives the total TEQ. • MCR showed decreasing trend with increasing total TEQ values. Overall more highly exposed people tend to have lower MCR values for the MI and NZ dataset, but not for NHANES. • Age and total TEQ are positively correlated. In the NHANES dataset two groups of age > or < 45 years can be distinguished with persons < 45 years showing generally lower DCL levels and higher MCR values.
Future perspec-tives / Outlook	• For all three groups, the MCR values were larger than in previous investigations of MCR of different mixtures, indicating a greater need for CRA for DLCs. A single substance RA based on the largest contributor only would underestimate the total TEQ by a factor of 2-6. • In the case of occupational or local sources of exposure, the impact of performing a CRA compared to single substance RA decreases. • Only 2-5 of the DLCs make significant contribution to the total TEQ. It might thus be sufficient to focus the CRA on the 5 highest ranking DLCs.

A.8 Cross-sectorial mixtures from consumer product and environmental exposure

ID	15
Title	Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment (Kortenkamp & Faust, 2010)
Journal	International Journal of Andrology
Authors	Kortenkamp, A., Faust, M.
Year	2010
Background & Objectives	There is widespread exposure to anti-androgens. Substances of concern include certain phthalates, pesticides and chemicals used in cosmetics and personal care products. However, chemicals risk assessment normally does not take account of the effects of combined exposure although a disregard for combination effects may lead to underestimations of risks. For this reason, this work aims at assessing the feasibility of conducting cumulative risk assessment, where the focus is on considering the effects of exposure to multiple chemicals, via multiple routes and pathways.
Substances	Anti-androgenic chemicals: a total of 15 substances including phthalates and other chemicals
Exposure Scenario	Human exposure from all known sources and by all known routes and pathways
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Exposure of human to anti-androgenic chemicals via all kind of exposure. The key components are known. No data on the hazard of the mixture itself. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? No information 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? Phthalates and agents capable of inducing the androgen insufficiency syndrome were grouped together.
Information sources	• Human exposure estimates from literature and publicly available assessment reports. • Reference doses for anti-androgenicity from literature
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	• Data were taken from peer-reviewed literature, or from publicly available reports of the European Scientific Committees and international regulatory bodies.

	• A distinction was made between median human intake value and intake value for highly exposed population groups.
Hazard Assessment	• Only toxicological endpoint with relevance to anti-androgenicity were considered • Dose describing "point of departure" normally used for RA (NOAELs, Benchmark doses) were taken from the peer reviewed literature and combined with uncertainty factors to derive acceptable level (AL); ADI were used when existing and derived from toxicological endpoint with relevance to anti-androgenicity
HRA	The HI approaches was used: -HQ was calculated for each chemical i ($HQ_i=EL_i/AL_i$) -$HI=\Sigma HQ$ EL: exposure level AL: acceptable level (e.g ADI)
Overall summary of outcome	• The cumulative risks from anti-androgen exposures exceed acceptable levels for people on the upper end of exposure levels. The value obtained for median exposures to the 15 substances can be judged tolerable (HI=0.38), whereas the value obtained for highly exposed population reaches 2.01. In this case, butyl paraben alone made up 50% of the HI. • Those results suggest that combined exposures to anti-androgens have reached levels of concern, especially among highly exposed groups of the population. • The authors suggest that risk reductions can be achieved by limiting exposures to the plasticizer diethyl hexyl phthalate, the cosmetic ingredients butyl- and propyl paraben, the pesticides vinclozolin, prochloraz and procymidone and bisphenol A.
Future perspec-tives / Outlook	• One assumption underlying the use of the HI approach is that the joint action of anti-androgens can be approximated by dose-addition; however synergism was observed with a mixture of androgens with diverse mode-of-action for particular endpoints; further work should be done to know whether this is a phenomenon of concern that should be taken into account in RA. • The summing of HQs implies that human population is exposed to each of the listed chemicals at the same time, which might not be very likely, especially in the high-intake scenario. Information about the co-occurrence of several chemicals in one and the same individual would be needed. • Significant knowledge gaps exist that prevent from arriving at definitive conclusions, i.e the absence of appropriate *in vivo* toxicity data about large numbers of *in vitro* androgen receptor antagonists. This assessment was restricted to chemicals where information about *in vivo* anti-androgenic effects was available, however many more substances with known human exposure are likely to contribute to cumulative anti-androgenic risks. • At this stage, too little is known about correlations between *in vitro* AR anti-agonists and their ability to induce disruption of male sexual differentiation *in vivo* to make meaningful extrapolations • p,p'-DDE and BDE 99 are highly lipophilic and build up in human tissues. By using intake values to calculate HQs these accumulating effects are not taken into consideration, thus the effective internal dose of these substances may be higher than suggested and the resulting risks may have been underestimated. To deal with this

	issue, it would be necessary to employ a different dose metric, and to relate intake values for all chemicals to their corresponding tissue concentrations. The data necessary for such calculations are currently not available.

