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Future water quality monitoring – Adapting tools to deal with mixtures of pollutants in water resource management

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Abstract

Environmental quality monitoring of water resources is challenged with providing the basis for safeguarding the environment against adverse biological effects of anthropogenic chemical contamination from diffuse and point sources. While current regulatory efforts focus on monitoring and assessing a few legacy chemicals, many more anthropogenic chemicals can be detected simultaneously in our aquatic resources. However, exposure to chemical mixtures does not necessarily translate into adverse biological effects nor clearly shows whether mitigation measures are needed. Thus, the question which mixtures are present and which have associated combined effects becomes central for defining adequate monitoring and assessment strategies. Here we describe the vision of the international, EU-funded project SOLUTIONS, where three routes are explored to link the occurrence of chemical mixtures at specific sites to the assessment of adverse biological combination effects. First of all, multi-residue target and non-target screening techniques covering a broader range of anticipated chemicals co-occurring in the environment are being developed. By improving sensitivity and detection limits for known bioactive compounds of concern, new analytical chemistry data for multiple components can be obtained and used to characterize priority mixtures. This information on chemical occurrence will be used to predict mixture toxicity and to derive combined effect estimates suitable for advancing environmental quality standards. Secondly, bioanalytical tools will be explored to provide aggregate bioactivity measures integrating all components that produce common (adverse) outcomes even for mixtures of varying compositions. The ambition is to provide comprehensive arrays of effect-based tools and trait-based field observations that link multiple chemical exposures to various environmental protection goals more directly and to provide improved *in situ* observations for impact assessment of mixtures. Thirdly, effect-directed analysis (EDA) will be applied to identify major drivers of mixture toxicity. Refinements of EDA include the use of statistical approaches with monitoring information for guidance of experimental EDA studies. These three approaches will be explored using case studies at the Danube and Rhine river basins as well as rivers of the Iberian Peninsula. The synthesis of findings will be organized to provide guidance for future solution-oriented environmental monitoring and explore more systematic ways to assess mixture exposures and combination effects in future water quality monitoring.

List of acronyms

AA – annual average
AOP – Adverse outcome pathways
BQE – Biological quality elements
CIS - Common European implementation strategy
DG SANCO –Directorate General for Health and Consumer Protection of the European Commission
EDA – Effect-directed analysis
EQS – Environmental quality standards
EROD – Ethoxyresorufin-O-deethylase
EU – European Union
GC-MS/MS – Gas chromatography coupled with double mass spectrometry
GFP – Green fluorescent protein
GST – Glutathione sulfotransferases
HPCCC – High performance counter current chromatography
KE – Key event
LC-HRMS/MS – Liquid chromatograpy of high resolution coupled with double mass spectrometry
MAC – Maximum allowed concentrations
MIE – Molecular initiating event
MoA – Mode of action
PAH – Polycyclic aromatic hydrocarbons
PNEC – Predicted no-effect concentration
RBSPs – River basin specific pollutants
TU – Toxic units
WFD – Water Framework Directive

1 Introduction

The monitoring of freshwaters with the goal of safeguarding environmental water quality in Europe so far has focused on the evaluation of the ecological and chemical status of water bodies. For the ecological status biological and hydromorphological quality elements are considered, while the chemical status is judged based on consideration of a few selected compounds (EU Dir 2000/60, EU Dir 2013/39). The established techniques for the biological quality elements rely on phytoplankton, macrophytes, phytobenthos, benthic invertebrate, and fish fauna recordings (EU Dir 2000/60). These monitoring efforts are carried out on a wide scale and at regular intervals, such that the ecological status is the aggregate of occurrence and abundance information. The chemical status, on the other hand, is derived from information on analytically determined concentrations of priority pollutants in different compartments such as water, sediment and biota, which are compared against Environmental Quality Standards (EQS) (EU Dir 2008/105, CIS GD 27, 2011). Complementary efforts include emission monitoring, effluent testing for acute toxic effects, and risk management measures for specific products, such as buffer zones for pesticide application or product labelling for pharmaceuticals or consumer products.

Despite the enormous efforts, the picture that emerges regarding ecological and chemical status is still incomplete, fragmented, and with contradictory assessments of the situation. There is general consensus that the target of “good ecological status” defined in the Water Framework Directive (WFD) will not be reached for the majority of European water bodies within the anticipated timeframes (EEA, 2012). Among the causes for this failure the contribution of chemical contamination, however, remains unclear, although efforts to assess chemical monitoring results point to a contributory role of chemical contamination (Malaj et al. 2014). Overall, about 40% of European water bodies (EC COM 673, 2012) still have an unknown chemical status as not even the monitoring of the EU-wide priority substances has been performed. From a management perspective the legacy compounds are of diminishing importance, due to decreasing use of these substances (many are regulated or banned) and the growing awareness that many other chemicals occur and may cause adverse effects in the aquatic environment. The occurrence of anthropogenic chemicals in the environment appears indeed to be widespread and the detection of mixtures of contaminants seems to be the rule rather than the exception (Kolpin et al. 2002, Loos et al. 2009). While elaborated hazard assessments leading to environmental quality standards are performed for priority pollutants, this is not the case for most other chemicals that have been recently detected. This is why these may be referred to as contaminants of emerging concern (EPA, http://water.epa.gov/scitech/cec/).

