KEMI
Swedish Chemicals Agency

An additional assessment factor (MAF)
– A suitable approach for improving the regulatory risk assessment of chemical mixtures?

REPORT 5/15

kemikalieinspektionen.se

KEMI
Swedish Chemicals Agency

The Swedish Chemicals Agency is supervisory authority under the Government. We work in Sweden, the EU and internationally to develop legislation and other incentives to promote good health and improved environment. We monitor compliance of applicable rules on chemical products, pesticides and substances in articles and carry out inspections. We review and authorise pesticides before they can be used. Our environmental quality objective is A Non-toxic Environment.

ISSN 0284-1185. Article number: 361 159.

Order print from Arkitektkopia AB, Box 11093, SE-161 11 Bromma.
Order tel: +46 8 505 933 35, Order fax: +46 8 505 933 99, e-mail: kemi@cm.se.

Preface

The Swedish Chemicals Agency has been assigned by the Swedish Government to produce a national action plan for a toxic-free everyday environment: Action plan for a toxic-free everyday environment 2011 – 2014 – protect the children better. The work with the action plan will continue until 2020.

Efforts are now going on in several areas, both in Sweden, within the EU and internationally and often in cooperation with other authorities. Reducing chemical risks in the everyday environment is one step towards attaining the Swedish Parliament´s environment quality objective A Non-Toxic Environment, which is the objective that we are responsible for.

Within the framework of the action plan, the Swedish Chemicals Agency compiles knowledge in our report and PM series elaborated by experienced colleagues, researchers or consultants. In this way, we present new and essential knowledge in publications which can be downloaded from the website www.kemikalieinspektionen.se.

An area of concern is the fact that in everyday life humans and the environment are constantly exposed to a mixture of chemicals, while under current chemical regulations, the risk from exposure to chemicals is usually assessed for individual chemicals only. The main objective of the present report was to explore the possibilities to use an additional Mixture Assessment Factor (MAF) to take possible combination effects into account in risk assessments.

The report was written by professor Thomas Backhaus, Department of Biological and Environment Sciences, University of Gothenburg. Project leader and contact person at the Swedish Chemicals Agency was Dr Gunilla Ericson.

The views expressed in this report are those of the author and do not necessarily represent the position of the Swedish Chemicals Agency.

Innehåll

Summary

Purpose of this review and critical analysis is to explore the possibilities of using an additional Mixture Assessment Factor (MAF) in order to account for the increased risk that a chemical mixture poses in comparison to the risk caused by each of its components. Although various reports and discussion papers suggest MAFs between 10 and 100 for arbitrary mixtures it is concluded that, given the huge diversity of relevant exposure scenarios, a sufficiently protective and at the same time not overly conservative generally applicable MAF is almost impossible to define. However, a MAF might be a suitable approach to account for mixture effects, if applied within pre-defined boundary conditions and in specific mixture scenarios.

A consensual and adequately protective MAF has to be based on conceptual and empirical knowledge on the toxicology and ecotoxicology of chemical mixtures. This in turn implies that the relevant uncertainties that are supposed to be covered by a given MAF are specified. The most important uncertainties for mixture risk assessment are: (i) incomplete knowledge of the compounds present and/or their concentrations, (ii) incomplete knowledge on the hazard profiles of the compounds present, (iii) possible synergistic interactions, (iv) the sole use of Concentration Addition (CA) for assessing the mixture, instead of mixture models specifically tailored towards the mixture in question.

A MAF might be most easily applied to mixtures whose chemical composition and concentrations are known to an extent similar to that of individual substances. We previously demonstrated that a factor of 'n' (=the number of compounds in the mixture) is sufficiently protective under the assumption that (i) the mixture behaves according to CA, and (ii) no individual mixture component is present at a concentration that poses an individual risk. In this report, four different exposure scenarios with mixtures of 15-42 compounds were further analyzed. The results indicate that (i) single-substance oriented risk management and mitigation substantially lowers the overall risk of the mixture, but (ii) is insufficient to ensure protection against mixture effects. After successful single-substance risk mitigation measures, (iii) the Maximum Cumulative Ratio seems an adequate approximation for a MAF, which ranged from 2 to 17, again highlighting its dependence on the specific exposure scenario under investigation.

It will therefore be critical for further explorations on the relationship between single substances and mixture risks to group and delineate 'archetypal' exposure scenarios. If chemical co-occurrence is structured by human actions, i.e. by economic, social, and technical influences, and by the properties of the receiving environmental compartments (sorption properties, biotic transfers, mass flows, etc) ‘archetypal’ mixtures might be defined for the various exposure scenarios. These priority mixtures might overcome the limited informative value of the current lists of individual priority pollutants and might provide an empirical basis for adequately sizing scenario-specific MAFs.

However, it should be noted that a MAF might not always be compatible with the tiered approach to risk assessment that is currently at the core of many regulatory frameworks. This is a consequence of the fact that a MAF at least partly reflects the uncertainty of the exposure to a mixture, which is often the result of the joint activities of several actors. An individual actor might, under these circumstances, not be able to reduce the uncertainty (and hence the MAF) in a given complex exposure scenario. This is in contrast to the uncertainties encountered during the

assessment of a single chemical, which can be reduced by an individual actor by supplying additional data for the compound in question.

Applying a MAF during the risk assessment of individual substances is conceptually identical to reducing the critical value of the risk quotient (PEC/PNEC, respectively DNEL/Exposure ratio) from 1 to a lower value. However, not only does the complexity of exposure scenarios make it difficult to agree on an appropriate size of a generic MAF. Additionally, the problem remains that appropriate risk management and mitigation measures might need to be developed for scenarios in which many actors contribute to an overall risk with chemical emissions that have an individual risk quotient below 1. Especially in highly developed countries with a functioning system of single-substance risk assessment and management, such scenarios are getting increasingly important, particularly near population centers and areas with high industrial activities.

As a consequence, the risk quotient of a chemical should not only be viewed as a measure of risk in itself, but primarily as a measure of the contribution of a compound to the overall risk in an exposure scenario. Overcoming the notion that the use of a chemical with a risk quotient below 1 ensures chemical safety even in complex exposure scenarios is critical. In fact, this might be more important for improving regulatory frameworks than using a MAF for decreasing the numerical value of said risk quotient.

Sammanfattning

Syftet med denna rapport är att kritiskt analysera och beskriva kunskapsläget samt undersöka möjligheterna att använda en extra säkerhetsmarginal vid riskbedömning av kemikalieblandningar (MAF - Mixture Assessment Factor) för att ta hänsyn till den ökade risk en kemikalieblandning utgör relativt den risk de enskilda ämnena utgör.

Trots att mängder med rapporter och artiklar föreslår MAFs mellan 10-100 för en godtycklig blandning så är det i princip omöjligt att definiera ett generellt MAF som är tillräckligt säkert och samtidigt inte överdrivet konservativt för alla tänkbara blandningar och relevanta exponeringsscenarier. Dock, om ett MAF används inom ett begränsat område och i en specifik blandningssituation kan det vara ett lämpligt arbetssätt.

Ett adekvat skyddande MAF måste baseras på vetenskapliga begrepp och empirisk kunskap om kemiska blandningars toxikologi och ekotoxiologi. Detta i sin tur förutsätter att de osäkerheter man räknar med ska täckas av en MAF är specificerade. De viktigaste osäkerheterna för att utvärdera risker med en blandning är: (1) ofullständig kunskap om vilka ämnen som utgör blandningen och/eller deras koncentrationer, (2) ofullständig kunskap om deras miljöfarlighet, (3) möjliga synergistiska interaktioner, (4) att bara använda koncentrationsaddition för att utvärdera risken med en blandning istället för blandningsmodeller som skräddarsys för den aktuella blandningen.

En MAF appliceras lättast på en blandning där sammansättningen och koncentrationerna är kända i samma omfattning som för de individuella substanserna. Vi har tidigare visat att en faktor "n" (=antal substanser i blandningen) är tillräckligt skyddande under förutsättning att (1) blandningen är predikterbar enligt CA, och (2) ingen av de enskilda ämnena förekommer i koncentrationer som gör dem riskabla enskilt. I denna rapport, har fyra olika exponeringsscenarier med blandningar med 15-42 ämnen analyserats ytterligare. Resultatet visar att (1) riskhantering baserad på enskilda ämnen och begränsningar väsentligt minskar risken även för blandningen, men (2) det är otillräckligt för att säkert säga att det skyddar mot kombinationseffekter. Efter att ha begränsat riskerna med de enskilda ämnena, (3) Maximum Cumulative Ratio verkar ge en hyfsad approximation för en MAF i intervallet 2 till 17, återigen belyser detta att det är beroende av det specifika exponeringsscenariot som utvärderas.

För att vidare utforska förhållandet mellan risken för enskilda ämnen och blandningar är det kritiskt att gruppera och avgränsa typiska exponeringsscenarier. Om kemikalier förekommer tillsammans baserat på mänsklig aktivitet, t.ex. ekonomiska, sociala eller tekniska anledningar, och av egenskaper hos den mottagande miljön (sorptionsegenskaper, massflöden etc.) kan olika typiska blandningar definieras för olika exponeringsscenarier. Dessa prioriterade blandningar kan lösa problemet med det begränsade informationsvärdet som finns i aktuella listor över prioriterade ämnen och kan ge en empirisk bas som ger en adekvat dimensionering av scenario-specifika MAFs.

Det bör dock noteras att ett MAF inte alltid är kompatibelt med det stegvisa sättet att göra riskbedömningen som för närvarande utgör kärnan i flera regelverk. Detta är en följd av det faktum att en MAF åtminstone delvis reflekterar osäkerhet av exponeringen till en blandning vilket oftast är resultatet av flera olika aktörer. En enskild aktör kan, under dessa förhållanden, inte reducera osäkerheten (och därmed MAF) i ett komplext exponeringsscenario. Detta står i

kontrast till de osäkerheter som förekommer i bedömningen av enskilda ämnen, dessa kan reduceras av en enskild aktör genom att ta fram ytterligare data för det aktuella ämnet.

Att applicera en MAF vid riskbedömningen av enskilda substanser är konceptuellt likställt med att reducera det kritiska värdet på riskkvoten (PEC/PNEC, DNEL/Exposure ratio) från 1 till ett lägre värde. Komplexiteten i exponeringsscenarier gör det emellertid svårt att komma överens om ett lämpligt värde på ett allmänt MAF. Dessutom kvarstår problemet att lämplig riskhanterings och riskreducerande åtgärder måste utvecklas för scenarier där många olika aktörer bidrar till risken med utsläpp av kemikalier med individuella riskkvoter lägre än 1. Särskilt i länder med välutvecklade och fungerande system för riskbedömning och hantering av enskilda ämnen kan dessa scenarier vara betydelsefulla, särskilt så i tätorter och områden med hög industriell aktivitet.

Som en följd ska riskkvoten för en kemikalie inte bara ses som ett mått på risken som sådan men primärt också som ett mått på bidraget från ämnet till den totala risken i ett exponeringsscenario med blandningar. Det är av yttersta vikt att få bort det allmänna begreppet att en riskkvot under 1 försäkrar att en kemikalie är säker att använd även i komplexa exponeringsscenarier. Det kan till och med vara så att detta är viktigare än att använda en MAF för det minskade värdet på riskkvoten.

1 Glossary of terms

a.s.	active substance in a plant protection product, a biocide product or a pharmaceutical product
Antagonism	A mixture is said to behave antagonistically, if its joint toxicity is lower than expected by a pre-defined mixture toxicity concept (usually CA or IA).
AF	Assessment Factor.
CA	Concentration Addition.
DNEL	Derived No Effect Level, "the level of exposure above which humans should not be exposed" (ECHA, 2008a).
EC50	The concentration that causes 50% of the maximum effect in a defined biotest.
EDA	Effect-directed assessment.
EQS	Environmental quality standard.
ERA	Environmental risk assessment.
ETR	Exposure Toxicity Ratio, i.e. PEC/ECx (EFSA, 2013).
HRA	Human Health Risk Assessment.
IA	Independent Action, also termed "Response Addition".
IF	Interaction Factor, an additional mixture assessment factor that is specifically discussed to account for synergistic interactions in a mixture (Backhaus, 2013).
LOEC	Lowest Observed Effect Concentration, the lowest tested concentration that did cause a statistically significant effect in an experiment.
MAF	Mixture Assessment Factor, synonymous to MUF (Mixture Uncertainty Factor), discussed in detail in this document.
MATS	Mixture Assessment Triggering Substances defined as "substances which indicate that the mixture requires an assessment which goes beyond the isolated assessment of the substances themselves." by Bunke (2014).
MCS	Multi-constituent substances. These are chemically defined, with the main constituents making up between 10% and 80% (w/w) (ECHA, 2012). The guidance furthermore explains the difference between a MCS and a mixture as follows: "The difference between mixture and multi-constituent substance is that a mixture is obtained by blending of two or more substances without chemical reaction. A multi-constituent substance is the result of a chemical reaction." (footnote 12, page 21).
MCR	The ratio between the maximum toxic unit and the sum of the toxic units of all mixture components.
MPC	Maximum permissible concentration. "The MPC is a concentration of a substance in air, water, soil or sediment that should protect all species in ecosystems from adverse effects of that substance. A cut-off value is set at the fifth percentile if a

	species sensitivity distribution of NOECs is used. This is the Hazardous Concentration for 5% of the species" (Janssen, 2004).
NC	Negligible concentration. "The NC represents a concentration causing negligible effects to ecosystems. The NC is derived from the MPC by dividing it by 100. This factor is applied to take into account possible combined effects." (Janssen, 2004).
NHANES	National Health and Nutrition Examination Survey, implemented by the US Department of Health and Human Services.
NOEC	No Observed Effect Concentration, the highest tested concentration that did not cause a statistically significant effect in an experiment.
PEC	Predicted Environmental Concentration.
PNEC	Predicted No Effect Concentration, "the concentration of the substance below which adverse effects in the environmental sphere of concern are not expected to occur" (ECHA, 2008).
POD	Point of Departure, a concentration within the range of tested concentrations, used as a starting point for extrapolations to lower concentration levels in risk assessment, such as NOECs, NOELs, BMDLs, ECx values.
RCR	Risk Characterization Ratio, synonym to RQ (risk quotient).
REACH	Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals.
RQ	Risk Quotient, the ratio between exposure (e.g. PEC or measured concentration) and the POD (point of departure).
Synergy	A mixture is said to behave synergistically, if its joint toxicity is higher than expected by a pre-defined mixture toxicity concept (usually CA or IA).
TIE	Toxicity Identification and Evaluation.
UVCB	Materials of unknown or variable composition, complex reaction products or biological materials, that cannot be sufficiently defined by their chemical composition, as the number of constituents is relatively large, the composition is to a significant part unknown, or the variability of composition is large (ECHA, 2012).