ID	16
Title	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 wastes water effluents
Journal	Environmental Sciences Europe
Authors	Paul Price, Xianglu Han, Marion Junghans, Petra Kunz, Chris Watts, Dean Leverett
Year	2012
Background & Objectives	In 2010, Cefic has published a decision tree for the RA of chemical mixture, based on concepts taken from a number of published approaches including those developed by the joint group of three non-food Scientific Committees to the European Commission (SCs), the World Health Organisation/International program on Chemical Safety (WHO/IPCS), and recent publication on new quantitative tools (Maximum Cumulative Ratio, MCR), use of the Threshold of Toxicological Concern (TTC) in the assessment of risk from combined exposure. This paper applies the CEFIC decision tree to real world examples of exposures to multiple chemicals, for both human health and environmental risk assessment.
Substances	559 mixtures analysed for up to 222 substances measured in surface water samples (362) and effluent samples (197). The samples contained detectable levels of 2 to 49 substances, reported from water monitoring programmes in Europe, and include a wide range of inorganics, and polar and non-polar organic chemicals.
Exposure Scenario	Exposure via surface water or effluent from wastewater treatment plants (WWTPs)
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Exposure from surface water or water effluent. Key component known, according to monitoring data. No data available on the hazard of the mixture itself. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, data are coming from monitoring data 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes, data are coming from monitoring data 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? No assessment groups

Information sources	• Exposure: From monitoring data programme in Europe. Include seven data sets, differing in the number of compounds analysed in each samples and the water surveyed. • Reference Values: literature and internet based search.
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	• Similar approach for HRA and ERA • Conservative assumption: - Sampled surface water is assumed to be used directly as a water supply; individuals would be exposed from the consumption of drinking water. - A 10-fold dilution of the effluent has been assumed before the water would be used as drinking water. - A consumption of 2l per day for a 60 kg adult has been assumed.
Hazard Assessment	• It has been assumed that none of the components have non-additive interaction • MOA have not been researched therefore an additive models was used as the default assumption HH: If the RVs were not available, the Cramer classes provided an alternative source of conservative estimate of oral toxicity in order to determine the HI (WHO Tier 0). ERA: RVs available to determine HI (WHO Tier 1).
HRA	The HQ/HI approach is used: HQ=Dose/RV; HI=ΣHQ • If HI>1 the MCR is determined • For non-detected chemicals (NDs), which might be present at level <LOD, two assumptions has been made: -NDs=0 -NDs=LOD/$2^{0.5}$
ERA	Similar approach as for Human RA, with HQ=Concentration/RV
Overall summary of outcome	• For HH effects, 2% of the mixtures were of concern, 98% had a HI<1. For ERA, 68% of the mixture were of concern with one or more substance that had an individual HQ>1, 19% of the mixture had a HI<1, and about 12% were predicted to have toxicity of concern that would not have been identified unless a combined assessment has been performed (HI>1 but HQ<1). This means that the HH effects of the combined measured substances would have been sufficiently addressed by chemical-by-chemical approaches and had little need for a separate assessment of the combined exposure, which is not the case for ERA. • The majority of the toxicity came from one chemical in 44% of the case (HH) and 60% of the exposure (ERA). • The tree identified chemicals where data on the MOA would be most useful in refining an assessment.
Future perspec-tives / Outlook	• Chemicals with exposure levels exceeding their RVs, which would be subject to a refined chemical-specific RA, were not considered in this case-study. • The assumption of a 10-fold dilution of the effluents can be wrong for small rivers under low-flow condition; in addition, for rivers

	receiving multiple discharges the receiving water might already contain one or more of the compounds from discharges that occur upstream

