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Advanced methodological framework for the identification and prioritisation of contaminants and contaminant mixtures

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[1] Document history

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I. Summary

This deliverable report presents a proposal for an advanced methodological framework for identifying priority pollutants and priority mixtures of pollutants in European freshwaters. The proposal aims to tackle major shortcomings of current prioritisation procedures under the EU Water Framework Directive (WFD). These are:

(i) For most aquatic pollutants the high data demands for a conclusive risk assessment cannot be met. Significant risks from so-called emerging pollutants may remain undetected. The WFD does not include an effective mechanism to close such knowledge gaps. The introduction of a watch-list mechanism for up to 14 substances provided an improvement but no fundamental change to this situation.

(ii) Individual pollutants are assessed as if they would occur in isolation, largely ignoring the fact that they are part of complex multi-constituent mixtures. Environmental quality standards that have been established for single priority pollutants may not be sufficiently protective against mixture effects. Regulatory approaches for effectively tackling the problem are missing.

The development of the proposed advanced framework is based on a thorough examination of all available concepts and methods for both (i) the regulatory assessment of risks from chemical mixtures and (ii) the integration of such mixture risk assessment approaches into prioritization procedures. None of the available approaches provides a comprehensive solution for the complex problem. Each approach has some specific advantages but also suffers from severe limitations. As the best possible way forward, this report therefore proposes a framework which integrates all available lines of evidence (LOE) on significant risks. This includes evidence from

(i) integrated modelling of co-exposure and resulting mixture risks,

(ii) chemical monitoring, in combination with so-called component-based approaches (CBA) for mixture risk assessment and driver identification,

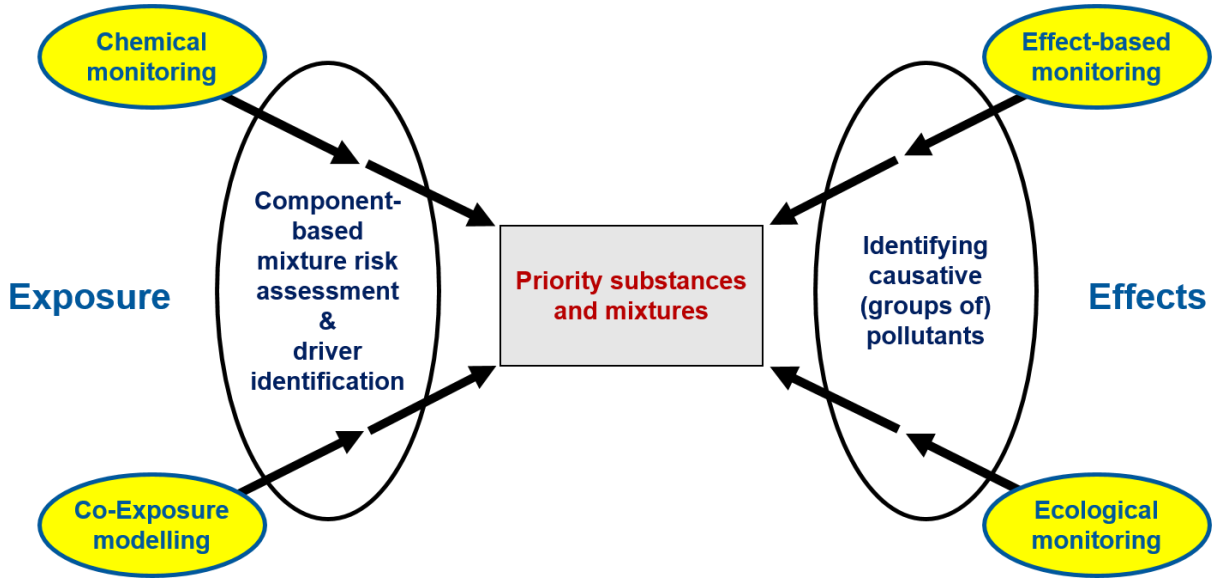
(iii) effect-based monitoring (*in vitro* or *in vivo* whole mixture testing in the lab or onsite), in combination with effect-directed analysis (EDA) or related methods for the identification of causative (groups of) pollutants,

(iv) ecological monitoring (field observations on so-called biological quality elements), in combination with any possible indications on causative (groups of) pollutants.

Where one or more lines of evidence identify groups of pollutants presenting a significant risk, these should be subject to prioritisation for risk reduction measures. Where appropriate, such groups may be reduced to few mixture components or even one single component which can be demonstrated to explain most of the overall risk, so-called drivers of mixture risks. Wherever conclusive evidence on significant risks and resulting needs for risk reduction cannot be reached because all possible LOEs are somewhere blocked by significant data or knowledge gaps, mixture components of potential concern are not left unnoticed but they are prioritised for further research and testing.

Some elements of the advanced methodological framework may be readily applicable under the existing WFD. Full implementation, however, requires changes in the legal text.

II. Graphical summary



III. Contents

IV. Tables, Figures, and Boxes

V. Abbreviations and symbols

AbwV	Abwasserverordnung (German waste water ordinance)
ADI	acceptable daily intake
AF	assessment factor
AhR	aryl hydrocarbon receptor
AL	acceptable level
BEQ	bioanalytical equivalent concentration
BMD	benchmark dose
BP	biocidal products
BPR	Biocidal Product Regulation
BQE	biological quality element
c	concentration
CA	concentration addition
CAG	common assessment group
CBA	component-based approach
CEU	Council of the European Union
CLP	classification, labelling and packaging of substances and mixtures (Regulation (EC) 1272/2008)
CMR	carcinogenic, mutagenic, or toxic for reproduction
COMMPS	combined monitoring-based and modelling-based priority setting scheme (as established by Decision No 2455/2001/EC)
CRC	concentration response curve
CYP	cytochrom P450
D	deliverable
d	dose
DA	dose addition
DNEL	derived no effect level
DOW	description of work (of the SOLUTIONS project)
DRC	dose response curve
E	effect
EBM	effect-based monitoring
EBMT	effect-based monitoring tool[1]
EBTV	effect-based trigger value[1]
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
ECx	effect concentration for the effect x (e.g. EC50)
ED	endocrine disrupter
EDA	effect-directed analysis

[1] Other SOLUTIONS documents use the abbreviation *EBT* for '*effect-based tools*' (for monitoring), but in the literature cited in this report, the same abbreviation is also used for '*effect-based trigger value*'. For clarification and for avoiding any confusion, throughout this report the longer abbreviations EBMT and EBTV are used for *effect-based monitoring tools* and *effect-based trigger values*, respectively.

EDx	effect dose for the effect x (e.g. ED50)
EFSA	European Food Safety Authority
EFSA	European Food Safety Authority
EIFAC	European Inland Fisheries Advisory Commission (of the FAO)
EL	exposure level
Eq.	equation
EQS	environmental quality standard (as defined under the WFD)
ERA	environmental risk assessment
EU	European Union
F	concentration response function
F^{-1}	inverse concentration response function
FAO	Food and Agriculture Organization (of the UN)
FRAM	Center for future chemical risk assessment and management strategies at Gothenburg University (https://fram.gu.se/)
GA	General Assembly (of the SOLUTIONS consortium)
HBGV	health based guidance value
HC5	hazardous concentration for 5% of the species
HI	hazard index
HQ	hazard quotient
HRA	human health risk assessment
IA	independent action
ICPS	International Programme on Chemical Safety
ID	internal deliverable
JRC	Joint Research Centre of the European Commission
LOEL	lowest observed effect level
MAF	mixture assessment factor
MCR	maximum cumulative ratio (MCR) (as defined by Price et al. 2012)
MCS	multi-constituent substance (as defined under REACH)
MEC	measured environmental concentration
mix	mixture
MM	mixed model
MoA	mode of action
MRA	mixture risk assessment
MS	Member States (of the EU)
msPAF	multisubstance potentially affected fraction
NEC	no effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NORMAN	Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances (http://www.norman-network.net/)
OECD	Organisation for Economic Co-operation and Development
p	proportion
PAH	polycyclic aromatic hydrocarbon

PBDE	poly-brominated diphenyl ether
PBT	persistent, bioaccumulative, and toxic
PEC	predicted environmental concentration
PICT	pollution induced community tolerance
PNEC	predicted no effect concentration
POD	point of departure (LOEL, NOAEL, or NOEC)
PODI	point of departure index
PPP	plant protection products
PPPR	plant protection products Regulation (Regulation (EC) No 1107/2009)
QO	quality objective
QS	quality standard
QSAR	quantitative structure activity relationship
RBSP	river-basin specific pollutants (as defined under the WFD)
REACH	registration, evaluation, authorisation and restriction of chemicals (Regulation (EC) No 1907/2006)
REP	relative effect potency
RQ	risk quotient
RV	reference value
SCCS	Scientific Committee on Consumer Safety (of the EC)
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks (of the EC)
SCHER	Scientific Committee on Health and Environmental Risks (of the EC)
SSD	species sensitivity distribution
SSG	single species group
STE	spatial, temporal and extent of PNEC exceedances approach (as proposed in Carvalho et al. 2016).
TEF	toxic equivalency factor
TEQ	toxic equivalent (dose or concentration)
TER	toxicity exposure ratio (as defined under the PPPR)
TG	technical guidance
TGD	technical guidance document
TTC	threshold of toxicological concern
TU	toxic unit
TUS	toxic unit summation
UN	United Nations
US EPA	Unites States Environmental Protection Agency
UVCBs	materials of unknown or variable composition, complex reaction products or biological materials (as defined under REACH)
WEA	whole effluent assessment
WFD	Water Framework Directive (Dir 2000/60/EC)
WG	working group
WHO	World Health Organization
WMA	whole mixture approach
WMT	whole mixture testing
WP	work package

1 Introduction

1.1 Aims and scope

This deliverable report explores options for integrating mixture risk assessments (MRA) into prioritisation procedures under the EU Water Framework Directive (WFD). This includes considerations about closing knowledge gaps about so-called '*emerging substances'*, i.e. existing pollutants which may make significant contributions to overall mixture risks, but for which further investigations on exposure and individual toxicity are required.

This report provides an overview of all available concepts and methods that may be used for achieving the aim of integrating MRAs into prioritisation procedures. This includes concepts for regulatory mixture risk assessments (sections 2), concepts for setting environmental quality standards (EQS) for mixtures (section 3), and concepts for integrating mixture risk assessments into prioritisation procedures under the WFD (section 4). The overview provides both explanations of the state-of-the-art and SOLUTIONS proposals for further advancement.

As a main result, the overview shows that no single approach is able to tackle all problems associated with the complex issue of developing prioritisation procedures that take account of mixture risks. Every possible option provides special advantages but also suffers from severe limitations. As the best possible way forward, this report therefore proposes an advanced methodological framework which integrates different concepts and methods in a four-lines-of-evidence-approach (section 5). The advanced framework is not intended to rule out existing approaches but aims to integrate existing procedures for single substances prioritisation with novel methodological elements for identifying mixtures that present significant risks as well as mixture components which may dominate such risks, so-called '*drivers*' of mixture risks. Some elements of the advanced methodological framework may be readily applicable under the existing WFD. Full implementation, however, requires changes in the legal text, as finally explained in section 6.

As an outflow from the '*Concepts*' sub-project, this deliverable is focused on conceptual issues of identifying and prioritising significant mixture risks. However, it draws on experimental and computational methods which are developed in the SOLUTIONS sub-projects on '*Tools*' and '*Models*', respectively, including the testing of such novel methods in case studies. The methodological details are documented separately in the corresponding deliverables from the Tools and Models sub-projects, in particular D9.1 on effect-based tools for whole mixture testing (WMT) and experimental approaches for driver identification, D13.1 on linking ecological status monitoring with the identification of possible chemical causes of detrimental effects, and D18.1 on so-called component-based (modelling) approaches (CBA) for mixture risk assessment and driver identification.

This report is confined to considerations about the advancement of prioritisation procedures under the scope of the WFD. Prioritisation procedures under the European Drinking Water Directive are separately addressed in a dedicated deliverable D3.1 on abatement of drinking water pollution. Furthermore, the scope of this report is confined to considerations about mixture risk identification and ranking. Technical and socio-economic considerations on efficient risk reduction measures are beyond scope. And finally, this report is confined to considerations about the prioritisation of existing

pollutants and pollutant groups. Ways to the anticipation of future pollution and potentially resulting risks to or via the aquatic environment are separately outlined in deliverable D6.1 on '*pollution of tomorrow and options to act*'.

The development of tools and models in SOLUTIONS is focussed on the detection of risks for organisms in the aquatic environment and on risks for humans via fish food and drinking water consumption. As a consequence, the same applies to the proposed advanced framework for prioritisation. An extension to the prioritisation of pollutants and pollutant mixtures endangering terrestrial predators via secondary poisoning, such as fish-feeding birds and mammals, is possible in principle, but requires additional methodological advancements.

1.2 Legal provisions and existing prioritisation approaches

As a '*strategy against pollution*', Article 16 of the EU Water Framework Directive (WFD) (Dir 2000/60/EC) requires the risk-based identification of '***priority substances***'. The idea is to make risk reduction efforts most efficient by focussing them on those water pollutants that present the highest risks. The aim is to reduce pollution levels below so-called environmental quality standards (EQS). EQS "*means the concentration of a particular pollutant or group of pollutants in water, sediment or biota which should not be exceeded in order to protect human health and the environment*" (WFD, Article 2(35)). Currently, EQS values are defined for 45 EU-wide priority substances. In addition, EU Member States are required to identify river-basin specific pollutants (RBSP) and to set corresponding quality standards on a national or a transboundary river-basin-wide level.

The prioritisation procedures which have been established by the European Commission under these legal provisions suffer from two major shortcomings (Heiss and Küster 2015, Dulio and Slobodnik 2015, Faust and Backhaus 2015, Brack *et al*. 2017):

(i) For most aquatic pollutants the high data demands for a conclusive risk assessment, for risk ranking and for EQS setting, cannot be met.

As a consequence, significant risks from emerging pollutants may remain undetected. The WFD does not include an effective mechanism to close such knowledge gaps. The introduction of a watch-list mechanism for up to 14 substances in 2013 (see below) provided an improvement but no fundamental change to this situation.

(ii) Individual pollutants are assessed as if they would occur in isolation, largely ignoring the fact that they are part of complex multi-constituent mixtures.

Mixtures usually pose a higher risk than the individual components alone. As a consequence, EQS for single pollutants may not be sufficiently protective if toxicants occur jointly as part of multi-component mixtures. In 2014, the practical relevance of the problem was highlighted by a study led by the European Commission's Joint Research Centre (JRC). Mixtures of 14 or 19 priority pollutants at EQS levels were shown to cause significant effects in several bioassays (Carvalho et al 2014). In principle, the need to consider mixture risks was already

well recognised during previous prioritisation exercises (Daginnus *et al.* 2011). However, regulatory approaches for effectively tackling the problem are still missing.

SOLUTIONS set out to develop tools, concepts, and approaches for tackling these problems. SOLUTIONS work builds on previous achievements of the NORMAN network on emerging substances (http://www.norman-network.net/) and on the existing Commission approaches to WFD enforcement as detailed in the following.

1.2.1 Commission approaches

The WFD came into force in 2000. The first list of priority pollutants (Annex X to the WFD) was established in 2001 (Decision No 2455/2001/EC) and corresponding EQS have been laid down in 2008 (Dir 2008/105/EC). In 2013, the list was amended and the EQS were revised (Dir 2013/39/EU). For the forthcoming second review, Commission services have performed preparatory work (Carvalho *et al*. 2016).

For the first prioritisation exercise, the Commission used a "*combined monitoring-based and modelling-based priority setting scheme*" (COMMPS), which was developed in collaboration with the Fraunhofer Institute (Klein *et al*. 1999). The COMMPS procedure was "*designed as a dynamic instrument (…) open to continuous improvement and development*" (Decision No 2455/2001/EC, Recital 17). Refinements made for the first review are described in James *et al*. (2009) and Daginnus *et al*. 2010. For the ongoing second review, the principle of the combined approach shall be maintained, but the monitoring-based part is suggested to include a novel approach to risk ranking, the so-called "*spatial, temporal and extent of PNEC exceedances approach*" (STE) (Carvalho *et al*. 2016), which has been developed by Von der Ohe *et al*. (2011). For EQS setting, a detailed Technical Guidance Document has been developed (EC 2011a).

Applicability of the Commission approaches is limited to a small fraction of aquatic pollutants. For most pollutants, the available hazard or exposure data do not satisfy the WFD Article 16 requirements for a risk-based ranking. In 2013, the prioritisation approach was complemented by a so-called watch list mechanism. Substances or groups of substances on the watch list are subject to temporary EU-wide monitoring "*for the purpose of supporting future prioritisation exercises*" (Dir 2008/105/EC, Article 8b as amended by Dir 2013/39/EU). The initial watch list was established in 2015 (Commission Implementing Decision (EU) 2015/495). It was limited to 10 substances, two of which are groups of substances, including two estrogens[2] and five neonicotinoid insecticides[3]. In 2018, the list was revised for the first time (Commission Implementing Decision (EU) 2018/840). Five single substances were removed. They were replaced by two other single substances and a group of three macrolide antibiotics[4]. During future revisions, the current number of eight substances and groups of substances is allowed to be increased stepwise up to a maximum of 14 (Article 8b(1) of Dir 2008/105/EC).

[2] The natural hormone 17-Beta-estradiol (E2) and its degradation product estrone (E1)

[3] Imidacloprid, thiacloprid, thiamethoxam, clothianidin, and acetamiprid

[4] Erythromycin, clarithromycin, and azithromycin

To justify the inclusion of selected macrolides and neonicotinoids as groups in the watch list, the Commission explicitly pointed to "*the fact that substances with the same mode of action could have additive effects*" (Recital 9 of Commission Implementing Decision (EU) 2018/840). The joint monitoring of substances in such groups provides an important step towards the assessment and ranking of mixture risks.

1.2.2 The NORMAN approach

The NORMAN approach seeks to remove the huge knowledge gaps about emerging substances. Pollutants which do not meet the data requirements for risk assessment and EQS setting under the WFD are no longer ignored. They are

- assigned to a number of action categories and
- prioritised for action within these categories.

Possible actions include toxicity testing, chemical monitoring, and improvement of analytical methods. A brief overview of the methodology is given in Dulio and Slobodnik (2015). A detailed description is provided in Dulio and Von der Ohe (2013).

The NORMAN approach is confined to single substance assessments and the procedure builds on evidence from chemical monitoring as the crucial starting point.

The approach suggested in this report goes a step further, not only by taking mixture toxicity into account, but also by including other lines of evidence into prioritisation procedures, such as results from co-exposure modelling and effect-based monitoring. However, initiatives such as NORMAN are highly important to pave the ground for component-based mixture risk assessments, which rely on the availability of exposure and toxicity data for single substances, as detailed in section 2.2.2 below.

1.3 SOLUTIONS prioritisation workshops

Prioritisation is a cross-cutting issue, requiring trans-disciplinary efforts and the collective expertise of scientists and regulators. The proposal for an advanced methodological framework developed in this report draws on an exchange of opinions and ideas with external partners. This exchange was facilitated by the SOLUTIONS stakeholder board and in particular by three dedicated workshops with invited external experts.

The first SOLUTIONS workshop on prioritisation methodologies was held in Paris in 2014, organised jointly with the NORMAN network. The workshop provided an overview on the state-of-the-art and derived recommendations for improvement. All presentations are publicly available via the NORMAN website at http://www.norman-network.net/?q=node/156. As an outflow, three opinion pieces were published (Heiss and Küster 2015, Dulio and Slobodnik 2015, Faust and Backhaus 2015).

The second SOLUTIONS prioritisation workshop specifically explored options for integrating mixture risk assessments into prioritization procedures under the WFD. It was held in Gothenburg in 2017,

jointly organised with the FRAM Center for Future Chemical Risk Assessment and Management Strategies at Gothenburg University. Discussions at the workshop were focussed on three main questions:

(i) How to identify '*priority mixtures*', i.e. pollutants typically co-occurring and presenting significant joint risk widespread and frequently?

(ii) How to identify '*drivers of mixture risks*', i.e. mixture components that explain most of the overall risk?

(iii) How to set Environmental Quality Standards (EQS) for priority mixtures?

All presentations are publicly available via the FRAM website at http://fram.gu.se/Outreach/Conferences+and+workshops/joint-workshop-fram---solutions-2017-. Opinion papers emerging from the discussions are in preparation for publication.

The third SOLUTIONS workshop on prioritisation methodologies was recently held in February 2018, again in Gothenburg. In contrast to the first two workshops, which were symposia with more than 90 people, many invited lectures, and parallel discussion groups, this third event was a small discussion meeting of SOLUTIONS partners with invited external experts from the regulatory arena, 25 participants in total. SOLUTIONS partners presented their draft ideas about the envisaged advanced methodological framework and placed them for debate. The proposal was well received, and the feedback obtained from the invited external experts is reflected in the final proposal presented in this report.

1.4 Key terms

'*Pollutants*' and the similar term '*contaminants*', '*mixtures*' and the related terms '*co-occurrence*', and '*co-exposure*', as well as '*prioritisation*' are the key terms of this deliverable report. For the sake of clarity and to avoid any possible ambiguities, the understanding of these terms is explicitly defined in the following. Other important terms denote concepts and ideas which are subject to ongoing debates about the most appropriate interpretation and operationalisation, such as '*priority mixtures*' and '*drivers of mixture risks*'. These are explained in the dedicated sections below.

'*Pollutants*' vs '*contaminants*'

In the title of this deliverable (as it was contractually fixed in the DOW), the terms '*contaminants*' and '*contaminant mixtures*' are used in the general meaning of potentially harmful anthropogenic chemicals. Unfortunately, however, the terms could be misunderstood by readers familiar with the terminology of the European chemicals legislation. '*Contaminant*' is no term used in the WFD, but it has a specific legal meaning under EU food law[5] which was not intended to be the focal point here.

[5] Under Council Regulation (EEC) No 315/93, '*contaminant*' "*means any substance not intentionally added to food which is present in such food as a result of the production (…), manufacture, processing, preparation,*

To avoid such confusion, the term '*contaminant*' is replaced by '*pollutant*' throughout the text of this report. The term '*pollutant*' is used within the meaning of Article 2 of the WFD to denote substances introduced, "*as a result of human activity, (…) into the air, water or land which may be harmful to human health or the quality of aquatic ecosystems or terrestrial ecosystems directly depending on aquatic ecosystems (…)*[6]." Most pollutants may also become food contaminants, but the considerations in this report are not limited to such pollutants which actually meet both definitions.

'*Mixtures*', '*co-occurrence*', and '*co-exposure*'

The term '*mixtures*' is used in this deliverable report to denote any '*co-occurrence*' of pollutants in environmental media, such as air, water, sediments, soil, or in biota, including the human body. Such '*co-occurrence*' may result from

(i) releases of chemicals that are legally registered as single substances on the EU market, but which are mixtures in themselves, so-called *multi-constituent substances* (MCS) and *materials of unknown or variable composition, complex reaction products or biological materials* (UVCBs) as defined under REACH[7],

(ii) releases of intentionally prepared mixtures that are placed on the EU market as chemical products (formerly denoted as *preparations*[8]),

(iii) joint releases of chemicals from a single source, such as a production, transportation, consumption, recycling, or waste (water) treatment process, or the

(iv) coincidental mixing of chemicals released from various sources and reaching environmental media, environmental organisms, and humans through multiple routes of exposure.

Type (i) and type (ii) mixtures, and partly also type (iii) mixtures are subject to regulations under different pieces of EU law (Kortenkamp, Backhaus, Faust 2009; EC 2012; Kienzler at al. 2014). Type (iv) mixtures represent the main focus of regulatory concern. In 2012, the Commission concluded that "*within the framework of EU legislation, there is no mechanism for a systematic, comprehensive and integrated assessment of mixture effects taking into account different routes of exposure and different product types"* (EC 2012). This continues to apply.

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treatment, packing, packaging, transport or holding of such food, or as a result of environmental contamination" (Article 1).