2 Background

The following background provides a brief overview of approaches for the risk assessment of chemical mixtures, focusing especially on recent developments in European chemical regulation. A classification system for chemical mixtures will be suggested in order to help systematize the application of MAF's in different exposure scenarios. A summary of the use of assessment factors for standard single-substance assessments will prepare the ground for the later discussion of the pros and cons of establishing an assessment factor specifically for risk assessing chemical mixtures. Finally, the uncertainties encountered specifically during the risk assessment of chemical mixtures are summarized at the end of this chapter.

2.1 Risk Assessment of Chemical Mixtures

Specific risk assessment and regulation of chemical mixtures is needed in particular for two reasons: First, the risk of a mixture typically exceeds the risk of each individual mixture component. This leads, secondly, to the so-called "something from nothing" phenomenon (Silva, 2002): even when it is ensured that all compounds of a mixture are present at concentrations that are considered safe from a regulatory perspective, the resulting mixture can still cause substantial toxic effects. These two characteristics have been demonstrated for different types of chemical mixtures, investigating different endpoints and exposure settings (reviewed by Kortenkamp, 2009). Consequently, the setting of quality standards and thresholds for individual chemicals is a necessary first step, but, taken alone, is insufficient for ensuring a non-toxic environment and the protection of human health.

The scientific state of the art in mixture toxicology and ecotoxicology has been reviewed in a series of reports and peer-reviewed publications, e.g. Kortenkamp, 2009; ECETOC, 2011; SCHER, SCENIHR & SCCS, 2012. A consensus seems to be emerging that the concept of Concentration Addition (CA) can serve reliably in a first-tier approach for predicting and assessing the joint toxicity of chemicals in the environment and human health. CA is therefore used for assessing chemical mixtures within REACH (Regulation (EC) No 1907/2006, in the form of the 'hydrocarbon block method'), the Biocide Regulation (EU) No 528/2012 and the Pesticide Regulation (EC) No 1107/2009. It is also discussed as a means for assessing mixture toxicities in the technical guidance document for setting EQS values (EU Commission, 2011) in the context of the WFD (Directive 2000/60/EC). This report therefore assumes, if not specifically mentioned otherwise, that CA is a valid approach to extrapolate from the hazard or risk of an individual compound to the hazard or risk of a defined mixture.

The characteristics of CA and its use for chemical risk assessment have been discussed in detail by US EPA, 2007; Kortenkamp, 2009; Hutchinson, 2011; Meek, 2011; Backhaus, 2012; SCHER, SCENIHR & SCCS, 2012; Kienzler, 2014. In the context of the following discussion it might be important to highlight the following four characteristics:

1) CA can only be applied to a mixture whose chemical composition is known and only if the individual toxicities (PODs) for all components are at hand.
2) CA builds on the notion that the compounds in a mixture do not interact and share a similar mode of action.
3) According to CA the mixture always poses a higher risk than each individual compound.

4) According to CA the mixture always poses an interim hazard, i.e. the POD of the mixture is somewhere between the POD of the most and the POD of the least toxic compound. The exact quantitative relationship between the PODs of the mixture and its components depends on the mixture ratio.

2.2 Mixture classification

It should be noted that the concept of 'a single substance', in the sense of a collection of molecules with the same structure, is purely theoretical. In practice, even a substance of highest grade usually contains 0.1-0.01% impurities – which might be (eco)toxicologically highly relevant. In fact, REACH and the CLP Regulation use an even broader definition of a substance (Art 3(1) of REACH, Art 2(7) of CLP): "*Substance means a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.*" In order to provide a practically useful framework for chemical risk assessment, REACH uses the term "well defined substance" versus "UVCB substance". "Well defined substances" are further divided into "mono-constituent substances", with the main constituent present at 80% (w/w) or more, and "multi-constituent substances", with the main constituents being present between 10 and 80% (w/w) (ECHA, 2012, p. 15).

This implies that (a) the chemical composition of a substance in the sense of REACH and the CLP might change from production to production batch, and (b) the "well defined substance" that is produced in a chemical production plant might differ substantially from the chemically defined, pure, substance that is monitored in the environment or a human body.

Two fundamentally different classes of chemical mixtures need to be distinguished:

Class A: defined mixtures, for which the chemical composition is known to an extent that is comparable to that of individual substances, and
Class B: chemically partly or completely unknown mixtures.

Kortenkamp and coworkers (2009) have furthermore classified chemical mixtures in relation to their chemical complexity and emission/imission patterns. On these basis, Table 1 provides an extended mixture classification, which is used in the present report.

2.3 Assessment Factors in Chemical Risk Assessment

Any risk assessment only provides a simplified excerpt of reality and assessment factors (AFs) are used to account for various data gaps. For example, the following sources of uncertainties are supposed to be accounted for by an overall AF during the environmental risk assessment of an industrial chemical that falls under REACH (ECHA, 2008, p. 19):

- intra- and inter-laboratory variation of toxicity data;
- intra- and inter-species variations (biological variance);
- short-term to long-term toxicity extrapolation;
- laboratory data to field impact extrapolation.

		Example
Class A: Defined Mixture	Type I: Mixtures that are legally considered a single substance, but which are actually mixtures from a chemical perspective	Multi-constituent substances (MCSs)
	Type II: Products that contain more than one chemical	Formulated pesticide, biocide or pharmaceutical product
	Type III: Chemicals jointly emitted from production sites, during transport, consumption or recycling processes	Pharmaceuticals and excipients monitored in the effluent of a production plant
	Type IV: Chemicals that coincidently occur together in environmental media (water, soil, air), food items, biota and human tissues, as a result of emission from various sources, via multiple pathways	Analytically monitored WFD priority pollutants in a river
Class B: Incompletely defined Mixture	Type I: Substances that are mixtures themselves	Natural fragrances, UVCBs
	Type II: Products that contain more than one chemical, without being chemically fully characterized	Herbal medicinal product
	Type III: Chemicals jointly emitted from production sites, during transport, consumption or recycling processes	Effluent from a wastewater treatment plant
	Type IV: Chemicals that coincidently occur together in environmental media (water, soil, air), food items, biota and human tissues, as a result of emission from various sources, via multiple pathways	All chemicals that co-occur in a river at a given moment in time

Table 1: Classification of mixture types.
This is an extended version of the mixture classification from (Kortenkamp, 2009).
It should be emphasized that mixture effects are not mentioned in this list as a source of uncertainty that standard AFs account for.

If the so-called base set of data (acute toxicity for algae, daphnids, fish) is available, an overall AF of 1 000 is used to calculate the PNEC for the freshwater environment. An additional AF of 10 is used to extrapolate between freshwater and marine organisms (ECHA, 2008). Two issues are critical for discussing Mixture Assessment Factors (MAFs) in the following: First, there is no guidance given on which proportion of the overall AF is attributable to which of the four sources

of uncertainty. Second, the extrapolation exclusively relates to the *hazard* assessment. AFs are also used in all other regulatory frameworks to account for uncertainties during the hazard assessment, but not for the exposure assessment. Instead of using AFs, an exposure assessment starts with a worst-case exposure scenario in the first tier, assuming no degradation of the compound in question, high emission rates in a confined area, etc.

During human health risk assessment, REACH uses the following AFs for assessing systemic toxicity (ECHA, 2008a):

- interspecies differences
  - correction for differences in metabolic rate per body weight (allometric scaling): 1.4 – 7, depending on the tested species
  - remaining differences: 2.5
- intraspecies differences
  - worker: 5
  - general population: 10
- differences in duration of exposure
  - sub-chronic to chronic: 2
  - subacute to chronic: 6
  - subacute to sub-chronic: 3
- issues related to dose-response 1
- quality of whole database 1

This is far more fine-grained that the broad overall AF used during the environmental assessment and for each source of uncertainty a separate default AF is given. The overall AF used for a particular assessment is then the result of multiplying all individual AFs. However, again, AFs only account for uncertainties of the hazard estimates, but not of the exposure estimates.

2.4 Uncertainties in the Risk Assessment of Chemical Mixtures

The hazard or risk assessment of a mixture is obviously subject to all uncertainties that already play a role during the hazard and exposure assessment of an individual chemical substance. However, several additional uncertainties are encountered during the hazard and risk assessment of chemical mixtures, and several biases might impact the overall assessment (Table 2). It warrants specific notice that most of these uncertainties might lead to an underestimation of the actual toxicity of the mixture. Empirical evidence clearly suggests that the two uncertainties that might lead to a risk/hazard overestimation (using CA for a mixture not entirely composed of similarly acting components, and antagonistic interactions) are quantitatively often neglectable (Kortenkamp, 2009). In contrast, several of the uncertainty that might lead to risk underestimations are huge, as further discussed below. For any discussion of a MAF it is important to clearly specify which uncertainties such an additional assessment factor is supposed to account for.

Source of Uncertainty / Bias	**Consequences**	**Discussed and described in e.g.**
The simultaneous presence of compounds as mixtures is ignored	Risk underestimation	Kortenkamp (2009); ECETOC (2011); SCHER, SCENIHR & SCCS (2012)
A mixture not entirely composed of similarly acting substances is assessed by CA	Hazard/risk overestimation, unless the concentration-response curves of the individual compounds are exceedingly flat	Junghans (2006); Kortenkamp (2009); Altenburger (2013)
Antagonistic interactions of the components in a mixture that is assessed by CA	Hazard/risk overestimation	Kortenkamp (2009)
Synergistic interactions of the components in a mixture that is assessed by CA	Hazard/risk underestimation	Kortenkamp (2009); Backhaus (2013); Altenburger (2013); Cedergreen (2014)
Insufficient (eco)toxicological knowledge on the mixture components to calculate the CA-expected toxicity.	Hazard/risk underestimation if the compounds with insufficient knowledge are simply ignored in the assessment. Otherwise the bias of the mixture toxicity assessment depends on the quality of the (eco)toxicological modeling and data bridging that is applied (e.g. QSAR estimates)	Backhaus (2010); Altenburger (2013)
Not all components included in the CA-based (eco)toxicity assessment of a complex exposure situation	Hazard/risk underestimation	Escher (2013); Tang (2014); Tang (2014a)

Table 2: Sources of uncertainty specific for the hazard and risk assessment of chemical mixtures.

These uncertainties are specific for the assessment of mixtures. That is, they come on top of the uncertainties encountered during the assessment of the individual mixture components.

3 Review and assessment of the Mixture Assessment Factors suggested in the literature

A specific mixture assessment factor (MAF, also sometimes termed "MUF", Mixture Uncertainty Factor) can account in principle for any of the uncertainties listed in Table 2. The following compilation of MAFs that are suggested in the scientific literature is therefore structured along their suggested application for Class A and Class B mixtures. Chapter 5 then analyses four class A mixtures, in order to explore the relation between the previously suggested MAF of 'n' (Backhaus, 2010) and a sufficiently conservative MAF for realistic mixture scenarios. A discussion of the pros and cons of the different MAF types and their application for the risk assessment of chemical mixtures is then provided in chapter 8.

3.1 Extrapolation from the risk of a single substance to the risk of a defined (Class A) mixture, assuming the mixture behaves according to CA

Even if the chemical composition of the mixture of interest is well known, the (eco)toxicological knowledge for several compounds might be insufficient to estimate its CA-predicted hazard/risk. In a previous report for KEMI (Backhaus, 2010) we discussed a possible MAF of 'n', i.e. the number of components in the mixture. It can be mathematically proven – under the assumptions that (i) the mixture behaves according to CA, and (ii) no component is present at a concentration above its POD – that a MAF of 'n' is sufficiently protective. This has also been further deliberated in a subsequent work for the German Environmental Agency (Altenburger, 2013).

The broader implications of using a MAF of 'n' have also been discussed by Hutchinson (2011):

> *Backhaus et al. (2010) suggested the use of a default mixture assessment factor for use in PNEC derivation. Whether this is actually needed in all cases is unclear, and the scientific basis would need to be carefully explained (eg should different factors be applied to acute and chronic data sets?). Simply increasing the level of precaution in the PNEC is likely to identify more scenarios as posing a risk. There therefore needs to be a consideration of the contribution of other areas of uncertainty to the assessment factor as well as possibilities for refinement should a risk be identified. In this regard, Chapman et al. (1998) encouraged the use of experimental evidence rather than defaulting to safety (assessment) factors to compensate for lack of information. Although this is a reasonable suggestion, the limits of testing requirements for regulatory purposes and large number of chemicals supplied may restrict the extent to which it can be put into practice. This is an issue that could warrant further review. […]*
>
> *Where suitable evidence exists, it is possible at a practical level to take account of additional toxicity caused by mixture effects by increasing the size of the assessment factor in the PNEC derivation for individual substances (eg as suggested by Backhaus et al. 2010). Further work could help provide examples, providing a flexible approach which considers the mode of action and highlight additional factors that may need to be considered on a case-by-*

case basis (eg comparing acute lethality due to narcosis versus chronic effects due to a specific mode of action such as endocrine disruption) (ECETOC 2007). However, given the limited empirical evidence for generic mixture effects on a wide scale, a blanket default approach to prospective risk assessment (eg under REACH) is expected to be inefficient and over-precautionary at the present time. In addition, the relevance of the other uncertainties that are addressed by the assessment factor would need to be considered, to ensure that any revised threshold was based on the best available scientific evidence (Chapman et al. 1998). In some cases, it may be possible to use toxic equivalency factors instead, provided that a suitable evidence base exists as is the case for dioxins and furans (Defra 2002) and other well-defined classes of environmental contaminants.

The strategy to apply a factor of 'n' to account for mixture components with an unknown (eco)toxicity is used in the upcoming EFSA guidance for pesticide risk assessment in edge-of-field scenarios. In section 10.3.7. "Simplified approaches for mixture risk assessment", (EFSA, 2013) the following is stated:

If no synergistic effects are indicated and the ETR values of the individual a.s. (ETRi) contained in the formulation are below the relevant trigger value, the mixture RA can follow a simplified approach: if all ETRi . ETR trigger/n (n= number of a.s.) the mixture also fulfils the authorisation criteria and the procedure can be stopped. Care must be taken that the PECi values considered in ETRi are identical to those defined relevant for the mixture RA (i.e. PECmix = sum of PECi).

This approach has been further taken up in a recent publication by Frische and coworkers on strategies for the environmental hazard and risk of pesticide mixtures (Frische, 2014):

[…] it is then asked whether the default assumption of Concentration Addition could in fact lead to the indication of an unacceptable risk. This is not the case if all individual TER-values (TERxi) exceed the corresponding trigger values by a factor of n, whereby n denotes the number of relevant mixture components included in the calculation. Thus, where this criterion is fulfilled (all TERxi C trigger times n) the procedure can also be stopped: the mixture fulfills the authorization criteria."

In contrast, the current, transitional, guideline on biocide mixture risk assessment recommends against the use of a MAF for the assessment of biocidal products (mixtures of type AII), ECHA (2014):

Using a specific safety factor, e.g. the MAF for mixtures has been dismissed, mainly since it would be difficult to scale such a factor for all different kinds of biocide product types.