The European Commission became aware of the problem of chemical mixtures (Council Conclusions 2009), and in its communication on the combination effects of chemicals (EC COM 252, 2012) describes the challenges requiring scientific support. In principle, tools for analysing and assessing combined effects from defined mixtures have been well studied and documented over the past decades (e.g. Kortenkamp and Altenburger 2011) and suggestions about how component-based predictive environmental risk assessment may be performed are presented (e.g. Backhaus and Faust 2012). Thus, the existence of combined effects is a fact and the principal means of addressing them are known (EC 2011). The challenge now is to develop systematic ways of addressing chemical mixtures in environmental assessment (EC COM 252, 2012).

The EU-funded SOLUTIONS project (http://www.solutions-project.eu/) takes up this challenge for water quality assessment and monitoring by undertaking to improve monitoring strategies and combining them with modelling efforts based on pre-market data (Brack et al.

2015). Here we outline our strategies for analysing and assessing chemical mixtures for water quality monitoring purposes. We intend to explore three options for identifying and developing systematic approaches to accommodate for contaminant mixtures in water quality assessment (Fig 1). Firstly, we test the hypothesis that it is possible to identify mixtures whose compositions are representative for specific sites or typical for specific sources and are thus amenable to component-based mixture assessment. Secondly, we elaborate means of identifying batteries of bioanalytical assays that allow comprehensive assessment of impact of mixtures on water quality. Finally, we combine effect-based and chemical analytical tools to probe causal links between mixture occurrence and combined effects and to support the identification of drivers of mixture toxicity.

Fig. 1: Challenges to deal with mixtures of pollutants in water quality monitoring and to provide management solutions

The major questions of combination effects of chemicals (EC COM 252, 2012) with regard to their impact on water quality assessment and the above mentioned strategies will be studied in the context of case studies at the river Danube (de Deckere et al 2012, Grund et al. 2011, Liska et al. 2008), the Rhine catchment (Hollender et al 2009, Ter Laak et al 2010) and for rivers of the Iberian Peninsula (Muñoz at al, 2009, Navarro-Ortega et al. 2012). Investigations will be based on existing data and experimental studies. Moreover, these case studies will be utilised to complement and jointly evaluate results from modelling and measurement-based approaches (Brack et al. 2015).

2 Identification of priority mixtures

The Scientific Committees of the Directorate General for Health and Consumer Protection (DG SANCO) have emphasised that ‘in view of the almost infinite number of possible combinations of chemicals […] focus on mixtures of potential concern is necessary’ (EC 2011). A number of criteria were proposed for consideration, including co-occurrence at individual concentrations below but close to acceptable levels, indications for similar action, and the potential for toxicological interactions. Additional criteria, such as scale of exposure (EC COM 252, 2012), co-occurrence of transformation products or source attributions might be considered. In general, if bias towards known contaminants is to be reduced, this task requires on the one hand multi-residue target and non-target screening techniques to cover mixtures occurring in the environment more comprehensively. On the other hand, improvements leading to lower detection limits for known bioactive compounds are also needed as for some of the newly established water priority substances (Table 1) it is currently virtually impossible to analytically determine compounds at the very low EQS concentrations set for them in the WFD.

Table 1: Environmental Quality Standard (EQS), annual average (AA) and maximum allowable concentrations (MAC) set for the newly established WFD priority substances in inland and other surface waters* (EU Directive 2013/39/EU). Unit: µg/l, nomenclature as in the legal reference

It is therefore the goal of the SOLUTIONS project to improve chemical analytics both with respect to capabilities to screen for more compounds and to improve present detection limits. Subsequently, the data from case studies will be utilised to investigate the co-occurrence of components. To identify mixtures of priority, two data evaluation strategies will be pursued.

Firstly, we will try to identify patterns of co-occurring compounds and correlate them to site characteristics, land use or specific contamination sources. Secondly, to support the assessment of detected mixtures, toxicity data gaps will be filled through modelling and subsequent hazard quotient formulation. The results will be used in component-based mixture toxicity extrapolations to identify mixtures of potential toxicological concern (Price et al. 2011).

The significant analytical gaps regarding the detection limits of compounds with very low PNECs or EQS (Table 1) in environmental media and/or biota require novel concepts in the sampling and clean-up of samples. With a given sensitivity of chemical analytical techniques, detection limits can be improved by accumulating and concentrating compounds from larger volumes of water, e.g. either by passive sampling or by large volume solid phase extraction. Table 2 lists the approaches that are pursued to this end and summarizes the existing experience within the SOLUTIONS consortium.