ID	17
Title	Determining the maximum cumulative ratios for mixtures observed in ground water wells used as drinking water supplies in the United States
Journal	Environmental Research and Public Health
Authors	Xianglu Han and Paul Price
Year	2011
Background & Objectives	Data from water samples taken from groundwater wells from the public water system across the USA 1993-2007 were used. These samples have been analysed for a wide variety of chemicals including PPPs, VOCs, metals and other inorganics. The aim of this study was to further explore the usefulness of the MCR (Maximum Cumulative Ratio) and to investigate in detail (1) the pattern of the MCR and its ranges when applied to different types of samples/mixtures, (2) to explore the relationship between the MCR, number of substances in a mixture (n) and HI, and (3) to detect the impact of non-detects on the MCR values.
Substances	Dataset for 932 samples of ground water with measured compounds (number in brackets) being major ions (11), trace elements (23), PPPs and their metabolites/degradates (83), and VOCs (85). Not all 200 substances were analysed in all samples. 58 of the 200 substances were never detected and therefore the cases study focused on the remaining 142 compounds. Samples were excluded from further assessment if any of the 3 highest ranking chemicals in mean HQ was not measured (option 1) or if any of the first 6 highest ranking chemicals in mean HQ was not measured (option 2). Furthermore only mixtures including at least 5 compounds measured were included in the assessment. 2 options to deal with compounds below the LOD were compared.
Exposure Scenario	Exposure via ground water used for human consumption as drinking water without prior treatment as worst case assumption.
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Exposure from groundwater used as drinking water. Key component known, according to chemical analysis data. No data available on the hazard of the mixture itself. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes. 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP?

	No assessment groups
Information sources	• Hazard information (permitted doses PD) were taken from US EPA, ATSDR databases and other sources. • Exposure data from USGS groundwater monitoring data set
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	• Assumption: - groundwater directly consumed as drinking water (i.e. without prior treatment) - drinking water consumption rate 2L/day, 100% oral absorption, body weight 60 kg
Hazard Assessment	• Chronic RfD (for non-PPPs). For PPPs chronic Population Adjusted Doses (PADs) were used, and acute PADs if no chronic PAD was available. • No MoA and grouping considered Concentration addition assumed for all components using HI
HRA	• The HQ/HI approach is used : HQ=Dose/PD (PD=permitted dose) $HI=\Sigma HQ$ • If HI>1 the MCR is determined • For non-detected chemicals (NDs), which might be present at level <LOD, two assumptions have been made: -NDs=0 -NDs=LOD/$2^{0.5}$
Overall summary of outcome	• MCR has a negative correlation to HI (i.e. for mixtures with high HI the effect is driven by fewer compounds). • The effect of in- or excluding non-detects has a large influence on MCR for mixtures with small HI, but little impact on MCR for mixtures with HI>1. • A positive correlation of MCR with the number of analytes n was shown for both cases considering and not considering non-detects. E.g. in samples with 5-10 detects the MCR ranged from 1.0-2.0, while in samples with 15-25 detects the MCR range was 1.0-5.0. • The average MCR in all samples was 2.2-3.1, indicating that HI of most mixtures are dominated by just a few chemicals. • MCR values decreased with increase in toxicity (fewer compounds driving the risk in more toxic mixtures).
Future perspec-tives / Outlook	• The authors state that the toxicity of environmental mixtures is usually dominated by a relatively small number of components, • The MCR is a useful tool for screening and ranking on where mixture effects need to considered and where a single substance RA might be sufficient.

ID	18
Title	Example Case study B: Tier 0 – Substances potentially detectable in surface water - Annex B (Meek et al., 2011)
Journal	Regulatory Toxicology and Pharmacology 60 S1-S14
Authors	Boobis, Budinsky, Crofton, Emry, Felter, Mihlan , Mumtaz, Price, Solomon, Zaleski
Year	2011
Background & Objectives	Surface water represents a real-world example of a complex mixture. Many of the substances present do not have established chronic health standards or health-based guidance values, indeed, for some of the components there might be little or no information on their toxicity. Investigation of these mixtures using higher-tier assessments would require considerable resources and a significant number of data. The intent of this case study is to illustrate the potential utility of applying the threshold of toxicological concern (TTC) approach in a Tier 0 assessment to prioritize the need for further evaluation of a chemical mixture.
Substances	Data are based on surface water monitoring data, but to create an example a similar hypothetical mixture of 10 compounds was created. The 10 chemicals are form different classes (fragrances, pesticides, surfactants, personal care products, solvents, petrochemicals)
Exposure Scenario	Human exposure via the consumption of water is the considered exposure pathway.
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Data are available from surface water monitoring but no data on the hazard of the mixture itself are available. Human exposure via the consumption of water is the considered exposure pathway. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes. For the purpose of the case study it is assumed to be possible via the consumption of surface water as drinking water. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes. The 10 substances used for the case study were detected in the same survey. They are therefore assumed to occur simultaneously and continuously. 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? \
Information	• Exposure data available from monitoring of surface water. • Use of TTC