[6] In addition to human health and environmental safety, the WFD includes other protection goals such as damage to material property, or impairment of amenities and other legitimate uses of the environment, which are out of the scope of this deliverable report.

[7] Regulation (EC) No 1907/2006

[8] From 1 June 2015, Directive 1999/45/EC on dangerous preparations was repealed by Regulation (EC) 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP) which entered into force in 2009. In the new legislation, the term "*preparation*" was replaced by "*mixture*". However, in other pieces of EU legislation, such as the PPPR, the term "*preparation*" continues to be used.

'*Co-exposure*' of organisms may result from the co-occurrence of pollutants in the same environmental medium, such as water or sediments, or from the simultaneous or sequential uptake of pollutants via different routes, such as food and water, resulting in a mixture of pollutants co-occurring in an environmental organism or in a human body. Considerations on resulting mixture risks in this paper are not confined to any of these specific phases of co-occurrence of pollutants but include them all.

In a very narrow sense, the assessment of the co-occurrence of substances in water, sediment, or biota may refer to a specific point in time only. In a somewhat wider sense, it may be understood to include all substances to which an organism may be exposed over a certain period of time simultaneously or sequentially. From a toxicological perspective, the very narrow understanding is inappropriate. Both simultaneous and sequential exposure must be taken into consideration, unless there is sufficient time between independent exposure events ensuring a full recovery of an individual or a population. The development of detailed rules for a regulatory distinction between simultaneous, sequential, and independent exposure events remains subject to future work. As a pragmatic and precautionary default assumption, sequential exposures during a certain time window may be assessed like simultaneous exposures. The definition of appropriate time windows may strongly depend on the generation time of a species and needs further research work.

'*Prioritisation*'

This report is focused on '*prioritisation*' in the sense of Article 16 of the WFD. This means that

(i) the **objects** for prioritization are chemically defined surface water pollutants or groups of pollutants, in particular pollutants co-occurring as a mixture,

(ii) the **criteria** for prioritization are indicators of significant risks to the aquatic environment or via the aquatic environment to humans or terrestrial organisms, and

(iii) the **purpose** of prioritization is to focus risk reduction measures on (groups of) pollutants presenting the highest risks.

It is well recognized that the Article 16 strategy alone is insufficient to achieve the ultimate WFD aim of a good status of European surface waters (Brack et al. 2017). To tackle the problem, a number of complementary prioritization approaches are used or discussed for use, with differing objects, criteria, and purposes, such as the prioritization of contaminated sites for remediation actions, or the prioritization of waste water discharges for treatment or improvement of treatment, or the prioritisation of chemicals with certain hazardous properties (such as CMRs or endocrine disrupters) for preventive exposure reduction measures. In addition, special prioritization approaches may apply under related legal acts such as the Directives on drinking water (Council Dir 98/83/EC), bathing water (Dir 2006/7/EC), or urban waste water (Council Dir 91/271/EEC). Such complementary prioritization efforts are beyond the scope of this report. The same applies to technical and socio-economic considerations about the most appropriate risk reduction measures for priority pollutants or priority groups of pollutants.

2 Concepts for regulatory mixture risk assessments (MRA) and mixture EQS setting

The integration of mixture risk assessments (MRA) into WFD prioritisation procedures presupposes the existence of generally accepted rules and approaches for performing regulatory MRAs under the framework of the European chemicals and environmental legislation. At the current stage, however, this is still an evolving issue. Under the WFD, no explicit legal requirements and no particular guidance for performing MRAs exist. Under some few other pieces of European legislation, such as the Regulations on plant protection products (PPPs)[9] and biocidal products (BPs)[10], clear legal mandates for performing MRAs have been established. The development of agreed principles and procedures for translating these normative requirements into practical assessments, however, still is an ongoing task. Where guidance documents have already been completed, they appear to be fragmented and not fully consistent in terminology and assessment rules, as detailed in section 2.2.3 below. Uniform principles and harmonised methodologies for performing human health risk assessments (HRA) and environmental risk assessments (ERA) of combined exposures to multiple chemicals are missing. Closing this gap is the ambitious goal of a '*MixtTox*' working group (WG) which was set up by the European Food Safety Authority (EFSA) in 2016[11], and which is supported by contributions from SOLUTION partners. However, results of EFSA's MixTox WG are not expected to become available before the end of the SOLUTIONS project. In addition, in accordance with EFSA's remit, the resulting guidelines may be focussed on chemical mixtures in the human food chain. This aspect overlaps but is not congruent with the scope of the WFD.

Given this background, this section provides a brief introduction into existing concepts and approaches for MRA, and a short overview on the state of regulatory use of such concepts. In addition, we make suggestions for their future use in the context of the WFD. In particular, we propose uniform principles and a common tiered framework for the use of so-called component-based approaches (CBA) for HRA and ERA of pollutant mixtures in European surface waters, as explained in the following.

Regulatory approaches to the problem of mixture risk assessment fall into two basic categories: the so-called whole mixture approach (WMA) (section 2.1) and the so-called component-based approach (CBA) (section 2.2). The WMA means that the mixture of concern is experimentally tested as if it were a single substance. The composition of the mixture is usually unknown or not exactly known. The CBA, in contrast, means that the expected toxicity of a mixture is calculated on the basis of toxicity data for individual mixture components by using models of joint action. The composition of the mixture must be exactly defined. As detailed in the following, WMA and CBA both have some special advantages and limitations. As a consequence, WMA and CBA should not be regarded as rival

[9] Regulation (EC) No 1107/2009

[10] Regulation (EU) No 528/2012

[11] Further information on EFSA's 'MixTox' initiative is available at https://www.efsa.europa.eu/en/topics/topic/chemical-mixtures

but as complementary approaches, which need to be integrated in an intelligent way for identifying priority pollutants or priority mixtures most effectively.

2.1 Whole mixture approach (WMA)

In general, whole mixture approaches (WMAs) do not play a prominent role in the debate about the assessment of mixture risks under EU chemicals legislation. The EU legislation requires prospective assessments of chemical risks or chemicals safety, prior to their marketing, use, or release of chemicals and chemical products. To this end, testing requirements for single chemicals and chemical products have been well defined under various pieces of legislation, such as REACH, PPPR, BPR, etc. On the policy level, there is a high resistance against any substantial widening of toxicological testing requirements for prospective risk assessment purposes, both for ethical and for economic reasons. On the science level, the laws of combinatorics dictate that a systematic testing of the almost infinite number of possible combinations of pollutants is practically impossible and hence must be confined to a tiny fraction of well selected sample cases.

As a consequence, existing initiatives for the implementation of prospective MRAs under European chemicals legislation basically aim to achieve the goal without additional experimental testing of whole mixtures, as far as possible. They are focussed on component-based modelling approaches as detailed in section 2.2 below. However, for retrospective assessments under media-oriented pieces of European environmental legislation, such as the WFD, the situation is different, as explained in the following.

2.1.1 Opportunities for WMAs under the WFD

Media-oriented pieces of European environmental legislation require the monitoring of the actual status of pollution of air, water, and soil. The monitoring results shall trigger remedial or preventive actions where damage is seen or where the risk for actual damage is found to be unacceptably high due to exposure levels exceeding quality standards. To this end, so-called '*effect-based monitoring*' is generally agreed to be a highly valuable complement to chemical monitoring (Wernersson et al 2014), and for mixture risk assessments both are informative. Chemical monitoring provides exposure information which must be combined with toxicological data for assessing resulting mixtures risks by means of CBAs. Effect-based monitoring, in contrast, provides immediate information on actually existing risks or even acute effects. For targeting risk reduction measure, however, the causative chemicals remain to be identified by appropriate methodologies, such as effect-directed analysis (EDA) or plausibility cross-checking with results from component-based assessments on the basis of chemical monitoring data.

As a regulatory tool, effect-based monitoring has long been used for so-called whole effluent assessments (WEA) from industrial sites or sewage treatment plants (STPs) in a number of EU member states (MS). For surface water monitoring, effect-based monitoring is not yet a well-established regulatory approach. However, there is strong move towards the strengthening of effect-

based surface water monitoring under the WFD (Wernersson et al 2014), either as a complement to chemical surveillance and operational monitoring or as a trigger for investigative chemical monitoring (as defined in Annex V of the WFD). The development is strongly supported by SOLUTIONS research on appropriate effect-based tools for monitoring and the design of a suitable test battery (Brack et al 2017, Neale et al 2017). This research work has been performed in the *Tools* sub-project. The results are documented in the final deliverable D9.1 and they flow into the outline of the advanced methodological framework proposed in section 5 below

For the purpose of this deliverable report, the term '*effect-based monitoring*' (EBM) is understood in a narrow sense, meaning the whole mixture testing of real environmental samples or extracts from environmental samples with *in vitro* screens, whole organisms, or even assemblages of organisms, such as periphyton communities, collectively denoted as '*effect-based monitoring tools'* (EBMTs)[12] here. The experiments may be conducted in the lab or onsite, such as fish caging experiments. The important condition is that they are conducted under controlled conditions which ensure a causal relationship between the (usually unknown) chemical exposure and the biological effects observed. Otherwise, EBM data may be inappropriate for deriving MRAs.

In a wider sense, the term '*effect-based monitoring*' has been suggested to include three main categories of EBMTs: '*bioassays*', '*biomarkers*', and '*ecological indicators of chemical pollution*' (Wernersson et al 2014). These categories were defined as follows: '*bioassays*' measure toxicity *in vitro* or *in vivo* under controlled laboratory conditions on cellular or individual levels, respectively; '*biomarkers*' are biological responses observed in individual organisms in the field, typically on a cellular or molecular level; and '*ecological indicators'* measure variations at higher levels of biological organisation levels, i.e. populations and communities. In this categorisation, '*bioassays*' comply with the narrow understanding of '*effect-based monitoring*' defined above for this report, '*biomarkers*' and '*ecological indicators'* usually may not. They are field measurements under usually non-controlled exposure conditions and the responses seen are not necessarily pollution-induced but may often also be caused by other stressors. There are some exceptions, such as pollutant-specific biomarkers and PICT as an ecological indicator for pollution induced community tolerance. Such exceptions are subsumed under the understanding of '*effect-based monitoring*' for this report. Otherwise, biomarkers and ecological indicators are subsumed under the term '*ecological monitoring*' as a separate approach explained in the following.

In addition to a monitoring of the '*chemical status'*, the WFD requires monitoring of the '*ecological status*'. This is mainly operationalised in terms of composition and abundance of so-called biological quality elements (BQEs). BQEs are species groups such as phytoplankton, large algae, macrophytes, macroinvertebrates and fish. Adverse effects seen on such BQEs are of primary importance because they directly show that WFD protection goals are not achieved and that risk reduction measures may be required. Unfortunately, however, this information is not directly usable for MRAs. The simple reason is the fundamental difference between BQE surveys and effect-based monitoring (in the narrow sense defined above). EBMTs signal effects of pollution. There is no doubt about the causal

[12] Other SOLUTIONS documents use the shorter term '*effect-based tools*' and the corresponding abbreviation *EBT*. However, in the literature cited in this report, the abbreviation EBT is also used for '*effect-based trigger value*'. To avoid any confusion, throughout this report the longer abbreviations EBMT and EBTV are used for *effect-based monitoring tools* and *effect-based trigger values*, respectively.

link between EBMTs and pollution. Functional relationships between (mixture) concentration and strength or frequency of observed responses can be determined, at least in principle. In contrast, detrimental effects on BQEs observed in the field may be caused by various pressures such as eutrophication, hydromorphological changes, climate changes, etc. etc. Whether chemical pollution plays a role or doesn't play a role is usually not immediately clear.

To make data from ecological status monitoring useful for performing MRAs and for prioritising chemicals or groups of chemicals, novel approaches are needed which examine the hypothesis of pollution-induced effects by means of (eco-)epidemiological types of methodologies. As a result, they may reveal correlations between pollution and adverse effects on BQEs. Causality, however, can only be established by means of complementary considerations of other lines of evidence.

Basically, the same situation may apply to data from biomarker studies, as defined above. For the purpose of this report, we therefore subsume assessments of biomarkers and BQEs under the term '*ecological monitoring*' and use it with a more general meaning than '*ecological status*' under the WFD. Under the WFD, ecological indicators are confined to responses seen on supra-individual levels, which by definition does not apply to biomarkers. However, both biomarkers and BQEs may provide indications for actual or potential effects on aquatic ecosystems which may be shown to correlate with pollution but for which causality must be proven by other means. All assessment situations which share these commonalities are covered by '*ecological monitoring*' as an umbrella term used in this report.

The use of EBM data for MRA is a special application of the principle of whole mixture approaches. The use of ecological monitoring data, however, goes beyond the classical categories of WMAs and CBAs for MRAs. Eco-epidemiological thinking is added as a novel element and combined with the classical elements into a multiple-lines of evidence approach. This is a novel way of approaching the MRA problem. The SOLUTIONS sub-project *Tools* explored the feasibility of such an approach. The results have been documented in the final deliverable D13.1 and flow into the outline of the advanced methodological framework proposed in section 5 below.

2.1.2 Advantages and limitations of WMAs

Whole mixture testing approaches have two major advantages which make them an indispensable element of the available '*tool box*' for MRAs:

(i) Whole mixture testing is the only reliable way for detecting significant mixture risks from unknown components in real environmental samples.

Illustrative examples from SOLUTIONS case studies are the identification of previously unnoticed drivers of mutagenicity (Muz et al 2017a) and antiandrogenic activity (Muschket et al 2018) in the German rivers impacted by wastewater effluents from a chemical-industrial area.

(ii) Whole mixture testing is the only reliable way for detecting unknown synergistic (or antagonistic) effects of mixture components at relevant environmental concentrations.

The terms '*synergism*' and '*antagonism*' are used here to denote toxicodynamic or toxicokinetic interactions between mixture components that result in significant deviations of the actual joint toxicity from predictions based on so-called '*non-interaction models*' or '*additivity models*' which are used for component-based assessments, as detailed in section 2.2 below. Synergistic interactions, i.e. more-than-additive mixture effects, provide a concern for HRA and ERA. Antagonistic interactions are not critical in this context, and hence not further considered in this report.

An illustrative example are previously unrecognized synergistic mutagenic effects of carboline alkaloids and aromatic amines which were revealed in a sample from the river Rhine by a SOLUTIONS case study (Muz et al 2017b).

Unfortunately, these strong advantages of WMAs contrast with a number of substantial limitations. These include:

(i) WMAs provide a 'spotlight' type of assessment, only applying to a mixture exactly composed as the tested one. A change in the concentration ratio of mixture components may already require a new testing, as does any change in the number and nature of components. '*Reading-across*' from a tested mixture to an untested mixture of similar composition requires additional assumptions, such as those underlying models of joint action that are used for CBAs (see below).

(ii) Routine application of WMAs is limited to selected samples and endpoints, both due to resource restrictions and ethical constraints. Frequent testing is possible with a selected battery of short term assays only, such as in vitro screens, assays with micro-organisms, or acute (non-animal) toxicity tests. Testing of chronic mixture toxicity, which is particularly important for regulatory MRAs, must be confined to selected cases, in particular where aspects of animal welfare come into play.

(iii) For prioritizing pollutants or pollutant mixtures for risk reduction measures, whole mixture testing alone is insufficient. Where WMAs signal significant risks, this just provides the starting point for a search for the causative agents. To this end, additional efforts are required, such as performance of an EDA.

To remove these limitations as far as possible, WMAs must be complemented with CBAs.

2.2 Component-based approach (CBA)

Most component-based approaches to mixture risk assessment are based on either of two basic models for joint action, '*concentration addition*' (CA) and '*independent action*' (IA). Mixed modelling (MM) approaches combine both models in a common assessment procedure. Collectively, these types of models are often denoted as '*non-interaction models*' or '*additivity models*'.

Unfortunately, a generally agreed terminology for mixture toxicology does not exist. The following sub-section 2.2.1 therefore provides an exact definition of the mentioned models which are important for regulatory assessments. The sub-sequent sub-sections provide brief overviews of the

data requirements and (2.2.2) the regulatory use of these models (2.2.3). Sub-sections 2.2.4 and 2.2.5 provide proposals for uniform principles and a common tiered framework for component-based MRAs under the WFD, respectively. Sub-section 2.2.6 finally summarises advantages and limitations of CBAs.

2.2.1 Models for predicting the toxicity of mixtures

Concentration addition (CA) and toxic unit summation (TUS)

Concentration addition (Loewe and Muischnek 1926), also denoted as '*dose addition*' (DA), assumes that mixture components have a similar mode of action (MoA). As a consequence, a mixture component can be replaced totally or in part by an equal fraction of an equi-effective concentration of another component without changing the overall effect $E(c_{mix})$. This is what the mathematical formulation of the model says. In Tab. 1, it is given for both two-component mixtures and multicomponent-mixtures in Eqs. 1 and 2, respectively. It can be transformed into a prediction of effect concentrations of mixtures ECx_{mix} as given by Eq. 3.

For risk assessment purposes, an algebraic equivalent of the CA is formula is frequently used, the so-called toxic unit summation (TUS) (see Tab. 2 in section 2.2.3). Toxic units (TU) are potency adjusted concentrations. The absolute concentrations (or doses) c_i of mixture components i are divided by reference concentrations causing the same effect X, i.e. the equivalent effect concentrations (or doses) ECx_i of single substances i. Hence, $TUS = \Sigma TUx_i = \Sigma(c_i/ECx_i)$. Originally, a 50% effect level (X = 50% and $ECx_i = EC50_i$) was suggested to be used for TUS calculations (Sprague 1970). However, the approach can also be applied to any other effect level X. If the sum of toxic units is one ($\Sigma TUx_i = 1$), the total effect of the mixture is expected to equal the value X. If the sum of toxic units is larger or smaller than 1, the total effect is expected to be larger or smaller than X, respectively.

Independent action (IA)

Independent action (Bliss 1939), also denoted as '*response addition*', assumes that mixture components contribute to a common endpoint via dissimilar and fully independent chains of reactions. As a consequence, the individual effects can be considered to be independent events in a probabilistic sense. Under the additional assumption that the susceptibilities of the individuals of an at-risk-population to different dissimilarly acting components are not correlated, the mathematical definition of the concept for a binary mixture given by Eq. 4 in Tab. 1 was derived by Bliss (1939)[13]. It can be extended to any number of mixture components as given by Eq. 5 in Tab. 1 and explained in detail in Faust et al. (2003). The model cannot be transformed into an explicit term for the prediction of effect concentrations of mixtures (ECx_{mix}) but the formulation given by Eq. 6 must be solved numerically. IA typically predicts a lower toxicity than CA, i.e. risk assessments based on IA are usually less conservative than CA-based assessments, as detailed in section 2.2.3 below.

[13] More sophisticated versions of the IA model have been suggested to include an additional coefficient r, intended to denote the degree of possible correlations between the susceptibilities of the individuals of an at-risk-population to different dissimilarly acting components (Bliss 1939). However, predictions may only be derived from such versions by making special assumptions on the correlation coefficient r. Therefore, only the basic version of IA with the simple assumption of r = 0 has gained practical relevance.

Table 1: Mathematical formulation of models for predicting the toxicity of chemical mixtures

	Concentration Addition (CA)		Independent Action (IA)	
Two-component mixtures	$E(c_{mix}) = X, \; if \; \frac{c_1}{ECx_1} + \frac{c_2}{ECx_2} = 1$	[1]	$E(c_{mix}) = E(c_1) + E(c_2) - E(c_1) \bullet E(c_2)$	[4]
Multi-component mixtures	$E(c_{mix}) = X, \; if \; \sum_{i=1}^{n} \frac{c_i}{ECx_i} = 1$	[2]	$E(c_{mix}) = 1 - \prod_{i=1}^{n} (1 - E(c_i))$	[5]
Transformations[a] for the prediction of effect concentrations	$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{F_i^{-1}(x_i)} \right)^{-1}$	[3]	$X = 1 - \prod_{i=1}^{n} (1 - F_i(p_i \bullet (ECx_{mix})))$	[6]

Notation

c_i = individual concentration of substance i in a mixture with n components ($i = 1...n$)

c_{mix} = total concentration of substances 1...n in the mixture ($c_{mix} = c_1 + c_2 ... + c_n$)

ECx_i = effect concentration of substance i, i.e. the concentration of substance i that causes the effect X if applied individually ($c_i = ECx_i$ if $E(c_i) = X$)

ECx_{mix} = effect concentration of the mixture, i.e. the total concentration of substances 1...n in a mixture that contains the mixture components in a given concentration ratio $p_1 : p_2 ... : p_n$ and causes the total effect X ($c_{mix} = ECx_{mix}$ if $E(c_{mix}) = X$)

$E(c_i)$ = individual effect of substance i if present in the concentration c

$E(c_{mix})$ = total effect of the mixture with the total concentration c_{mix} if the mixture components are present in the concentration ratio $p_1 : p_2 ... : p_n$

X = definite value for the effect E

p_i = relative proportion of substance i expressed as a fraction of the total concentration of substances in the mixture ($p_i = c_i / c_{mix}$)

F_i = concentration response function of substance i ($E_i = F(c_i)$)

F_i^{-1} = inverse concentration response function of substance i ($c_i = F^{-1}(E_i)$)

Effects E denote the relative intensity or frequency of a response parameter (defined as fraction of a maximum possible value) and thus can only take values between 0 % and 100 %: $0 \le E \le 1$.

If effects E are not considered as a function of concentrations c but of doses d, all formulas apply in an equivalent way (all c replaced by d) and CA is denoted as dose addition (DA).

[a] see Faust et al. 2003 for a full explanation of transformations

Mixed model (MM)

Mixed model approaches, also referred to as integrated modelling or two-stage procedures, are combinations of CA and IA. The components of a mixture are grouped according to their modes of action. CA is assumed for sub-groups of similarly acting mixture components, and IA is assumed

between such groups. Completely similar action (CA) and completely dissimilar action of all mixture components (IA) are the two possible extreme cases of the MM approach. If the components act partly similar and partly dissimilar, the MM approach predicts an intermediate toxicity within the "prediction window" defined by extreme cases of CA and IA. While CA and IA require single substance toxicity data only, application of the MM approach additionally needs good knowledge of the MoAs of all mixture components.

2.2.2 Data requirements for mixture toxicity predictions

For calculating mixture toxicity predictions by means of CA or IA, toxicity data must be available for all mixture components. Both models imply that all the individual toxicity data refer to the same endpoint, ideally determined in the same assay under identical conditions of exposure. For pragmatic reasons, regulatory approaches to mixture risk assessments deviate more or less from this demand for strictly identical test endpoints, as detailed in section 2.2.3 below.

It is important to notice that exact data requirements for the prediction of effect concentrations of mixtures are considerably different for CA and IA. In general, calculations under the assumption of IA necessitate much higher data requirements than applications of the formula for CA.

If CA is assumed, the prediction of effect concentrations of mixtures necessitates that equivalent effect concentrations of single substances are put into the formula (Eqs. 1 to 3 in Tab. 1). If, for instance, EC50 values are available for all mixture components, the expectable EC50 of the mixture can be readily calculated. Correspondingly, for the calculation of EC10 values of a mixture, EC10 values of single substances are required, and so on. These calculations of expectable effect concentrations can be done for any concentration ratio of mixture components, without any change in these requirements for necessary input data.

Generally higher data requirements of IA result from the fact that the concept does not operate with effect concentrations (ECx_i) but with the intensity or frequency of individual effects ($E(c_i)$), as explained above. For the numerical determination of the expectable effect concentration of a mixture (ECx_{mix}) by means of the transformed IA formula (Eq. 6 in Tab. 1), it is absolutely necessary that the effects of single substances can in each case be determined for exactly that individual concentration ($p_i \bullet ECx_{mix}$) which is present in a mixture that is expected to cause the total effect X under the assumption of IA.

In general, these complicated conditions mean that good knowledge of the concentration response functions (F_i) must be available for all the individual toxicants that are present in a mixture. These functions must provide valid estimates of single substance effects in relevant concentration ranges. These relevant concentration ranges, as well as the corresponding individual effects, become lower and lower with decreasing ratios between individual concentrations and with decreasing total concentrations of mixture constituents. However, the smaller the individual effects are that are entered into the calculation, the higher are the statistical data requirements that have to be met for a proper estimation of such low individual effects.