The number of analytical methods developed for targeted determination of emerging contaminants has experienced rapid growth over recent years and continues to increase which has led to the discovery of new environmental contaminants, metabolites and transformation products. Major gaps remain with respect to the identification and elucidation of the structure of known and unknown components of complex environmental mixtures potentially composed of tens of thousands of components. Two recent studies (Malaj et al. 2014, Moschet et al. 2014) demonstrated that more comprehensive analytical compound screening may substantially alter the assessment of surface water quality. In the study of Moschet et al. (2014), five Swiss riverine catchments were sampled during spring and analysed for the occurrence of some 250 components, mainly pesticides and biocides. AA-EQS exceedances for 19 compounds occurred in 70% of the water samples. This observation would have escaped attention when restricting the assessment to priority components only. Malaj et al. (2014) provide evidence that compounds occurring in European freshwaters even for routinely monitored chemicals such as γ-hexachlorocyclohexane, atrazine, cyanide, chlorpyrifos, chlorfenvinfos, or diuron at their detected concentrations may be close to hazardous concentrations at many sites. A second finding was that the outcome of risk assessment critically depends on the number of compounds analysed: often, apparently low environmental risk associates with a limited number of monitored chemicals. After these proof-of-principle investigations, subsequent steps should therefore address the question of how to assess the totality of hazardous contamination in a reliable way while at the same time keeping efforts at a realistic level. To address this issue a focus on priority mixtures that might be derived from chemical analytical information is a promising approach. Priority mixtures identification based on the analytical data is, in our perspective, not limited to sets of defined chemicals at specified concentrations but rather an analysis of patterns is needed as described above.

SOLUTIONS looks for answers regarding better coverage of detectable and unidentified compounds by establishing non-target screening workflows and a set of interacting compound identification tools which integrate GC-MS/MS and LC-HRMS/MS technology with computer tools for retention, fragmentation, hydrogen-deuterium exchange and toxicity prediction and database for mass spectra. More details concerning the roads taken are summarised in Table 2.

Table 2: Chemical analytical problems addressed in the SOLUTIONS project to support priority mixture identificatio

Once we obtain more comprehensive data on the occurrence of multiple chemicals in

freshwaters by means of targeted, multi-residue, and screening chemical analytical efforts, the subsequent issue will be to find out whether mixture patterns can be elucidated. In order to identify potentially repetitive mixture patterns, analytical data for detected compounds could be subjected to data clustering. An exemplary effort is illustrated in Figure 2.

Here, out of 396 organic compounds that were analysed and quantified in water samples from five small rivers of the Rhine catchment (Moschet et al. 2014), 141 chemicals were found to occur above their detection limits in at least one of the rivers. The data was hierarchically clustered (distance method = ”Euclidian”, clustering method = ”Ward”) according to the site of occurrence and the detected concentrations. At this coarse level, groups of chemicals with high, moderate and low concentrations can be determined and site-specific occurrences become obvious. Using this approach for comparing more sites including additional chemical, toxicological (e.g. hazard ratios), or site-specific information may be advanced to allow characteristic toxicological signatures to be correlated with the different human activities such as the cultivation of grains, orchards or meadows as opposed to urban, domestic, or industrial influences. Moreover, the scale of occurrence of mixtures and archetypical versus river basin-specific pollutants may be derived.

Fig 2: Heatmap of concentrations for 141 chemicals reported in Moschet et al. (2014) in five rivers, clustered to identify occurring mixture patterns. MDL=minimum detection limit

Efforts such as those from Malaj et al. (2014) and Moschet et al. (2014) not only provide wider coverage of priority pollutants and currently used pesticides than previously available, but also demonstrate that the detectable concentrations may raise concern for unwanted biological effects. To study the significance, temporal and spatial scale of occurring concentrations, complementary comparison with toxicity information for the detected compounds should help. Subsequently, any concentration-response-relationship information can feed into component-based mixture toxicity modelling approaches (Altenburger et al. 2004, Altenburger and Greco 2009) to derive estimates of resulting combined effect. The results of these combined effect estimates may in turn prove to be suitable for the development of a novel perspective for identification of river basin-specific pollutants and for advanced EQS settings for priority mixtures.