sources	
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	• Assumed that surface water is directly consumed without treatment • Worst case by choosing exposure of children and lifetime chronic exposure using maximum detected levels.
Hazard Assessment	It was assumed for the case example that no data would be available and the TTC was applied using ToxTree.
HRA (Tier 0)	• Concentration Addition was assumed using the HI (HI=sum of HQ) • Resulting HI was 0.2
Overall summary of outcome	• Given the conservative choices made to address the uncertainties, a HI<1 is considered to trigger no need for higher tier analysis.
Future perspec-tives / Outlook	• This hypothetical case study demonstrated the utility of using the TTC approach as a Tier 0 assessment tool for chemical co-exposures.

ID	19
Title	Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale (Malaj et al., 2014)
Journal	Proceedings of the National Academy of Sciences of the United States of America
Authors	Egina Malaj, Peter C. von der Ohe, Matthias Grote, Ralph Kühne, Cédric P. Mondy, Philippe Usseglio-Polatera, Werner Brack, Ralf B. Schäfer
Year	2014
Background & Objectives	To investigate new spatial scales in chemical RA and to achieve a RA of organic chemical on the continental scale, including 4000 European monitoring sites. To compare the chemical risk with the ecological status of the site, when possible
Substances	Organic chemicals. Data are based on surface water monitoring data
Exposure Scenario	Exposure of aquatics organisms (fish, invertebrates, and algae, represented *by Pimephales promelas*, *Daphnia magna*, and *Pseudokirchneriella subcapitata*, respectively).
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Data are available from surface water monitoring but no data on the hazard of the mixture itself are available. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, as this is based on monitoring data. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes, as this is based on monitoring data. 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? No assessment groups
Information sources	• Exposure data available from monitoring of surface water (Waterbase dataset of the European Environmental Agency). • Hazard data collected from database
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	• Measured concentration of 223 chemicals for 4001 sites distributed over 91 European river • The chemical concentrations (µg/l) for each monitoring site were reported as mean (C_{mean}), and maximum (C_{max}) annual values, typically used to characterize chronic and acute exposure, respectively.
Hazard	• Short term toxicity values were collected for each chemical and

Assessment	each of the three species: (*P. promelas* (96 h); *D. magna* (48 h); and *P. subcapitata* (48–96 h). In a sequential order, LC_{50} values were compiled by using experimental, predicted, or baseline (from the octanol–water partitioning coefficient) toxicity data. • Those toxicity data allowed the calculation of risk threshold for each organisms group, defined as: 1) Acute risk threshold (ART): *1/10 of the LC_{50} values for each of the three standard test organisms* 2) Chronic risk threshold (CRT): *1/1,000, 1/100,* *and 1/50 of the LC_{50} values for invertebrates, fish, and algae, respectively*.
RA for aquatic organisms	• Chemical risk (CR): the CR index for each organism group per river basin was calculated: $CR_{j,o,b} = N_{j,o,b}/N_{total,b,}$ *N : number of sites for which one of the chemical concentrations exceeded the risk threshold j (ART or CRT) for each organism group o within a river basin b,* *N_{total}: total number of sites within that river basin.* • Maps of distribution of the chemical risk (divided into 5 classes from low to high CR) in Europe were created. • To compare with the ecological status of the sites another approach was used: For each site within a rivers basin for which an ecological status was available: 1) C_{max} was compared to the ART; 2) C_{mean} was compared to the CRT. As concentrations exceeding these thresholds may cause acute and chronic ecological effects, respectively. Those sites were divided into three classes: (i) Chemical concentration > ART, *sites acutely affected by chemicals*; (ii) Chemical concentration > CRT, but <ART, *sites chronically affected by chemicals;* (iii) Chemical concentrations < CRT, *sites with no or negligible risk from chemicals*. The frequency of sites with high or good ecological status was calculated per class.
Overall summary of outcome	• Organic chemicals were likely to exert acute lethal and chronic long-term effects on sensitive fish, invertebrate, or algae species in 14% and 42% of the sites, respectively. • Of the 223 chemicals monitored, pesticides, tributyltin, polycyclic aromatic hydrocarbons, and brominated flame retardants were the major contributors to the chemical risk (pesticides were responsible for 81%, 87% and 96% of the observed exceedances of the ART for fish, invertebrates and algae respectively) • The risk of potential acute and chronic long-term effects increased with the number of ecotoxicologically relevant chemicals (ARCs) analysed at each site. • As most monitoring programs considered in this study only included