The average ratio between individual concentrations and the total concentration of mixture components decreases with an increasing number of mixture components. As a consequence, the data requirements for a valid use of the IA model increase. This can for example be illustrated for a situation where the individual effects of the constituents contributing to the total effect of a mixture are assumed to be identical. IA would predict a total effect of 50 %, if two substances are combined in concentrations that would each cause around 30 % individually. If the number of mixture components is increased to 10, however, the same total effect of 50 % is already expected to occur, if the individual effects entered into the IA formula each have a value of only 6.7 %. For these ranges of low individual effects, valid estimates of corresponding effect concentrations are required. If this requirement cannot be met, the IA concept cannot provide valid predictions of mixture toxicity. With multi-component mixtures with high numbers of constituents, these data requirements may often be impossible to meet in practice.

2.2.3 Regulatory use of CBAs

As already pointed out above, the risk assessment of chemical mixtures for human health and the environment is not a well-established standard element of EU chemicals legislation, but it is a developing issue. In 2012, the European Commission presented a summary of the state of affairs in a Communication to the Council on "*The combination effects of chemicals*" (EC 2012). That communication was the Commission's formal response to an invitation of the Council "*to assess how and whether relevant existing Community legislation adequately addresses risks from exposure to multiple chemicals from different sources and pathways, and on this basis to consider appropriate modifications, guidelines and assessment methods*" (CEU 2009). The response from the Commission was based on a corresponding opinion of the three Scientific Committees of the Commission (EC 2011b) and an earlier state-of-the-art report contracted by the Commission (Kortenkamp et al. 2009). In 2014, the Commission's Joint Research Center prepared an updated comprehensive overview on regulatory requirements and available guidance for the assessment of mixtures (Kienzler et al. 2014, 2016). Ongoing initiatives for the removal of remaining conceptual and methodological challenges and the harmonisation of approaches have been established on different organisational levels, such as EFSA[14], OECD[15], and WHO[16].

In the EU, explicit requirements for considering mixture effects of chemicals from multiple sources were first introduced in the Regulation on Maximum Residue Levels (MRLs) of pesticides in food and

[14] See the introductory part for section 2 above for further information on EFSA's 'MixTox' initiative

[15] A project team under the working parties on hazard and exposure assessment of the Organisation for Economic Co-operation and Development (OECD) is developing a guidance document on the assessment of risks from the combined exposures to multiple chemicals (http://www.oecd.org/chemicalsafety/environment-health-safety-news.htm). An overview of the project is available at http://www.euromixproject.eu/wp-content/uploads/2017/05/5_OECD-Combined-Exposure-Project-EuroMix-May-2017-E-Leinala.pdf.

[16] As a part of the International Programme on Chemical Safety (IPCS), the World Health Organization (WHO) developed a *Framework for Risk Assessment of Combined Exposures to Multiple Chemicals*. An informal *Combined Exposure Group* continues the activities. Information on the framework development and follow-up initiatives is available at http://www.who.int/ipcs/methods/harmonization/areas/aggregate/en/.

feed in 2005[17]. Since then, the European Food Safety Authority (EFSA) has been working on the development and the implementation of a corresponding methodology. In the USA, the development started earlier. During the last 30 years, the Environmental Protection Agency (US EPA) and other national authorities spent considerable efforts on the development of guidelines and methodologies for the risk assessment of chemical mixtures (Syberg et al. 2009; Teuschler et al. 2004; US EPA 2000, 2007). However, in the USA, legal requirements for assessing mixture risks are confined to the protection of human health, while the corresponding activities in the EU include both risks for human and for the environment.

Tiered approaches starting from CA as a default assumption

The US EPA developed an approach to component-based risk assessments which is dependent on modes of actions (MoAs) of mixture components (US EPA 2000). Concentration addition is assumed for mixtures of substances with a similar mode of action. Independent action is assumed for mixtures of dissimilarly acting substances. And a mixed model (MM) is assumed for mixtures of substances with partly similar and partly dissimilar modes of action. From a scientific perspective this appears to be a sound approach. From a regulatory perspective, however, this approach leads into unsolvable problems. For many environmentally pollutants, knowledge about modes of action is insufficient or totally missing, thereby rendering the approach practically inapplicable for many realistic exposure scenarios. In addition, the high data demands for appropriate applications of IA and mixed models can often not be met with the single substance data that are typically available to regulatory authorities.

As a pragmatic and precautious way out of this dilemma, tiered approaches have been suggested that start from the assumption of CA for all mixture components, regardless of MoAs, as a reasonable worst case estimate. If this indicates a significant risk, refined MoA-based assessments may be conducted where the necessary data are available. Alternatively, precautionary measures may be taken. This way of thinking has guided ecotoxicological MRAs for quite some time already (e.g. ECETOC 2001, 2011a), but in the human arena it was introduced by a WHO working group in 2011 only (Meek at al. 2011). This development prepared the ground for discussing consistent and coherent approaches across the disciplinary borders (ECETOC 2011b). The first generic framework for both human and environmental MRA was proposed by the European Commission's Scientific Committees (EC 2011b). The most recent and most refined example of a generic decision tree was developed by Price et al. (2012). A SOLUTIONS proposal for an advanced tiered framework for application under the WFD is provided in section 2.2.5 below.

[17] Regulation (EC) No 396/2005

The use of CA as a pragmatic and precautionary default assumption can be justified by a combination of four arguments (EFSA 2013c):

- Data requirements for a proper application of CA are much easier to fulfil than for IA or MM.
- Usually, the assumption of CA provides a more conservative estimate of mixture toxicity than the alternative assumption of IA. Theoretically, the reverse situation is possible (Fig. 1 C) but the practical relevance of such a situation has yet not been demonstrated.
- Synergistic effects that significantly exceed the CA expectation are exceptions and not the rule, at least for multi-component mixtures.
- The CA assumption is conservative, but not vastly over-conservative. Typically, the "prediction window" between CA and IA is not very wide (Fig. 1). For realistic assessment situations it will rarely exceed an order of magnitude on the concentration axis. Typically, it is much smaller. Even with mixtures composed of up to 100 chemicals, predicted effect concentrations of the mixture derived from CA and IA may usually differ by a factor of less than 5 only, as explained theoretically and demonstrated practically in Chapter 13.4 of Kortenkamp et al. (2012).

Pragmatic simplifications of CA

In regulatory practice, even the data requirements of CA may still be unfulfillable. As a consequence, a number of pragmatic simplifications have been derived from the original CA concept. Partly they have already become established procedures under specific pieces of legislation in the EU or in the US. A selection of three prominent examples is given in Tab. 2: the *point of departure index* (PODI) (Wilkinson et al. 2000) and the *hazard index* (HI) (Teuschler and Hertzberg 1995), which both were originally invented for HRA, and the *summation of PEC/PNEC ratios* which was first suggested for the derivation of water quality objectives by Calamari and Vighi in 1992.

A common feature of such approaches is that they basically make use of the CA formula as a calculation rule. However, they use input data that deviate more or less from the strict requirements of the original concept, but which may be easier available to a regulator. Therefore, they are collectively denoted as *CA-based* approaches in this report.

Typically, such approaches make use of the CA model in the formulation as a summation of toxic units (as explained in section 2.2.1 above). In contrast to the original definition of toxic units, however, equivalent effect concentrations are replaced by other hazard indicators, and actual concentrations of mixture components may be replaced by various exposure estimates, such as PEC values as defined under REACH. Collectively, all these ratios between measured exposure levels (MEC) or predicted exposure levels (PEC) and any indicators of hazardous or regulatory acceptable exposure levels are denoted as risk quotients (RQ) in this report. As a common principle, CA-based approaches typically sum up such risk quotients. Compliance or deviations of the sum of RQs from a value of 1 (or 1 multiplied by an assessment factor) is used a decision criterion, as shown in Tab. 2 for the selected examples.

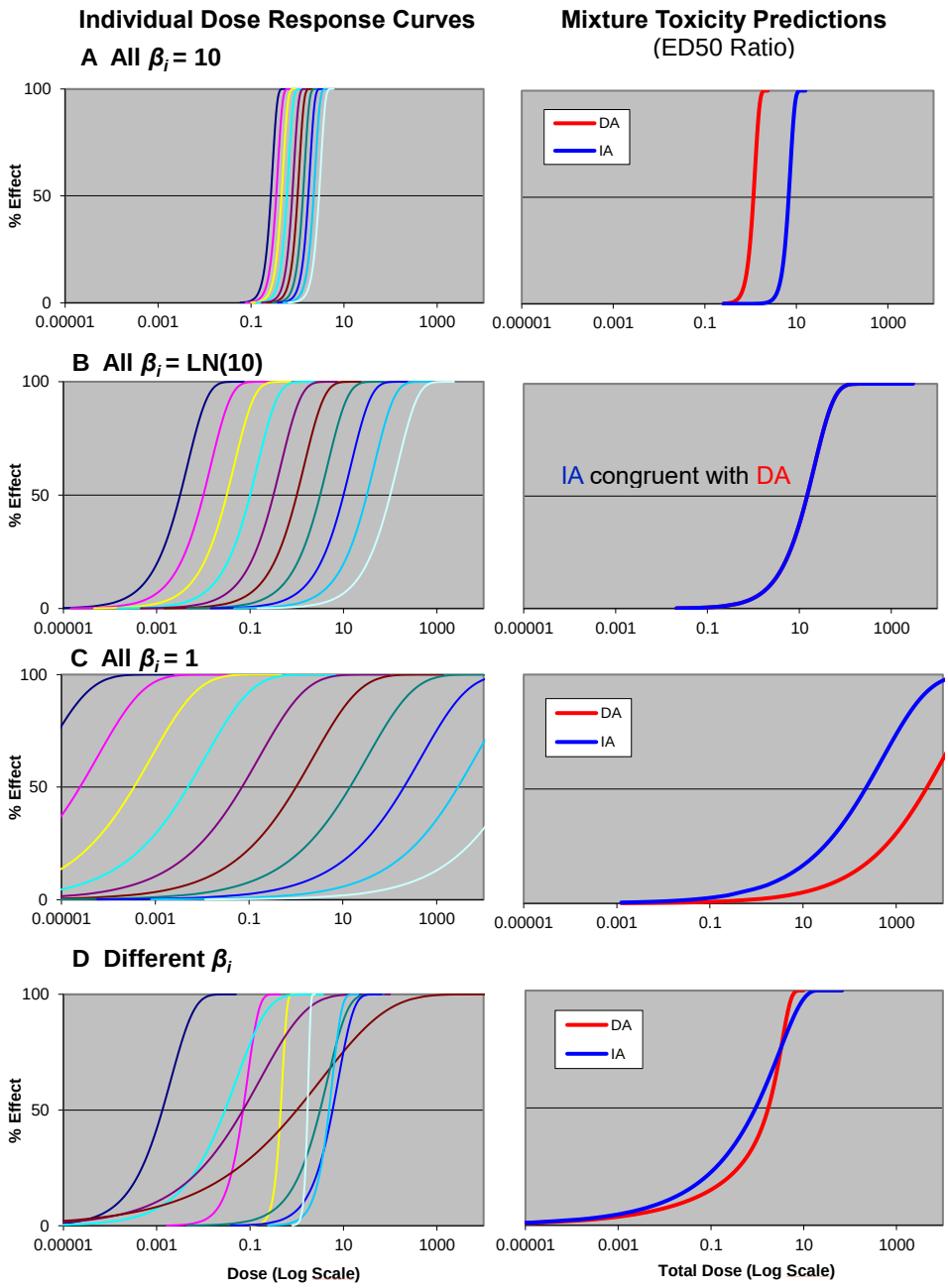

Figure 1: Illustration of the quantitative differences between independent action (IA) and dose addition (DA)

For a given number of components in a given dose ratio, the difference depends on the slopes of the individual dose response curves (DRCs). Individual DRCs of 10 hypothetical mixture components are shown on the left, the resulting ratios between predictions by IA and DA are shown on the right. The individual DRCs are described by the Weibull model $E_i = F_i(d_i) = 1\text{-}\exp(-\exp(\alpha+\beta_i \bullet \log_{10}(d_i)))$. Different scenarios are depicted for the slope parameter β_i: steep curves (A), intermediate steepness (B), very shallow curves (C), and a mixed situation (D). Resulting mixture toxicity predictions by DA and IA are shown for mixtures containing the 10 components in the ratio of their individual ED50 values. (From Kortenkamp et al. 2012, Chapter 13.4).

Table 2: Regulatory approaches to mixture risk assessments derived from the concept of concentration addition

Approach	Assessment term	Notes
CA (DA) *Concentration (Dose) Addition*	$E(c_{mix}) \leq x$ if $\sum_{i=1}^{n} \frac{c_i}{ECx_i} \leq 1$	Procedure termed Toxic Unit Summation (TUS) $\frac{c_i}{ECx_i} = TU_i = ToxicUnit$
PODI *Point of Departure Index*	No significant effect if $\sum_{i=1}^{n} \frac{EL_i}{POD_i} \leq 1$	EL = Exposure Level POD = LOEL, NOAEL, NOEC
HI *Hazard Index*	No reason for concern if $\sum_{i=1}^{n} \frac{EL_i}{AL_i} \leq 1$	EL = Exposure Level AL = Acceptable Level = ADI, DNEL, …
PEC/PNEC Summation	No unacceptable risk if $\sum_{i=1}^{n} \frac{PEC_i}{PNEC_i} \leq 1$	PEC = Predicted Environmental Concentration PNEC = Predicted NEC

Notation for the formulation of CA as given in Tab. 1

Instead of equivalent effect concentrations, the PODI uses estimates of so called *points of departure* (PODs) as input data, such as *no observed effect concentrations* (NOEC), *no observed (adverse) effect levels* (NO(A)EL) or *lowest observed effect levels* (LOEL). Thus, the simplifying pragmatic assumption is, that NOEC values or other PODs are approximately equivalent to a uniform low effect level X, such as an EC10 for instance, while in fact they may typically correspond to effect levels between 10 and 30 % (Moore & Caux 1997). In addition, PODs may refer to different toxicological endpoints. Thus, the PODI approach introduces two sources of possible prediction differences between the original CA model and the CA-based approach: inexactly defined low effect levels and merging of endpoints.

The hazard index (HI) goes a step further by using regulatory acceptable levels (AL) as input data, often also denoted as regulatory reference values (RV). Examples are the acceptable daily intake (ADI) or the derived no effect level (DNEL) for humans. These values are derived from PODs by applying *assessment factors* (AF), also denoted as *uncertainty factors*, or *extrapolation factors*. The HI calculation aims to derive an estimate of an acceptable level for a mixture. A practical advantage of this approach is that AL values may be readily available to regulators, while it may be demanding to retrieve the original toxicological data behind them. However, with assessment factors being included in the input data, a third source of potential prediction differences between the original CA concept and the pragmatic CA-based approach is introduced. As an alternative to this approach, it has therefore been suggested to calculate first a mixture toxicity indicator that does not include any assessment factors, such as the PODI, and then to apply a single assessment factor to the calculated

mixture toxicity value, just as it is done in single substance assessments (Wilkinson et al. 2000). If the assessment factors that are included in AL values are the same for all mixture components, both approaches yield the same result, otherwise they differ.

The PEC/PNEC summation may be considered as a conceptual equivalent to the (human-oriented) HI for the broader purpose of environmental mixture risk assessments. The three principal differences from the original CA model are the same, but the degree of the deviation may even be larger. The basic reason for this is the fact that PNECs do not aim to protect one species group only (such as humans) but all species in the environment. As a consequence, they may be based on toxicity data for completely different species groups, such as fish, daphnids and algae. As a consequence, the assessment factors used for PNEC derivation also aim to cover a broader spectrum of uncertainties than those used for HRA. They include (i) intra- and inter-laboratory variation of toxicity data, (ii) intra- and inter-species variations (biological variance), (iii) short-term to long-term toxicity extrapolation, and (iv) laboratory data to field impact extrapolation (ECHA 2008, p.17). The actually applied factors may indeed vary between 1 and 10 000. Depending on the data situation, the PNECs of two substances with almost identical toxicity may differ by orders of magnitude. And *vice versa*, two substances with very different toxicological profiles in different species may have the same PNEC. Such conditions may have vastly disturbing effects on mixture toxicity predictions.

For the reasons explained, CA-based approaches may be justified as worst case estimates for filtering out mixtures of potential concern. For obtaining conclusive evidence on significant mixture risks, and in particular for ranking mixture risks for prioritization purposes, however, they may be insufficient or even misleading. Full transparency of actual input data therefore is an important requirement for ensuring the reliability of component-based MRAs. As far as possible, the potentially disturbing effects of differing endpoints, differing assessment factors, and differing effect levels should be removed from the calculations.

Implementation guidelines

Where specific legal requirements for mixture risk assessments are already in place under EU law, competent authorities are working on the development of guidance for the implementation of these requirements. For the human health risk assessment of chemical mixtures, this process has not yet resulted in any formal implementation guideline. For environmental MRAs, however, a number of guidance documents has been released for application under the Plant Protection Product Regulation (PPPR)[18] and the Biocidal Product Regulation (BPR)[19]. Under the PPPR, procedures for mixture assessment have been included in the risk assessment guidelines for birds and mammals (EFSA 2009, section 2.5 and Appendix B), for aquatic organisms (EFSA 2013b, section 10.3; 2015, section 10.2), for bees (EFSA 2013a, section 8), and for non-target terrestrial plants (EFSA 2014, sections 8.1, 8.2, and Appendix F). Under the BPR, a dedicated guidance document on mixture toxicity assessment has been set up (ECHA 2014). It applies across the different types of biocidal products and in addition to

[18] Regulation (EC) No 1107/2009

[19] Regulation (EU) No 528/2012

corresponding specific guidelines for specific types of biocidal products, such as the guidance on disinfection by-products (DBPs) (ECHA 2016).

In detail, the level of guidance for mixture risk assessments differs considerably between these documents and the suggested terminologies and tiered approaches are not fully consistent. As a common feature, however, they are all based on the default assumption of concentration addition. The ecotoxicological mixture risk assessments refer to the same effect levels as usual single substance assessments, i.e. typically EC50 for short-term and EC10 (or other low effect indicators) for long-term risk assessments. NOECs may be used as a surrogate for a common low effect concentration such as EC10. The tiered guideline procedures typically start with a pragmatic simplification of the original CA concept, such as a summation of PEC/PNEC ratios, as a worst-worst case assumption. If this signals an unacceptable risk, the analysis is taken forward towards compliance with the conceptual premises of CA, as far as possible with available data. If concerns about a significant risk cannot be ruled out by this way, experimental testing of the mixture is considered as the ultimate option for clarification.

Where such experiments are conducted for testing the validity of CA-based predictions, the uncertainties and variabilities of both measured and predicted toxicity values of mixtures must be taken into account and the degree of precision of predictions that is required for regulatory purposes must be defined. In other words, rules and methods are needed for distinguishing between regulatory significant and regulatory insignificant deviations of observations from predictions. Up to now, the guidance for mixture toxicity assessment of biocidal products is the only one which defines a quantitative criterion for this assessment: "*synergistic effects are effects of a mixture which are greater than that predicted by CA by a factor of 5 or more*" (ECHA 2014, p. 7)[20]. Preceding reviews of available data had shown that stronger synergistic deviations from the CA-expectation are rarely observed for the aquatic toxicity of active ingredients of pesticidal and biocidal products (Belden et al. 2007; Altenburger et al. 2014)

2.2.4 Uniform principles for component-based MRAs

Considering the WFD protection goals and the regulatory state-of-affairs outlined in the preceding sections, mixture risk assessments under the WFD should

- ensure consistency with assessments performed under other pieces of European chemicals and environmental legislation, and
- apply a harmonized approach for both human and environmental MRAs.

To this end, we propose a set of uniform principles for the component-based regulatory assessment of environmental risks of mixtures of chemicals. It is derived from an earlier more specific proposal

[20] The formulation is somewhat ambiguous, because it refers to *"effects*" and not to *effect concentrations*. However, from the context it can be safely assumed that the "*factor of 5*" is meant to apply to differences in predicted and observed effect concentrations of mixtures ($EC_{x_{mix}}$ in the Eqs. given in Tab. 1) and not to differences in absolute or fractional *effects* (5 times 50 % effect, for example, would result in 250 % response, which is nonsense).

for common principles of environmental MRAs performed under the PPP and BP Regulations (Altenburger et al. 2014) which has flown into corresponding implementation guidelines (Frische et al 2014, ECHA 2014).

The suggested principles include:

- Environmental safety requirements for mixtures should neither be higher nor lower than the corresponding requirements for single substances.
- Procedures for the environmental safety assessments of mixtures should follow the principle of a tiered approach. For any specific type of regulatory assessment situation, it should be examined how different methods and criteria could most efficiently be combined in a tiered decision tree. To this end, the methodologies should ensure that proceeding from lower to higher, more data demanding tiers is only required, if there is a substantial chance that this may alter the regulatory conclusion in terms of acceptability or unacceptability of mixture risks.
- CA is considered to provide a reasonable default assumption for the joint toxicity of environmental chemicals, provided that there are reasons to assume that all relevant mixture components are included in the calculation.
- As input data, the original scientific concept of CA requires effect concentrations (or doses) that refer to the same biological effect in the same species under identical test conditions. For regulatory use, however, pragmatic simplifications and assumptions are unavoidable. This may refer to the merging of data for different test conditions, endpoints and species and to the use of NOEC or NOAEL values as a surrogate for quantitative estimates of low effect concentrations. In any case, the potential additional errors that may be introduced by such deviations from the original concept should be made transparent. Where possible they should be removed in a stepwise manner.
- IA and mixed models (MM) are much more data demanding and bear a higher risk of underestimating the actual mixture toxicity than CA. Therefore, the use of IA and MM should be restricted to situations where knowledge about MoAs and dose response relationships of mixture components supports the proper usage of these models.
- Where evidence is available for synergistic interactions of known mixture components resulting in significantly more-than-additive joint effects at relevant exposure levels, the CA default assumption may be dropped in favour of a special approach tailored to the individual case.
- Where CBA-based assessments point to an unacceptable risk, experimental testing of the mixture may be considered as an ultimate option for clarification, unless practical, ethical, economical or other regulatory considerations argue against such a decisive experiment.

In line with these principles, SOLUTIONS suggests a common tiered framework for component-based HRA and ERA of pollutant mixtures in European surface waters, as detailed in the following section.

2.2.5 Common tiered framework for component-based HRA and ERA of chemical mixtures under the WFD

SOLUTIONS WP 18, part of the *MODELS* sub-project, works on a tiered framework for the component-based assessment of human and ecological risks from exposure to multi-component pollutant mixtures in European surface waters. An initial version of the tiered approach was presented in the internal deliverable ID M5.1. In accordance with the DOW, the proposal was confined to MRAs for humans and fish. However, it can easily be expanded to include MRAs for any other single species groups. The conceptual outline of such an extended version is given in Tab. 3. In ID M5.1, the conceptual outline is complemented by a detailed decision tree. In addition to assessing the significance or insignificance of the overall mixture risk, the detailed decision tree includes (i) the identification of drivers of the overall mixture risk (see section 4.2 below), and (ii) the identification of mixture components which should be subjected to further toxicity testing because they may make a significant contribution to the overall risk but the available toxicity data is insufficient for clarification.

Currently, the procedure is tested and refined by means of trial runs with both chemical monitoring data from the SOLUTIONS sub-project cases *CASES* and co-exposure modelling data generated by the SOLUTIONS '*modelling train*'. The final version will be presented in D18.1. In addition to single species MRAs, WP18 is also working on the advancement of methods for MRAs for aquatic species assemblages on the basis of species sensitivity distributions (SSDs), the so-called msPAF approach. They will be included in the final framework proposal as a complementary approach which is suitable for ERA but not for HRA of mixtures.