A recent report by the German Environmental Agency (UBA) (Bunke, 2014) suggested to use a PEC/PNEC ratio (termed "RCR") of 0.1 for so-called "Mixture Assessment Triggering Substances" (MATS) as a trigger to initiate mixture risk assessments. A MATS is defined as "*a*

substance which causes concern, because it already occurs in relevant concentrations in the environment in relation to its inherent ecotoxicity" Unfortunately, no specific suggestions are provided how a MATS can be operationalized. The use of a MAF is then discussed in section 2.5.3 "Mixture Assessment Factors" of the report:

> *For regulatory handling of mixture effects there are proposals to apply a "mixture assessment factor" (MAF) to the single substances (see, e.g., section 2.2.5). Other assessment factors, as currently used under REACH to calculate reference points (like the PNEC) for the single substances, do not cover the possible influences of co-exposures. This would mean an additional "safety factor" to take account of simultaneous exposure to multiple substances. Therefore the use of a MAF means a reduced value for a PNEC or the MAF could equally be interpreted as a separate multiplier extending the formula to calculate an RCR:*
>
> *RCR= PEC/ MAF x PNEC < 1 (!)*
>
> *for all (known) substances within a mixture (for higher tiers the MAF would be included accordingly to other indices).*
>
> *However, MAFs are a rather imprecise way to handle risks from mixtures,*
>
> - *as they do not take into account of the specific data on the substances present in the assessed mixture (the factor does not vary depending on the concentration and the identity of the substances in a specific mixture),*
> - *the appropriate size of a MAF to correspond to assumed concentration additivity depends on the size of the mixture (number of included substances), for which it is used.*
>
> *As the specific data on the substances in the assessed mixture are not looked at, the MAF is often proposed in the case that not all of the relevant ingredients of the assessed mixture are known. This will be the case for environmental mixtures or other coincidental mixtures, where the establishment of MAFs is a very notable proposal.*
>
> *The appropriate size of the MAF could be linked to the typical number of substances (n) occurring in a mixture, because, for concentration addition, the (mathematically justified) MAF is equal or smaller than n in order to adequately account for such addition effects. Note that, more precisely, only those substances need to be accounted for, which contribute to a common ecotoxicological endpoint (e.g., only those substances need to be considered which a toxic to fish). As derived "from episodic findings", some authors (Price et al., 2010; Price and Han, 2011) argue that only few major substances may contribute significantly to the mixture effects with much lesser contributions by the other constituents, which may argue for a somewhat smaller MAF compared to the full number of constituents of the mixture. KEMI (2010) report a proposal in the Netherlands, where an assessment factor of 100 is applied to derive so-called "negligible concentrations". This factor should also provide a safety margin for combined toxicity (It is not stated, where this factor is implemented into regulatory practice).*

The advantage of the MAF-approach is the easy handling, once the size is fixed. The overall application would usually provide a relevant margin of safety to significantly reduce concerns from mixture effects. However, the scientific justification of the size of this factor is poor in case of mixtures with flexible size. Very often a factor of 10 is proposed as a minimum yet relevant quantification for a MAF.

For the general use of such a factor under REACH the consequences would be too far reaching, as all discharges into the environment would have to be reduced to an RCR of, e.g., 0.1. This may be regarded as not proportionate without further substance specific justification. A more targeted use of the MAF could be considered: this may be realised in other regulatory frameworks (provision of selected quality standards which include the mixture assessment factor for selected substances) or it may be possible under REACH, if criteria are provided, for which substances a MAF assignment is proportionate.

The possibility to use a MAF in the context of class A mixtures is mentioned without further discussion by Kienzler (2014):

As possible supplementary elements, mixture assessment factors (MAFs) and whole mixture testing can be considered. MAFs are sometimes proposed if a tiered approach is not feasible, and used as an additional assessment factor to calculate a PNEC for all those single substances known to be present within the mixture.

Le (2012) mentions a MAF in the specific context of metal mixtures:

[…] a specific mixture assessment factor is not employed in the current chemical-by-chemical risk assessment. The main reason is the lack of a validated approach for derivation of such a factor. Metal toxicity, as applied in this thesis, is determined by the accumulation of metals at the biological surface, which is a function of the stability constants of the metals and other metals in the environment. Consequently, effects of one metal on the accumulation of another metal at the biological surface are influenced by the difference in their stability for biological ligands. Therefore, an uncertainty factor based on the difference in the affinity constants between different metals in mixtures may improve the risk assessment of metal mixtures.

3.2 MAFs to account for synergistic interactions in a defined (class A) mixture

Sarigiannis (2012) mentions a MAF of 10 to protect against unexpected synergistic interactions, however, without providing specific recommendations for or against its use in risk assessment:

One way to deal with the general lack of knowledge about interactions in a cumulative risk assessment context is to use an additional uncertainty factor accounting for potential synergy effects […] Increasing the uncertainty factor by a factor of 10 and thus accounting for interactions of chemicals in a mixture would cover a tenfold increase in mixture toxicity due to interactions between

the mixture components. Not much is known about the significance and extent of synergy effects and it is unclear whether an uncertainty factor of 10 would be protective or over-protective. Currently no specific assessment factor for mixtures is employed in the traditional chemical-by-chemical risk assessment.

Feron and colleagues (2005) come to similar conclusions:

> *"In the case of similar action with interaction, it may or may not be possible to draw quantitative conclusions depending on the data. In theory, here exposure limits for the individual substances do not provide sufficient protection, regardless of whether the result of the interaction can be expressed in figures, except when the (supra-additive) interaction appears to be negligibly small. One might consider the use of uncertainty factors but there is no scientific basis for this."*

Backhaus and coworkers (2013) discuss an additional assessment factor of 2 to account for synergistic interactions between the components of biocidal products:

> *We therefore suggest to initially penalize CA-based assessments with an additional assessment factor, termed "IF" (Interaction Factor), in particular if no ecotoxicity data for the product in question are at hand. This factor shall account for the possibility of synergistic interactions (higher mixture toxicity than predicted due to chemical, toxicokinetic and/or -dynamic interactions).[…] as the general chance of underestimating the risk by more than a factor of 2 seems to be low for the majority of cases, an IF of 2 currently seems sufficiently protective.*

This has also been taken up in a presentation by Porsbring (2011) at a Special Science Symposium on biocide risk assessment.

In their transitional guideline for mixture toxicity assessment, ECHA (2014) considers a 5-fold higher risk than predicted by CA acceptable:

> *As the default assumption of non-interactive joint action is concentration addition (see point 2), synergistic effects are effects of a mixture which are greater than that predicted by CA by a factor of 5 or more*

This is in line with similar rules put forward by EFSA for pesticide mixtures (2013):

> *The observed and calculated mixture toxicities are considered in agreement if the MDR is between 0.2 and 5. This convention is in line with a proposal currently brought forward for the authorisation of biocidal products under the auspices of ECHA. In such a case, make use of the measured mixture toxicity (ECxppp) in the RA (at least, if mixture compositions in the study and at PECmix are compatible, see section 10.3.6).*

3.3 Extrapolation from the risk of a single substance to the risk of an undefined (Class B) mixture

A compilation of the suggestions for a MAF that is supposed to account for the fact that any environmental compartment or organism, including humans, is always exposed to more compounds than those that are detected even in broad, intense monitoring surveys is provided in the following and summarized in Table 3.

A MAF of 100 is used in the Netherlands to extrapolate from the Maximum Permissible Concentration (MPS) of a compound to its Negligible Concentration (NC), Jannsen (2004), van Vlaardingen (2007). The factor is supposed to account for mixture effects in the environment, Jannsen (2004):

> *The negligible concentration (NC) represents a value causing negligible effects to ecosystems. The NC is derived from the MPC by dividing it by 100. This assessment factor takes into account combination toxicity (VROM, 1989a,b). There is no equivalent to the NC in the EU-RAR.*

A workshop with participants from European academia and regulatory authorities on combination effects of endocrine disrupters was organized under the auspice of the Danish EPA and the Nordic Council of Ministers in 2010 in Copenhagen (Tørsløv, 2013). It was concluded that:

> *In order to deal with substances that are regulated under different regulatory regimes, it was proposed that about 10% of the safe dose or exposure to an ED should be allowed within each area of regulation. This could prevent cumulative exposure from different routes, e.g. food, water, environment. For instance in food, the maximum residual level for an ED should be set at a factor of 10 below the allowed intake of the substance to take into account contributions to the same mode of toxic action from other substances.*
>
> *Furthermore, it was generally agreed that combination effects could be addressed in human health risk assessment and environmental risk assessment by allowing that each substance could only contribute with a maximum of 10% to the total risk (i.e. a maximum Risk Characterisation Ratio of 0.1) or by introducing an extra Mixture Assessment Factor (MAF) of 10 in human health risk assessments and a factor of 100 in environmental risk assessments.*

A follow-up workshop on the "Road to regulation of endocrine disruptors and combination effects" was organised by the Danish Environmental Protection Agency and the Nordic Council of Ministers in 2013, in which the conclusions of the previous workshop were re-emphasized (Petersen, 2014):

> *Along these lines, the Nordic proposal of reducing the allowed RCR from 1 to 0.1 under REACH – or in general reducing the allowed limit values to 1/10 of the tolerable daily intake, was discussed. It was in general agreed that this way forward is still valid.". This would correspond to a MAF of 10 for the protection of human health.*

This strategy was also mentioned in the corresponding summary brochure (Nordic Council of Ministers, 2012):

> *Only a part (e.g. 10 %) of the "safe dose" should be allowed within each area of regulation*

A MAF of 100, termed "UF-mix" was suggested by the Pesticide Action Network in 2011, Muilerman (2011):

> *To fully account for mixture toxicity and include all negative effects on humans and wildlife, and also include the (often synergistic) effects of natural stressors, an extra uncertainty factor, the UF-mix is needed, estimated to be at a level of 100. The UF-mix should be implemented as soon as possible, to address the neglect of the past.*

Kortenkamp (2007) also briefly analyzes the possibilities of using a MAF:

> *If no data or information is available, it was considered to apply a default mixture assessment factor, making certain assumptions about the likely number of chemicals in the mixture.*

Silins and coworkers (2011) briefly discuss a MAF, but do not recommend a specific factor:

> *Another approach to assess any uncertainty with mixtures and to protect against possible mixture effects would be to use a safety factor, a mixture assessment factor (US EPA 2000). This factor could be used if not all components are identified and if concentrations or concentration ratios are unknown. This has been suggested as a possible approach within this context: however it is not clear how much this approach has been used in practice.*

Unfortunately, the author of the present report was not able to identify any suggestion for a MAF in the quoted EPA guideline from 2000 (US EPA, 2000), the supplementary guidance for conducting health risk assessment of chemical mixtures.

Area	Size	Reference
Environment	100	Janssen, 2004; van Vlaardingen, 2007
Environment	100	Tørsløv, 2013
Human health	100	Muilerman, 2011
Human health	10	Tørsløv, 2013, Petersen, 2014

Table 3: Suggested Mixture Assessment Factors for the protection of human health or the environment against the toxicity of class B mixtures (incompletely known chemical composition).

3.4 Miscellaneous

Reference values such as ADIs, DNELs or PNECs are set with a measure of precaution. This has been interpreted that they should be "*best viewed as lower-confidence limits of estimates of dose that are protective for that chemical*" (Price, 2009). Based on this line of reasoning, it has then been argued in the same publication that "*In the case of mixtures, using the DNELs for each mixture component results in an overestimation of toxicity, since the probability that all components of a mixture are as toxic as their DNELs becomes very small as the number of mixture components increases.*"

Following this line of reasoning would argue for lowering the assessment factors used in single substance assessments before the resulting PODs are used for mixture risk assessment. However, the argumentation fails to realize a fundamental property of CA: applying the concept does not inflate the overall uncertainty, but in fact *averages* it; CA is simply the weighted harmonic mean of the single substance PODs, weighted for their concentration in the mixture. This is illustrated in the following simple example:

The PNEC of a 2-compound mixture can be estimated using CA (Backhaus, 2012) as follows:

$$PNEC_{mixture} = 1 \Big/ \frac{p_1}{PNEC_1} + \frac{p_2}{PNEC_2}$$

Purely for the sake of keeping the example as simple as possible it is assumed that the lowest NOEC for both compounds is 1 and that they are both present in the same concentration (i.e. 50% of the mixture is made up by substance 1 and 50% by substance 2). If the same AF of 10 is used for both substances to calculate the individual PNECs, the resulting mixture PNEC is 1/(0.5/0.1+0.5/0.1)=0.1. That is, not surprisingly, the mixture has the same PNEC of 0.1 as both individual compounds.

If it is now assumed that for the second compound an AF of 100 is used, the resulting PNEC of the mixture is 1/(0.5/0.1+0.5/0.01)=0.02. That is, the mixture PNEC with AF_1=10, AF_2=100 is only a factor of 5 lower than the mixture PNEC calculated with AF_1=10, AF_2=10. This clearly shows that the individual uncertainties are not added, but instead averaged.

Price and colleagues (2009) furthermore argued that *The safe dose of a mixture determined by an independence model, $mDNEL_i$, would be the lowest value of $mDNEL_i$ for the mixture's components.* Mathematically speaking this can be expressed as (equation 3 of Price, 2009)

$$mDNEL_i = \min(\frac{DNEL_i}{F_i})$$

With $mDNEL_i$ denoting the mixture DNEL, $DNEL_i$ the DNEL of compound *i* and F_i the fraction of that compound in the mixture ($0 < F < 1$). Accordingly, the DNEL of the mixture is always higher than the DNEL of each mixture component, i.e. the mixture is assumed less toxic than each of its components. This notion is based on the No Addition case (see discussion in Kortenkamp, 2009), i.e. the assumption that the total toxicity of a mixture equals the toxicity of the most toxic compound at the concentration at which it is present in the mixture. However, no experimental examples of the No Addition case can be found in the (eco)toxicological literature, except from the almost trivial case of a toxic compound that is mixed with inert substances such as water. It is hence unclear whether the outlined situation has actual practical relevance.

Furthermore, it has been argued that the difference in species sensitivities to a mixture is smaller than the differences in species sensitivities to the individual compounds (Pedersen, 1996). This is, again, a result of the fact that a mixture averages the properties of its components. The practical result would be source of uncertainty summarized as "biological variance" (see discussion on the use of assessment factors in standard single-substance oriented assessments, page 13) is smaller for a mixture than for the individual substances.

4 Extrapolation from the risk of a single substance to the risk of a defined (Class A) mixture – Analysis of selected exposure scenarios

We have previously proven mathematically that a MAF of 'n' is sufficiently protective under the assumptions that (i) the toxicity of the mixture can be described by CA, and that (ii) no individual compound is present at a concentration at which it presents an individual risk, i.e. all individual toxic units (=RQ) are below 1 (Backhaus, 2010, Altenburger, 2013). A MAF of 'n' describes the relation between the RQ of a mixture and its components for the worst case scenario in which all components are present at equal toxic units, minimally below 1, so that no single substance risk is indicated but the mixture risk is maximal.