3 Impact of mixtures

Chemical monitoring of water quality accounts for quantitative assessments of the occurrence and fate of known contaminants in water bodies and thus facilitates the management and remediation of defined compounds. The ultimate goal of water quality management under the WFD, however, lies in the provision of good ecological and chemical status. Thus, analytically undetected but toxicologically relevant compounds, transformation products and mixture effects may be overlooked in an approach that is purely based on chemical analytical measurements. It is suggested that bioanalytical tools can improve the environmental impact assessments (CMEP 2014, Escher and Leusch. 2012, Malaj et al. 2014). A second goal in SOLUTIONS, therefore, is to advance and apply bioanalytical methods to see whether improved impact assessment of mixtures is within reach. The simultaneous exposure of organisms to different compounds may not necessarily mean that combined effects are evoked at detectable levels (Altenburger et al. 2004). This may be due to individual components acting differently and it may be due to the relation between the dose-dependency of components and the concentrations found in the mixtures which may not give rise to

detectable contributions (EC 2011). A way forward for mixture impact assessment for field situations may be seen in devising bioanalytical tools that are tailored for specific mixture assessment objectives.

Bioanalytical tools are defined here as assays which capture key events (KE) of biological reactions following experimentally controlled or observed chemical exposure and molecular initiating events (MIE) in an organism, detected at the level of the cell, organism, population or community and possibly leading to adverse outcomes. Moreover, these tools can inform us about the existing toxic pressure for biological systems if employed *in situ*. The first large scale attempts have recently been made to address the use of various bioassays for mixture impact analysis of surface waters (Escher et al. 2014, Carvalho et al. 2014). Subsequently to demonstrating that effects of mixtures seem to be relevant in various environmental settings, different management perspectives can be distinguished. The management problem may need (i) diagnostics, i.e., identifying the biological receptor that is affected by mixture exposure; (ii) forensics, i.e., elucidating the causes of an emerging adverse effect and their responsible source; or (iii) status assessments, i.e., allocating the contribution of chemicals to an impaired ecological status and delivering a prognosis for the development of the water quality.

The underlying conceptual thinking in the SOLUTIONS project for benchmarking the studied bioanalytical tools with respect to their contributions for the different mixture impact questions will be based on a modified version of the concept of adverse outcome pathways (AOPs)(Ankley et al 2010, OECD 2013) as illustrated in Fig 3. In distinction to the AOP concept we here deal with mixtures, where it is conceived that no longer individual molecular initiating events but rather measures of common adverse outcome are required to capture potential mixture impacts (EFSA 2013). We thus define key events as those observations that integrate several potential MIEs. This would comprise simultaneous observation of activation or inhibition of various nuclear receptors but also detecting alterations of biotransformation which under mixture exposure can provide indication for unexpected combined effects. In the AOP at the next level of biological complexity cellular stress responses and subsequently organisms fitness measures are observed.

Effect-based tools summarise all the various cell- or organism-based bioassays that typically are performed in the lab to characterize environmental samples. Effect-based tools with response detection on the molecular, subcellular or cellular levels are believed to aggregate the combined effects of similar bioactive components for the specific responses they are designed to capture. For diagnostic or forensic tasks arrays of tools will have to be designed to cover different biological effect qualities, while for surveillance tasks where a defined receptor is to be protected, individual tools might provide effective impact detectors.

Effect-based tools that detect apical organism responses are easily related to toxicologically consented adverse effects and thus lend themselves to applications in chemical environmental hazard assessment. Mixture impact assessment is currently well capable of assessing the combined toxicity of similar and dissimilar acting components at the organism level (Altenburger and Greco 2009), whereas understanding the translation of mixture responses observed in molecular and cellular assays and more apical and regulatory-relevant assays remains a formidable research challenge (Altenburger et al. 2012). Therefore, by linking the responses from the different organisational levels through the integrated use of bioassays representing the molecular, cellular, organism and population level we aim to improve our understanding of potential biases in the existing effect detection tools.

Finally, ecological tools are employed to bridge toxicological effect findings as understood for individual organisms and chemical mixtures from the effect-based tools, to field observations of compromised ecological structure and function. Two perspectives are pursued here, on the one hand for selected effects, such as exposure stimulated metabolism we perform *in situ*

studies on feral fish (Brinkmann et al. 2013, Boettcher et al. 2010) (table 3) while on the other hand we will deploy trait-based approaches to investigate community-level effects of chemical contaminants. Trait-based approaches are used increasingly to derive correlations between the occurrence of species traits and exposure to (mixtures of) chemicals, but also to distinguish between chemical stress effects and impact of other major pressures, e.g. hydromorphological alterations or eutrophication. If mode of action (MoA)-specific species traits can be identified, biomonitoring data could be used as a marker for chemical stress at the aggregating MoA level. This assessment can also be used to identify the chemicals likely to pose the highest ecological risks (Van den Brink et al., 2013).