The suggested workflow is divided into three main tiers in which the distorting influence of different assessment factors present in regulatory values is successively removed, and increasingly sophisticated assumptions about modes of action are introduced:

- Tier 1 MRA, using regulatory values including EQS values in conjunction with the hazard index method (HI), including conceptual equivalents such as ∑PEC/PNEC,
- Tier 2 MRA based on the assumption of CA or DA of all mixture components contributing to a common endpoint (adverse outcome) in the same species or species group, regardless of modes of action,
- Tier 3 MRA, using the mixed model approach, i.e. CA (or DA) for sub-groups of similarly acting mixture components (contributing to a common endpoint by a common MoA) and independent action (IA) between such groups, including completely similar (CA or DA) and completely dissimilar action (IA) as the two possible extreme cases of the MM approach.

Considering the practical difficulties in fulfilling the data demands, the Tier 2 and Tier 3 assessments are each sub-structured into a number of sub-tiers. This sub-tiering is designed to evaluate whether a definite conclusion can be reached without completely fulfilling all data requirements that apply to the respective main tiers. Detailed explanations of this sub-structural details of the scheme have been provided in ID M5.1 and will be included in the final D18.1.

The development of the tiered scheme started from existing proposals for generic decision trees for both human and environmental MRAs, in particular the most recent and most refined proposal by Price et al. (2012). In addition, existing proposals for non-generic MRA schemes were also considered, in particular to specific decision trees which have been developed for regulatory MRAs for aquatic species on the basis of single substance data that are routinely generated under different pieces of EU chemicals legislation. This applies to assessments based on REACH data (Backhaus and Faust 2012), to assessments under the PPP Regulation (EFSA 2013b, section 10.3; partly based on Altenburger et al. 2014), and to assessments under the complementary biocidal products Regulation (ECHA 2014; partly based on Backhaus et al. 2013). Those decision trees were tailor-made for the specific regulatory context and cannot be generalized for the purpose of MRAs under the WFD. However, care was taken to ensure that the proposed generic decision tree follows the same principles and represents an approach that is consistent with those specific guidelines.

The proposed tiered framework both adopts and omits a number of elements from the Price et al. scheme and it adds several new features. A detailed description and an explanation of all commonalities and differences is provided in ID M5.1 and will be included in the final D18.1. Important novel features are:

- Full coverage of all possible data situations for regulatory decision making (Tiers 1 to 3).
- Clear and fully transparent differentiation between worst case assessments derived from simplified CA-based approaches (Tier 1) and assessments based on the original scientific concept of CA (as explained in section 2.2.3 above) (Tier 2).
- Clear decision criteria for situations when unacceptable risks occur, and when refinement of the analysis should cease.
- Clear separation of the identification of unacceptable mixture risks from the identification of drivers of the overall risk, as explained in further detail in section 4.2 below.

The assessment of mixture risks in terms of being regulatory significant or insignificant entails a need for defining assessment factors (AF) for deriving a regulatory acceptable level from a component-based prediction of a low effect level of a mixture (see column *'assessment rule*' in Tab 3). Consented rules for such AFs for MRA do not exist. However, considering the established rules for applying AFs in single substance risk assessment, in particular those for EQS setting (EC 2011a), it may be possible to derive default AFs for MRAs which ensure an equivalent protection level for both single substances and mixtures, as suggested as guiding principle in section 2.2.4 above. D18.1 will include a corresponding proposal, alongside with the final version of the proposed tiered framework.

Table 3: Conceptual outline of a tiered component-based MRA for humans and aquatic single species groups (SOLUTIONS WP 18)

Tier	Assessment concept		Input data	Method	Advantage over preceding tiers	Assessment rule	Decision logic
	Assumption	**Specification**					
Premise: mixture of concern defined in terms of components i and exposure levels EL_i for humans ($EL_{i,HUMAN}$) and for aquatic single species groups ($EL_{i,SSG}$) such as fish, daphnids, algae							
1	**DA (or CA)** for all mixture components	Worst case risk estimations following from conservative input data not exactly meeting model requirements	**RVs**	HI		No significant risk if HI ≤ 1	Assessment moved to the next tier if a significant risk cannot be ruled out by a reasonable worst case estimate (of the maximum expectable risk)
2A			**Species(-group) specific (lowest) PODs**	PODI	No distortion by varying AFs. No merging of incomparable species	No significant risk if (PODI x AF) ≤ 1	
2B			Species(-group) specific (lowest) **BMDs or low ECs**	BMD- or low-EC-based PODI	No distortion by ill-defined effect levels	No significant risk if (BMD- or ECx-based PODI x AF) ≤ 1	
2C		Exact modelling of maximum expectable risks	**Endpoint-specific** BMDs or low ECs	TUS (for low-effect levels *x*)	No aggregation of toxicity data across different endpoints	No significant risk if (∑TUx x AF) ≤ 1	
3A	**Mixed Model (MM)**, i.e. DA (or CA) for groups of similarly acting mixture components, IA between groups	Limit value calculations of minimum expectable risks following from incomplete specifications of IA model parameters and/or insufficient knowledge on MoAs		Assumption of No Addition of single substance toxic units (TUx_i) as a (lower) limit value for IA-based predictions	Expansion of the one-sided conservative MRA to a two-sided "Prediction Window" between minimum and maximum expectable risk	Significant risk if $TUx_i > 1/AF$	Assessment stopped if a significant risk is demonstrated for a reasonable best case scenario (of the minimum expectable risk)
3B			Endpoint-specific BMDs or low ECs **+ MoA information**	TUS within MoA group *j* for endpoint *k* and assumption of No Addition between groups, as a (lower) limit value for MM-based predictions	Available knowledge on MoAs taken into account	Significant risk if $\sum TUx_{j,k} > 1/AF$	
3C			**Dose (or concentration) response functions** (for common endpoints)	Assumption of completely independent MoAs for all mixture components as an extreme case of MM	All parameters of the IA model specified; IA-based predictions of BMD_{mix}^{IA} and/or $EC10_{mix}^{IA}$ calculated (where available data allow to do so)	▪ Significant risk if $BMD_{mix}^{IA} < (\sum EL_{i,HUMAN}) \times AF$, and/or $EC10_{mix}^{IA} < (\sum EL_{i,SSG}) \times AF$ ▪ Inconclusive evidence if available information on slope of DRCs or CRCs is insufficient but Tier 2C assessments give a reason for concern	As before. Assessment also stopped in case of serious data gaps on slope of DRCs (or CRCs)
3D		Best possible mixture risk modelling	Dose (or concentration) response functions **+ MoA information**	State-of-the-art MM-based calculation of low-effect doses or concentrations of the mixture (BMD_{mix}^{MM}, $EC10_{mix}^{MM}$); MRA derived by means of an AF	Prediction Window narrowed down to a precise mixture toxicity prediction and definite MRA derived (where available knowledge and data allow to do so)	▪ Significant risk if $BMD_{mix}^{MM} < (\sum EL_{i,HUMAN}) \times AF$, and/or $EC10_{mix}^{MM} < (\sum EL_{i,SSG}) \times AF$; ▪ No significant risk otherwise, provided MM is applicable; ▪ Inconclusive evidence if MM is inapplicable but Tier 2C assessments give a reason for concern	Component-based MRA completed

2.2.6 Advantages and limitations of CBAs

The reliability of assessments and rankings obtained by the component-based modelling approach crucially depends on two important pre-requisites: (i) all relevant mixture components are included in the calculations, and (ii) toxicodynamic or toxicokinetic interactions between mixture components that result in strong deviations of the actual joint toxicity from non-interaction models are absent or at least insignificant. Whether these conditions really apply, can ultimately be verified by means of the experimental whole mixture testing approach only.

The component-based modelling approach is readily applicable (i) to an unlimited number of exposure scenarios, (ii) to any endpoint, and (iii) to both prospective and retrospective assessments (iv) at low costs and (v) with no ethical constraints. In theory, these are five big advantages of CBAs over WMAs. In practice, however, realization of these advantages is strongly limited by dependence on the availability of the necessary input data, i.e. single substance exposure and toxicity information for all relevant mixture components and all relevant endpoints.

An illustrative example for underestimations of mixture risks which may result from neglecting relevant mixture components in routine chemical monitoring has been provided by Moschet and co-workers (2014). They performed a component-based mixture toxicity calculation on the basis of a comprehensive screening of polar organic pesticides in Swiss surface waters, including 249 original compounds plus 134 transformation products. The results were compared with scenario calculations based on routine monitoring data which are typically confined to a selection of 15-40 pesticides. The comparison revealed, that MRAs on the basis of the limited routine monitoring data underestimate the cumulative pesticide mixture risk by a factor of 2 on average.

Techniques for both chemical monitoring and co-exposure modelling are rapidly developing and the SOLUTIONS sub-projects *Tools* and *Models* make a significant contribution to this. As a result, the data situation for applying CBAs is continuously improving on the exposure side, but huge gaps remain on the hazard side. Missing toxicity data for single substances are the bottleneck. Surrogate data, generated by means of QSARs, read-across, endpoint-to-endpoint extrapolations, or similar methods, are important for prioritizing mixture components for further testing but usually they are insufficient for conclusive MRAs. The uncertainty is too high and they are largely confined to estimates of acute toxicity, inappropriate for chronic MRAs.

In addition, even with the best available chemical monitoring strategies and the most advanced exposure modelling techniques, a clear risk remains for overlooking pollutants which make a significant contribution to overall mixture risk, as has been repeatedly demonstrated by means of EDA studies, including examples generated in SOLUTIONS (Muz et al 2017a, Muschket et al 2018). Cases of unknown synergistic interactions between mixture components may further aggravate the problem (Muz et al 2017b).

Thus, with the aim to reduce the possibility of overlooking relevant mixture components and underestimating overall mixture risk, WMAs and CBAs must not be regarded as rival but as complementary approaches, which should be used in an integrated fashion for making MRAs most efficient, effective, and reliable.

3 Environmental quality standards (EQS) for mixtures

The regulatory assessment of mixture risks entails a need for establishing acceptable levels (AL) of exposure to mixtures, i.e. levels at and below which mixture risks may be considered regulatory insignificant. Under the WFD, we may shortly denote them as '*mixture EQS*'.

Fortunately, the development of rules for deriving mixture EQS does not have to start from scratch. There is a long-standing debate on the issue, which is briefly reviewed in section 3.1. More than 30 years ago, the need to define quality standards for mixtures was already well recognised. However, it was only six years ago, that a short guidance on calculating quality standards '*for substances occurring in mixtures*' for the first time has been included in the revised Technical Guidance (TG) for deriving EQS (EC 2011a, chapter 7).

With this guidance, a technical basis was created for the possible prioritisation of mixtures and the corresponding setting of mixture EQS, as detailed in section 3.2. So far, however, no practical use of this possibility has been made. In addition, the guidance leaves substantial room for improvement. Recommendations for such improvements are developed in section 3.3.

3.1 Brief history of EQS for mixtures

In 1987, the European Inland Fisheries Advisory Commission (EIFAC) of the FAO[21] came to conclude that for toxicants with a similar mode of action (MoA), "*their additive joint action may necessitate the setting of water quality criteria for this group as a whole and not on the basis of individual compounds*" (EIFAC 1987). This conclusion resulted from an updated review of the aquatic toxicity of multi-component mixtures. The conclusion was a complete revision of an earlier EIFAC opinion on the issue. It marked a paradigm change in regulatory aquatic toxicology.

Seven years earlier, in 1980, EIFAC had prepared its initial review of effects of mixtures of toxicants on freshwater fish and other aquatic life. In those times, the experimental evidence on combined effects from toxicants at low individual concentrations was poor. As a consequence, EIFAC initially assumed that quality criteria derived from single substance assessments would also be protective for combined exposures (EIFAC 1980). The need to change this position seven years later came from a number of pioneering studies on the multi-component mixture toxicity of non-reactive organics with an unspecific "narcotic" mode of action (Könemann 1980; Hermens et al 1984, 1985; Broderius and Kahl 1985). They provided first experimental indications for significant combined effects from mixture components at concentrations which do not cause significant effects when applied individually. During the subsequent 20 years, the evidence was strengthened by studies with improved experimental designs, for different endpoints and modes of actions, and for both mixtures of similarly and dissimilarly acting components (see Kortenkamp et al 2007 for a review).

[21] Food and Agriculture Organization of the United Nations (UN)

Five years after EIFAC's recognition of the need to define quality standards for mixtures, Calamari and Vighi (1992) were the first to make a proposal for a practical implementation of the idea. As a quality objective for a mixture QO_m, they suggested that the following condition must be met:

$$\sum_{i=1}^{n} \frac{c_i}{QO_i} < 1 \qquad [7],$$

with c_i denoting the individual concentrations of toxicants and QO_i the individual quality objectives (equivalent to EQS as nowadays defined under the WFD). The approach is based on the assumption of CA and it is conceptually similar to the HI approach or the PEC/PNEC summation as defined in Tab. 1 in section 2.2.3 above. However, as an important additional condition, Calamari and Vighi suggested that the objective should only apply to groups of toxicants with a common mode of action. As criteria for establishing such common MoA groups they proposed that substances in a group should have a congeneric molecular structure and the toxicity should be describable by a common QSAR. As examples for such groups with a common mixture EQS they suggested chlorophenols, chlorinated aliphatic hydrocarbons, and organophosphorus compounds.

The idea of classifying pollutants into common MoA groups sounds simple. To find a consensus on such grouping for the purpose of regulatory MRAs and EQS setting, however, has turned out to be an almost unsolvable task, at least in the short term. The debate has now continued over decades and progress towards effective solutions is made only at a glacial pace. The discussion is hampered by diverging views on the appropriate definition of common MoAs and by huge knowledge gaps about MoAs for many endpoints in many species groups. Often it amounts to nothing more than a consensual call for more research on the issue. For the purpose of regulatory MRAs, tiered approaches have therefore been invented as a way out of the dilemma, as already explained in section 2.2.3 above. However, whether and how such tiered approaches may also be used for the derivation of mixture EQS remains an issue of debate, as discussed in section 3.3 below.

Thirteen years after EIFACs insight into the need for mixture EQS, the EU WFD came into force in 2000. The WFD created an umbrella for harmonized EQS across all EU Member States. EQS have a broader scope than the earlier water quality objectives. As already explained in the introductory section, they are supposed to be not only protective for aquatic ecosystems, but also for wildlife and humans that are exposed via drinking water and fish consumption. This further complicates a possible MoA-based grouping of pollutants for EQS setting. It entails a need for aligning such an approach with corresponding efforts of EFSA for classifying food contaminants into so-called common assessment groups (CAGs) on the basis of toxicological profiles (EFSA 2013d).

While in general, prioritisation and EQS setting under the WFD has been largely focused on single pollutants, a few important exceptions apply. The most prominent exemption is the EQS for 29 dioxins and dioxin-like compounds in biota which is defined in terms of the sum of toxic equivalents (TEQs), i.e. individual concentrations multiplied by toxic equivalency factors (TEFs) established by the World Health Organisation (WHO). In addition, there are three more cases where an EQS refers to a sum of concentrations of selected congeners or isomers of a parent compound: six poly-brominated diphenyl ethers (PBDEs), four cyclodiene pesticides, and four DDT isomers (Directive 2008/105/EC, as last amended by Directive 2013/39/EU).

24 years after the EIFAC recognised a need for establishing mixture EQS, a short chapter on the "*Calculation of QS for substances occurring in mixtures*" was for the first time included in the technical guidance (TG) for deriving EQS under the WFD (EC 2011a, chapter 7). The chapter set a starting point, but may need some expansion and refinement for supporting practitioners effectively, as detailed in the following sections 3.2 and 3.3. Up to now, it has obviously not been used for establishing any EQS for mixtures. Although EQS include HRA and ERA by definition (see above), the existing guidance on quality standards for substances in mixtures is totally confined to consideration of aquatic mixture toxicity.

3.2 The existing guidance for mixture EQS setting

The existing guidance offers three approaches: a toxic unit summation (TUS) approach, a toxic equivalency factors (TEF) approach, and an approach based on the so-called PETROTOX model. All these are different applications of the CA model. The full guideline text on TUS- and TEF-based EQS setting for mixtures is displayed in Box 1. The PETROTOX model is a very special tool for assessing complex petroleum products. It is based on the CA assumption in combination with a QSAR model for so-called 'narcotic action' or 'baseline toxicity'. A detailed discussion of this special tool is omitted from this deliverable report and a focus is made on the more general TUS- and TEF-based approaches suggested in the guidance.

Apart from summing up relative concentrations of mixture components, such as TUS and TEF, mixture EQS may also be set in terms of sums of absolute concentrations. This approach has been in fact been used for PBDEs, cyclodienes, and DDT isomers (see above). Strangely, no word is said on this option in the guidance.

TUS, as originally defined in the literature is an algebraic equivalent of the CA model (see Tab. 2 above). In the guidance, however, the approach is suggested to be applied in a way where equivalent effect concentrations are replaced by individual quality standards QS_i and these are further equated with PNECs (see Box 1). Thereby the approach becomes formally equal to the approach suggested by Calamari and Vighi. However, whether the approach shall only be used for mixtures of similarly acting components, as Calamari and Vighi suggested, or whether it may be applied to any defined mixture, regardless of individual MoAs, is not explicitly said in the guidance.

In contrast, the suggested TEF approach is explicitly said to be applicable to groups of substances with a similar MoA only. The suggested TEF approach is an implicit simplification and generalisation of the TEF approach developed by the WHO for dioxins and dioxin-like compounds (see above). The WHO derived TEFs from distributions of relative effect potency (REP) calculated from experimentally determined effect concentrations or effective doses for AhR-mediated biochemical and toxic responses, preferably EC50 or ED50 (Van den Berg et al 2006). In contrast, the guidance for mixture EQS setting defines TEFs in terms of relative PNECs. The TEF for a mixture component is suggested to be calculated by dividing the PNEC for this constituent by the lowest PNEC of any substance in the group of similarly acting substances under consideration. As already explained in sections 2.2.3 and 2.2.5 above, such PNECs may include differing assessment factors and may apply to different

endpoints. Thus, there is a conceptual difference between the TEF approach suggested in the guidance and the TEF approach established by WHO.

Box 1: Excerpt from the Technical Guidance for Deriving EQS (EC 2011a).

Full text of the initial part of Chapter 7 on TUS and TEF approaches for mixture EQS derivation. Remaining text of the chapter on the PETROTOX model omitted.

7. CALCULATION OF QS FOR SUBSTANCES OCCURRING IN MIXTURES

For well-defined mixtures, ie those with a well defined qualitative and quantitative composition, the toxic unit (TU) approach (e.g.Altenburger and Greco 2009) may be used to calculate the EQS. A Toxic Unit (TU) is defined as the ratio of the exposure concentration to the effect concentration for a specific medium (e.g. water). A TU for each constituent$_i$ in a substance / group of substances should be calculated as,

$$TU_i = \frac{C_{w,i}}{QS_i}$$

Cw,i Concentration in water of the constituent i

QSi PNEC for the constituent i

To estimate the toxicity of the mixture, the TU_i for all constituents in the mixture/group of substances are summed.

$$TU_{mixture} = \Sigma TU_i$$

When the $TU_{mixture}$ equals one or is greater than one, the mixture is expected to be above the threshold (ie QS).

EQSs may be defined for grouped substances that exert a similar mode of action and may be expressed according to the concept of Toxic Equivalent [TEQ] concentrations in environmental samples. The Toxic Equivalency Factor [TEF] is the fraction of the PNEC of constituent$_i$ divided by the lowest PNEC measured or calculated for a constituent that belongs to the group of substances being considered (Di Toro, 2000).

$TEQ = \Sigma_n (TEF_i * c_i)$

TEFi Toxic Equivalency factor for constituent i

Ci concentration of constituent i

The TU concept is equivalent to the Toxic Equivalency Factors (TEFs) for PCB's, PCDD's and PCDF's for humans and wildlife which were agreed by the World Health Organization (WHO) in 1997 and have been revised for dioxin-like compounds by the WHO in 2005, including criteria to take substances into the TEQ concept (Van den Berg *et al*. 1998, 2006)

Unfortunately, the guidance tends to obscure such differences rather than making them clear and transparent. The guidance states that "*The TU concept is equivalent to the Toxic Equivalence Factors (…) which were agreed by the World Health Organisation ….*". However, apart from the differences

introduced by equating PNECs with equivalent effect concentrations in the guideline, the sentence is not even true for the original concepts. Concentration additivity is an important prerequisite of the TEF concept but they are not the same. Mathematically, the TEF approach and the TUS approach are only equivalent under the important additional assumption that concentration response curves of mixture components are parallel. Otherwise REPs may be different for every effect level, and the two approaches may yield different estimates of acceptable exposure levels for mixtures.

3.3 Recommendations for improvement

The Commission's Scientific Committees reviewed the guidance prior to publication and they signalled that they saw a need for improvement (EC 2010). However, at that time, definite suggestions for such improvements were postponed to first await the Committees opinion on the "*Toxicity and Assessment of Chemical Mixtures*", which was under development. The work on that opinion was completed at the end of 2011. The final opinion (EC 2011b) outlined principles and a generic tiered approach to mixture risk assessments, but it did not specifically address EQS setting for mixtures. In particular, it remained an open issue for debate whether and how the principle of a tiered assessment of mixture risks could or should be used for the purpose of EQS setting. The envisaged specific proposal for improvement of the guidance for mixture EQS setting remained undeveloped. The corresponding chapter 7 of the TG for deriving EQS has been left unchanged since then.

At the 3rd SOLUTIONS workshop on prioritisation methodologies, a dedicated discussion group made a fresh attempt to review the guidance and to outline ways for improvement. In general, the group agreed that the guidance provides a first step into the right direction but requires revision and refinement to comply with the state-of-the-art and to become really helpful for users. In detail, the group did not aim to reach a consensus statement, but some main lines of agreement came out from the discussions. Considering the arguments raised and the views expressed in conjunction with the status of available concepts and approaches for regulatory MRAs as reviewed in the preceding sections, a number of recommendations for improvement of the guidance for mixture EQS setting can be derived. They are detailed in the following sections, grouped into general recommendations (section3.3.1), and recommendations for component-based mixture EQS setting (section3.3.2). In addition, the group considered options for effect-based mixture EQS setting (section3.3.3) and possible alternatives to mixture EQS setting (section 3.3.4).

Just as the existing guidance, the following recommendations for component-based mixture EQS setting presume that a priority mixture has already been defined in terms of the number and nature of components. Just the derivation of a safe exposure level for such a pre-defined mixture is considered. The basic question, however, how such a priority mixture may be identified in the first place, is left open. It is separately addressed afterwards in section 4.1.

3.3.1 General recommendations

Basically, the scope of the mixture EQS guidance should be widened and conceptual premises should be made explicit, as further detailed in the following.

- **SCOPE: Integrate HRA and ERA**

To be consistent with EQS setting for single substances, the guidance for deriving mixture EQS should cover all protection goals of the WFD, including risks to humans and terrestrial species via drinking water and secondary poisoning. The scope should not be left (implicitly) confined to mixture risks for aquatic species. The guidance should distinguish between common principles and approaches, which should generally govern EQS setting for mixtures, and specific approaches, which may apply to specific endpoints or specific types of mixtures only. Where not self-evident, compliance of specific approaches with overarching principles should be explained. The uniform principles and the common tiered framework for MRA proposed in sections 2.2.4 and 2.2.5 above may provide a basis for establishing generic rules for mixture EQS setting under the WFD.