In contrast to this theoretical worst case scenario, several exposure assessments of realistic scenarios have been presented in the open literature in which the RQ values of environmentally realistic mixtures are very unevenly distributed (Junghans, 2006; Price, 2012a; Price, 2014; Backhaus, 2014). Under these conditions, only a comparatively few compounds 'drive' the mixture risk and even a MAF that is substantially lower than 'n' is sufficiently protective.

It has been previously argued that risk management and mitigation should first focus on the 'drivers of mixture toxicity', in particular on compound(s) whose individual RQ exceeds 1 (Price, 2012). But even if successful risk mitigation measures are implemented that lower the RQ of all compounds below 1, the mixture toxicity can still be substantial, depending on the number of compounds considered and on the actual size of the individual RQ value. Or, in other words: although the presence of a compound at a concentration exceeding its POD (=toxic unit > 1) should trigger appropriate single-substance oriented measures so that the risk of the concerned compound is sufficiently mitigated, the remaining mixture might still pose an unacceptable risk.

Four different mixture scenarios are probed in the following in order to get a first idea of a suitable MAF for realistic exposure scenarios, in relation to the worst-case MAF of 'n'. Two of the scenarios are relevant for environmental risk assessments, two for the assessment of human health. They were selected because the underlying monitoring data are either published in the peer-reviewed literature or in publically accessible databases and the accompanying information on the hazard of the mixture components was available to the study author, so that a CA-based analysis could be implemented. All scenarios reflect real or at least realistic exposures:

1) A pesticide mixture that was found in a Swedish stream in an agricultural area as part of the Swedish national monitoring program of pesticides. Exposure data were taken from (SLU, 2014), EQS values from (KEMI, 2008).
2) A mixture of pharmaceuticals monitored in a STP effluent. Exposure data are documented in (Andreozzi, 2003), ecotoxicity data for all detected pharmaceuticals were compiled by (Backhaus, 2014).
3) A mixture of anti-androgens at concentrations and mixture ratios relevant for human health. Exposure and hazard data for human health were taken from (Kortenkamp, 2010).
4) A mixture of organic air pollutants to which inhabitants of major European cities are exposed. Exposure and hazard data for human health were taken from (de Brouwer, 2014).

The assessment was implemented using the following algorithm:

1) For mixture 2 an assessment factor of 1 000 was applied to EC50 from studies on algal toxicities, reflecting the standard AF used within REACH to extrapolate to the PNEC using the base-set of data. For the other mixtures, the PODs reflect critical thresholds for human health (reference values) or the environment (EQS's) and were hence not further adjusted prior to the mixture analysis.
2) All compounds present in the mixture at an individual toxic unit > 1 were set to a toxic unit of 0.99. This reflects a successful single-substance oriented risk management and/or mitigation, as requested by current regulatory frameworks.
3) The maximum tolerable concentration of the mixture is the concentration at which the sum of all individual toxic units equals 1. Consequently, the actual sum of toxic units for a particular mixture gives the factor by which the total concentration of the mixture exceeds the tolerable mixture concentration. Decreasing the mixture concentration by this factor, subsequently termed $MAF_{Defined}$, would ensure that no unacceptable risk is caused by that particular mixture.

The analysis of the four mixtures is presented in Table 4 and Figure 1. It shows that (i) the higher the initial risk of the mixture, the higher the mixture toxicity even if a successful single-substance oriented risk management and mitigation is assumed. (ii) in all cases the mixture risks exceed 1 even if a successful single-substance oriented risk management and mitigation is assumed (iii) a MAF of 'n' is overprotective by a factor 2.5- 7.7, (iv) a realistic, scenario-specific MAF lies between 2 and 17, (v) the MCR increases as a result of single-substance oriented risk management and mitigation, (vi) the adjusted MCR gives a good approximation for a realistic MAF.

Those results clearly point to the scenario-specific nature of a MAF, which in turn calls for a broad systematic evaluation of published exposure scenarios, in order to gain a better

understanding of the range of realistic MAF values. In particular the finding that the MCR might provide a good approximation for a realistic MAF might warrant further exploration. This, however, would require substantial additional efforts, in particular to compile, quality-check and assess the data on the human health or environmental hazards of each individual mixture component for the compartment / biota in which they were measured. The lack of a publically available database with a compilation of quality-checked hazard profiles of compounds from different regulatory frameworks becomes painfully obvious here.

Type of mixture	Area	Number of components	POD[1]	Mixture risk quotient[2]	MCR[3]	Mixture risk after adjustment (=$MAF_{Defined}$)[4]	MCR[5]after adjustment	Details and Reference
Pesticide mixture monitored in a Swedish stream near an agricultural area	Environment	42	Environmental quality standards (EQS)	136	3.6	16.80	17	Monitoring data from site “M42”, downloaded from SLU (2014), sample from 4. Nov. 2012, EQS values from KEMI (2008)
Mixture of pharmaceuticals in a STP effluent	Environment	18	1/1 000 of the EC50 for algal growth & reproduction	48	1.2	4.65	4.7	Backhaus (2014), Scenario from STP M1-I, details in Andreozzi (2003)
Mixture of anti-androgens	Human health	15	Reference values for anti-androgenicity	2.01	2.01	2.00	2.00	High intake scenario from Kortenkamp (2010), reference doses from Kortenkamp (2010)
Mixture of organic air pollutants	Human health	29	Reference values	4.33	2.9	3.78	3.9	De Brouwere (2014), exposure scenario from EXPOLIS study

Table 4: Mixture Assessment Factors (MAF) sufficiently protective against the joint toxicity of realistic mixtures.

[1] POD: Point of Departure, i.e. the hazard estimate used to calculate the individual toxic unit, reference values, EQS values, PNECs
[2] Estimated using Concentration Addition. The value gives the sum of all individual toxic units
[3] Maximum Cumulative Ratio, i.e. sum(TU)/max(TU)
[4] Adjustment: all individual RQs exceeding 1 were set to 0.95, reflecting successful single substance oriented risk management and mitigation.
[5] Maximum Cumulative Ratio, i.e. sum(TU)/max(TU)

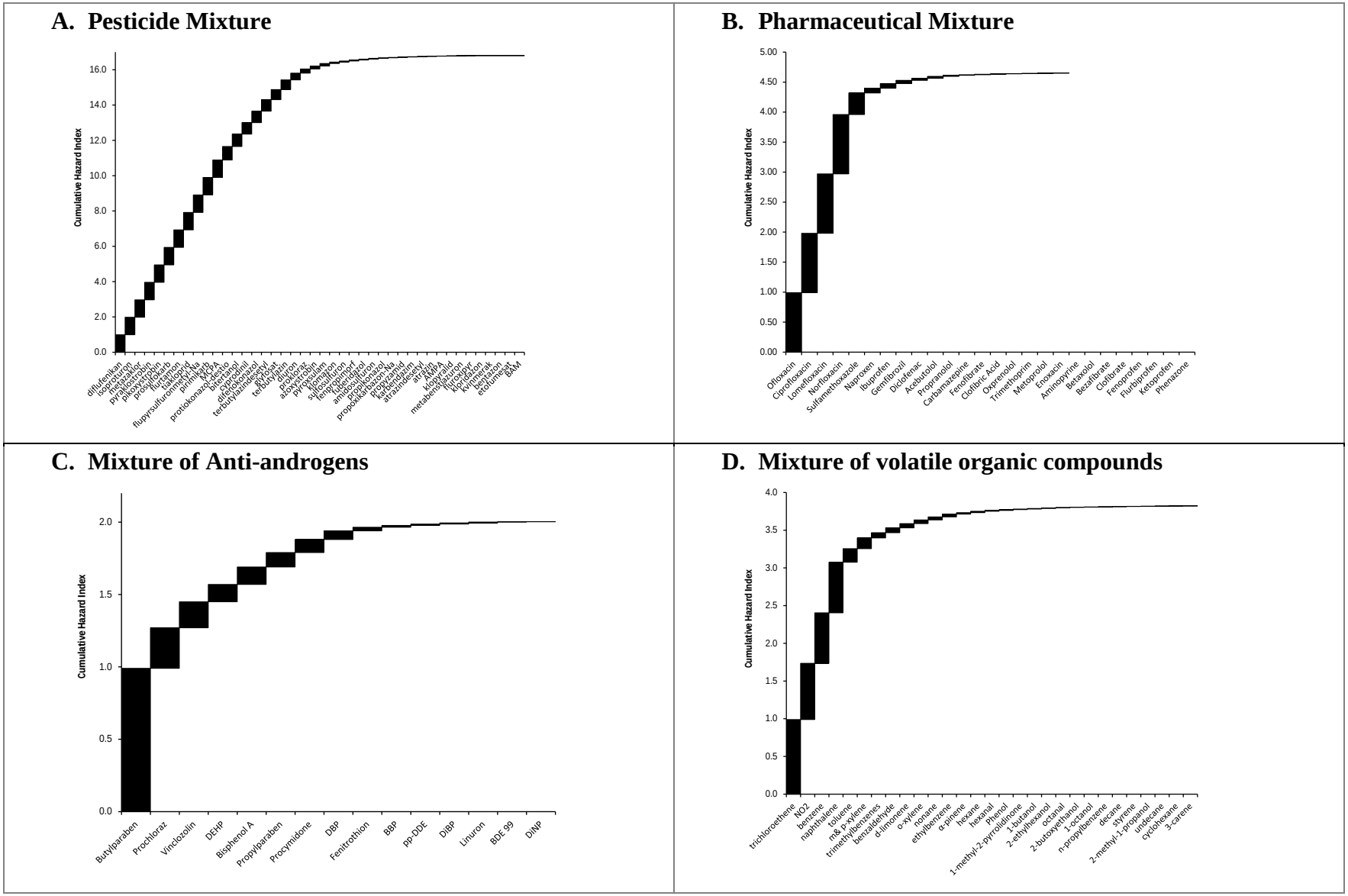

Figure 1: Distribution of toxic units for the four selected mixture scenarios.

A: Ecotoxicological evaluation of a pesticide mixture monitored in a Swedish stream near an agricultural area; B: Ecotoxicological evaluation of a mixture of pharmaceuticals in a STP effluent; C: Toxicological evaluation of a mixture of anti-androgens; D: Toxicological evaluation of a mixture of organic air pollutants. For details see text and Table 4. All individual toxic units that exceed 1 in the actual mixture have been set to 0.99 for the analysis. The plotted distribution hence reflects the situation after a successful single-substance oriented risk management and mitigation.

5 Selected examples of studies to gauge the relation between toxicity of an environmental sample (Class B mixtures) and the joint toxicity of identified pollutants therein

Environmental samples typically contain a complex mixture of chemicals with an unknown composition (Class B, Typ IV). Two complementary approaches are used in order to explore to toxicological or ecotoxicological impact of these exposures: (i) Toxicity Identification and Evaluation (TIE), and (ii) broad analytical screenings using *a priori* knowledge on emission patterns. TIE strategies employ a combination of biological and chemical-analytical approaches in order to successively simplify the environmental sample, identify the chemicals present and quantify their contribution to the overall (eco)toxicity, see review e.g. in (Brack, 2011). That is, a TIE study starts with a Class B, Typ IV mixture and uses a stepwise approach in order to establish the ecotoxicologically relevant Class A, Typ IV mixture for that scenario. TIE studies are therefore dependent on the availability of a sufficient amount of the original sample for the fractionation, identification and chemical confirmation of the mixture components, as well as pure standards of all these substances in sufficient amounts to run confirmatory bioassays. The final (eco)toxicological confirmation step in a TIE study compares the observed toxicity of the original sample with either the observed toxicity of an artificial mixture that is composed of the identified pollutants in the concentration and exposure ratio, or with the CA-predicted toxicity of that artificial mixture. Grote (2005) established the “index of confirmation quality” (ICQ) as the ratio between the observed toxicity (ECx values such as EC50, EC10, etc.) of the environmental sample and the observed or predicted toxicity of the artificial mixture that is made up of all identified toxicants. An ICQ of 1 hence indicates that all toxicants have been identified; an ICQ below 1 indicates that the artificial mixture cannot fully explain the toxicity of the sample and either additional unidentified toxicants are present, or synergistic interactions occur between the mixture components. An ICQ exceeding 1 indicates that the sample is less toxic than the artificial mixture, e.g. because of a limited bioavailability of the compounds in the original sample.

TIE studies have been successfully applied in a range of studies with mixtures of 2 to up to approximately 15 compounds with specific modes of action that can be (eco)toxicologically evaluated in bioassays with microorganisms (mainly algae and bacteria) or cell-based *in vitro* tests. Grote (2005), for example, observed ICQ values between 0.1 and 1 when comparing the algal toxicity of whole sediment extracts from the Bitterfeld area in Germany and the Brofjorden area in Sweden with the algal toxicity of a 9-, respectively 7-compound mixture that was composed of the chemicals identified at each site (Figure 2). Table 5 gives a summary of the results from similar studies. ICQ values typically vary between 0.1 and 10, indicating that identified compounds typically do not explain less than 10% of the observed toxicity of environmental samples.

Sample type	Compounds	Bioassay and Endpoint	ICQ	Reference
Wastewater	10 resin acids, monoterpenes and fatty acids	Nitrification inhibition assay with *Nitrobacter spec*	0.83, based on the observed toxicity of the artificial mixture (EC50 values)	Svenson, 2000
Sediment	2 mixtures, 7, resp. 9 PAH's, methyl-parathion	Inhibition of reproduction (green algae, *Scenedesmus vacuolatus*)	0.1 – 1.0, depending on effect level (based on CA, IA and observed toxicity of the artificial mixture)	Grote, 2005
Sediments	8 different mixtures of 1-6 PAH's, triclosan, lipophilic organics	Inhibition of reproduction (green algae, *Scenedesmus vacuolatus*)	0.1 – 1.0, depending on effect level, based on observed toxicity of the artificial mixture. Majority between 0.5 – 2	Bandow, 2009
Polar bear plasma extracts	3 mixtures of 6 hydroxylated penta-, hexa-, and hepta-chlorbiphenyls and 9 PFAS derivates	Transthyretin-binding assay (competition assay with thyroxine)	0.4-0.47, based on the observed toxicity of the artificial mixture (EC50 values)	Simon, 2013
Sediments	6 mixtures of PAHs,	EROD induction in fish cells	0.3-3.0, depending on effect level, based on observed toxicity of the artificial mixture.	Schulze, 2012
Additional TIE studies that did not allow to calculate ICQ values include Kammann (2004); Booij (2014);				

Table 5: ICQ values from ecotoxicological TIE studies.