Fig 3: Conceptual framework for bioanalytical tools illustrating their place in an adverse outcome pathway network elucidated by mixture exposure and indicating the potential roles of bioanalytical tools in mixture impact assessment

A variety of bioanalytical tools will be explored in this project (Table 3) for their capabilities to aggregate mixture effects of chemicals irrespective of the presence of possibly unknown chemicals, or variability in the mixture composition. The list is not comprehensive but comprises (i) *in vitro* nuclear- and cell-reporter assays that indicate intracellular presence of contaminants or detect specific receptor- or aggregated stress responses, (ii) standard toxicological organism-based bioassays that detect apical responses in fish, daphnia and algae and directly relate to established biological quality elements (BQE), and (iii) ecology-oriented bioindicators using biomarker responses in individuals or community function (pollution-induced community tolerance), or trait-based composition information. The bioanalytical tools to be applied in the SOLUTIONS project are further specified in Table 3 regarding their properties, perspective and the existing experience.

Table 3: Bioanalytical tools used in the SOLUTIONS project to improve the impact assessment of mixtures for diagnostic, forensic and ecological quality purposes

Bioanalytical tools in their totality and in future arrays could thus help determine the impact of mixtures with respect to distinct water quality management questions. Moreover, if proven workable, this approach could possibly link multiple chemical exposure assessment directly to specific environmental protection goals.

4 Identification of mixture toxicity drivers

Despite the presence of mixtures of multiple compounds in environmental media and samples, theoretical considerations and experimental findings suggest that the overall risk may be driven by only a few mixture components (Altenburger et al. 2004, Backhaus and Karlsson 2014, Price et al. 2012). The European Commission considers the development of methodologies for the identification of such drivers of mixture toxicity a research priority (EC COM 252, 2012). One of the major challenges in the assessment of complex environmental mixtures therefore is the identification of those chemicals that contribute significantly to observed effects. Furthermore, routinely detected chemicals often cannot explain observed biological responses (e.g., Escher et al. 2013) which points to a mismatch between these assessment approaches. This mismatch may be resolved through joint efforts from both disciplines for the different lines of evidence, e.g., by linking chemical monitoring and biological effect and monitoring data by traits-based or effect-directed approaches.

Effect-directed analysis (EDA) may help to identify novel and unexpected compounds that may cause adverse effects on biota and human health (Brack et al. 2008). The principle of EDA is to reduce natural samples to less complex mixtures or individual compounds by bioassay-directed fractionation of environmental samples so that relevant toxicants can be isolated and identified. The approach has been demonstrated as useful in several instances (Brack 2011, Houtman et al. 2007, Thomas et al. 2009) and will be advanced and applied on water, sediments and fish from selected sites in the river basins of Danube, Rhine, and beyond. Current limitations of EDA due to laborious and time-consuming procedures will be addressed by SOLUTIONS. This includes specific investigations on the application of EDA for monitoring, structure elucidation of unknown polar compounds, increasing the number of bioanalytical endpoints, and the application to food chain accumulation and thus secondary poisoning.

The approach pursued is illustrated in Figure 4. SOLUTIONS will develop a tiered protocol to identify river basin-specific pollutants that can be considered drivers of mixture toxicity. To date, the monitoring of contaminants according to WFD is restricted to chemical analytical monitoring of individual chemicals. In the first tier this information can be used for the establishment of MoA that are known to be relevant in specific water bodies or river basins supporting a MoA- or BQE-specific default approach e.g. based on the summation of toxic units of the components (Backhaus and Faust 2012). This approach already goes beyond the current WFD approach and provides a first set of chemical target screening-based candidate drivers. The MoA information also helps to complement chemical monitoring with multi-endpoint (eco)toxicological screening and allows for the identification of mismatches between candidate drivers and multiple biological effects. If unexplained biological effects occur, WFD-like chemical target monitoring is extended in tier 2 by multi-target and non-target screening in order to achieve a more comprehensive picture of contamination patterns. In combination with (eco)toxicological screening, this provides the basis for a novel approach called virtual EDA to identify chemical signals that are correlated with effects from background signals. Virtual EDA has been suggested as a term by Eide et al. (2002) and has been recently evaluated in a proof of concept study for the characterisation of chemicals responsible for mutagenic effects in a river impacted by an industrial effluent (Hug et al., in prep.). The approach reduces the complexity of mixture components through the use of multivariate statistics and pattern recognition methods on samples for several sites as a virtual decomposing approach which should direct the focus of subsequent more elaborated identification efforts to a subset of sites. SOLUTIONS will test this approach in case studies on contaminated samples from the Danube and Rhine river basins. Still unexplained mixture effects will be addressed through higher tier EDA studies (tier 3) as a site-specific approach, which will also be used to validate the results of virtual EDA at specific sites.

The identification of unknown compounds using mass spectrometry data remains a major bottleneck in many disciplines (Creek et al. 2014, Scheubert et al. 2013) and often hinders the successful completion of EDA studies (Schymanski et al. 2009). Efforts in SOLUTIONS will therefore focus on the development of methods for generating and pre-selecting toxicant candidate structures from the given analytical and effect information as indicated in Table 2. The structure elucidation approaches also include efforts for the integration of prediction of transformation, toxicity, physico-chemical properties, MS fragmentation and chromatographic retention.