- **CONCEPTUAL PREMISES: Make assumptions about MRA approaches explicit**

Risk assessment and EQS setting cannot be separated. Deriving mixture EQS is dependent on the way MRAs are performed. Unfortunately, a guidance for performing MRAs under the WFD does not exist, as already stressed repeatedly. Under these conditions, the existing guidance for mixture EQS setting makes implicit assumptions about the way MRAs may be performed. It implies that (i) a CBA is taken, (ii) CA is assumed to explain mixture toxicity, and (iii) CA may be pragmatically simplified for the purpose of EQS setting by replacing effect concentrations with PNECs. Given the scientific and regulatory state-of-the-art of using CBAs summarised in section 2.2 above, assumptions (i) and (ii) may be considered as being already widely accepted. Assumption (iii), however, is critical. It may mean that an initial worst-case assumption in a tiered MRA is considered as a sufficient basis for EQS setting, with no need and no option for refinement. This is a questionable approach and more appropriate procedures may be available, as detailed below. Hence, if maintained, such critical assumptions should be clearly explained and well justified. Amending the text of the existing chapter on mixture EQS setting may be an option to achieve this. A better way, however, might be to address the issue more fundamentally by creating the missing guideline for MRA under the WFD. Considerations and proposals in the preceding sections 2.1 and 2.2 should provide a platform for such a development.

3.3.2 Component-based mixture EQS setting

The guidance on component-based mixture EQS setting offers much room for improvement. This includes terminological precision, definition of data requirements, choice of assessment approaches

and endpoints, transparency of extrapolations, inclusion of MRA methodologies for species assemblages, and ways of expressing EQS for mixtures, as detailed in the following.

- **TERMINOLOGY: Improve precision and remove inconsistencies**

The terminology is confusing as already indicated in section 3.2 above. It lacks precision and it is inconsistent with other parts of the same guidance document, with other pieces of EU regulations and implementation guidelines, and with the cited scientific literature. Terms which denote differing concepts and approaches are mixed up. PNECS are equated with quality objectives although chapter 1.3 of the same guidance document clearly stresses that these are conceptually different terms. A PNEC may be adopted as a quality objective, but a quality objective is not necessarily a PNEC. On top of this inconsistency, PNECs are even equated with equivalent effect concentrations for common endpoints, both for the purpose of summing up risk quotients (RQs) and for establishing TEFs. Such a fuzzy use of these terms ignores that PNECS for different substances may refer to completely different endpoints and may differ by orders of magnitude due to differing assessment factors applied.

The fuzzy use of terms apparently mirrors a lack of awareness of the substantial differences between the original scientific CA concept and the various CA-based approaches such as HI, PODI, MEC/PNEC or PEC/PNEC summation, etc. However, for the appropriate use of tiered frameworks for MRAs, such as the one proposed in section 2.2.5 above, a careful distinction of such terms is absolutely crucial. Otherwise, the substantial differences between different tiers and resulting assessments may all become blurred.

- **DATA: Define minimum requirements**

The quality and quantity of available single substance toxicity data dictate which CBAs may be applicable and which not, as explained in section 2.2.2 above. They predetermine whether a component-based MRA is possible at all and which level of a tiered framework can be reached without further research and testing. For decision taking in terms of mixture EQS setting, it is crucial to define the data that are both necessary and sufficient. Otherwise refinement of MRAs may be continued *ad infinitum*.

As a principle, the same data requirements should apply to both EQS setting for single substances as well as for substances in mixtures. This appears to be self-suggesting and ensures consistency. Such data requirements have been laid down in the WFD and the guidance document for EQS setting. In detail, there may be some room for improvement, but in principle there is no need for inventing new data requirements for the purpose of MRAs. For assessing aquatic mixture risks, this means that toxicity data (acute EC50 or LC50 or chronic NOEC) must at least be available for the so-called 'base set' of taxa, i.e. algae (or macrophytes), daphnia (or representative organisms for saline waters) and fish. For HRA, it means that PODs such as NOAELs, or derived acceptable levels such as ADIs may be used.

These minimum requirements have immediate consequences for MRAs and the derivation of mixture EQS. For aquatic mixture risks, the assessment can in any case be performed on a species group specific level (tier 2 in the Backhaus and Faust 2012 scheme, tier 2A or higher in the scheme proposed in Tab. 2 above). There is no need to start with a worst-case tier I assessment. A merging of PNECs across completely different species groups as suggested in the existing guidance is not necessary. Distorting effects of differing assessment factors may be avoided. The same may apply to human MRA if PODs are immediately available or if the PODs from which ADIs or other acceptable levels have been derived can be retrieved. Otherwise it may not be possible to go beyond a tier I HI approach for humans.

A second consequence of these minimum requirements is that known mixture components for which these minimum data sets are not available cannot be included in the mixture EQS setting. They should be prioritised for further testing.

It is important to notice here that knowledge of MoA is no data requirement, neither for EQS setting under the WFD nor for any other assessments of risks and derivations of safe exposure levels under any other piece of EU chemicals legislation. Where available, such knowledge may be used to refine mixture EQS setting, but it must not be a prerequisite for decision taking. The tiered scheme proposed in Tab. 2 supports this requirement.

- **ASSESSMENT APPROACHES: Choose the best possible level**

Minimum data requirements determine which assessment level of a tiered framework can be achieved at least. But this does not necessarily mean that mixture EQS setting should be rigidly and uniformly based on that minimum level. Following the proportionality principle In EU legislation, a flexible approach should be more appropriate. Where the available data allow to go for a higher tier, that opportunity should be used. This would mean to ensure chemicals safety with the least possible restrictions.

EQS setting must be performed on the basis of the data available to the authorities at the time of setting. There is no mechanism to "*demand the commissioning of new studies*" and "*exceedance of the EQS will not normally trigger a refinement of the standard*" (EC 2011a, p.11). Chapter 1.3 of the guidance for EQS derivation stresses that these are important conceptual differences between EQS setting under the WFD and the estimation of acceptable levels under other pieces of EU chemicals legislation such as PNECs under REACH and TER values (toxicity exposure ratios) under the PPPR. Given these conditions, the considerations above mean a simple answer to the question how a tiered MRA should be used for EQS setting: chose the highest level that is achievable with all available data.

- **ENDPOINTS: Filter out the critical ones**

"*The concept of an overall threshold (…) that protects all receptors and routes is a feature of EQS derivation that does not normally apply in chemical risk assessment*" (EC 2011a, p.11). This is another conceptual difference highlighted in the TG for deriving EQS. As a consequence, numerous specific

QS (called "*temporary standards*") for specific species groups (called "receptors") and exposure routes must first be derived before final EQS values are selected as overall standards. This procedure is demanding for single substances already. Extending the approach to mixtures may multiply the necessary efforts. To manage the task efficiently, it is therefore advisable to design early filters and ranking steps which allow to focus efforts on those species groups and endpoints which may be most sensitive and hence critical for final EQS setting. To this end, tiered procedures may provide valuable tools. Where initial worst-case estimates do not signal any significant mixture risks for certain species groups and compartments, such as freshwater or sediment, they can be safely ignored for the remaining part of the exercise. This consideration provides a second answer to the question how tiered MRA approaches can be used for EQS setting.

- **EXTRAPOLATIONS: Define assessment factors for deriving mixture EQS**

For deriving single substance EQS from available single substance toxicity data, a number of assessment factors (AF) have been laid down in Annex V to the WFD and in the TG for deriving EQS. These AF may be inappropriate or insufficient for deriving mixture EQS. A complementary set of AF for mixture EQS setting is needed which ensures an equivalent protection level against risks from exposure to both single substances and mixtures.

The established AFs integrate different extrapolation steps into single figures, such as acute to chronic, species to species, and lab to field extrapolations. For the purpose of MRAs, however, it may be necessary to separate the different extrapolation steps. The reason is that the available data sets for different mixture components may be inhomogeneous. For example, if only an acute LC50 is available for assessing the fish toxicity of component *A* but a chronic NOEC for component *B*, an acute-to-chronic extrapolation is necessary for component *A*. This first extrapolation step provides comparable toxicity indicators which can then be used for a component-based MRA. The calculation may result in an estimate of a NOEC of the binary mixture of components *A* and *B* for fish in the lab. A second extrapolation step may then be performed for estimating a safe level for any fish in the field, just in the same way as it is usually done for single substances.

- **SPECIES ASSEMBLAGES: Consider the use of SSD-based approaches**

For single substances, the TG for deriving EQS offers two possible methods for estimating safe levels for organisms in the environment, the '*deterministic*' and the '*probabilistic*' approach. The deterministic approach means that the lowest credible toxicity datum is selected, followed by application of an AF between 1 and 1000. The probabilistic approach means that an estimate of the hazardous concentration for 5% of the species (HC5) is derived from SSD (species sensitivity distribution) modelling and an AF between 1 and 5 is applied. The probabilistic approach is preferred but the data requirements are much higher than for the deterministic approach. A minimum of 10 NOEC or EC10 values from different species covering at least 8 taxonomic groups is necessary (EC 2011a, p.41).

SSD-based methods are not only available for single substances but also for mixtures. The so-called msPAF (multi-substance potentially affected fraction) approach applies the CA, IA, and MM approach to the risk assessment for species assemblages by replacing concentration response functions with species sensitivity distribution functions (de Zwart and Posthuma 2005). As a consequence, the inclusion of the msPAF approach into the guidance document should be considered. Whether the data requirements for all mixture components should be the same as for SSD-based single substance assessments remains to be clarified. If the same data demands regarding SSDs are applied to ms-PAF, the applicability of the approach would remain limited to mixtures of some few intensively studied aquatic pollutants.

- **EXPRESSING EQS FOR MIXTURES: Consider the required level of precision**

EQS are order of magnitude estimates. The assumptions and extrapolations included in the derivation procedures do not allow any higher precision. This fact should be kept in mind when considering different options for expressing EQS for mixtures, as detailed in the following.

Under the WFD, an EQS "*means the **concentration** of a particular pollutant or group of pollutants in water, sediment or biota which should not be exceeded …*" (Article 2(35)), as already explained in section 1.2 above. Applied to a mixture, this means that an EQS is an acceptable sum of concentrations of individual components. This acceptable sum, however, usually varies with the concentration ratio of mixture components, unless they would all be equally potent and have identical concentration response curves. As a consequence, a general definition of an EQS for a given set of components in any possible concentration ratio cannot be given in terms of an absolute sum of concentrations, but only in terms of a sum of relative concentrations. This may be a sum of toxic units under the assumption of CA, or a sum of other risk quotients, such as MEC/PNEC, under simplifying pragmatic approaches derived from CA, as explained in section 2.2.3. above. Alternatively, sums of toxic equivalent concentrations could be used, if there are reasons to assume that the components have parallel concentration (or dose) response curves and CA applies.

These conditions complicate the regulatory setting of mixture EQS and the compliance control. If a mixture EQS would be defined as a sum of RQs (say $\Sigma RQ_i \leq 1$) (which has not been done up to now), the legal establishment would require the definition of consented hazard indicators for all mixture components (such as NOECs or PNECs) which would have to be used as denominators of the risk quotients for calculating the actual sum. If a mixture EQS is defined as a sum of TEQs, the legal establishment requires the definition of agreed RPFs, as has been done for dioxins and dioxin-like compounds in a tremendous multi-national effort under the umbrella of the WHO (van den Berg et al. 1998, 2006).

In view of these complications, the question arises under which conditions simplifications may be possible and justifiable. It is self-suggesting to consider the expression of a mixture EQS as a sum of absolute concentrations, as currently in place for PBDEs, cyclodienes, and DDT isomers. This is easier to understand, to establish legally, and to control practically. The approach provides sufficient protection if it is based on a worst-case assumption, i.e. all mixture components are assumed to be as potent as the most potent one and the acceptable level of that most potent component is set to

apply as a limit for the acceptable sum of all concentrations too. But of course, the approach may not only be sufficiently protective but also highly over-protective. However, this may only apply, if the potencies of the mixture components differ vastly, say several orders of magnitude. In cases where NOECs or other potency indicators of mixture constituents do not differ largely, say less than an order of magnitude, the simple way of expressing a mixture EQS in terms of a sum of absolute concentrations may be considered as a pragmatic and well justifiable way forward.

The reason behind is, that acceptable total concentration levels of a mixture under the assumption of CA vary between the acceptable individual levels of the most and the least potent mixture component, depending on the concentration ratio. Hence, where the individual potencies of the mixture components differ by less than an order of magnitude, the difference between an acceptable level derived from CA and an acceptable level derived from the simple assumption that all components are as potent as the most potent one, will also always differ by less than an order of magnitude.

The guidance should reflect on the required level of precision and define conditions under which a simplified expression of mixture EQS in terms of a sum of absolute concentrations are acceptable or recommendable and prescribe the appropriate way of derivation. As a suggestion, sophisticated ways of deriving and expressing mixture EQS may be considered superfluous, where the resulting figures differ from a simpler and protective approach by less than an order of magnitude.

The checking of compliance with a mixture EQS defined as the sum of concentrations of mixture components or the sum of risk quotients requires that all components are monitored together. This may significantly compromise the practical applicability of mixture EQS. For this reason, alternative approaches need to be considered too (see section 3.3.4 below).

3.3.3 Perspectives for effect-based mixture EQS setting

Effect-based monitoring tools are no elements of chemical status assessment under the current WFD. Consequently, the existing guidance for mixture EQS setting does not include any considerations on the alternative or complementary use of whole mixture testing approaches.

However, there is a move towards a methodological paradigm change, as explained in section 2.1.1 above. SOLUTIONS makes a strong plea for using EBMTs as a complement to chemical monitoring tools. This may remove some shortcomings of chemical monitoring and support a better integration of ecological and chemical status monitoring. Concerning the definition of quality objectives, this raises the question whether EBMTs should just remain auxiliary means, guiding or supporting chemical monitoring, but basically not touching upon the existing system of chemicals-based water quality assessments, or whether, as a possible further development, the establishment of effect-based quality standards may be considered, in addition and complementary to chemically defined EQS or as an alternative.

The WFD aims to protect waters and depending ecosystems as a heritage (Article 1 in conjunction with Recital 1) and to restore at least a '*good status*' where deterioration has occurred. Where the (unknown) mixture of pollutants present in a water sample causes significant effects in a relevant

bioassay at relevant exposure levels, this certainly provides a strong indication that the status is in fact not good. Thus, it appears to be self-suggesting to define quality standards in terms of effects which should not be detectable with standardised assays, such as short-term algal, daphnids, or fish-embryo toxicity. To take account of acute-to-chronic ratios, species-sensitivity differences and fluctuating exposure levels, an acceptable level may be defined in terms of a concentration factor. For example, a revised legislation could require that an extract from a surface water sample which contains the pollutants in a ten- or hundred-fold concentration should yet not cause any significant toxicity.

Such a way of defining an effect-based EQS would be a conceptual reversal of the WMT approach which has since long been taken by some Member States for assessing the quality of waste waters (see section 2.1.1 above). In Germany for instance, dilution factors that are needed for rendering waste waters non-toxic in a number of biological assays (such as fish, algae, daphnids or bacteria) are used to establish emission limit values for discharges from certain industrial sectors (as laid down in the AbwV[22]). However, for defining effect-based EQS under the WFD, a '*reverse dilution factor approach*', or better to say a '*concentration factor approach*', would encounter two problems. Firstly, it may be insufficient for prioritisation purposes, and secondly it would require a substantial revision of the current legal text.

Under the current WFD, EQS setting is directly coupled to the prioritisation of pollutants or groups of pollutants for risk reduction measures. Effect-based EQS would provide a means for directly monitoring the achievement of WFD protection goals and for prioritising polluted sites, but for targeting risk reduction measures they would need to be complemented by methods for identifying the causative agents, as already pointed out in section 2.1 above. This would mean to replace the current rigid prioritisation scheme, which starts from an *a priori* defined list of candidate pollutants, by a more flexible tiered or combined approach, where biological effects may trigger a search (i) for causative chemicals, (ii) for the sources of these chemicals and (iii) for the most appropriate risk reduction measures. From a scientific perspective this may sound simple and straightforward. From a regulatory perspective, however, it may mean a substantial overhaul of the current "*Strategies against pollution of water*" as laid down in Article 16 of the WFD.

In addition to these procedural considerations, there is a very clear and simple reason why the existing WFD provides very limited room for a possible effect-based EQS setting, if any. This is the current legal definition of EQS. EQS are "***concentrations** of (…) priority substances in surface water, sediments or biota* (Article 16(7) in agreement with Article 2(35)), as already explained in sections 1.2 and 3.3.2 above. If revising the legal text is ruled out as an option, the only imaginable way of meeting this narrow EQS definition with biological testing may be to express the results in terms of equivalent concentrations of a reference compound, somewhat similar to the component-based TEF approach which has been established for dioxins (see section 3.2 above). However, this would presume a well-defined group of known chemicals with a common MoA, preferably from a common source, which can be detected with a specific assay that is essentially insensitive to any other

[22] Verordnung über Anforderungen an das Einleiten von Abwasser in Gewässer (Abwasserverordnung - AbwV) (Ordinance concerning the requirements for the discharge of waste water to surface waters – Waste water ordinance)

(unknown) component of real pollutant mixtures in waters. This approach would certainly not be suitable for use with apical endpoints, such as algal reproduction or daphnids immobilisation, which may be affected by almost any pollutant, if concentrations are high enough.

Current efforts for implementing EBM approaches under the WFD are indeed largely focussed on MoA-specific *in vitro* screens. Concerning acceptable or non-acceptable levels of responses seen in such assays, however, there is a growing consensus that these should be defined by a novel type of threshold, the so-called '*effect-based trigger value*' (EBTV)[23], distinct from the existing definition of EQS which could be left untouched. EBTVs shall be used to classify the pollution status of waterbodies in terms of acceptable or non-acceptable. Thus, for monitoring purposes they are functionally equivalent to EQS, but differently determined. The idea is to define EBTVs in terms of so-called '*bioanalytical equivalent concentrations*' (BEQs). BEQs express the response of a bioanalytical assay in terms of an equivalent concentration of a reference compound. If the EBTV is set to a properly chosen BEQ value, the protection level for the tested mixture should be equivalent to the protection level provided by the EQS for the reference compound.

The development of such an EBTV approach is most advanced for steroidal estrogens (Kase et al 2018, Könemann et al 2018). The development was driven by the fact that three estrogens were placed on the WFD watch list in 2015 (see section 1.2.1 above), but the chemical analytical methods available in most Member States were insufficiently sensitive for monitoring these hormones at the very low concentration levels which are toxicologically relevant. Thus, the primary aim was to use biological assays as a replacement for physico-chemical detectors. MRA was a secondary goal.

Certainly, considerable progress has been achieved with the development of the EBTV approach for agents exerting estrogen-receptor mediated effects. This may be regarded as a successful test case, which paves the way for the rapid implementation of effect-based approaches for other substance groups too. However, there are also good reasons to assess the prospects with some caution. For steroidal estrogens, the development of the EBTV approach was favoured by a combination of conditions which may not apply in the same ideal way to other groups of substances. In addition to (i) a lack of appropriate chemical-analytical methods for detection, this includes (ii) a congeneric structure, (iii) a common specific MoA, (iv) a common chronic endpoint of concern (reproductive toxicity) which is not testable in short-term apical assays, (v) common sources and exposure pathways (excretion by women, common transport with household waste water, and release into the aquatic environment mainly via waste water treatment plants), and (vi) the fact that the substances had already been identified as candidates for prioritisation and (vii) provisional EQS values had been derived beforehand.

Independent from the prospects for realisation in the short or long term, implementation of the EBTV approach for a number of specific MoAs certainly would be an important step forward to a better protection from mixture risks. However, alone it will not provide a sufficient solution to the problem. This is not only due to our limited knowledge about MoAs of many substances in many

[23] In the relevant literature, the shorter abbreviation *EBT* is used. However, EBT is also used as an abbreviation for *effect-based tools* for monitoring, both in other SOLUTIONS documents and parts of the cited literature. To avoid any confusion, throughout this report the longer abbreviations EBTV and EBMT are used for *effect-based trigger values* and *effect-based monitoring tools*, respectively.

species which hampers assay development. It is also not only due to the fact that economic constraints may limit the number of assays in a battery for routine testing. But it is particularly true for mixtures of components which contribute to a common adverse outcome via different MoAs, which can only be seen with assays for apical endpoints, not with MoA specific screens. Algae and aquatic macrophytes, for example, are often seen to be significantly affected by photosystem-II (PS II) inhibitor herbicides and biocides, but they are also very sensitive to a number of other aquatic pollutants. Hence, a water quality assessment for aquatic primary producers which is solely based on a PS II-inhibitor assay may significantly underestimate the overall mixture risk, and risk management measures which exclusively focus on PS II inhibitors may fail to achieve the goal of a good status for algae and higher aquatic plants. This is not just a theoretical example, but the practical relevance has been demonstrated in a number of Swiss streams (Langer et al 2017).

As a best possible way forward, it may therefore be advisable to design a test battery which includes both, apical short-term assays and MoA-specific in vitro screens. Apical assays, where they provide indications for population relevant in a short term, at low costs, and with no ethical constraints, such as tests with algae, daphnids, fish embryos, and bacteria. MoA-specific in vitro screens, where they provide indications for potential chronic effects which cannot be seen in apical short-term assays, in particular for endpoints of high concern, such as CMR and ED properties. A corresponding proposal is developed in the TOOLS sub-project (see SOLUTIONS deliverable D9.1).

Effective use of such a test battery under the WFD will require the establishment of corresponding threshold values for discriminating between acceptable and not acceptable levels of response seen with the assays. This may either require a revised definition of EQS or the introduction of a novel complementary type of threshold, such as the suggested EBTV approach. Revision of the EQS definition means to change the legislation. Whether the same applies to the establishment of EBTVs, or whether it may be possible to introduce them without amending the WFD, may be a legal case to clarify. In any case, the creation of a solid legal basis for effect-based water quality assessments should be the preferable way forward. To this end, recommendations have been formulated in Brack et al 2017. The issue is further detailed in section 6 below.

3.3.4 Alternatives to mixture EQS setting

The setting of EQS for mixtures turns out to be a surprisingly complicated issue. Summing up some risk quotients or performing some quick bioanalytical assays sounds simple from a scientific perspective. But when it comes to legal implementation and enforcement, lack of data, lack of consented procedures, and lack of standardised assays turn out to represent significant hurdles. It becomes understandable, why no mixture EQS have been established so far, although the issue has been discussed for more than 30 years now and despite the fact that an official guidance for deriving mixture EQS has been issued already seven years ago. The entire regulatory system is well designed for dealing with single substances, but not for coping with mixtures. A change has started to occur, but will take time to bed in, in particular because it needs coordination and harmonisation across the whole body of EU chemicals and environmental legislation.

Given these difficulties and the poor prospects for a rapid change, the question arises whether simpler alternatives to mixture EQS setting may be available. The only option which is occasionally discussed as a possible solution, is to integrate MRAs into the setting of EQS for single substances. Essentially, the idea boils down to the application of an additional assessment factor in single substance EQS derivation. The factor shall account for the fact that the toxicity of the pollutant under consideration may be enhanced by the simultaneous presence of other substances which contribute to the same adverse outcome. In other words, the acceptable level for a single pollutant is lowered in the presence of other jointly acting compounds. For short, the approach has been denoted as the '*MAF*' option, with MAF standing for '*mixture assessment factor*', but it can also be found under other terms in the literature. The crucial and yet unresolved problem is to define an appropriate numerical value for such a MAF, a value which has broad acceptance and is scientifically well justified. This is further detailed in section 4.3 below.

Interestingly, the existing guidance is entitled "*Calculation of* ***QS for substances occurring in mixtures***" (see Box 1). This phrasing seems to suggest that the quality standard for a single substance may be set differently if the substance occurs in a mixture. However, this idea is actually not addressed in the text but it is confined to the setting of an EQS for the mixture as a whole. The MAF option is not mentioned. For a future revision, it may be advisable to make the issue explicit and to clarify why it is not considered.

4 Concepts for integrating mixture risk assessments into prioritization procedures

Basically, three different concepts have been suggested which may be useful for integrating mixture risk assessment into prioritisation procedures under the WFD:

- the identification of '*priority mixtures* '
- the identification of '*drivers of mixture toxicity'*, and
- the establishment of a '*mixture assessment factor*' (MAF).

All these are '*concepts under construction*' so to say. None of them is fully worked out and consensually agreed. None of them was specifically invented to be used for prioritisations under the WFD. They originate from the general debate about possible ways for dealing with mixture risk under the EU framework of chemicals and environmental legislation, in particular risks from coincidental mixtures of multiple environmental pollutants or food contaminants from different sources and pathways.