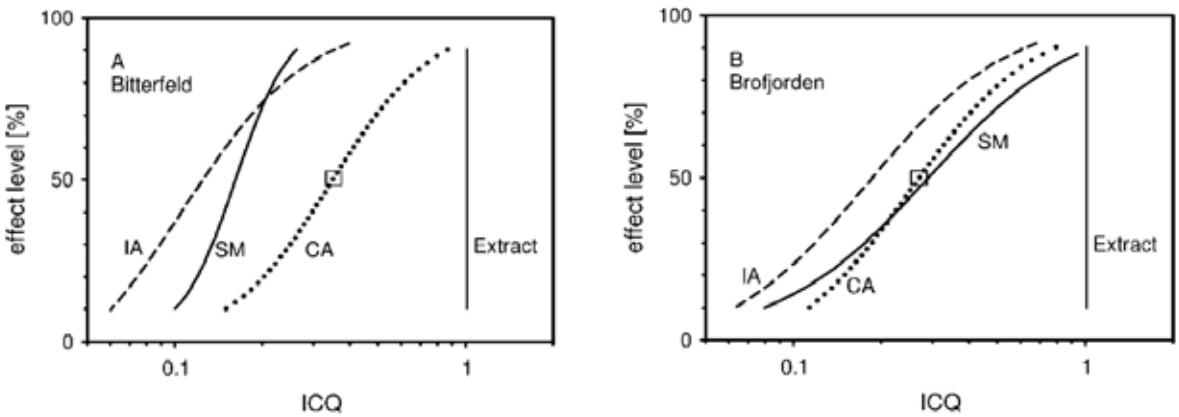

Figure 2: ICQ of mixtures identified in sediments from Bitterfeld (Germany), and Brofjorden (Sweden). From (Grote, 2005).

IA: Expected mixture toxicity of the artificial mixture, composed of the 9-, respectively 7-compounds found at Bitterfeld and Brofjorden according to Independent Action; CA: Expected mixture toxicity according to Concentration Addition. SM: Observed toxicity of the artificial mixtures. For details see text and the original publication.

An alternative to the TIE approach is to start with a broad analytical screening, potentially guided by information on emission sources or with the chemicals that are compiled in priority lists of relevance for the investigated compartment. Such a study has, for example, been recently published by Escher (2013), see Figure 3A. The authors analyzed the overall toxicity of various water samples from the urban water cycle in Australia (sewage to surface water to drinking water), and analyzed the occurrence of 269 micropollutants, compiled from various regulatory lists. The compounds that were detected by a range of chemical-analytical methods were used to compose so-called “iceberg” mixtures, i.e. artificial mixtures containing the identified compounds in their respective concentrations and mixture ratios. Figure 3 shows that in general less than 0.1% of the observed toxicity (baseline toxicity and oxidative stress) could be explained by the confirmed pollutants.

These results are in sharp contrast with a similar study from Tang (2014), which analyzed the algal toxicity of similar water samples in comparison to the corresponding artificial “iceberg” mixtures comprising several herbicides, Figure 3B. In this study the authors could explain between 37% and 135% of the toxicity of the water samples. The different outcomes of both studies are most likely caused by the initial selection of chemicals considered for the mixture experiments. In other words, the broad regulatory lists of chemicals used in the study by Escher (2013) might in fact only poorly reflect actual co-occurrences of chemicals in the water samples, a hypothesis that is supported by the fact that only 5 to a maximum of 48 of the total 269 chemicals were found. In contrast, the number of herbicides that qualify as potential water pollutants was only 12 in the samples analyzed by Tang (2014), and the toxicity parameter that was used (inhibition of photosynthesis yield) is closely coupled to the presence of herbicides.

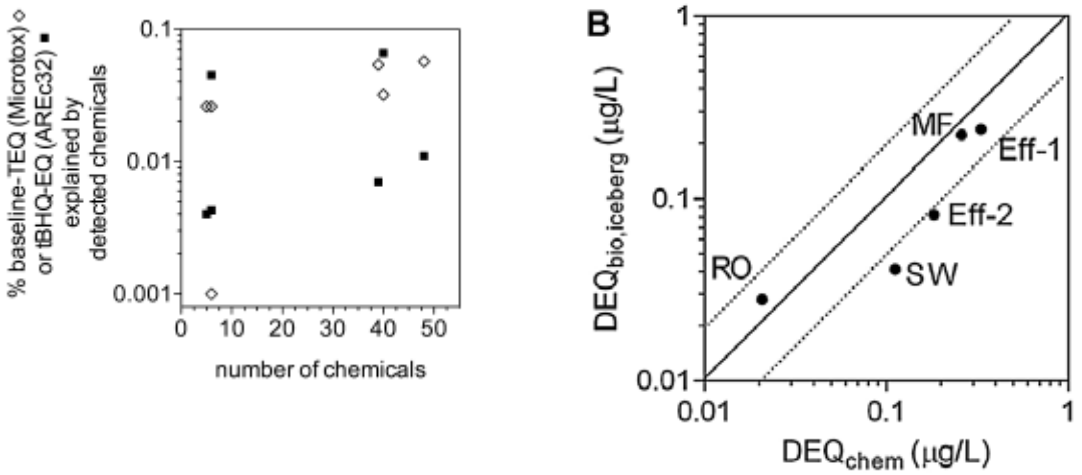

Figure 3 Relation between total toxicity of various water samples and the toxicity explained by artificial mixtures of the identified toxicants present.

A: Fraction of the total toxicity of various water samples (sewage, effluent, drinking water) that can be explained by the detected 5-48 chemicals. Less than 0.1% of the total toxicity can be ascribed to the joint toxicity of the known compounds. Open symbols: baseline toxicity, analyzed with the Microtox assay (bioluminescence inhibition of in the marine bacterium Vibrio fischeri). Closed symbols: oxidative stress, analyzed with human mammary MCF7-derived reporter cell lines (AREc32 cells). From Escher (2013).

B: Relationship between the observed algal toxicity (inhibition of photosynthesis yield) of extracts from various surface water types and the toxicity of the artificial mixture composed of the detected compounds, therein, termed "iceberg mixture". The iceberg mixture explained between 37% and 135% of the total toxicity of the different water types. Eff-1: secondary treated wastewater effluent (influent to MF); MF: after microfiltration; RO: after reverse osmosis; Eff-2: secondary treated wastewater effluent; SW: storm water.

From Tang (2014).

6 Exposure Assessment

An overview of the current state of the art in exposure science is given in a recent report by the National Research Council (NRC, 2012). Meek (2013) identified the following four guiding questions for the problem formulation phase of mixture exposure assessment:

1. What is the nature of data on exposure? For example, are the key components known?
2. Is exposure likely, taking into account the context?
3. Is there a likelihood of co-exposure within a relevant timeframe?
4. What is the rationale for considering compounds in an assessment group?

Question 1 and 2 are general exposure related questions, and question 4 prepares the ground for the later hazard assessment. It is question 3 that points to *the* critical issue for the exposure assessment of chemical mixtures: the assessment, which compounds co-occur in sufficient proximity in space and time so that their toxicological or ecotoxicological impacts might add up. An exposure assessment might approach this problem from either of two perspectives: chemical- or receptor-oriented. A chemical oriented perspective estimates the concentration and likelihood of co-occurrence of a pre-defined selection of substances, while a receptor oriented perspective puts a certain environmental compartment or biota (the receptor) into focus.

The problem formulation phase of a chemical-oriented exposure assessment begins with a list of potential mixture components (e.g. compounds monitored in an emission source, found in a chemical product, detectable by a certain analytical method, compounds that are used for similar purposes, subject to a common regulatory framework and/or are chemically similar). The identification of emissions sources is often a critical element in the problem formulation (see e.g. Rice (2008), during which location and nature (point or diffuse source, anthropogenic or natural, continuous or intermittent emission, etc.) will be determined. These activities result in a list of candidate substances which is then used to define actually occurring mixtures, either by exposure modelling or monitoring efforts.

A chemical-oriented approach follows in principle the standard approach for exposure monitoring that is outlined in REACH and similar Regulations and Directives, with the additional challenge to identify the temporal and spatial proximity and hence the likelihood of co-occurrence. It might be interesting to notice that such extended exposure assessments might go beyond the capabilities of individual companies: while they might be able to implement a mixture-aware exposure assessment for specified emission sources in the context of the Industrial Emissions Directive, the information necessary to assess the exposure to type IV mixtures on a regional scale requires a broader overview of the emissions taking place, which might be only available to regulatory authorities.

An alternative to the chemical-oriented approach is to take the receptor-oriented perspective, i.e. to start the assessment with the definition of the exposed entity that is to be assessed (an environmental compartment, or a particular species such as humans). This perspective is central for the “exposome” approach that is currently gaining momentum for the exposure assessment of humans, but also other selected species (e.g. polar bears, Simon, (2013)) to chemical mixtures.

The term “exposome” was coined by Wild in 2005 and describes the totality of the life-long exposure as a conceptual framework for understanding the environmental context of adverse health impacts (Wild, 2005). It aims to provide a “*comprehensive description of lifelong exposure history*” (Wild, 2012). Actually, the exposome concept goes beyond the mere

exposure to chemical mixtures, but also considers diets, age and lifestyle in order to draw the full picture of cumulative effects of environmental exposures during the lifecycle, from conception to death. The “public health exposome” provides a conceptual model to generate and test hypotheses about the underlying causality between adverse health outcomes in the general public and cumulative exposure to multiple stressors (Juarez, 2014). This is consistent with the recent trend to go beyond assessing exposure (and risk) of chemical mixtures alone, but to also consider the joint effect of chemicals with non-chemical stressors, see e.g. Zartarian (2010), Sexton (2011), Williams (2012).

This receptor perspective is also pivotal for the ecosystem perspective of the Water Framework Directive and the Marine Strategy Framework Directive, but is underused in prospective chemical risk assessment frameworks such as REACH, the biocide or the pesticide Regulation.

Chemical-oriented as well as receptor oriented assessments, can be implemented either by using various exposure models, or strictly retrospectively, based on chemical monitoring – and various combinations of both approaches. It is not uncommon that a particular study is actually assessing only the exposure of a certain receptor to a certain group of chemicals, e.g. the exposure of humans to pesticide mixtures.

6.1 Modeling-based approaches

Any approach that models the exposure to chemical mixtures has to (1) assess the likelihood of different compounds co-occurring; (2) integrate the exposure of the selected receptor (environmental compartment, certain biota) through the different pathways, reflecting the probability of exposure by any given pathway and the timing of exposures; and (3) preserve the links between spatial, temporal, and demographic aspects of exposure for defined individuals or population members.

It is a critical question whether chemical co-occurrence is random or structured; and, if it is structured, which chemicals associate with one another. If chemical co-occurrence is sufficiently structured by human actions, i.e. by economic, social, and technical influences, and by the properties of environmental compartments (sorption properties, biotic transfers, mass flows, etc) certain “typical” mixtures might be prevalent in certain exposure scenarios. For example, the time spent in traffic which has a major impact on human exposure to mixtures of volatile organic pollutants. Tornero-Velez(2012) used approaches that were previously used in ecological research to model the co-occurrence of certain species at a given location in order to model the occurrence of pesticide mixtures in French childcare centers. Such approaches might, if sufficiently validated and generalizable, prove valuable tools for simplifying the exposure assessment of chemical mixtures.

REACH and similar regulatory frameworks base their exposure assessment on a chemical-by-chemical analysis, driven by exposure scenarios and, most of the time, multimedia fate models based on mass-balance modeling, see discussion in MacLeod (2010). They usually start with standardized worst-cases assumptions such as the assumption that no biotransformation takes place, that high volumes are emitted and that emissions occur in close temporal and spatial proximity. This strategy allows tiering by replacing these worst-case assumptions a step-by-step with more realistic parameters. However, before such a chemical oriented approach becomes useful for mixture exposure assessments, additional data need to be available on chemical co-occurrence in time and space. Such studies are currently often restricted to mixtures from a common emission source or chemical group (often also defined by a similar use pattern).

Modeling of chemical mixtures seems more commonly implemented for human exposure assessment. The US EPA Office of Pesticide Programs for example uses a whole range of models for assessing cumulative exposures of humans to pesticides and the resulting risks[6]:

1. Dietary Exposure Evaluation Model (DEEM). The model is based on the food consumption data from NHANES, residue data from the different food commodities, and toxicological information (NOAELs etc), and aims to assess cumulative exposures and risks mainly of chemicals found in the diet. DEEM can be used to estimate total exposure of the overall U.S. population as well as defined subgroups, classified by age, gender or ethnicity. The Commodity Contribution Analysis of DEEM is used to identify the contribution of residues in individual foods to the overall estimate of dietary exposure. An implementation of DEEM is freely available as the "DEEM-FCID/Calendex Software", http://www.epa.gov/pesticides/science/deem/ Calendex is a supporting software tool for conducting an aggregate exposure assessment, i.e. for combining dietary and residential (non-dietary) exposures.
2. The Stochastic Human Exposure and Dose Simulator (SHEDS) A popular-science version of the tool is described at http://epa.gov/ord/sciencematters/august2011/sheds.htm [accessed 1. Feb. 2015]
3. The Cumulative and Aggregate Risk Evaluation system (CARES), which conducts cumulative human-health oriented exposure and risk assessments for pesticides. CARES was originally developed by CropLife America, an umbrella organization that represents pesticide producers in the US (http://www.croplifeamerica.org). The US EPA, and others, contributed scientific expertise to the software development. The software is currently taken care of by the ILSI Research Foundation (http://www.ilsi.org/ResearchFoundation/Pages/CARES.aspx [accessed 1 Feb 2015]. The CARES program and its source code are publicly available.
4. The model for assessing exposure, risk and benefits to elements of people's diets and living environment (LifeLine) is a probabilistic model of aggregate and cumulative exposures to pesticides and other chemicals which are applicable to the general US and Canadian populations. The tool allows to characterize population-based aggregate and cumulative exposures and risks from pesticide residues in food and drinking water. The software is maintained by the LifLine group http://www.thelifelinegroup.org/ [accessed 1 Feb 2015] and is freely available.

All the models implement the following steps (Williams, 2012): (1) they simulate an individual and their activity patterns throughout the day; (2) they combine activity information, consumption patterns, residue concentrations, and exposure factors; and then, (3), estimate population-level exposures using probabilistic sampling.

6.2 Retrospective, monitoring-based approaches

A plethora of studies on the co-occurrence of chemical in various environmental compartments are available in the literature. Within a European environmental context the

[6] It should be emphasized that the following descriptions of the different models were compiled from the respective websites and peer-reviewed literature. It was beyond the scope of the presented report to download, install and test-run the different software packages.

most prominent example might be the monitoring of priority pollutants in the context of the WFD, whose data are collected in the European Water Information (WISE). The WISE-WFD database will contain data from River Basin Management Plans reported by EU Members States according to article 13 of the Water Framework Directive, but is currently not yet publically available (WISE, http://www.eea.europa.eu/data-and-maps/data/wise_wfd, accessed Feb 2015].