	a subset of these chemicals, this assessment likely underestimates the actual risk. Moreover, the results also depend on monitoring practice: a dense monitoring network and the inclusion of most ecotoxicologically relevant chemicals trigger a higher risk. • Chemical risk strongly depended on the land use in the upstream catchments of the monitoring sites. • Increasing chemical risk was associated with deterioration in the quality status of fish and invertebrate communities. Those results clearly indicate that chemical pollution is a large-scale environmental problem and requires far-reaching, holistic mitigation measures to preserve and restore ecosystem health
Future perspec-tives / Outlook	• There is a theoretical risk predicted based on the exposure concentrations monitored. This risk is increasing with the number of chemicals, as CA is the model used. However, in this study no mixture testing has been done and therefore it is not possible to compare the real toxicity with the predicted toxicity. • Those results are probably underestimating the risk for the following reason: 1) The significantly increasing trend of the CR with the number of ARCs that were analysed suggested that the acute and chronic risks would be higher if more ARCs were analysed. River basins with more than 15 ARCs analysed exhibited generally higher chemical risks. 2) For 18% of the analysed chemicals, in the majority of cases (>50%), the reported LOQ (smallest concentrations that can be reliably quantified) values were above the CRT. Thus, analytical measurements with higher sensitivity are required. 3) Whereas pesticides are designed to acutely affect invertebrates and algae, fish typically suffer from compounds affecting development, fitness, or reproduction (e.g., by endocrine disruptors), which are not covered here, but might increase the risk to fish communities 4) Other considerations could increase the chemical risk: (i) chemicals usually occur in mixtures, which might exhibit stronger combined adverse effects (ii) transformation products may be more ecotoxicologically potent than their parent compounds (iii) current monitoring relies on point grab water samples at monthly or quarterly intervals, which are very likely to underestimate the real maximum concentrations For a more realistic prospective risk assessment, monitoring programs should be designed to measure at least all ARCs, unless there is strong evidence that a specific ARC is ecotoxicologically irrelevant in a basin. However, emerging chemicals other than those frequently monitored are likely to be present in ecotoxicologically relevant concentrations in water samples and should be progressively identified and included in monitoring programs.

ID	20
Title	Should the scope of human mixture risk assessment span legislative / regulatory silos?
Journal	Science of the Total Environment
Authors	Evans RM, Martin OV, Faust M, Kortenkamp A.
Year	2016
Background & Objectives	Based on the fact that most of current chemical legislation addresses potential risks based on single substance assessments, it was investigated whether there is a concern that this approach is not sufficiently protective. The need for a mixture risk assessment (MRA) spanning different regulatory sectors is discussed based on two aspects: (1) evidence that combined effects have been shown for chemical mixtures containing substances regulated under different legislation and (2) evidence for human co-exposure to chemicals regulated under different legislation. One case study example is included to illustrate the potential risk, based on data published by Schlumpf et al 2010.
Substances	UV filters, fragrances, parabens, phthalates, organochlorine pesticides, PDBEs, and PCBs
Exposure Scenario	- Exposure of breast-fed children through human milk
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Exposure data from human biomonitoring of breast milk. Range of POPs and cosmetic product ingredients measured. No measured data on the hazard of the mixture itself available. 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, data from human biomonitoring in human milk. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes, co-exposure of breast-fed children to chemicals detected in human milk. 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? Risk is calculated for the whole range of compounds as one group, but also for individual subgroups based on chemicals classes.
Information sources	• Exposure data from Schlumpf et al 2010 from mother/child cohorts where for the first time a large number of POPs and cosmetic product ingredients were measured • Hazard information: reference doses collected from authorities and literature as in Schlumpf et al 2010
MIXTURE ASSESSMENT/METHODOLOGY	