The terms '*priority mixtures*' and '*drivers of mixture toxicity'* were created in 2012 in the Communication from the Commission to the Council on "*The combination effects of chemicals – Chemical mixtures*" (EC 2012). In contrast, the MAF approach originates from diffuse sources. For a long time, the underlying idea has been mentioned sporadically in the literature with different wordings, but rarely discussed in detail. The acronym MAF was coined in a review of available options for mixture risk assessments under REACH, prepared for the Swedish Chemicals Agency (Backhaus et al 2010). The concept has already been introduced in section 3.3.4 above.

The following sections 4.1, 4.2, and 4.3 examine whether and how these three generic concepts could be developed into specific approaches for prioritizations under the WFD.

4.1 Priority mixtures

The number of possible combinations of pollutants in the environment is almost infinite, but apparently not all of them present a significant risk. The exact composition of pollutant mixtures may be different at every site and every point in time, but there may be typical co-exposure patterns of major mixture components which occur widespread and frequently. Given these presumptions, it should be possible to sort out mixtures of high concern and to prioritise them for risk reduction measures. Essentially, these were the implicit assumptions that the Commission made when announcing the establishment of "*an ad hoc working group (…) to promote the integrated assessment of* ***priority mixtures****, …*" and to "*develop, by June 2014, (…) technical guidelines to promote a consistent approach to the assessment of* ***priority mixtures*** *across the different pieces of EU legislation*" (EC 2012, p.9).

Apparently, this was over-ambitious and no significant progress towards these high aims has actually been achieved. However, SOLUTIONS took up the challenge to advance the idea for the special field

of water pollution, both in conceptual and in methodological terms. Conceptual aspects of identifying priority mixtures of water pollutants are addressed in this section, based on discussions held at the 2nd SOLUTIONS prioritisation workshop. Advancements of experimental and computational methods for identifying priority mixtures are detailed in the corresponding deliverables from the *TOOLS* and *MODELS* subprojects, in particular D9.1, D13.1, D18.1 (see section 1.1 above).

The use of the concept of '*priority mixtures*' in the context of the WFD entails a need for an appropriate normative definition, in terms of legal aims and requirements, that fits with the WFD. A corresponding proposal is provided in the following section 4.1.1. In addition, operational criteria are needed which translate the normative provisions into measurable indicators and practical decision rules. To this end, existing proposals are reviewed in the sub-sequent sections 4.1.2 and 4.1.3 and suggestions for specification and advancement are made. Further, important aspects of a systematic identification of priority mixtures which are not or not sufficiently covered by the existing proposals are summarised in section 4.1.4. Finally, possible accompanying measures against so-called regrettable substitution are briefly discussed in section 4.1.5.

4.1.1 Normative definition under the WFD

The aim of prioritization under the WFD is to make risk reduction measures efficient by targeting those substances and mixtures that present the highest risks. This has an important implication: components of priority mixtures must be chemically defined. Otherwise sources and exposure routes cannot be identified and measures for risk reduction cannot be taken. As a consequence, effect-based tools may be well suited for detection and monitoring of mixture risks but must be complemented by methods for the identification of causative agents.

Given these premises and considering the current provisions and procedures for the prioritization of single substances, a priority mixture within the meaning of the WFD may be defined as a group of pollutants that

(i) co-occur[24] in the aquatic environment (in water, sediments, or biota),

(ii) jointly represent a significant risk to or via the aquatic environment, and

(iii) exceed an acceptable level (mixture EQS) widespread and frequently (EU wide or river-basin wide) and to a considerable degree.

Criteria (i) and (ii) define the candidate mixtures for prioritisation. Criterion (iii) defines the (main) aspects considered for risk ranking and the selection of priority mixtures from a candidate list. It is an extension of principles used for single pollutants rankings (Von der Ohe et al. 2011, Carvalho et al. 2016) to mixtures. Other aspects, such as PBT or CMR properties, may be included in the risk-based selection process as a distinction between the wider term "priority mixtures" and specific mixtures of "priority hazardous substances", as defined under the WFD.

[24] Definition of co-occurrence is given in section 1.4.

This definition implies that

(i) EQS for priority mixtures can be set on the basis of agreed mixture risk assessment procedures, defining the borderline between "significant" and acceptable mixture risks,

(ii) available data and procedures allow to rank significant mixture risks in terms of higher or lower threats, and widespread or rare exceedance of acceptable cumulative exposure levels, and

(iii) tools for monitoring the success of reduction measures and compliance with mixture EQS need to be developed if not yet available.

4.1.2 Operational criteria suggested by SCHER/SCENIHR/SCCS[25]

To translate the suggested normative definition into measurable indicators and practical decision rules, two main types of operational criteria are needed:

(i) criteria for characterizing situations of **co-occurrence** or co-exposure in terms of the number, nature, and sources of mixture components, their concentrations and concentration ratios, and the frequency of their occurrence on temporal and geographical scales,

(ii) criteria for assessing and ranking mixture toxicity and **mixture risks** resulting from situations of co-exposure in terms of significance, severity, and frequency of exceedance of acceptable levels.

Suggestions for such operational criteria have been made by the European Commission's Scientific Committees SCHER, SCENIHR, and SCCS in 2011 (EC 2011b). The Committees' suggestions were generic, not specific for any special piece of EU chemicals legislation such as the WFD, and including all possible exposure routes, not confined to exposures to and via the aquatic environment. Since then, not much progress has been made on the issue, except for the maximum cumulative ratio (MCR) approach suggested by Price and co-workers (2012), which is separately addressed in the next sub-section 4.1.3.

The relevant section from the Committees document is displayed in full length in Box 2. In the following, brief reflections are provided on each of the suggested criteria. To this end, the criteria are grouped into criteria for co-exposure assessment and criteria for mixture risk assessment, and quoted in shortened form. As the criteria are generic, the comments basically reflect whether they are relevant for the special subject of pollutants in aquatic systems, and if so, whether they require corresponding specifications, modifications or extensions.

[25] SCHER - Scientific Committee on Health and Environmental Risks, SCENIHR - Scientific Committee on Emerging and Newly Identified Health Risks, SCCS - Scientific Committee on Consumer Safety (of the European Commission)

Box 2: Excerpt from the opinion of the Commission's scientific committees on the "*Toxicity and Assessment of Chemical Mixtures*" (EC 2011b, p.35-36).

Full text of the response to Question No. 4 of the corresponding request of the Commission, referring to appropriate criteria for prioritising mixtures.

Question 4 – Given that it is unrealistic to assess every possible combination of chemical substances, what is the most effective way to target resources on those combinations of chemicals that constitute the highest risk for man and the environment?

In view of the almost infinite number of possible combinations of chemicals to which humans and environmental species are exposed some form of initial filter to allow a focus on mixtures of potential concern is necessary. The following criteria are proposed for consideration:

- Human and/or environmental exposure at significant levels (e.g., close to the HBGVs, DNELs or PNECs for several components).
- Chemicals that are produced and/or marketed as multi-constituent substances or commercial mixtures with several components and/or active ingredients and/or substances of concern (i.e. as defined by EU legislation, e.g. REACH, CLP, pesticides and biocidal products legislation, food law, *etc.*).
- Potential serious adverse effects of one or more chemicals at the likely exposure levels.
- Likelihood of frequent or large scale exposure of the human population or the environment.
- Persistence of chemicals in the body and/or in the environment. High persistence/bioaccumulation would be a property of importance.
- Known information of potential interaction at levels of human and environmental exposure.
- Predictive information that chemicals act similarly such as (quantitative) structure activity relationships and structural alerts.
- Particular attention should be paid to mixtures for which one or more components are assumed to have no threshold for its effects such as genotoxic carcinogens; a MOE or a lifetime cancer risk approach could be applied.

Exposure to one or more components approaching the threshold levels for adverse effects would mean that the mixture should be given priority for assessment. A TTC-like approach can be used to eliminate combinations that are of no concern [for details on the applicability of a TTC approach for the assessment of chemical mixtures see Boobis *et al.* (2011) and Price *et al*. (2009)].

For the environment, attention should be paid to mixtures of chemicals, individual components of which approach the PNEC. The TTC model may be not appropriate for biological communities, where a threshold of concern may be several orders of magnitude different for different taxonomic groups of organisms (from bacteria to vertebrates). In some cases, for very sensitive taxonomic groups, even very low concentrations which are difficult to assess or measure may be not negligible.

In view of the difficulty and time needed to retrieve or generate an appropriate dataset for hazard characterisation and exposure estimates, a tiered approach, such as proposed by the WHO/IPCS (2009b) or EFSA (2008), may be considered (for details on the tiered approach, see Methodology section 3.4). The identification of the data gaps after the application of the tiered approach should determine the extent of testing of chemical mixtures and study design.

Reference "WHO/IPCS (2009b)" refers to a draft version of the tiered scheme which was later published by Meek et al 2011. Other references as provided in the reference list for this report.

Comments on suggested criteria for characterizing situations of co-occurrence or co-exposure

- Criterion: *"**Chemicals (…) produced (…) as (…) commercial mixtures…**"*

Commercial mixtures are certainly an important aspect for consideration, but they are just one out of a number of possible reasons for the co-ocurrence of pollutants in the aquatic environment. Commercial mixtures are a priority for considerations where humans or other organisms come into direct contact with them. However, when considering environmental media such as water and its associated species and '*receptors*', a look at commercial mixtures does not lead far. Components of commercial mixtures stay together at the point of emission, but disperse when released into the environment. As a result, we have different mixtures at different locations. Hence, for aquatic mixture risk assessments, all sources and all transport routes of pollutants must be taken into consideration.

- Criterion: *"**Likelihood of frequent or large scale exposure…**"*

Under the WFD, frequent and widespread occurrence is the most important criterion for selecting priority substances and groups of substances from those presenting a significant risk. Consequently, it is included in the normative definition of a priority mixture as suggested above. The major obstacle to effective implementation is missing exposure data for many pollutants.

- Criterion: *"**Persistence of chemicals in the body and/or in the environment. High persistence/bioaccumulation would be a property of importance**."*

Persistence and bioaccumulative potential of a chemical determines where it will be found in environmental media and in the food chain. Chemicals with these properties will tend to occur together. Hence, substances meeting both the persistent (P) and the bioaccumulation (B) criterion certainly deserve priority for co-exposure assessments.

For the subsequent assessment of resulting mixture risks, toxic (T) properties must be considered additionally. For substances meeting all three criteria, P, B and T, conventional regulatory risk assessments on the basis of risk quotients such as MEC/EQS or PEC/PNEC are considered to be too uncertain due to unpredictable long-term effects of bioaccumulation and the practical difficulties to reverse such accumulation (EC 2003). Under the WFD, priority substances with PBT properties are called *'priority hazardous substances'* (Article 16 in conjunction with Article 2). By definition they are a special sub-group of *'priority substances*'. This sub-group is not only subject to risk reduction measures but the ultimate aim is to stop any discharges, emissions and losses. The same should apply to mixtures of such substances, as indicated in the normative definition proposed above.

The WFD does not include any definition of PBT criteria, but recital 28 of the daughter Directive 2008/105/EC on EQS refers to the corresponding criteria laid down under the REACH Regulation (EC) No 1907/2006, under the old biocidal products Directive 98/8/EC, and under the old *Technical Guidance Document for Risk Assessment* (EC 1996) in support of the old Commission Directive 93/67/EEC on principles for risk assessment of substances notified under the old Council Directive 67/548/EEC on CLP of dangerous substances. In practice, this means that the PBT criteria defined in

Annex XIII to the REACH Regulation are now used to determine whether priority substances under the WFD are *'priority substances*' or *'priority hazardous substances'*.

As laid down in Annex XIII to the REACH Regulation, substances fulfilling the toxicity criterion (T) are substances with a long-term NOEC in water organisms of less than 0.01 mg/L or substances with CMR properties or other evidence for chronic toxicity in humans. These restrictions may mean that the single substance PBT assessments may be insufficient to include all relevant mixture components and to cover all relevant risks from mixtures of substances fulfilling the P- and B-criteria. For example, mixture risks from secondary poisoning of fish eating birds may be overlooked because bird toxicity is not included in the PBT assessment. In addition, substances which have a higher aquatic NOEC than 0.01 mg/L may still make significant contributions to the overall aquatic risk from mixtures of substances with P- and B-properties. As a consequence, co-exposure to substances with P- and B-properties should in any case trigger assessments of resulting mixture risks, not *a priori* limited to mixture components meeting the T-criterion as defined under REACH.

Comments on suggested criteria for assessing and ranking mixture toxicity and mixture risks

- Criterion: *"**Human and/or environmental exposure at significant levels (e.g., close to the HBGVs, DNELs or PNECs for several components).**"*

Under the WFD, HBGVs (such as the ADIs), DNELs and PNECs are used to derive EQS values (EC 2011a) which define the borderline between regulatory significant and insignificant risks.

The formulation "*close to*" leaves some room for interpretations. It may mean *'slightly above'* or *'slightly below'*, but the implications are different. If mixture components exceed the regulatory borderline, they are candidates for prioritisation under the WFD anyway, and the same should apply to mixtures of such substances.

The criterion is more important to consider, if it should aim to capture situations where a significant mixture risk may result from components not presenting a significant risk individually, because they are just below the regulatory borderline of concern. Where several mixture components are present at concentrations *'slightly below*' individually acceptable levels, the assumption of CA suggests a high possibility for a significant mixture risk, and the components '*closest to'* such individually acceptable levels can be expected to make the highest contribution to the expectable overall toxicity in this situation (apart from components already exceeding individual threshold values). If "*close to*" is further specified in terms of a quantitative default assumption (e.g. ≥ 10% of individual EQS), this criterion may be viewed as a simplification of the driver concept. It may be considered as a simple non-exclusive criterion for filtering out candidate priority mixtures. The bottleneck for effective use of such a criterion, however, is missing data on acceptable levels (such as DNELs or PNECs) for many aquatic pollutants and endpoints.

The phrasing used by the Committees suggests a component-based approach (CBA) for assessing whether "*exposure at significant levels*" occurs. Under the WFD, however, whole mixture testing by means of EBMTs may become an important complementary approach for assessing whether real

environmental exposures are actually close to levels causing significant effects (or even already above), as detailed in sections 2.1 and 3.3.3 above.

- Criterion: *"**Potential serious adverse effects of one or more chemicals at the likely exposure levels.**"*

Again, the wording leaves room for interpretations, but presumably the criterion is intended to denote situations were one or more mixture components present a significant risk already individually, i.e. they are already above individual EQS or equivalent levels. Hence the overall mixture risk must be expected to be even higher and certainly regulatory significant (unless antagonistic interactions should occur at relevant exposure levels). Mixtures meeting this criterion should be most easily identifiable, at least easier than mixtures meeting the previous one.

Under the WFD, substances presenting significant risks individually are candidates for prioritization (Article 16). The existing selection procedures, however, do only consider the extent and the frequency of exceedance of individually acceptable levels in isolation. They do not consider whether several candidate chemicals co-occur in the same water sample, as already explained in section 1.2 above. This applies to both, the selection procedures that have been used by the Commission (Klein et al 1999, James et al 2009, Daginnus et al 2010, Carvalho et al 2016) and to the NORMAN approach for prioritizing emerging pollutants (von der Ohe et al. 2011, Dulio and von der Ohe 2013). Where reliable information on the co-occurrence of candidate priority substances or priority substances is available from chemical monitoring or modelling studies, such mixtures may be easily identified as candidate priority mixtures. However, for assessing and ranking the overall risk, the question arises whether other mixture components which do not exceed individual threshold values may make significant additional contributions. The issue is considered in further detail in section 4.1.3 below.

As for the previous criterion, the complementary use of EBMTs and CBAs may be a powerful approach for assessing whether real environmental exposures have a "*potential" to cause "serious adverse effects*".

- Criterion: *"**Known information of potential interaction at levels of (…) exposure.**"*

Fortunately, there is little evidence for significant synergistic (more than concentration-additive) interactions between constituents of multi-component mixtures at low concentrations which are considered acceptable for individual pollutants under the WFD (Boobis et al 2011, Cedergreen 2014). However, there may be exceptions and there may be situations of relatively high exposures where toxico-kinetic interactions may occur.

Therefore, known cases of synergistic interactions of water pollutants certainly deserve particular attention when it comes to the prioritisation of mixtures. Prominent examples of concern are strong synergistic effects of fungicides such as the imidazole prochloraz with insecticides such as alpha-cypermetryn (Norgaard and Cedergreen 2010) and parathion (Levine and Oris 1999), which are often sprayed together. The latter have been shown to modulate CYP1A expression and to inhibit cytochrome P450 enzyme activity (Levine et al 1999) and may thus interfere with the metabolism of the insecticides. Another example are potentials for synergistic mutagenic effects which have

recently been demonstrated to result from the co-occurrence of ubiquitous carboline alkaloids with frequently occurring industrial aromatic amines (Muz et al. 2017b), as already mentioned in section 2.1.2. above.

- Criterion: ***"Predictive information that chemicals act similarly such as (quantitative) structure activity relationships and structural alerts"***

The criterion of chemicals acting "*similarly*" is diffuse, and the Committee's proposal does not include an exact definition of the term. Ideally, the definition of a priority mixture should include all those components of an environmental mixture that make a significant contribution to a common adverse effect, irrespective of whether they do so by the same, by similar, or by different modes of action. Structural similarities of chemicals, however, usually provide indications for a common MoA. Therefore, it is important to be aware that the suggested criterion may identify a sub fraction of relevant mixture components only. Hence, it must not be regarded as an exclusion criterion. Absence of structural indications for similar action does not necessarily mean that substances do not contribute to a common (eco)toxicological endpoint. And vice versa, structural similarity does not necessarily coincide with (eco)toxicological similarity, of course. Nevertheless, provided that these reasons for caution are not disregarded, positive structural indications for similar (eco)toxicological properties certainly provide a valuable trigger for assessing the resulting mixture risks.

If "*similarly*" acting is understood in terms of a common MoA, the suggested criterion is basically identical with the approach to mixture EQS derivation proposed by Calamari and Vighi in 1992 already (see section 3.1). Since then, computational (eco)toxicology has made significant progress. For predictively assessing risks of chemicals to aquatic organisms, a considerable body of (Q)SAR and read-across approaches has accumulated. The main limitation is that most of them refer to acute toxicity, not to chronic effects which may be decisive for EQS setting for single substances and mixtures. However, if combined with acute to chronic explorations, they may still provide valuable tools for filtering out mixtures of potential concern.

In a tiered framework for component-based mixture risk assessments under the WFD, as suggested in section 2.2.5 above, single substance toxicity estimates derived from structural indicators are an important means for bridging initial gaps in experimental data. They serve to filter out mixtures of potential concern and to prioritise components of such mixtures for further testing. For reaching a definite and consensually acceptable regulatory conclusion that a mixture presents a significant risk which may require risk reduction measures, however, the evidence should be backed by experimental findings and not rely on computational methods only. Thus, structural indications for similar (eco)toxicological properties may be considered as a valuable element of a toolbox for prioritising mixtures rather than a sufficient criterion for identifying priority mixtures in its own.

The current list of WFD priority substances already includes a few groups of structurally similar compounds for which common EQS values have been defined, such as dioxins and PBDEs, as already mentioned in section 3.1 above. From a scientific perspective, however, there are many more structural groups of pollutants which give a reason for concern and for which MRAs should be conducted, such as quaternary ammonium compounds used as antimicrobials (Li and Brownawell

2010) or alkylbenzene sulfonates, a widely used group of anionic surfactants (Tamura et al 2017), just to mention two examples.

- Criterion: *"**Particular attention should be paid to mixtures for which one or more components are assumed to have no threshold for its effects such as genotoxic carcinogens; …**"*

Under the WFD, for the purpose of establishing EQS for substances for which "*a threshold level cannot be established (e.g. some genotoxic carcinogens)*", the applicable guidelines suggest to use "*risk values corresponding to an additional risk of, e.g., cancer over the whole life of 10^{-6} (one additional cancer incident in 10^{6} persons taking up the substance concerned for 70 years)*" (EC 2011a). Under these conceptual premises, mixture risks may be assessed by means of CBAs as for any other endpoint and where acceptable levels are expected to be exceeded, mixtures may be prioritized in the same way as for any other protection goal.

4.1.3 Operational criteria suggested by Price and co-workers

Price and co-workers (2012) suggested a classification of mixtures into three main groups, whereby the third group is further divided into two sub-groups. The classification is intended to support risk management decisions. To this end, each group is associated with different conclusions about appropriate risk management strategies. The classification is part of a tiered framework for mixture risk assessment (see section 2.2.5 above) which was developed under sponsorship of the chemical industry. It is derived from a CA-based approach, using the hazard index (HI) as decision criterion (see Table 2 in section 2.2.3 above). Individual risk quotients are denoted as '*hazard quotients*' (HQ) in the paper. The HI is the sum of the individual HQs. The ratio between the HI and the highest HQ of all mixture components is denoted as '*maximum cumulative ratio*' (MCR).

The groups are defined as follows:

- **Group I:** Concentrations (or doses) of one or more mixture components exceed individually acceptable levels (individual HQ >1) and hence present a concern. As a consequence, the mixture also gives concern (HI >1).
- **Group II:** Neither the individual components present a concern (all HQ <1) nor the mixture as a whole (HI <1).
- **Group III:** None of the mixture components presents a concern individually (all HQ <1), but the mixture as a whole does (HI >1).
    - **Group IIIa:** One mixture component provides the majority of toxicity (MCR <2, i.e. HQ of one component is >50% of the HI).
    - **Group IIIb:** No one chemical dominates the overall toxicity of the mixture (MCR >2, i.e. all individual HQ are <50% of the HI).

In general, the scheme may be helpful for structuring the problem and for considering appropriate risk management needs and options. In detail however, the criteria and the risk management conclusions that the authors suggest for each of the groups are disputable and may require refinement, as explained in the following:

- **Group I** mixtures are identified as a concern already on the basis of traditional chemical-by-chemical risk assessment. As a consequence, the authors conclude that "*efforts to refine the assessment of combined exposures or to reduce the receptors' exposure need to focus on the chemicals that are a concern before addressing risks from the combined exposures*".

  It is undisputable that pollutants exceeding individual acceptable levels (HQ >1) require risk reduction measures. However, completely disregarding the other mixture components until this has been achieved may be a rather ineffective way of reaching the ultimate protection goal, i.e. no significant mixture risk. If there are other mixture components simultaneously present which do not exceed individually acceptable levels (HQ <1) but which already sum up to a partial HI >1, even a total elimination of all individual substances of concern (HQ >1) will fail to achieve a good status where significant mixture risks are absent. Thus, in the interest of not wasting valuable time and steering risk reduction measures most effectively, the contributions of all mixture components should be taken into considerations from the very beginning.

  In addition, under the specific conditions of the WFD, it must be taken into consideration that not every mixture component exceeding individually acceptable levels is defined as a priority pollutant. It is only a selection of some few substances which occur widespread and frequently and present particularly high concerns. Thus, focusing on these only, clearly bears a high risk of overlooking important mixture components and underestimating total mixture risks.

- **Group II** *"exposures can be set aside as a low concern"* write the authors. This conclusion is obviously undisputable, unless other lines of evidence provide a reason for concern, such as empirical indications for more-than-concentration-additive (synergistic) interactions between certain mixture components.

- **Group IIIa** mixtures "*have one chemical that is responsible for the majority of the toxicity received by a receptor"* and the authors conclude that *"that chemical should be the focus of either refining the risk assessment or reducing exposure*".

  In fact, there are exposure situations where one single mixture component dominates the overall toxicity and where a focus of risk reduction measures on this single component may be sufficient to achieve the goal of no significant mixture toxicity. However, correctly identifying such a single '*driver*' situation by means of a component-based approach requires careful considerations of the appropriate quantitative criterion used. The authors use the phrase "*majority of the toxicity"* and they operationalize it in terms of an individual HQ that contributes more than 50% to the HI. This very simple approach is valid under specific conditions only. Otherwise, it may be completely misleading. For becoming generally valid it needs careful refinement.