However, the compounds that are highlighted as WFD priority compounds might only present a very small fraction of the toxic compounds relevant European freshwater systems. Moschet (2014) recently published a paper in which the consequences of intensified monitoring efforts were analyzed from a mixture perspective. Even though the study focused on pesticides only, and compared results with standard pesticide monitoring efforts (which go far beyond the few pesticides flagged as WFD priority compounds), the limitation of current monitoring and risk assessment practices became obvious: 30 to 50 pesticides and pesticide transformation products were detected per sample, and the CA-estimated ecotoxicity threshold was exceeded in each and every sample. Results from the Swedish pesticide monitoring program also indicate the occurrence of these compounds as mixtures and the highlight the ecotoxicological consequence of the resulting exposures, (Bundschuh, 2014).

Data from chemical monitoring in humans show similar patterns. The Center for Disease Control and Prevention (CDC) of the US Department of Health and Human Services conducts the National Health and Nutrition Examination Survey (NHANES) since the 1960s, with the aim to assess the health and nutritional status of adults and children in the United States (http://www.cdc.gov/nchs/nhanes.htm, accessed Feb. 2015). One product of the NHANES survey is the National Report on Human Exposure to Environmental Chemicals (http://www.cdc.gov/exposurereport/, accessed Feb. 2015), and the corresponding 4th report was published in 2009. The selection of chemicals for inclusion in the report is described in detail at http://www.cdc.gov/exposurereport/chemical_selection.html. Major criteria for an inclusion in the study were suspected exposure of at least parts the U.S. population, the potential consequences of an exposure for human health and the availability of analytical methods with sufficient accuracy, precision, sensitivity, specificity and throughput at an affordable cost.

Blood and urin samples were collected from a subset of the NHANES participants, and the last update (CDC, 2015) presents data for 265 chemicals. Not all of these could be analyzed for all samples, as the sample volumes were often insufficient. The report (CDC, 2009) states: "*Not all the chemicals in the Report are measured in the same individuals. Therefore, it is not possible to determine the fraction of all measured chemicals that were found at detectable levels in a given person.*", and no further information of chemical co-occurrence is provided.

However, the study by Woodruff and colleagues (2011), which focused on pregnant women, highlights the common exposure to chemical mixtures. The authors assessed the concentrations of 163 chemicals (heavy metals, PBDEs, PAHs, phthalates, organochlorine pesticides, cotinine) in blood, urine, and serum. 71 analytes were measured in parallel – and a median of 50 chemicals (range 35-60) of these were found in a given sample.

The Environmental Working Group (EWG) has tested a far broader range of chemicals (413 in total) in the umbilical cord blood of 10 newborn babies which were randomly selected from U.S. hospitals (Houlihan, 2005). An average of 204 (min 154 and max 231) compounds was found. Unfortunately, the study report does not provide an assessment of potential health effects, beyond general warnings.

In Germany the GerES (German Environmental Survey) has been carried out repeatedly since the mid-1980s, aiming to provide a representative population-level assessment of the extent, distribution and determinants of exposure to environmental pollutants (Becker, 2007), with the last implementation, GerES IV (2003-20006), focusing on children (Kolossa-Gehring, 2007). A total of 1.790 children aged 3-14 years from 150 sampling locations participated in GerES IV. Samples of blood, urine, tap water, house dust and indoor air were analysed for several heavy metals, organochlorine compounds, organophosphate metabolites, PCP and other chlorophenols, PAH and pyrethroid metabolites. Hearing tests, measurements of traffic noise and interviews to get exposure-related information were conducted (Becker, 2008). Although the study design (sampling regime) would allow to analyze the data from a mixture perspective, they are all presented strictly substance-by-substance (Becker, 2008).

In summary, the available monitoring studies highlight – not surprisingly – the common occurrence of complex mixtures in environmental compartments and in humans. But they also indicate the still persistent lack of awareness that the co-occurrence of chemicals is a critical issue for analyzing the risks to exposed populations, which cannot be assessed without an appropriate study design and documentation.

The Human Early-Life Exposome (HELIX) project is supposed to overcome these limitations (HELIX, 2015). HELIX is a European collaboration that aims to characterize the individual exposomes of children (Vrijheid, 2014). It is supposed to measure environmental exposures of up to 32,000 mother-child pairs and assess their impact on the growth, development, and health of the children. Smartphones will be employed to assess mobility and physical activity, and perform personal exposure monitoring. Classical biomarkers as well as novel “Omics” techniques will determine the metabolome, proteome, transcriptome, and epigenome as impacted by exposure to multiple chemicals. Toxicological endpoints include estimates for fetal and child growth, obesity, neurodevelopment, and respiratory outcomes.

The exposure assessment in HELIX is divided into two parts: (i) a general exposure, driven by residence (exposure to outdoor air pollutants, UV, and noise) (ii) an individual-based exposure (drinking water pollutants, indoor air pollution, pesticide uptake via food, POP and mercury exposure), estimated via questionnaires, and biomonitoring of individuals). The two parts will then be merged in order to estimate the individual exposomes.

The SOLUTIONS project (http://www.solutions-project.eu) is another European collaboration, specifically tackling the environmental consequences of exposure to chemical mixtures, with the professed aim to develop an inventory of River Basin Specific Pollutants for the Danube and Rhine river basins, to describe their occurrence as chemical mixtures and to assess their environmental impacts (Brack, 2014). TIE based approaches and extended monitoring efforts will provide the basis for the exposure assessment and a broad range of bioassays covering various populations of aquatic organisms (algae, invertebrates, fish, plants) will allow to provide detailed analyses of the resulting environmental impacts. Finally, SOLUTIONS will suggest appropriate abatement options.

Biomarker-based approaches are commonly used for retrospectively assessing chemical exposure and the resulting effects, they play a role in all the aforementioned research activities for the identification and confirmation of the (eco)toxicological impacts of an exposure to the mixtures in question. Their specific application for assessing the impacts of mixtures on human health is discussed in Silins (2011, 2011a). Biomarkers of exposure are preferably specific for the chemicals that an organism is exposed to (e.g. DNA adducts indicate exposure to alkylating agents, estrogen-receptor binding indicates exposure to xeno-estrogens), while biomarkers of effect often less specific. Biomarkers of effect could help to

identify the active components of the mixtures, they are therefore often employed as part of TIE studies.

7 Discussion

Assessment factors in chemical hazard assessment account for various sources of uncertainty, in order to limit the demand of experimental data and allow a quick, conservative assessment of whether there is ‘a case to answer’ that would warrant more in-depth studies. This discussion on the pros and cons of a Mixture Assessment Factor (MAF) is targeted towards the additional uncertainties that are encountered while assessing the risk of a chemical mixture (Table 2), the philosophy of single substance assessments is not a particular subject of the following text.

It became clear from the literature survey on assessment factors in the context of mixture hazard and risk assessments that three different uncertainty sources are being accounted for by the various MAF types. A MAF can be used to

1. account for the excess toxicity if a class A mixture is more toxic than predicted by CA (synergistic toxicity). This factor is, for the sake of having an unambiguous nomenclature, termed IF (Interaction Factor), in the following.
2. ensure that a class A mixture, i.e. a mixture for which at least the number of compounds is known, but for which (eco)toxicity data might be lacking for some of its components, does not pose a risk for human health or the environment.
3. ensure that a class B mixture, i.e. a mixture whose chemical composition is partly unknown) does not pose a risk for human health or the environment.

Additionally, it has been argued that mixtures have specific properties that actually lead to a *reduced* uncertainty, in comparison to single substance assessments. This is discussed in section 4.4.

The most appealing feature of using a MAF in order to account for the increased risk of a mixture is without doubt its simplicity. A MAF would not impact the hazard or risk assessment process *per se*, no additional experimental data or modeling efforts would be required. However, the relation between the total risk of a mixture and the risk of one of its individual components can, in principle, take any value, from almost 1 (if the scenario is dominated by that particular chemical) to infinity, if the component in question adds only a negligible toxic unit to a mixture of substantial toxicity and risk. In other words, a MAF can take any value, given the huge variety of exposure scenarios, unless additional boundary conditions are given for the mixture scenario. Table 6 gives an overview of the suggested sizes of the different MAFs, in relation to such boundary conditions. In the following discussion it is basically assumed that appropriate single-substance risk assessments and mitigations ensure that the toxic unit of each component is below 1, i.e. no single substance risk is indicated.

Any additional assessment factor would obviously lead to the conclusion of an elevated risk in comparison to the current substance-by-substance risk assessment that is in the center of prospective regulatory frameworks for chemical regulation, such as REACH, the Biocide Regulation or the Pesticide Regulations. This would require additional risk management and mitigation efforts in certain scenarios. It is certainly tempting to argue that any MAF is better than the current widespread ignorance of realistic exposure situations, where co-occurrences

Number of compounds known	All individual RQ's (toxic units) below 1	RQ's quantified	Information on interactions available	MAF
no	unknown	no	no	arbitrary value
yes	unknown	no	no	arbitrary value
yes	yes	no	no	number of mixture components*IF
yes	yes	no	interactions unlikely	number of mixture components
yes	yes	yes	no	MCR*IF
yes	yes	yes	interactions unlikely	MCR
yes	yes	yes	yes	case-by-case based on weight of evidence

Table 6: Suggested Mixture Assessment Factors (MAFs) in relation to the available knowledge on mixture composition.

RQ: Risk Quotient, MCR: Maximum Cumulative Ratio, IF: Interaction Factor.

of a multitude of chemicals in various biota and environmental compartments are common. However, a consensual and defendable implementation of a MAF will only be feasible if it is appropriately sized, i.e. specifically tailored towards a given exposure scenario.

Implementing a MAF would require a paradigm shift in the use of assessment factors in risk assessment, as it differs fundamentally from the AFs currently using during single substance assessment. The latter are used to bridge from the simplified conditions under which a chemical is hazard-assessed, based on the notion that under realistic condition the hazard might in fact be higher than indicated by the experimental data.

In stark contrast to the use of AFs during single substance assessments, a MAF accounts largely for uncertainties in the *exposure* assessment. In fact, based on the assumption that CA adequately describes the mixture behavior, the hazard of a mixture is lower than the hazard of its most potent compound, as CA is providing an average (the weighted harmonic mean) of the toxicity of all mixture components. The increased risk of a mixture – in comparison to the risk of even the most dominant compound, if assessed individually – is a result of the fact that the concentration of a mixture is always higher than the concentration of each component.

In contrast to hazard assessments, exposure analyses are based on the use of standard environments that are conservatively parametrized. For example, the regional environmental exposure assessment within REACH assumes a population of 20 million inhabitants in a region of 4 000 km^2 (ECHA, 2008c). Higher tier exposure estimates then populate the exposure models with more realistic estimates. This strategy provides conservative worst case exposure estimates, but at the same time highlights, again, the fundamental flaw of the current

guidelines: it is implicitly assumed that each and every of the several thousand chemicals that is listed in the REACH registry has its own separate 4 000 km^2 region available into which it is emitted.

It is a critical issue in this context that the idea of introducing a MAF is basically incompatible with the tiered approach to risk assessment that is central to prospective chemical regulatory frameworks. This is a consequence of the fact that a MAF reflects the uncertainty in the overall exposure to a mixture. This can be tackled by an individual actor, i.e. producer, importer or downstream user, only if the mixture in question is a chemical product or is emitted from a point source which is under control of the actor. But otherwise an actor has only a very limited possibility to reduce the uncertainty of a mixture evaluation by using higher tier assessments, as the mixture-specific uncertainties are the joint result of all activities of all actors taken together. This is in contrast to the uncertainties encountered in a single substance assessment, which can be minimized by an individual actor by producing additional data for the compound in question.

This reliance on sound exposure assessments emphasizes another shortcoming of the current implementation of substance-oriented regulatory frameworks (REACH, biocide and pesticide Regulations): a systematic compilation, dissemination and exchange of valid exposure information would be essential to gain a better understanding of which mixtures occur where and how often. Such a repository of exposure scenarios is, however, not available at the moment.

If the individual toxicities and the concentrations of the mixture components are known, it is largely agreed that CA provides a reliable first tier approach for assessing the toxicity of a mixture, see overview in section 3.1. However, it should be emphasized that despite substantial efforts and publication activities during the last years, the empirical data on the toxicity of mixtures are still quite limited and biased towards

- mixtures composed of a comparatively small number of compounds;
- mixtures composed of compounds from one particular chemical class (e.g. volatile organic compounds), use group (e.g. pesticides) or mode-of-action group (e.g. mixtures of (xeno)estrogens);
- mixture ratios that are selected in order to facilitate the comparison with conceptual predictions, but not in relation to actual environmental occurrences;
- studies in the freshwater environment

In view of these limitations, it might be suggested to use a specific IF that accounts for the uncertainty that stems from the sole use of CA, which is often the only option in a regulatory setting, given the available information on the (eco)toxicity of the individual compounds (Backhaus, 2012). Two uncertainties are critical in this scenario:

1) CA is known to <u>over</u>estimate the toxicity of most mixtures that are not composed of entirely similarly acting substances. Although empirical evidence on the amount of overestimation is still very limited, extensive simulation studies by Faust (2004) argue for this overestimation to not exceed a factor of 10, even for a mixture of 100 compounds, if the toxicity estimates relate to studies with algae and daphnids. However, for toxicity data from fish it was concluded that relevant overestimations (>= one order of magnitude) can occur already if more than 12 strictly dissimilarly acting compounds co-occur together and are estimated by CA instead of IA. Similar studies are absent from the field of human toxicology.
2) Synergistic interactions lead CA to <u>under</u>estimate the toxicity of a mixture. Such interactions can occur on a chemical, toxicokinetic or toxicodynamic level.

Chemical interactions are for example described in Dahllöf (2005). The authors describe that combinations of the antifouling biocides Zn-pyrithione and copper are noticeably more toxic than expected by CA (Bao, 2008), which is caused by the transchelation of Zn-pyritione in the presence of ionic copper to the more toxic Cu-pyrithione. An example of toxicokinetic interactions is provided by the synergistic effects of mixtures of various organophosporus and carbamate insecticides (Laetz et al. 2009). Organophosphate insecticides such as malathione are degraded after uptake by carboxylesterases, rendering them inactive. However, carbamates inhibit this biodegradation, which results in an increased cytochrome P450-driven bioactivation of the organophosphates to the corresponding oxon derivate and, consequently, to a synergistic toxicity of the mixture. Other toxicokinetic interactions have been described by Nørgaard (2010). Toxicodynamic interactions seem to be rare in comparison.

In a recent report to the German Environmental Agency, we tried to estimate the size of an IF for the environmental risk assessment of biocide products (Altenburger, 2013), concluding that an IF of 2 seems adequate in view of the empirical data at hand. However, it needs to be pointed out that experimental data on the quantitative consequences of synergistic interactions are extremely rare and that this analysis was mainly related to combinations of biocides. ECHA (2014) and EFSA (2013) take a deviation from CA-expected toxicities by a factor of five or more as an indication for synergistic interactions for mixtures involving biocides, respectively plant protection products. Cedergreen (2014) provides an overview of the available data on synergistic pesticide and biocide mixtures, concluding that synergistic mixtures are rare and were usually observed in 2-compound mixtures. Further empirical data and systematic evaluations on the quantitative consequences of synergistic interactions in more complex mixtures are certainly needed.