HRA	• A HI approach was used. Individual substance RQ were calculated, HI for different chemical groups and the HI for the whole mixture.
Overall summary of outcome	• HI>1 was identified for several chemical classes (i.e. organochlor pesticides and PCBs) • The overall HI for the whole mixture was 66, indicating a potential risk. • The different chemical components were mapped on different regulations and it is shown that some of them are covered under several pieces of legislation and the overall mixtures span a wide range of relevant regulatory silos.
Future perspec-tives / Outlook	• There is evidence underlining the co-exposure of humans to substances regulated under different "regulatory silos" and evidence of combined effects. • Several examples are shown of chemicals regulated under different legislation that elicit common effects (e.g. (developmental) neurotoxicants, substances potentially harmful to the developing brain). • Options to address a MRA across regulatory silos are discussed, e.g. extending the EFSA pesticide residue cumulative assessment group approach to other regulatory sectors.

ID	21
Title	Application of the maximum cumulative ratio (MCR) as a screening tool for the evaluation of mixtures in residential indoor air
Journal	Science of the Total Environment
Authors	Katleen De Brouwere, Christa Cornelis, Athanasios Arvanitis, Terry Brown, Derrick Crump, Paul Harrison, Matti Jantunen, Paul Price, Rudi Torfs
Year	2014
Background & Objectives	Four datasets of residential indoor air exposure were used to calculate HI and MCR based on chronic inhalation toxicity values.
Substances	Volatile Organic Carbons (VOCs) and NO_2 / residential indoor air
Exposure Scenario	Exposure to mixtures via residential indoor air (volatile organic carbons VOCs, and NO_2)
Problem Formulation (according to WHO/IPCS mixture assessment framework)	1. WHAT IS THE NATURE OF EXPOSURE? ARE THE KEY COMPONENTS KNOWN? ARE THERE DATA AVAILABLE ON THE HAZARD OF THE MIXTURE ITSELF? Exposure via indoor air; composition not fully know, only for monitored compounds, not data available on whole mixture 2. IS EXPOSURE LIKELY, TAKING INTO ACCOUNT THE CONTEXT? Yes, considering the time humans spent indoors. 3. IS THERE A LIKELIHOOD OF CO-EXPOSURE WITHIN A RELEVANT TIME FRAME? Yes. 4. WHAT IS THE RATIONALE FOR CONSIDERING COMPOUNDS IN AN ASSESSMENT GROUP? No grouping
Information sources	• Reference values retrieved by a structured review • Indoor air monitoring data from 5 European datasets including 1800 records
MIXTURE ASSESSMENT/METHODOLOGY	
Exposure Assessment	Exposure from monitoring data measuring VOCs and NO_2. Flemish school and home survey, OQAI French home indoor air study, EXPOLIS personal sampling and indoor residential air across European cities.
Hazard Assessment	Chronic inhalation RVs for non-cancer endpoints were collected from an array of sources (starting from authorities documents). For some data-poor substances, they were derived from occupational exposure limits. Chronic inhalation RVs could be identified for 44 substances. Large variations were found for RVs from different agencies ranging up to factor 300.
HRA	Calculating the HI using air concentrations/inhalation RVs Calculating the MCRs
Overall	• Average MCR was 1.8, with a range from 1 to 5.8. MCR was found to be small compared to the number of chemicals in the mixtures,

summary of outcome	indicating that generally the overall effect was driven by only a few chemicals. • MCR is significantly declining with increasing HI. • Large majority from Flemish school survey are categorised in the low concern group II, while Flemish homes to the concern for combined effects group III, and to the single substance concern group I. Most of the OQAI data are assigned to single substance concern group I. • Substances identified as biggest contributors were NO_2, trichloroethylene, acrolein, xylenes. These were however, not consistently measured in all the studies, so comparison of datasets and overall drivers is difficult. • Study shows that there are a significant number of cases where combined effects should be considered further and a chemical-by-chemical approach would be insufficient. However, the mixtures showing concern for combined effects were not those with the highest HIs. Highest HI values were observed for samples where single substances were dominating the overall risk.
Future perspec-tives / Outlook	• Personal measurements had generally a higher HI than indoor air measurements. Average ratio for HI was 1.5 (range 0.15-19). The use of indoor air versus personal monitoring could lead to some underestimation. • The choice of the RV had a large impact on the overall results. Using minimum RVs instead of the basic RVs moved most samples n to the group of single substance of concern I.

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