The reasons for this harsh criticism are as follows. With the aim to reduce the overall risk to an acceptable level, it is not sufficient to consider the fractional contribution of components to the HI alone, but it is also necessary to take the overall mixture risk level into account, i.e. the value of the HI. The authors criterion implies that any reduction of the HI by more than 50 % is sufficient to reduce the mixture risk to an acceptable level of HI ≤1. However, this is mathematically only true, if the actual HI does not exceed a value of 2. If the HI is 10, 90% reduction is required, if it is 100, 99% reduction is necessary for achieving an acceptable risk level. If it is 1000, we end up with a need for 99.9% reduction and so on. Considering huge fluctuations in exposure concentrations and the fact that acceptable levels are usually just order-of-magnitude estimates, HI values larger than 2 are not unrealistic. Recent simulation studies for realistic land use scenarios found HI values typically in the range between 0.1 and 100, outliers even reached HI levels up to 10,000 (Posthuma et al. 2018). On such a logarithmic scale, 50% reduction may be completely insufficient and hence more careful considerations are required before targeting risk reduction measures on a single component only.

- **Group IIIb** mixtures, where no one chemical dominates the joint effect of all components, are of concern because the HQs sum to an unacceptable level. This situation is a clear challenge for the existing regulatory system. Undoubtedly, there is no way to reduce the problem to conventional single substance assessment and management.

  Price et al do not detail a risk management option for group IIIb mixtures, but they conclude that "*These exposures are most affected by the default assumption that all chemicals follow addition models. Therefore, refining the assessment for these exposures should focus on determining the modes of action (MoAs) for the chemicals that drive the toxicity of exposures and using these data to refine the assessment.*"

  In principle, the need to refine an HI-based assessment as far as possible is undisputable. The requirement to clarify MoAs, however, may lead to a postponement of any regulatory decision *ad infinitum*. Generating knowledge on MoAs is certainly valuable, but the availability of such knowledge is no precondition for single substance risk assessments and risk management under any piece of EU law. Hence, introducing it as a necessary requirement for mixture risk assessment and management would mean to raise very high barriers for any precautionary action. As a way out, section 2.2.5 above suggests a tiered framework that leads to regulatory decisions by using the available data as best as possible. It may lead to a need for further single substance testing, which is doable, but it does not require the clarification of MoAs, which is an open-end research issue. Where such an assessment does not rule out concerns for significant mixture risks, experimental testing of the mixture of concern may be considered as the ultimate option for clarification.

Concerning the task of identifying priority mixtures, the important aspect introduced by the Price et al. paper is to distinguish between two basic situations:

a) significant mixtures risks which can be sufficiently managed by targeting risk reduction measures on a single component, and

b) mixtures risks which cannot be sufficiently tackled with conventional single substance approaches.

In the first situation, the need to identify priority mixtures becomes superfluous. In the second, it becomes urgent and essential. The criteria suggested by Price et al. for discriminating between the two cases are insufficient and require revision as explained above. The principle, however, is helpful.

The considerations about the Price at al. grouping criteria directly lead into the concept of identifying '*drivers*' of mixture risks. This is further detailed in section 4.2 below.

4.1.4 Towards a systematic identification of priority mixtures

Considering the existing proposals for identifying priority mixtures detailed in the two preceding sections, there are two major deficits:

(i) there is no clear concept or systematic strategy for revealing typical co-exposure patterns which present significant risks, and

(ii) there is a methodological constraint on component-based approaches, disregarding the advantages of a complementary use of both CBAs and WMAs.

Whether the concept of priority mixtures can be turned into something practically useful, crucially depends on the question whether it will indeed be possible to identify typical co-exposure patterns which present significant risks, widespread and frequently. Currently, this is a working hypothesis. SOLUTIONS explores this hypothesis on the basis of both monitoring and modelling data. Data analyses are still running. The results will be reported in Deliverable D18.1.

The operational criteria suggested by the Commissions scientific committees as well as by Price and coworkers, are largely focused on component-based approaches. The disadvantages of such a 'single-sided' methodology have been explained in detail in section 2 above. As a consequence, the methodological framework suggested in this report favors the integrated use of both CBAs and WMAs wherever possible.

4.1.5 Accompanying measures against regrettable substitution

Effective prioritization approaches should include accompanying measures for safeguarding against counter-productive effects of so-called '*regrettable substitution*' or '*bad substitution*'. This applies to both single substances and mixtures. In the context of the WFD, bad substitution may mean the replacement of a dangerous chemical with an equally dangerous or even worse substance or a mixture of substances with equi-effective or even more effective concentrations occurring in waters, sediments, or biota. As an unintended side-effect, market shifts towards bad substitutes may be triggered by any kind of regulatory black-listing. The prioritization of substances or mixtures for risk reduction measures under the WFD may be one of these. The unwanted effect may occur where different substances are readily available or may become readily available on the chemicals market for a common purpose with no significant difference in resulting environmental risks (or even

worsening the situation). Picking out one or few compounds from such a group may stimulate quick shifts to bad alternatives and render risk reduction measures useless.

Current prioritisation procedures under the WFD fail to avoid such false incentives for bad substitution. They consider water pollution as a rather static problem, largely disregarding flexibilities in the chemicals market. However, the WFD provides an opportunity for tackling both problems, mixture risks and bad substitution, with an integrated approach. Groups of priority pollutants may be defined to include both, substances actually co-occurring at a given site and during a certain period of time (mixtures) as well as substances that may be used as a replacement without changing the overall risk, both in quantitative and in qualitative terms. This could help to ensure that risk reduction efforts for priority substances or priority mixtures are not brought to naught by bad substitution of substances.

Working out such accompanying measures in detail is a rich field on its own and not further pursued in this report.

4.2 Drivers of mixture risks

Under realistic environmental conditions, the constituents of a multi-component mixture will usually not equally contribute to the overall hazard and risk. On the contrary, a growing body of empirical examples suggest that typically only a few compounds may explain most of the overall toxicity. This opens a way for reducing the complexity of identifying priority mixtures. Risk reduction measures should be most efficient, if they focus on the drivers. To achieve the protection goals both efficiently and sufficiently, the driver identification should meet two criteria:

(i) the drivers dominate the mixture risk, and

(ii) the removal or significant reduction of the drivers would render the remaining overall risk insignificant.

As already detailed in section 4.1.3, simple *ad hoc* definitions of drivers, such as substances explaining 50 % or 80 % or 90 % of the overall toxicity, may not be generally sufficient to meet these criteria. The actual overall risk must be taken into account, when distinguishing between drivers and non-drivers for effective risk reduction measures.

Drivers may be identified by experimental approaches (EDA) and by component-based approaches or by a combination of both. Details on methods and criteria for driver identification are worked in the *TOOLS* and *MODELS* subproject and documented in the corresponding deliverables.

To obtain reliable results and to ensure that risk reduction measures are not misguided, both approaches are demanding. In particular, data requirements for reliable driver identification by component-based approaches should not be underestimated. Reliable driver identification is different from making a reasonable worst-case mixture risk assessment, such as a hazard index calculation.

The first important reason for this may be differing assessment factors due to different data availabilities. Two substances may in fact be equally toxic but PNECS derived according to REACH rules may differ by orders of magnitudes, for example. If such PNECS are used for driver identification, the results may be completely misleading. While in fact both substances may equally contribute to the overall mixture risk, the one with a high assessment factor may be erroneously identified as a driver. Thus, a ranking of mixture components on the basis of lower tier risk quotients, such as hazard quotients or PEC/PNEC ratios is appropriate for identifying substances which require further testing because they may potentially be drivers of mixture risk, but they do not provide a sufficient basis for targeting risk reduction measures. They may be completely misguided and mean a waste of effort and resources.

The second important reason is that drivers are endpoint specific. For algae, the overall mixture toxicity of a water sample may be driven by completely different mixture components than for daphnids, fish or humans. For example, a sample may include PS-II inhibitors which drive algal toxicity and specific neurotoxicants which drive fish toxicity. Correspondingly, PNECS for the two types of components may have been derived from tests with algae and fish, respectively. If risk quotients are summed up which include these two types of differently derived PNECS, this means that it is implicitly assumed that a chimeric species may exist which combines the physiological features of both algae and fish and hence is most sensitive to the mixture. As a tier zero worst case estimate for sorting out mixtures of no concern, this is justifiable. As a basis for identifying mixture components on which risk reduction measures should be focused, it is simply nonsense.

The third important reason is that driver identification by CBAs is model dependent. The fact that mixture toxicity estimates by CA and IA do often not differ very much in terms of predicted effect concentrations for mixtures, does not mean that they lead to the same assumptions about the relative contributions of mixture components to the overall risk. Depending on the slope of concentration response curves, the rank order may indeed be completely reversed. Finally, if synergistic interactions between mixture components should occur, both approaches may be misleading. Before targeting risk reduction measures on a presumed driver, experimental verification of a component-based driver identification may therefore be taken into consideration.

4.3 The mixture assessment factor (MAF) option

The so-called '*MAF option*' could be a radical alternative to the complex issues of identifying priority mixtures and drivers of mixture toxicity. As already explained in section 3.3.4 above, it means to include considerations of potential mixture effects in the risk assessment of single substances, basically by means of a dedicated additional assessment factor. The approach is enticing for its pragmatism, but scientific reasoning for choosing a numerical value for a MAF is difficult, as detailed in a review prepared for the Swedish Chemicals Agency (KEMI 2015). Basically, it means to make an assumption about the number, the potency and the concentration ratios of pollutants co-occurring at a site which may contribute to a common adverse outcome.

The approach has been used for the derivation of environmental quality criteria for single substances in the Netherlands. The Dutch procedure includes the application of a factor of 100, which is

explicitly defined as a safety margin for protection from combination toxicity (van Vlaardingen and Verbruggen 2007, p.109). The factor is used to derive a so-called '*negligible concentration'* (NC) from a so-called '*maximum permissible concentration'* (MPC). The MPC is conceptually equivalent to a PNEC (Predicted No Effect Concentration). The NC is the long-term target value. Other practical applications of the MAF approach apparently do not exist.

More recently, use of the approach has been suggested for addressing combined effects of chemicals in the prospective environmental safety assessment under REACH (van Broekhuizen et al. 2017). A factor of 5 to 10 was suggested as a possible default value. The proposal is based on an msPAF analysis of Dutch monitoring data which indicates that typically no more than 5 to 10 chemicals dominate the overall toxicity. Under these conditions the assumption of CA implies that a MAF of 5 to 10 sufficiently safeguards against unwanted mixture effects.

Applying a MAF in single substance prioritisation procedures would first of all mean that more pollutants would exceed acceptable levels and hence be identified as candidate priority pollutants. If the MAF would be constant for all substances and endpoints, the relative ranking would remain the same, otherwise it would change.

Debating the issue at the 2nd SOLUTIONS prioritisation workshop showed that currently a consensual acceptance of a MAF approach under the WFD does not seem to be achievable. Hence it is not further considered in this report. However, with more and more evidence accumulating on typical co-exposure patterns, better justifications for a data-driven size of a MAF may become possible, and the situation may change.

5 Proposal for an advanced methodological framework

Developing prioritisation procedures that take account of mixture risks is a complex task. No single approach is available that provides a comprehensive solution to all the problems associated with the issue. Every possible option has some special advantages but also suffers from severe limitations. This is the basic outcome from the examination of all concepts for regulatory mixture risk assessment, mixture EQSD setting, and mixture prioritisation in the three preceding sections 2, 3, and 4.

Given this situation, the best possible way forward is to integrate different concepts and methods in a multiple-lines-of-evidence approach. To this end, a proposal for an advanced methodological framework for prioritisation is outlined in this section. The advanced framework does not rule out existing approaches for single substance prioritisation, but they are expanded and completed with novel methodological elements for identifying mixtures presenting significant risks as well as mixture components which may dominate such risks (Fig. 2).

The proposal is focussed on the identification of priority mixtures and drivers of significant mixture risks. The subsequent step of identifying the most appropriate risk reduction measures is beyond the scope of this deliverable. The same applies to possible accompanying measures for avoiding regrettable substation, as outlined in section 4.1.6 above). Following from the considerations in the preceding section 4, '*priority mixtures*' are understood as combinations of pollutants which present a significant risk, widespread and frequently. '*Drivers*' are mixture components on which risk reduction measures should be focused because (i) they dominate the mixture risk and (ii) their removal or significant reduction would render the remaining overall risk insignificant.

The development of the proposal is guided by the scientific state-of-the-art, not *a priori* limited to methods and approaches which regulatory authorities are required to use under the existing legal framework. Hence, full implementation of the proposed framework will require changes in the legal text which are outlined in the subsequent section 6.

5.1 Principles

The advanced methodological framework is suggested to be based on the following principles:

- **Integrating co-exposure modelling and chemical monitoring**

  The efficient characterisation of the co-exposure of organisms to multiple pollutants requires the complementary use of modelling approaches and chemical monitoring. The principle of such a combined approach is already embedded in the existing Commission approaches for single substance prioritisation (see section 1.2.1 above), but is now extended to mixtures.

  The weighting of evidence from both approaches and the design of efficient strategies for mutual validation remains a challenge. As a default, exposure modelling results should be regarded as predictive estimates which require verification by means of chemical or effect-based monitoring. To focus verification efforts on critical situations, exposure modelling

should be integrated with component-based MRA methodologies to filter out those exposure situations which may be expected to cause significant mixture risks. This is the approach explored in the SOLUTIONS 'model train' (Deliverables D14.1, D14.2, and D18.1).

- **Integrating mixture risk modelling and effect-based monitoring**

  The identification of significant risks from pollutant mixtures requires the complementary use of component based approaches (CBA) and whole mixture testing approaches (WMA). No single approach is able to solve all problems of mixture risk assessment and ranking. As detailed in section 2 above, CBA means the prediction of mixture toxicity and the predictive assessment of mixture risks on the basis of single substance toxicity data by means of models for the joint action of toxicants, such as '*concentration addition*' (CA) or '*independent action*' (IA). WMA in the context of the WFD means the biological testing of whole samples or extracts from water, sediment or biota, followed by effect-directed analyses (EDA) or other approaches for toxicant identification.

  The optimisation of strategies for interlinking both approaches for the efficient identification of priority mixtures or drivers of mixture toxicity remains a challenge. The process should start with the definition of a suitable test battery as brought forward by the SOLUTIONS sub-project *TOOLS* (Deliverable D9.1). Where effect-based monitoring data point to a significant risk, component-based approaches should be used to check whether measured or modelled co-exposure data provide a sufficient explanation, prior to performing an EDA as an ultimate step for clarification. Conversely, where component-based approaches indicate a significant mixture risk, experimental testing provides the ultimate means of verification. This may not be limited to assays of a routine monitoring test battery but may involve any relevant kind of toxicity testing.

- **Integrating multiple lines of evidence (LOE) on significant risks**

  To reduce the possibility of overlooking significant risks from single substances and mixtures, all possible lines of evidence should by explored for the detection of such risks and for the identification of the causative agents or mixtures. This multiple-lines-of-evidence (LoE) approach should include prioritisation procedures starting from:

  (i) ecological monitoring (field observations on so-called biological quality elements),

  (ii) effect-based monitoring (including *in vitro* and *in vivo* testing in the lab or onsite),

  (iii) chemical (co-exposure) monitoring (followed by component-based MRA), and

  (iv) modelling of co-exposure (followed by component-based MRA).

  The four lines of evidence use all possible ways of detecting candidate pollutants and pollutant mixtures but converge into a common ranking and selection step as detailed in section 5.4 below and graphically presented in Figs. 3-6.

- **Identifying priority mixtures and drivers of mixture toxicity**

  Where one or more lines of evidence identify groups of pollutants that typically co-occur and present a significant joint risk, these should be subject to prioritisation for risk reduction measures. There are several options for identifying such priority mixtures of aquatic pollutants and for setting mixture EQS that safeguard against adverse effects from such groups, as detailed in sections 3 and 4 above. Where appropriate, priority mixtures may be reduced to a few components, or even one single component, which can be demonstrated to explain most of the overall risk, so-called drivers of mixture risks, as detailed in section 4.2 above.

- **Prioritising mixture components for further research and testing**

  Wherever conclusive evidence on significant risks and resulting needs for risk reduction cannot be reached because a line of evidence is somewhere blocked by significant data or knowledge gaps on exposure or toxicity, substances or mixtures of potential concern are not left unnoticed but they are prioritised for further research and testing. This principle is adopted from the **NORMAN approach** and extended to the needs of mixture risk assessment, where knowledge gaps about relevant mixture constituents are particularly critical, especially for component based approaches.

  The requirement for bridging data gaps derives from the fact that CBAs require comparable and reliable data sets for all relevant mixture components. Techniques for bridging data gaps, such as QSAR, read-across, thresholds of toxicological concern (TTC) etc., can be used for first tier worst-case assessments of the overall mixture risk, but they may be inappropriate for risk ranking and for driver identification as explained in section 4.2 above. SOLUTIONS WP 18 works on an advanced decision tree for the prioritisation of mixture components for research and testing (Deliverable D18.1).

5.2 Novel methodological elements

Existing prioritisation methodologies mainly rely on two methodologies: targeted chemical monitoring and exposure modelling of single substances. The resulting measured (MEC) or predicted environmental concentrations (PEC) are divided by PNECs or other reference values to obtain risk quotients which are used for assessment and ranking (Fig. 2, top). In addition to these, the proposed advanced methodological framework makes full use of a whole array of novel or improved computational and experimental state-of-the-art methodologies which may collectively contribute to mixture risk identification and prioritisation, including:

- advanced sampling and extraction methodologies for both chemical analyses and biological testing,
- modern targeted and non-target analytical methods for multi-substances chemical monitoring,
- novel multi-substance co-exposure modelling techniques,

- a battery of effect-based monitoring tools including both apical short *in vivo* assays and high-throughput *in vitro* testing,
- on site testing and monitoring methodologies with both single species and species communities such as PICT studies (pollution induced community tolerance),
- eco-epidemiological and multiple-lines-of-evidence approaches for unraveling relationships between ecological status and chemical pollution,
- tiered component-based approaches for assessing mixture risks and identifying drivers, including both assessments for single species groups and for species assemblages, the so-called msPAF methodology (multi-substance potentially effect fraction of species),
- experimental and so-called 'virtual' effect-directed analysis (EDA) for identifying causative agents driving the effects seen with effect-based monitoring tools.

All these methods are detailed in the corresponding deliverables from the SOLUTIONS sub-projects *TOOLS* and *MODELS*.

5.3 General outline

Existing prioritisation methodologies are exposure-based and use two lines of evidence (LoE): prioritisation starting from chemical single substances monitoring and prioritisation starting from single substance exposure modelling (Fig. 2, top). To develop this scheme further into a framework that can cope with mixture risks and which minimises the risk of overlooking pollutants which make significant contributions to the overall risk, two main steps are necessary (Fig. 2, bottom):

I. The exposure-based approach is expanded to include methodologies which facilitate the assessment and ranking of mixture risks and the identification of mixture risk drivers. This comprises

   Ia) 'upgrading' of both modeling and chemical monitoring methodologies for delivering multi-substance co-exposure estimates, and

   Ib) establishing component-based MRA procedures as a core element for filtering out mixtures and drivers of concern from both modeling- and monitoring-based co-exposure information.

II. The exposure-based approach is complemented by an effect-based approach, bringing in two additional LoEs and thereby expanding the framework to a 4-lines of-evidence-approach. This includes

   IIa) the use of evidence on adverse effects from both effect-based monitoring tools and ecological monitoring as additional starting points for prioritisation procedures, and

   IIb) the use of a suite of methodologies for identifying causative pollutants and groups of pollutants as a second core element for filtering out mixtures and drivers of concern.

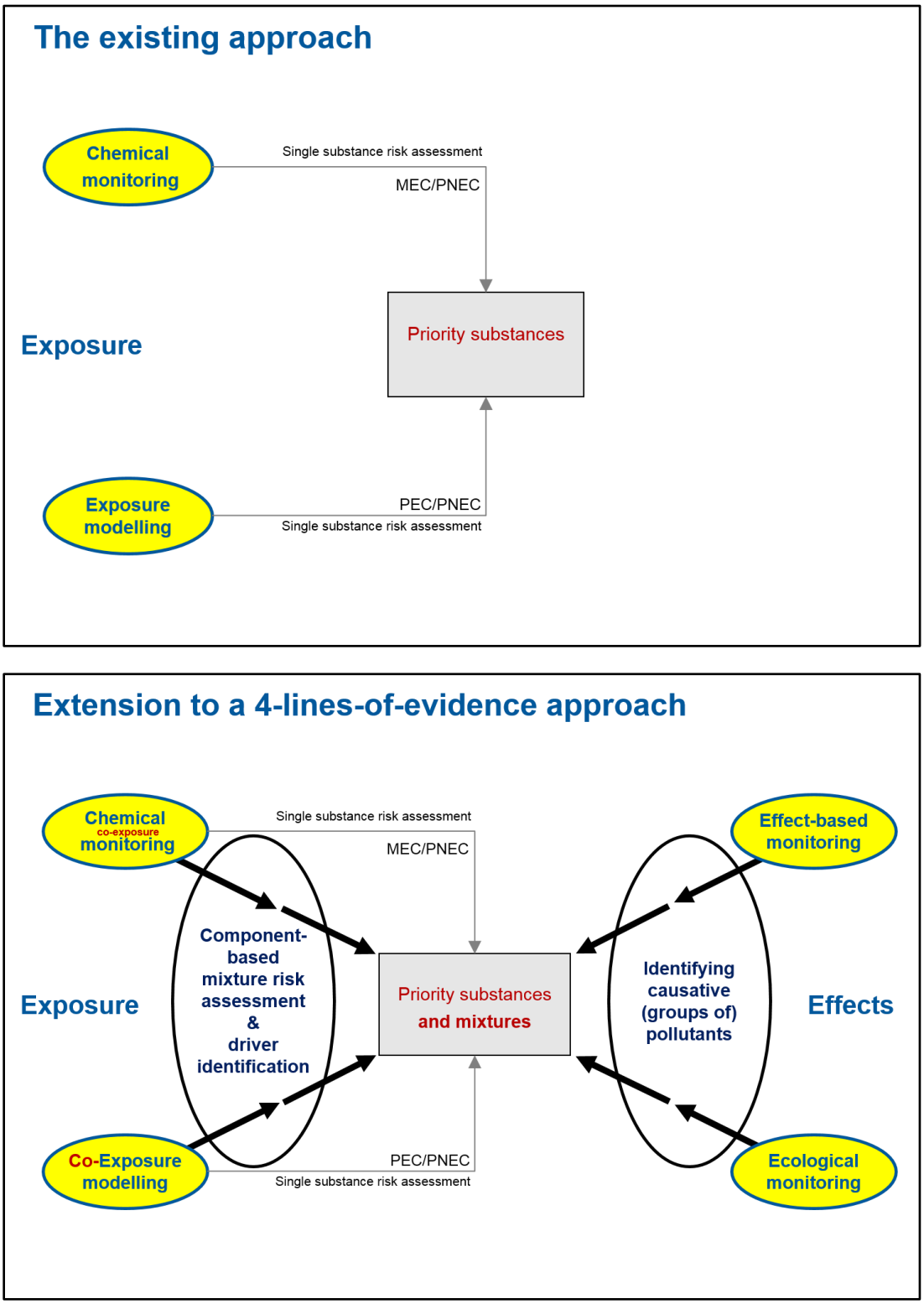

Figure 2: Extension of the existing prioritisation approach (top) to a 4-lines-of-evidence approach (bottom)

Component-based approaches (step I) are crucially dependent on the availability of single substance data, and they may underestimate risks resulting from unknown mixture components or synergistic interactions, as explained in section 2.2 above. The introduction of complementary effect-based prioritisation approaches (step ÍI) reduces the possibility of overlooking significant mixture risks as far as possible.