Boobis and colleagues (2011) analyzed the prevalence of synergistic interactions at relevant exposure levels in human-health oriented studies, finding mixture toxicity exceeding CA-predictions in 6 of 90 analysed studies, with a maximum IF of 4. Interactions are also accounted for during the human health assessment of chemical mixtures by the so-called Binary Weight of Evidence (BINWOE) approach of the US Department of Health, Agency for Toxic Substances and Disease Registry (ATDSR) (US Dep. of Health, 2001). ATDSR recommends that a qualitative weight of evidence analysis should be carried out if the sum of toxic units of the mixture increases above 0.1 (Pohl, 2003). This seems to imply the notion that synergistic mixture effects might increase the toxicity of a mixture by a factor of at maximum 10 above the CA-predicted toxicity. Also the US EPA has developed a similar approach to account for interactions (US EPA, 2000).

So far it is unknown how those two contrasting factors (overestimation *versus* underestimation) impact the overall predictive power of CA. All meta-analyses of the available data indicate that CA is quite accurate overall (Kortenkamp, 2009; Boobis, 2011; Cedergreen, 2014 and references therein), but it is unknown whether that is just a result of the strong bias in the available data. Mixtures composed entirely of metals seem a notable exception from the general trend, as those are prone to rather specific interactions.

An approach to estimate a suitable MAF for class A mixtures (mixtures with a defined composition) could be developed along the lines that were discussed in chapter 4.1. The four examples of realistic mixtures that were analyzed in the context of this report show that single-substance oriented assessments are a critical first step for risk management and mitigation, but, at the same time highlight that they are insufficient. A MAF of ‘n’, which is protective after a successful management of the individual substances seems slightly over-

conservative, but the degree of conservationism is different between the different scenarios: the actual MAF (=ratio by which the CA-expected toxicity exceeds its POD) is between a factor of 7.7 (mixture of air pollutants) and 2.5 (pesticide mixture) lower than the factor 'n'. In the four scenarios the adjusted MCR (=ratio between the sum of toxic units and the highest toxic unit under the assumption that a successful single substance oriented risk mitigation is implemented) seems to provide a good approximation of a MAF. Therefore, a broader in-depth evaluation of published monitoring data is needed, which would require a dedicated effort for compiling and assessing the necessary hazard profiles for all the individual mixture components.

The critical issue to explore in this context concerns the scenario-specificity of a MAF for class A mixtures. It is highly doubtful that an appropriate generic MAF can be established for chemical mixtures. The typical approach to base the size of an AF on a realistic worst-case scenario, which is used when establishing standard single-substance AFs for chemical hazard assessment, might not be appropriate for mixture assessment, as it will be hard to define a 'realistic worst case' pollution scenario, given that the even the freshwater environment alone comprises heavily polluted industrial sites and harbors as well as almost pristine nature reserves. Additionally, chemical pollution does not only differ quantitatively, but also qualitatively between different exposure scenarios. That is, certain chemicals that are priority pollutants in one scenario might be completely absent in another. It will hence be necessary to start compiling and systematizing archetypal pollution scenarios. In this context it might be worthwhile to systematically analyze how human activities and physico-chemical properties of environmental compartments structure chemical pollution. The study by Tornero-Velez (2012) on expectable exposures in childcare centers is a promising step in this direction.

A MAF, even if established 'only' for class A mixtures would allow for example to go beyond the current practice to set EQS values in the context of the Water Framework Directive only for individual priority compounds, or narrowly defined classes of chemicals from a certain chemical class, such as brominated diphenylethers (a class of brominated flame retardants).

Class B mixtures, i.e. mixtures with at least partially unknown chemical composition, pose even greater challenges. In addition to the issues discussed previously for class A mixtures, a MAF that is applied to class B mixtures would need to account for the unknown presence of compounds with an unknown toxicity. Given the diversity of exposure situations, which are characterized by the environmental compartment or species in question, magnitude, type and dynamics of chemical imissions, and the fate and effects of the chemicals involved, it is highly questionable whether setting such a generic MAF might be possible at all, for the simple reason that the involved knowledge gaps and uncertainties are colossal.

TIE studies have developed over the last decade into a powerful approach to define (eco)toxicologically relevant site-, or biota-specific pollution, and confirmatory studies have a promising explanative power: usually 10% or more of the toxicity of an environmental sample is explained by the identified compounds. This could argue for using a factor of 10 for extrapolating from the class A mixture in a given scenario to the corresponding class B mixture. Such a factor of 10 would, however, not ensure protection against the toxicity of a mixture *per se*, as it does not consider the relation between the risk of the individual components to the overall risk of the sample. TIE studies are currently mainly implemented for high-exposure scenarios with a comparatively low number of compounds, which often belong to the same chemically or mechanistically defined class and more empirical data are certainly needed. The SOLUTIONS project aims to fill in this gap (Brack, 2014).

Unfortunately, TIE studies are resource-demanding, limited by the availability of robust and sensitive bioassays and dependent on the availability of high purity standards of suspected pollutants, see discussions in e.g. Brack (2011). However, basing studies on existing single-substance based prioritization efforts instead of investing time and effort for investigating each exposure scenario also seems problematic. At least the studies by Escher (2013) seem to indicate that regulatory lists of priority pollutants, even if compiled specifically for the area of interest, might be of only very limited use for setting up the initial list of candidate mixture components to be included in the assessment.

Despite these massive empirical knowledge gaps and conceptual problems, several suggestions have been put forward to implement a MAF also for class B mixtures (see literature compilation in section 4.2). It should be pointed out that none of the suggestions seem to be based on a defendable conceptual basis or empirical data, except the fact that they obviously go in the right direction by improving the currently inadequate level of protection. But whether the suggested factors of 10 for human health and 100 for the environment are just right, overprotective or not large enough is entirely up for speculation. It is therefore highly doubtful that such a MAF might be consensual, given the vastly different priorities and risk perceptions of different stakeholders.

7.1 Conclusions and Recommendations

A MAF is a tool that is easy to implement into the current regulatory system, it increases the protectiveness of chemical risk evaluation and helps to overcome the erroneous focus on a compound-by-compound assessment that is prevalent in most of the current frameworks. However, given the immense diversity of exposure scenarios relevant for human health and the environment, it might not be possible to define a generally applicable MAF. A mixture assessment factor will only become practically useful if

(i) it is clearly specified which type of uncertainty a certain mixture assessment factor accounts for (e.g. risk of synergistic interactions, gaps in the knowledge of the (eco)toxicological profiles of the mixture components, or the possible presence of unknown compounds, see table 2 for a complete overview), and

(ii) if exposure situations can be grouped into scenarios of sufficient similarity in terms of mixture composition and concentration. That is, due to the interdependency of exposure and hazard assessment when evaluating the risks of chemical mixtures, a MAF will have to be more scenario-specific than the currently used single-substance oriented assessment factors. It will therefore be a major task for the near future to develop and delineate classes of exposure scenarios with sufficiently similar characteristics. There is a clear need to go from lists of priority pollutants to priority mixtures that represent “archetypal scenarios” for biota, including humans, and the various environmental compartments.

Applying a MAF during the risk assessment of individual substances is conceptually identical to reducing the critical value of the risk quotient (PEC/PNEC, respectively DNEL/Exposure ratio) from 1 to a lower value. However, basing regulatory decisions on such seemingly simple bright lines between “risk” and “no-risk” has already be thoroughly criticized by the National Research Council (NRC, 2009). Not only does the complexity of exposure scenarios make it difficult to agree on an appropriate size of a generic MAF. Additionally, the problem remains that appropriate risk management and mitigation measures need to be developed for scenarios in which many actors contribute to an overall risk with chemical emissions that have an individual risk quotient below 1. Especially in highly developed countries with a

functioning system of single-substance risk assessment and management, such scenarios are getting increasingly important, particularly near population centers and areas with high industrial activities.

As a consequence, the risk quotient of a chemical should not only be viewed as a measure of risk in itself, but primarily as a measure of the contribution of a compound to the overall risk in a given exposure scenario. This notion has fundamental consequences for chemical management, as it implies that no compound is completely 'risk-free', even if its risk quotient is well below 1. In one scenario the risk quotient of a certain compound might be perfectly acceptable, while in other, more 'busy' scenarios risk management measures are called for, in order to ensure adequate protection against the toxicity of the overall mixture. This implies that risk assessment approaches need to be diversified and become more scenario-specific, e.g. providing 'the' risk assessment for 'the' freshwater environment might simply be insufficient. This certainly calls for establishing a closer link between prospective, actor-oriented frameworks such as REACH, which often use quite general exposure scenarios, and retrospective, receptor-oriented frameworks such as the Water Framework Directive or the Marine Strategy Framework Directive that are inherently more scenario-specific.

Overcoming the erroneous notion that the use of a chemical with a risk quotient below 1 always ensures chemical safety is critical. In fact, this might be more important for improving regulatory frameworks than using a MAF for decreasing the numerical value of said risk quotient.

8 References

Altenburger R, Arrhenius Å, Backhaus T, Coors A, Faust M, Zitzkat D. 2013. Ecotoxicological combined effects from chemical mixtures. Part 1: Relevance and adequate consideration in environmental risk assessment of plant protection products and biocides. Report to the German Environmental Agency, UBA-Texte 92/2013, http://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/texte_92-2013_ecotoxicological_combined_effects_from_chemical_mixtures_part_1.pdf

Andreozzi R, Raffaele M, Nicklas P. 2003. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. Chemosphere, 50(10), 1319–30.

Backhaus T, Faust M, Blanck H. 2010. Hazard and Risk Assessment of Chemical Mixtures under REACH–State of the Art, Gaps and Options for Improvement. Report to the Swedish Chemicals Agency. http://www.kemi.se/Documents/Publikationer/Trycksaker/PM/PM3_10.pdf

Backhaus T, Faust M. 2012. Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environmental Science and Technology, 46(5), 2564–73.

Backhaus T, Altenburger R, Faust M, Frein D, Frische T, Johansson P, Kehrer A, Porsbring T. 2013. Proposal for environmental mixture risk assessment in the context of the biocidal product authorization in the EU. Environmental Sciences Europe, 25(1), 4.

Backhaus T, Karlsson M. 2014. Screening level mixture risk assessment of pharmaceuticals in STP effluents. Water Research, 49, 157-65.

Bandow N, Altenburger R, Streck G, Brack W. 2009. Effect-directed analysis of contaminated sediments with partition-based dosing using green algae cell multiplication inhibition. Environmental Science and Technology, 43(19), 7343–49.

Bao VWW, Leung KMY, Kwok KWH, Zhang AQ, Lui GCS. 2008. Synergistic toxic effects of zinc pyrithione and copper to three marine species: Implications on setting appropriate water quality criteria. Marine Pollution Bulletin, 57(6-12), 616–23.

Becker K, Conrad A, Kirsch N., Kolossa-Gehring M, Schulz C, Seiwert M, Seifert B. 2007. German Environmental Survey (GerES): Human biomonitoring as a tool to identify exposure pathways. International Journal of Hygiene and Environmental Health, 210, 267–69.

Becker K, Müssig-Zufika M, Conrad A, Lüdecke A, Schulz C, Seiwert M, Kolossa-Gehring M. 2008. German Environmental Survey for Children 2003/06 - GerES IV. Human Biomonitoring, levels of selected substances in blood and urine of children in Germany. Research Report 202 62 219 UBA-FB 001026 http://www.umweltbundesamt.de/sites/default/files/medien/publikation/long/3355.pdf

Booij P, Vethaak AD, Leonards PEG, Sjollema, SB, Kool J, De Voogt P, Lamoree MH. 2014. Identification of photosynthesis inhibitors of pelagic marine algae using 96-well plate microfractionation for enhanced throughput in effect-directed analysis. Environmental Science and Technology, 48, 8003–11.

Brack W. (ed). 2011. Effect-Directed Analysis of Complex Environmental Contamination. The Handbook of Environmental Chemistry, Vol. 15, Springer Publishers, Berlin, ISBN 3642267890

Brack W, Altenburger R, Schüürmann G, Krauss M, López Herráez D, van Gils J, Slobodnik J, Munthe J, Gawlik BM, van Wezel A, Schriks M, Hollender J, Tollefsen KE, Mekenyan O, Dimitrov, S, Bunke D, Cousins I, Posthuma L, van den Brink, PJ, et al. 2014. The SOLUTIONS project: Challenges and responses for present and future emerging pollutants in land and water resources management. The Science of the Total Environment, 503-504, 22-31.

Bundschuh M, Goedkoop W, Kreuger J. 2014. Evaluation of pesticide monitoring strategies in agricultural streams based on the toxic-unit concept - experiences from long-term measurements. The Science of the Total Environment, 484, 84–91.

Bunke D, Groß R, Kalberlah F, Oltmanns J, Schwarz M, Reihlen A, Reineke N. 2014. Mixtures in the Environment – Development of Assessment Strategies for the Regulation of Chemicals under REACH, Report 65/2014 for the German Environmental Agency (UBA) http://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/texte_65_2014_aust_hassold_mixtures_in_the_environment.pdf

CDC (Center for Disease Control and Prevention). 2009. Fourth National Report on Human Exposure to Environmental Chemicals, Report http://www.cdc.gov/exposurereport/pdf/fourthreport.pdf

CDC (Center for Disease Control and Prevention). 2015. Fourth National Report on Human Exposure to Environmental Chemicals Updated Tables, February 2015, http://www.cdc.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf

Cedergreen N. 2014. Quantifying synergy: A systematic review of mixture toxicity studies within environmental toxicology. PloS One 9, 5, e96580.

Dahllöff I, Grunnet K, Haller R, Hjorth M, Maraldo K, Petersen DG. 2005. Analysis, fate and toxicity of zinc- and copper pyrithione in the marine environment. Nordic Council of Ministers. Tema Nord Series, ISSN 0908-6692 http://norden.diva-portal.org/smash/get/diva2:701402/FULLTEXT01.pdf

De Brouwere K, Cornelis C, Arvanitis A, Brown T, Crump D, Harrison P, Jantunen, M, Price P, Torfs R. 2014. Application of the maximum cumulative ratio (MCR) as a screening tool for

the evaluation of mixtures in residential indoor air. The Science of the Total Environment, 479-480, 267–76.

ECHA (European Chemicals Agency). 2008. Guidance on information requirements and chemical safety assessment Chapter R.10: Characterisation of dose [concentration] -response for environment.

ECHA (European Chemicals Agency). 2008a. Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration] -response for human health.

ECHA (European Chemicals Agency). 2008c. Guidance on information requirements and chemical safety assessment Chapter R.16: Environmental Exposure Estimation.

ECHA (European Chemicals Agency). 2012. Guidance for identification and naming of substances under REACH and CLP.

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

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals). 2011. Development of guidance for assessing the impact of mixtures of chemicals in the environment. Technical Report 111.

EFSA (European Food Safety Authority). 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. The EFSA Journal, 11(7), 3290.