Fig 4: Principles of a tiered effect-directed analysis (EDA)

The diagnostic power of higher tier EDA will be addressed through efforts to adapt assays for

specific key events (see Table 3) as effect detectors for EDA. An array of screening assays potentially covering multiple species, MoAs and adverse effects (see Table 3 for details) will be deployed in the EDA approach.

Moreover, food chain accumulation will be approached exemplarily for fish tissue to investigate bioaccumulation and secondary poisoning through feed and food contaminated with complex mixtures of pollutants. Performing EDA on such tissue will aim to detect and identify bioavailable and bioaccumulative toxicants (Houtman et al. 2004), including metabolites formed in the organisms (Jeon et al. 2013).

5 Perspectives for solution-oriented mixture assessment

A central deliverable of the SOLUTIONS project is to generate guidance for the three mixture assessment challenges identified by the European Commission (EC COM 252, 2012), namely (i) the characterisation of priority mixtures, (ii) mixture impact assessment, and (iii) the identification of toxicity drivers. The need to tailor environmental monitoring tools towards contamination diagnosis in complex environmental matrices, however, is acknowledged on a worldwide scale. E.g. Environment Canada (2014) suggests guidance to use effect-based methods for aquatic effects monitoring from pulp and paper production. In Australia, where the water cycle is an issue with the perspective of reuse for humans, strict standards for a larger number of potential hazardous compounds have been formulated and it is suggested to link chemical and bioanalytical tools for water quality monitoring (Tang et al. 2014).

Thus the goals set out here should be of a wider interest. To achieve them we will provide documentation of the chemical analytical and bioanalytical tools and specify the approaches for the different needs in water quality monitoring and assessment. The various problems in current water quality management call for tailored approaches, which could provide solution-oriented mixture assessments. For instance, the identification of river basin-specific priority groups of pollutants (RBSPs) needs to be improved for river basin management plans, while risk assessment for unwanted effects calls for a more prominent role of bioanalytical tools. Mixture assessment is essential for water quality management, given the complexity of typical pollution scenarios. The tools that will be provided by the SOLUTIONS project shallfacilitate achieving this aim. The task is to operationalise the required mixture assessment, i.e. to tailor the available tools for the specific tasks laid out above. The SOLUTIONS project as a whole sets out to not only provide advanced methodologies for water quality monitoring, but also to deliver suggestions for testing requirements and data needs for carrying out mixture risk assessment and management in the context of the WFD. The last step will be performed in collaboration with the modelling, case studies and conceptual framework activities (Brack et al. 2014).

The NORMAN network (http://www.norman-network.net/) has recently proposed a novel risk assessment-based approach for prioritisation of water pollutants for improving water monitoring (Dulio and von der Ohe 2013, Brack et al. 2012). It suggests a strategy to cope with scarce data for individual compounds and to account for different management action categories. The scheme, however, remains limited to individual compound assessments. The tools developed and the data generated within the SOLUTIONS case studies may be used to amend such prioritisation schemes to address mixtures of contaminants of emerging concern and their impacts explicitly.

The larger vision of future water resource management and the contributions that can be anticipated, bears yet another level of perspectives. It is widely acknowledged that European water bodies are affected by multiple types of stress, such as water scarcity, morphological changes, and pollution. Addressing the joint effects from such multiple stressors in

management is limited by the currently available knowledge (Hering et al. 2014, Navarro-Ortega et al. 2014). Two international EU-funded projects, MARS (Hering et al. 2014) and GLOBAQUA (Navarro-Ortega et al. 2014), are addressing several primary and secondary stressors such as water flow extremes, thermal extremes, eutrophication, and impaired habitat morphology. The efforts in SOLUTIONS are clearly complementary and issues are easily identified where joint efforts could improve our mechanistic understanding of interactions between say low water flow and the impact of pollution. Also, as risk assessment, WFD status assessment and the understanding of ecosystem services follow different but related frameworks (Hering et al. 2014), we could gain improved coherence by providing better understanding of each of the frameworks. Finally, we could learn to consistently address scaling issues from the water body through the river basin up to the continental scale.

The revision of the WFD in 2019, the ongoing discussion on a common European implementation strategy (CIS), as well as the cycle of readjustments and refinements of river basin management planning (RBMPs) will be the outreach targets for our research activities. Timely provision of validated chemical analytical or bioanalytical tools, improved knowledge and useful decision support instruments will be vital for translating the various ideas into better practises. Moreover, an improved understanding of how mixture assessment may be performed could generate incentives for more coherent approaches in water resource management by providing the means for cross-compliance measures in environmental regulation.