The inclusion of evidence from ecological monitoring aims to achieve a better integration of the ecological and chemical status assessment under the WFD. Currently, they are performed totally in isolation, which is inappropriate for achieving the ultimate goal of a good water status most efficiently and effectively (Brack et al. 2017).

5.4 Lines of evidence (LoE)

In the following, the four lines of evidence are briefly explained in further detail.

Irrespective of the starting point, prioritisations for risk reduction measures are structured into three main steps:

I. the identification of "*individual pollutants or groups of pollutants presenting a significant risk*" (WFD, Article 16),

II. the risk-based ranking and selection of priority substances or mixtures, and

III. technical and socio-economic considerations of efficient risk reduction measures, including policy impact assessments.

Step I is different for every LoE. In step II, all four lines of evidence converge. Basically, the STE approach developed for single pollutants rankings (see section 1.2.1) can also be applied to mixtures identified by means of the proposed advanced framework. Step III is beyond the scope of this deliverable and hence not detailed.

5.4.1 LoE I – Prioritisation starting from ecological monitoring

Starting prioritisation from results of ecological monitoring in terms of observations of so-called biological quality elements (BQE) certainly is the most complex approach. However, where it leads to the identification of pollutants or pollutant mixtures of concern, the ecological relevance is undisputable. Therefore, where necessary and possible, this track should be pursued as outlined in Fig. 3.

The opening question is whether the ecological status is good. If the answer is yes, the exercise stops. Where BQEs indicate a bad status, the question whether this may, at least partly, be caused by chemicals needs to be clarified. As chemicals are typically only one of a large range of potentially responsible environmental factors, this is a challenging task. However, SOLUTIONS deliverable D13.1 demonstrates a methodological approach that is able to detect situations where chemical pollution explains a bad ecological status, at least in part.

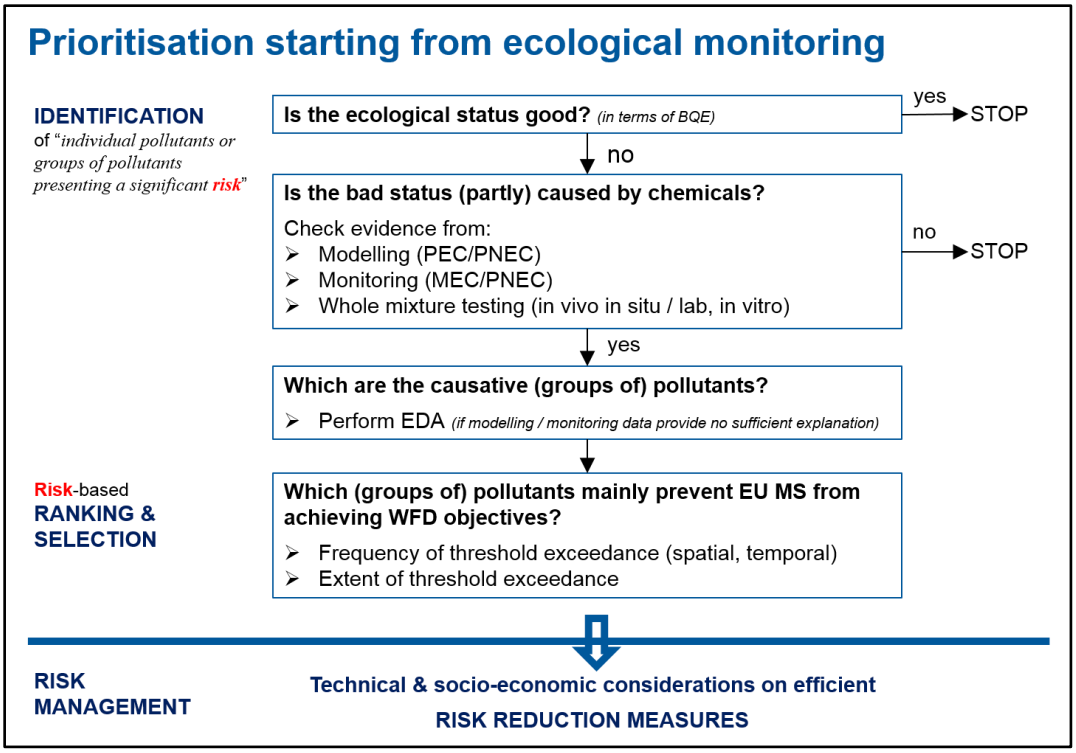

Figure 3: Prioritisation scheme starting from findings of ecological monitoring

The methodology does not work in all situations but in many. In general, the most important condition is the availability of a clear reference situation, i.e. a comparable site or another point in time at which pollution is significantly lower. The methodology is a weight-of-evidence approach. It works by integrating eco-epidemiological correlation analyses with the other three lines of evidence, i.e. the combined results from mixture risk assessments based on exposure modelling, chemical monitoring, and whole mixture testing, as far as available.

Typically, this weight-of-evidence approach is only able to provide an answer to the question whether pollutants are a contributing factor to a bad ecological status, but it is not sufficient to name the causative pollutants or groups of pollutants. Hence, the clarification of this question is the crucial next step. The identification of the causative substances may start with a refined component-based risk assessment on the basis of exposure modelling and chemical monitoring, but where this does not provide a sufficient explanation, an experimental effect-directed analyses (EDA) may need to be performed for ultimate clarification.

EDA is a well advanced state-of-the-art methodology to support monitoring of aquatic environments (Brack et al. 2016), but it is not yet established as regulatory tool for monitoring and prioritisation under the WFD. High expenses are often mentioned as a justification for reluctance. However, when discussing the issue at the 3rd SOLUTIONS prioritisation workshop, important counter-arguments were raised. First of all, expenses for prioritisation should be well invested money, because it helps to focus efforts on the right targets and thus may save an enormous amount of costs in the long run. In addition, costs for EDA may be compared to the high costs for toxicity testing of single substances. Under REACH, for example, such testing is triggered by tonnages brought on the market, which is

economically and pragmatically justified but toxicologically not necessarily the most sensible way forward. EDA, in contrast, is a tool for guiding efforts directly to those pollutants presenting the highest risks. Hence, it may well be worth the effort.

Where EDA or other methods achieve to identify pollutants or groups of pollutants as causative factors or co-factors of a bad ecological status, these should become candidates for prioritisation. The next and final step of the scientific part of the exercise then is to subject these candidates to a risk-based ranking procedure. This includes all pollutants and pollutants mixtures identified by any of the four lines of evidence and the criteria may be basically the same as those already applied for single substance prioritisation (see section 1.2.1, Von der Ohe et al. 2011, Carvalho et al. 2016), i.e. the frequency of threshold exceedances in both spatial and temporal terms as well as the extent of such threshold exceedances. The aim is to clarify which pollutants or groups of pollutants mainly prevent EU Member States (MS) from achieving the WFD objectives.

Finally, the results can be handed over to risk managers for technical and socio-economic considerations on efficient risk reduction measures

5.4.2 LoE II – Prioritisation starting with effect-based monitoring

When prioritisation starts from effect based monitoring, the second line of evidence (Fig. 4), then the important first step is to define an appropriate test battery, as brought forward by the SOLUTIONS sub-project TOOLS (Deliverable D9.1, Brack et al 2017, Neale et al 2017). As already explained in section 3.3.3, the test battery should include both apical short-term assays, such as tests with algae, daphnids and fish embryos, and *in vitro* mode of action screens which provide indications for potential chronic effects and effects on other species groups, in particular human health hazards.

If no indications for adverse effects are seen, the exercise stops. Otherwise, the next big step is the search for the causative pollutants or groups of pollutants, similar to the corresponding step in LoE I. For clarification, component-based mixture risk assessments on the basis of modeled or measured co-exposure information may be performed, as developed in SOLUTIONS WP 18 (Deliverable D18.1). If this does not provide a sufficient explanation, EDAs may need to be performed for the ultimate identification of those pollutants on which risk reduction measures should be focussed, as explained for LoE I.

The remaining steps are also the same as for LoE I, i.e. risk-based ranking and selection and finally forwarding the outcome to the identification of appropriate risk management options.

5.4.3 LoE III – Prioritisation starting with chemical monitoring data

The third line of evidence (LoE III) starts with chemical monitoring data (Fig. 5), as existing prioritisation procedures also do. For MRAs, however, the difference is that monitoring data must be generated and documented in a way that allows to assess the co-occurrence of defined pollutants at a given site in terms of concentrations and concentrations ratios for all known mixture components.

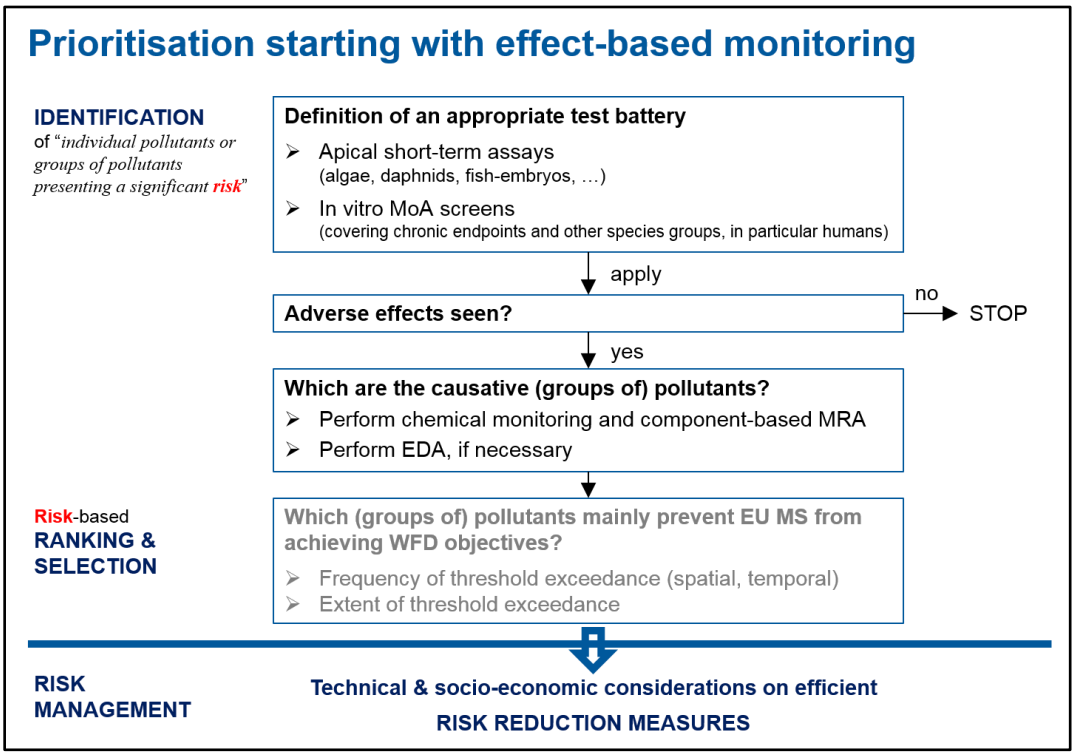

Figure 4: Prioritisation scheme starting from findings of effect-based monitoring

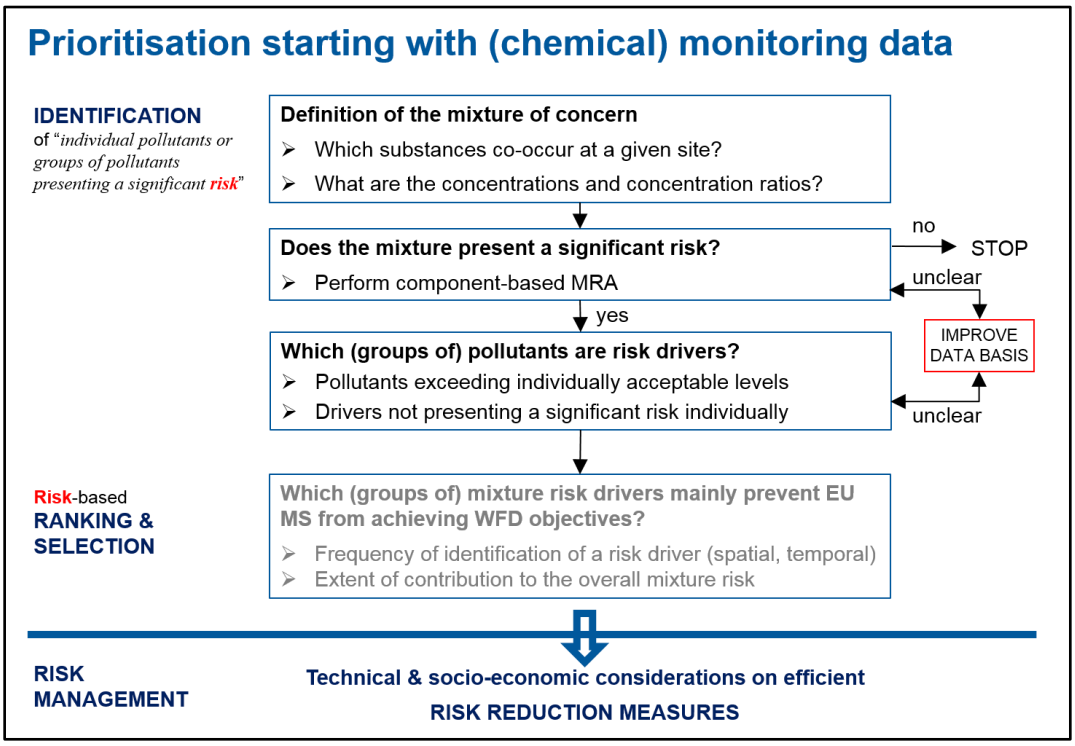

Figure 5: Prioritisation scheme starting from findings of chemical monitoring

This may sound trivial, but past monitoring campaigns were often not organized or not documented in a way that allows such a co-exposure assessment.

Where chemical monitoring provides such co-exposure scenarios, CBAs are required to assess the significance of expectable mixture risks as a second step. And where such mixture risks give a reason for concern, CBAs may also be used for identifying possible drivers of mixture risks as a third step. The procedure should use a tiered approach, as outlined in section 2.2.5 above and worked out in further detail in Deliverable D18.1. The tiered approach may require additional single substance testing where available data are insufficient for reaching conclusive evidence. The identification of drivers must include both, pollutants already exceeding individually acceptable levels as well as mixture components not presenting a significant risk on their own but making a significant contribution to the overall risk, as explained in sections 4.1.3 and 4.2 above.

The rest of the scheme is the same as for LoE I and II.

5.4.4 LoE IV – Prioritisation starting with co-exposure modelling

When prioritisation starts with co-exposure modelling (LoE IV), the initial procedure is essentially the same as with monitoring data (Fig. 6): the mixture of concern is defined and component-based MRAs and driver identification is performed.

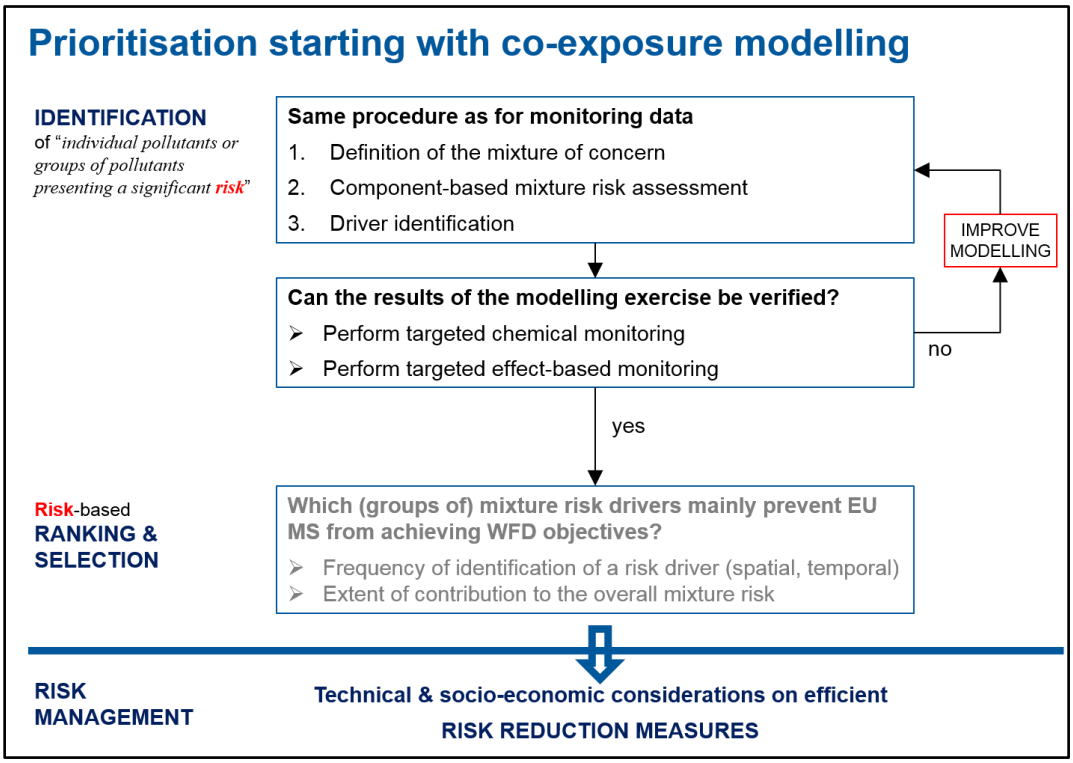

Figure 6: Prioritisation scheme starting from findings of co-exposure modelling

However, as the outcome is purely generated computationally, a verification step is necessary, before final conclusions can be drawn. Co-exposure modelling is a great advancement provided by the SOLUTIONS sub-projects MODELS. However, at the present status and with the currently available input data on chemicals use in the EU, modelled exposure concentrations may differ from available monitoring data by one or two orders of magnitude as detailed in the Deliverables D14.1 and D14.2. Therefore, where the modelling approach leads to indications of significant risks, targeted chemical or effect-based monitoring studies should be performed for confirmation. In designing such studies, it must be considered that actual concentrations of pollutants in surface waters may by fluctuating by orders of magnitude. In such cases, schedules for routine monitoring, as required under the WFD, may just provide snapshot measurements which may be insufficient for verifying or falsifying model predictions.

The remaining steps are the same as for the other LoEs.

6 Implementation

In the Communication on "*The combination effects of chemicals*", the European Commission once noticed that *"The requirement set down in the Water Framework Directive for water bodies to achieve good ecological status as well as good chemical status entails a focus not only on the concentrations of individual chemicals but also on their effects in combination"* (EC 2012). In general, this is certainly true. In detail however, the WFD provisions are very much focussed on conventional strategies for single substance prioritisation and monitoring. In fact, they leave little room for the introduction of novel methodologies that are needed for effectively addressing mixture risks. As a consequence, a full implementation of the proposed four-lines-of-evidence approach requires changes in the legal text. This is explained in the following section 6.1. In addition, effective use of mixture risk assessment approaches may suffer from data gaps which cannot be closed under the WFD, but which require overarching initiatives for strengthening mixture risk assessments across the various 'silos' of European chemicals legislation. This aspect is briefly highlighted in the final section 6.2.

6.1 Barriers for implementation under the existing WFD

For dioxins and dioxin-like compounds, the TEF approach has once been developed in a tremendous supra-national effort under leadership of the WHO. It was adopted under the WFD and used for a mixture EQS setting in terms of a sum of TEQs, as already explained in section 3. It is imaginable that the approach may be adapted or modified for other compound groups. Beyond this, however, any other way of effectively dealing with mixture risks under the WFD requires changes in the legal text. The following is needed:

- A broader definition of *priority pollutants.* It should include all substances that make a significant contribution to an unacceptable overall risk, irrespective of the fact whether they exceed individually acceptable levels or not.
- Comprehensive assessments of the chemical status, including all pollutants at a given site.

  Currently, EU wide priority pollutants and RBSPs are assessed completely in isolation. EU wide priority pollutants define the chemical status, while RBSPs are part of the ecological status assessment. In a real water sample, however, both kinds of pollutants co-occur. Hence for MRAs, this segregation is entirely non-sensical.
- Uniform principles for the prioritisation of pollutants and pollutant mixtures on different scales: EU wide pollutants, river basing specific pollutants, and site-specific pollutants.

  Currently, for example, PNECs or other reference values used by different Member States may differ by orders of magnitude (Vorkamp and Sanderson 2016). This renders comparable mixture risk assessments impossible.
- A clear legal mandate for the establishment of an effect-based monitoring system, which may be performed in parallel to chemical monitoring or as a trigger for chemical monitoring.

These special needs for amendments are part of a broader array of recommendations for revising the WFD with the aim to improve the achievement of its protection goals, as detailed in Brack et al. 2017.

6.2 Needs for strengthening MRA across regulatory silos

Any attempt for assessing and ranking risks of single aquatic pollutants and mixtures of pollutants is confronted with the problem of huge data gaps on the toxicity of thousands of substances. Basically, this applies to single substance prioritisation procedures, such as the NORMAN approach, in the same way as for the prioritisation of mixtures and mixture risk drivers. For initial screenings, these gaps can partly be closed by computational methods such as (Q)SAR, read across, etc. However, where these data-bridging methodologies lead to the identification of mixture components of concern, experimental toxicity testing is ultimately required for reaching conclusive evidence. Thus, there is a need for

- a clear legal mechanism for closing (eco)toxicological data gaps on candidate priority pollutants which have been identified by modelling approaches, read across, etc.

A similar problem applies to co-exposure modelling which suffers from the limited availability of reliable data on the amounts of chemicals used for certain purposes or emitted from certain processes.

The WFD does not include mechanisms for closing any of these data gaps. Such data are generated under other pieces of EU chemicals legislation such as REACH and various others. Hence, a solution is not possible under the WFD alone, but requires cross-cutting initiatives including all relevant pieces of EU chemicals legislation.

In principle, the problem was already well recognised when the initial list of priority substances was established 17 years ago, based on the '*combined monitoring-based and modelling-based priority setting*' (COMMPS) scheme (see section 1.2.1). In the Decision, the Commission noticed:

- *"The effectiveness of COMMPS is largely determined by the availability of relevant data. (...).* ***The purpose of Directive 2000/60/EC*** [the WFD] ***can only be fully achieved if full data availability is ensured by revising the Community legislation on chemical substances.*** ". (Decision No 2455/2001/EC, Recital 18)

At that time, REACH was under debate, which came into force six years later in 2007. Many stakeholders had high expectations that REACH would solve all the problems with data gaps for risk assessment. However, as REACH was finally designed, it does not deliver the quality and quantity of data required for conclusive (mixture) risk assessments under the WFD and other pieces of legislation. Most of the registration dossiers do not even meet basic quality requirements (Springer et al. 2015).

Thus, the problem of data gaps has not substantially changed since the WFD came into force 18 years ago. If the aim of identifying and prioritising mixture risks and drivers of such risk is taken serious, the system of European chemicals legislation needs rethinking. This does not only concern the specific

problem of missing data on use and toxicity of single chemical, but also a number of more general aspects of coordination between the WFD and other EU policies, EU legislation, and international conventions and agreements. This wider policy context is detailed in SOLUTIONS Deliverable **D7.1 on** "***Policy recommendations for maximum synergies between policy frameworks".***

7 Conclusions

- Integrating mixture risk assessments into prioritisation procedures under the WFD is possible. However, no single approach is able to tackle all the problems associated with the demanding task. Integration of all available concepts and methods into a four-lines-of-evidence approach is therefore the best possible way forward.
- The existing WFD does not allow to address mixture risks effectively. For a full implementation of the suggested four-lines-of-evidence approach, a revision of the legal text is necessary.
- Risk assessment and risk-based prioritisation is a data-hungry exercise. Already for the assessment of many single water pollutants, the limited availability of reliable toxicity data poses a serious problem. For conclusive mixture risk assessments, it becomes an even more severe bottleneck. In addition, co-exposure modelling suffers from the limited availability of reliable data on the amounts of chemicals used for certain purposes or emitted from certain processes. The WFD does not include mechanisms to close any of these data gaps. A solution is not possible under the WFD but requires cross-cutting initiatives including all pieces of EU chemicals legislation.

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