Escher BI, Van Daele C, Dutt M, Tang JYM, Altenburger R. 2013. Most oxidative stress response in water samples comes from unknown chemicals: The need for effect-based water quality trigger values. Environmental Science and Technology, 47, 7002–11.

EU Commission. 2011. Guidance Document No.27 - Technical Guidance For Deriving Environmental Quality Standards. Technical Report 2011-055.

Faust M, Scholze M. 2004. Competing Concepts for the Prediction of Mixture Toxicity: Do the Differences Matter for Regulatory Purposes? Report of the BEAM EU-Project, EVKI-CT1999-00012

Feron VJ, van Vliet PW, Notten WRF. 2004. Exposure to combinations of substances: a system for assessing health risks. Environmental Toxicology and Pharmacology, 18(3), 215-22.

Frische T, Matezki S, Wogram J. 2014. Environmental risk assessment of pesticide mixtures under regulation 1107/2009/EC: a regulatory review by the German Federal Environment Agency (UBA). Journal für Verbraucherschutz und Lebensmittelsicherheit., 9(4), 377-89.

Grote M, Brack W, Walter HA, Altenburger R. 2005. Confirmation of cause-effect relationships using effect-directed analysis for complex environmental samples. Environmental Toxicology and Chemistry, 24(6), 1420–27.

HELIX: Building the Early-Life Exposome. Centre for Research in Environmental Epidemiology (CREAL). http://www.projecthelix.eu [accessed 7 Jan 2015].

Houlihan J, Kropp T, Wiles R, Gray S, Campbell C. 2005. Bodyburden - The pollution in newborns. Report, The Environmental Working Group http://www.ewg.org/research/body-burden-pollution-newborns [accessed Maj 2015]

Hutchinson T, Dungey S. 2011. Environmental Risk Assessment of Chemical Mixtures in Aquatic Ecosystems: State of the Science & Evidence Gaps. CEFAS & Environment Agency

Report
http://randd.defra.gov.uk/Document.aspx?Document=10490_CefasEA2011Chemicalmixturesreportfor Defra_FINAL29June2011v2.pdf

Janssen MP, Traas TP, Rila JP, van Vlaardingen PL. 2004. Guidance for deriving Dutch Environmental Risk Limits from EU-Risk Assessment Reports of existing substances, RIVM Report 601501020/2004
https://rivm.openrepository.com/rivm/bitstream/10029/16457/1/601782001.pdf

Juarez PD, Matthews-Juarez P, Hood DB, Im W, Levine RS, Kilbourne BJ, Langston MA, Al-Hamdan MZ, Crosson WL, Estes MG, Estes SM, Agboto VK, Robinson P, Wilson S, Lichtveld MY. 2014. The Public Health Exposome: A Population-Based, Exposure Science Approach to Health Disparities Research. International Journal of Environmental Research and Public Health, 11(12), 12866–95.

Junghans M, Backhaus T, Faust M, Scholze M, Grimme LH. 2006. Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. Aquatic Toxicology, 76(2), 93–110.

Kammann U, Biselli S, Reineke N, Wosniok W, Danischewski D, Hühnerfuss H, Kinder A, Sierts-Herrmann A, Theobald N, Vahl HH, Vobach M, Westendorf J, Steinhart, H. 2004. Bioassay-directed Fractionation of Organic Extracts of Marine Surface Sediments from the North and Baltic Sea - Part II: Results of the biotest battery and development of a biotest index. Journal of Soils and Sediments, 5(4), 225–232.

Kienzler A, Berggren E, Bessems J, Bopp S, van der Linden S, Worth A. 2014. Assessment of Mixtures - Review of Regulatory Requirements and Guidance. JRC Report. ISSN 1831-9424 https://ec.europa.eu/jrc/sites/default/files/lbna26675enn.pdf

Kolossa-Gehring M., Becker K, Conrad A, Lüdecke A, Riedel S, Seiwert M, Schulz C, Szewzyk R. 2007. German Environmental Survey for Children (GerES IV) - First results. International Journal of Hygiene and Environmental Health, 210, 535–540.

Kortenkamp A. 2007. Exploring Novel Endpoints, Exposure, Low-dose- and Mixture-Effects in Humans, Aquatic Wildlife and Laboratory Animals. Final Report of the EDEN project. http://ec.europa.eu/research/endocrine/pdf/eden_final_report_en.pdf

Kortenkamp A, Backhaus T, Faust M. 2009. State of the Art Review of Mixture Toxicity. Report to the Commission of the European Union (Directorate General for the Environment), 2009. http://ec.europa.eu/environment/chemicals/pdf/report_Mixture%20toxicity.pdf

Kortenkamp A, Faust M. 2010. Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment. International Journal of Andrology, 33 (2):463-474.

KEMI (Swedish Chemicals Agency), Sammanställning av protokoll om riktvärden för växtskyddsmedel i ytvatten. Report 2008
http://www.kemi.se/Documents/Bekampningsmedel/Vaxtskyddsmedel/Protokoll_riktvarden_vaxtskyddsmedel.pdf

Laetz CA, Baldwin DH, Collier TK, Hebert V, Stark JD, Scholz, NL. 2009. The synergistic toxicity of pesticide mixtures: Implications for risk assessment and the conservation of endangered Pacific salmon. Environmental Health Perspectives, 117(3), 348–53.

Le TTY. 2012. Modelling bioaccumulation and toxicity of metal mixtures. PhD Thesis, FNWI, Radboud Universiteit Nijmegen. http://hdl.handle.net/2066/100831

MacLeod M, Scheringer M, McKone TE, Hungerbuhler K. 2010. The state of multimedia mass-balance modeling in environmental science and decision-making. Environmental Science and Technology, 44(22), 8360–64.

Meek MEB, Boobis AR, Crofton KM, Heinemeyer G, van Raaij M, Vickers C. 2011. Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework. Regulatory Toxicology and Pharmacology. 60(2 suppl 1), 1–14.

Meek MEB. 2013. International experience in addressing combined exposures: increasing the efficiency of assessment. Toxicology, 313(2-3), 185–9.

Moschet C, Wittmer I, Simovic J, Junghans M, Piazzoli A, Singer H, Stamm C, Leu C, Hollender J. 2014. How a complete pesticide screening changes the assessment of surface water quality. Environmental Science & Technology, 48(10), 5423–32.

Muilerman H. 2011. Preventing harmful effects of multiple chemical exposures on human health, a proposal of Pesticide Action Network Europe. Discussion Paper http://www.pan-europe.info/Campaigns/pesticides/documents/risk_assesment/PAN%20Europe%20proposal%20on%20mixtures%20Nov%202011.doc

NRC (National Research Council). 2009. Science and Decisions: Advancing Risk Assessment. Report. ISBN 978-0-309-12046-3.

NRC (National Research Council). 2012. Exposure Science in the 21st Century : A Vision and a Strategy, Report. ISBN 978-0-309-26468-6.

Nordic Council of Ministers. 2012. Chemical cocktails – a serious matter of concern. Brochure http://norden.diva-portal.org/smash/get/diva2:702829/FULLTEXT01.pdf

Nørgaard KB, Cedergreen N. 2010. Pesticide cocktails can interact synergistically on aquatic crustaceans. Environmental Science and Pollution Research International, 17(4), 957–67.

Pedersen F, Petersen GI. 1996. Variability of species sensitivities to complex mixtures. Water Science and Technology, 33(6) 109-119.

Petersen K, Lindeman B. 2014. Road to regulation of endocrine disruptors and combination effects. Report TemaNord 2014:529 http://dx.doi.org/10.6027/TN2014-529

Pohl, HR, Roney N, Wilbur S,Hansen H, De Rosa CT. 2003. Six interaction profiles for simple mixtures. Chemosphere, 53, 183–197.

Porsbring T. 2011. Effect assessment of biocide mixtures. Presentation at the Special Science Symposium on the “Environmental Risk Assessment of Biocides: Regulatory challenges and scientific solutions”, Society of Environmental Toxicology and Chemistry, Brussels http://sesss04.setac.eu/embed/sesss04/Tobias_Porsbring.pdf

Price P, Hollnagel HM, Zabik JM. 2009. Characterizing the noncancer toxicity of mixtures using concepts from the TTC and quantitative models of uncertainty in mixture toxicity. Risk Analysis, 1534–48.

Price P., Dhein E, Hamer M, Han X, Heneweer M, Junghans M, Kunz P, Magyar C, Penning H, Rodriguez C. 2012. A decision tree for assessing effects from exposures to multiple substances. Environmental Sciences Europe, 24(1), 26.

Price, P, Han X, Junghans M, Kunz P, Watts C, Leverett D. 2012a. An application of a decision tree for assessing effects from exposures to multiple substances to the assessment of human and ecological effects from combined exposures to chemicals observed in surface waters and waste water effluents. Environmental Sciences Europe, 24(1), 34.

Price, P, Zaleski R, Hollnagel H, Ketelslegers H, Han X. 2014. Assessing the safety of co-exposure to food packaging migrants in food and water using the maximum cumulative ratio and an established decision tree. Food additives & contaminants Part A, 31(3), 414–21.

Sarigiannis D, Hansen U. 2012. Considering the cumulative risk of mixtures of chemicals - a challenge for policy makers. Environmental Health, 11(Suppl 1), 18.

SCHER, SCENIHR, SCCS (Scientific Committee on Health and Environmental Risks Scientific, Scientific Committee on Emerging and Newly Identified Health Risks & Scientific Committee on Consumer Safety). 2012. Final opinion on: Toxicity and Assessment of Chemical Mixtures. ISBN 978- 92-79-3 0700-3 http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_155.pdf

Schulze T, Seiler TB, Streck G, Braunbeck T, Hollert, H. 2012. Comparison of different exhaustive and biomimetic extraction techniques for chemical and biological analysis of polycyclic aromatic compounds in river sediments. Journal of Soils and Sediments, 12, 1419–34.

Sexton K, Linder SH. 2011. Cumulative risk assessment for combined health effects from chemical and nonchemical stressors. American Journal of Public Health, 101 Suppl, S81–8.

Silins I, Högberg J. 2011. Combined toxic exposures and human health: biomarkers of exposure and effect. International Journal of Environmental Research and Public Health, 8(3), 629–47.

Silins I, Berglund M, Hanberg A, Boman A, Fadeel B, Gustavsson P, Håkansson H, Högberg J, Johanson G, Larsson K, Lidén C, Morgenstern R, Palmberg L, Plato N, Rannug A, Sundblad BM, Stenius U. 2011a. Human Health Risk Assessment of Combined Exposures to Chemicals, Report, Institute of Environmental Medicine, Karolinska Institute http://ki.se/sites/default/files/2011-3.pdf

Silva E, Rajapakse N, Kortenkamp A. 2002. Something from "Nothing" - Eight Weak Estrogenic Chemicals Combined at Concentrations below NOECs Produce Significant Mixture Effects, Env Sci Techn, 36 (8), 1751-56.

Simon E, van Velzen M, Brandsma SH, Lie E, Løken K, de Boer J, Bytingsvik J, Jenssen BM, Aars J, Hamers T, Lamoree, MH. 2013. Effect-directed analysis to explore the polar bear exposome: Identification of thyroid hormone disrupting compounds in plasma. Environmental Science and Technology, 47, 8902–8912.

SLU (The Swedish Agricultural University). 2014. Växtskyddsmedel i typområden på jordbruksmark och i åar http://jordbruksvatten.slu.se/pesticider_start.cfm [accessed Nov. 2014]

Svenson A, Sandén B, Dalhammar G, Remberger M, Kaj L. 2000. Toxicity identification and evaluation of nitrification inhibitors in wastewaters. Environmental Toxicology, 15, 527–32.

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

Tang JYM, Busetti F, Charrois JWA, Escher B. 2014a. Which chemicals drive biological effects in wastewater and recycled water? Water Res. 60, 289-299.

Tornero-Velez R, Egeghy PP, Cohen-Hubal, E. 2012. Biogeographical Analysis of Chemical Co-Occurrence Data to Identify Priorities for Mixtures Research. Risk Analysis, 32(2), 224–36.

Tørsløv J, Slothus T, Christiansen S. 2013. Endocrine Disrupters - Combination Effects. Report for the Nordic Council of Ministers, TemaNord 2011:537, ISBN 978-92-893-2238-6 http://www.diva-portal.org/smash/get/diva2:701545/FULLTEXT01.pdf

US EPA (United States Environmental Protection Agency), Risk Assessment Forum Technical Panel. 2000. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures EPA/630/R-00/002
http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20533

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

US EPA (United States Environmental Protection Agency). 2010. Recommended Toxicity Equivalence Factors (TEFs) for Human Health Risk Assessments of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Dioxin-Like Compounds, EPA/100/R 10/005
http://www2.epa.gov/sites/production/files/2013-09/documents/tefs-for-dioxin-epa-00-r-10-005-final.pdf

U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry. 2001. Guidance for the Preparation of an Interaction Profile
http://www.atsdr.cdc.gov/interactionprofiles/interaction_profile_guidance.pdf

van Vlaardingen PLA, Verbruggen E. 2007. Guidance for the derivation of environmental risk limits within the framework of "International and national environmental quality standards for substances in the Netherlands" (INS), RIVM Report 601782001
http://www.rivm.nl/en/Documents_and_publications/Scientific/Reports/2007/november/Guidance_for_the_derivation_of_environmental_risk_limits_within_the_framework_of_International_and_national_environmental_quality_standards_for_substances_in_the_Netherlands_INS_Revision_2007?sp=cml2bXE9ZmFsc2U7c2VhcmNoYmFzZT0yMjQyMDtyaXZtcT1mYWxzZTs=&pagenr=2243

Vrijheid M, Slama R, Robinson O, Chatzi L, Coen M, van den Hazel P, Thomsen C, Wright J, Athersuch TJ, Avellana N, Basagaña X, Brochot C, Bucchini L, Bustamante M, Carracedo A, Casas M, Estivill X, Fairley L, van Gent D, et al. 2014. The human early-life exposome (HELIX): Project rationale and design. Environmental Health Perspectives, 122(6), 535–44.

Wild CP. 2005. Complementing the genome with an "exposome": The outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiology Biomarker and Prevention, 14, 1847–50.

Wild CP. 2012. The exposome: From concept to utility. International Journal of Epidemiology, 41, 24–32.

Williams PRD, Dotson GS, Maier A. 2012. Cumulative risk assessment (CRA): Transforming the way we assess health risks. Environmental Science and Technology, 46, 10868–74.

Woodruff TJ, Zota AR, Schwartz JM. 2011. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. Environmental Health Perspectives, 119(6), 878–85.

Zartarian VG, Schultz BD. 2010. The EPA's human exposure research program for assessing cumulative risk in communities. Journal of Exposure Science & Environmental Epidemiology, 20, 351–58.

Swedish Chemicals Agency

Box 2, SE-172 13 Sundbyberg
+46 8 519 41 100

Visitors' and delivery address
Esplanaden 3A, Sundbyberg

kemi@kemi.se
www.kemikalieinspektionen.se