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Table 1: Environmental Quality Standard (EQS), annual average (AA) and maximum allowable concentrations (MAC) set for the newly established WFD priority substances in inland and other surface waters* (EU Dir. 2013/39/EU). Unit: µg/l, nomenclature as in the legal reference

	AA-EQS Inland surface waters	AA-EQS Other surface waters	MAC-EQS Inland surface waters	MAC-EQS Other surface waters
Dicofol	1.3×10^{-3}	3.2×10^{-5}	-	-
Perfluorooctane sulfonic acid and its derivatives (PFOS)	6.5×10^{-4}	1.3×10^{-4}	36	7.2
Quinoxyfen	0.15	0.015	2.7	0.54
Aclonifen	0.12	0.012	0.12	0.012
Bifenox	0.012	0.0012	0.04	0.004
Cybutryne (Irgarol)	0.0025	0.0025	0.016	0.016
Cypermethrin	8×10^{-5}	8×10^{-6}	6×10^{-4}	6×10^{-5}
Dichlorvos	6×10^{-4}	6×10^{-5}	7×10^{-4}	7×10^{-5}
Hexabromocyclododecane (HBCDD)	0.0016	0.0008	0.5	0.05
Heptachlor and heptachlor epoxide	2×10^{-7}	1×10^{-8}	3×10^{-4}	3×10^{-5}
Terbutryn	0.065	0.0065	0.34	0.034

* Inland surface waters encompass rivers and lakes and related artificial or heavily modified water bodies.

Table 2: Chemical analytical problems addressed in the SOLUTIONS project to support priority mixture identification

Problem	Approach	Method	Aim	References
Compound detection below EQS and estimation of time-averaged concentrations	Enrichment of trace compounds by time-integrative passive sampling	Partitioning and adsorption based passive sampling; Flow controlled passive sampling	Widen applicability of passive sampling by extending the method domain on emerging compounds and improve their performance in terms of limits of quantification and measurement uncertainty	Lohmann et al. 2012, Smedes and Booij 2012, Vrana 2012, Vermeirssen et al., 2013, Moschet et al., 2014
	Time-integrated sampling by *in situ* large volume solid phase extraction	Large-volume sampler for application *in situ* (e.g. at point sources or on monitoring ships)	Development of routinely applicable and commercially available technique with negligible compound -dependence of extraction efficiency; applicable for chemical and biotesting in parallel	Schulze et al. 2014
	Hydrodynamic counter current chromatography (HPCCC)	HPCCC-liquid-liquid partitioning	Improved enrichment and clean up as method improvements for wider use	Ignatova et al 2011
	On-line extraction and clean up methodology for LC	Turbulent flow chromatography	Automated on-line enrichment technique and clean up	Lopez-Serna et al. 2012
Inadequate coverage of environmental mixture components	Automated workflows for sensitive, informative and routinely applicable target and non-target screening techniques	GC- and LC-HR MS/MS techniques with innovative software tools and parameter prediction	Detection, identification and semi-quantification of larger numbers of chemicals at the same time including unknowns	Schriks et al. 2010, Vadillo and Barceló 2012, Schymanski et al 2014, Krauss et al., 2010
	Structure elucidation procedures for environmental trace contaminants and transformation products by systematic integration of analytical information from GC-	Workflow integrating analytical techniques and the use of innovative databases	Identification of new chemicals including transformation products and other unknowns in various matrices	Zonja et al 2014, Huntscha et al., 2014, Schymanski et al 2014, Gerlich and

	and LC-HRMS/MS with, prediction tools for retention, MS fragmentation, hydrogen-deuterium exchange and mass spectral and compound databases	and software in a consensus lines of evidence approach		Neumann 2013, Hug et al., 2014
Total contaminant concentrations in sediment do not reflect the exposure, i.e. biologically accessible concentration, because of unknown uptake capacity of sediments	Availability-based approach for the assessment of sediment contamination using equilibrium partitioning passive sampling ; both non-depletive (chemical activity) and depletive (accessible)	A release isotherm is recorded by equilibrations at different sampler – sediment ratios providing both the level in pore water and the accessible concentration.	Obtaining measured concentrations from sediment samples that allow spatial comparison and conversion into units applicable in other matrices (water, lipid) for comparison between environmental compartments.	Reichenberg and Mayer 2006 Smedes et al., 2013
Detection and unraveling of internal contamination of biota with trace contaminants	In tissue passive sampling to assess internal exposure to environmental mixture	Silicone thin-films as 'chemometers' equilibrated in intact tissues	Measure of the complex mixtures present in tissue while leaving the matrix behind	Jahnke et al., 2009 and 2014
	Parallel detection of multiple contaminants and selected biomarkers	LC-MS/MS screening approaches for contaminants and marker proteins	Integrated assessment of contamination and biochemical response	Yang et al., 2015
	Improved sample clean-up for determination of biota concentrations	Selective extraction and clean-up for lipid removal	Solving matrix problems for the detection of a broad set of emerging pollutants	Huerta et al., 2013 Navarro-Ortega et al 2012,

Table 3: Bioanalytical tools used in the SOLUTIONS project to improve the impact assessment of mixtures for diagnostic, forensic and ecological quality purposes

Biological level	Biosystem	Response observation	Indication of	Project aim	Method reference
Key events	Feral fish	EROD activity, bile PAH metabolites	internal exposure	*in situ* exposure	Brinkmann et al. 2013
		GST activity	internal exposure		Kammann et al. 2014
	Mammalian and Fish cells	EROD activity	dioxin-like	EDA detector	Creusot et al. 2013a
		nuclear receptor activation /inhibition	estrogen/ anti-estrogen		Creusot et al. 2013b
	Mammalian and Yeast cells	nuclear receptor activation /inhibition	androgen/ anti-androgen		Jalova et al. 2013
	Mammalian cells	fish nuclear receptor inhibition	corticosteroid/ anti-corticoid		Kugathas and Sumpter 2011
	Isolated enzyme	acetylcholine-esterase activity	neurotoxicity	EDA detector	Holth and Tollefsen 2012
Cellular responses	*E. coli*, yeast	gene expression, alterations on proliferation of gene	stress-response activation	EDA detector	Zhang et al. 2011, Su et al., 2014
	Salmonella typhimurium	Ames test using diagnostic strains	mutagenicity	EDA detector	Umbuzeiro et al. 2011, Reiferscheid et al. 2012
	Mammalian cell line	p53 activation	genotoxicity	adaptive stress response	Knight et al. 2009, Yeh et al. 2014
		Nrf2 protein in AREc32 activation	oxidative stress		Wang et al. 2006, Escher et al. 2012
		NF-kappaB activation	inflammation as immune response		Knight et al 2009

	Fish cells	Immune gene modulation	immune-competence		Segner et al. 2012
Organism responses	Zebrafish embryo	estrogenic cyp 19a1b-GFP activation	estrogen/anti-estrogen	validation of cellular response indication; EDA detector	Brion at al. 2012, Fetter et al. 2014
	Medaka embryo	estrogenic choriogenin-GFP activation	estrogen/ anti-estrogen		Kurauchi et al. 2005
		androgenic spiggin-GFP activation	androgen/ anti-androgen		Sébillot et al. 2014
	Xenopus embryo	thyroid THbZIP-gfp activation	thyroid/ anti-thyroid	EDA detector	Fini et al. 2007
	Algae	growth, transcriptome	apical effects, MOA	effect diagnostics	Nestler et al. 2012
	Daphnids	motility, transcriptome, metabolome	apical effects, MOA		Meland et al. 2011, Williams et al. 2011
	Zebrafish embryo	development, transcriptome	apical effects, MOA		Büttner et al. 2012, Klüver et al. 2011
		thyroid disruption	endocrine activity		Schmitt et al. 2012
	Abramis abramis	histopathology	organ toxicity		Wolf et al. 2010
Community responses	Algal biofilms	community tolerance measured as ^{14}C-uptake and biofilm formation kinetics	ecological mode-of-action	*in situ* effects	Blanck 2002, Pesce et al. 2010
	Invertebrates	alterations of trait composition	ecological mode-of-action		Van den Brink et al. 2011

EDA – effect-directed analysis
MOA – mode-of-action

Figure captions

Fig. 1: Challenges to deal with mixtures of pollutants in water quality monitoring and to provide management solutions

Fig 2: Heatmap of concentrations for 141 chemicals reported in Moschet et al. (2014) in five rivers, clustered to identify occurring mixture patterns. MDL=minimum detection limit

Fig 3: Conceptual framework for bioanalytical tools illustrating their place in an adverse outcome pathway network elucidated by mixture exposure and indicating the potential roles of bioanalytical tools in mixture impact assessment

Fig 4: Principles of a tiered effect-directed analysis (EDA)

Fig 1

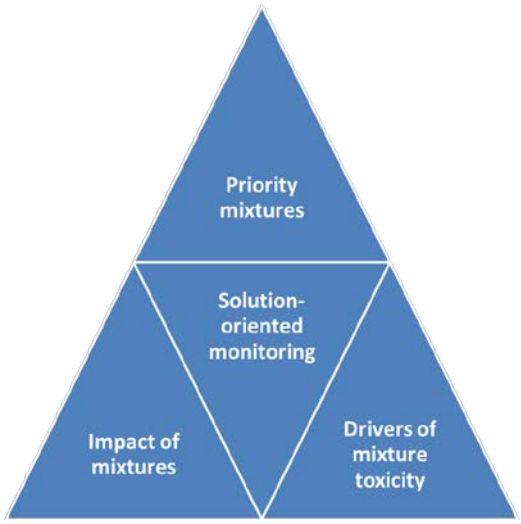
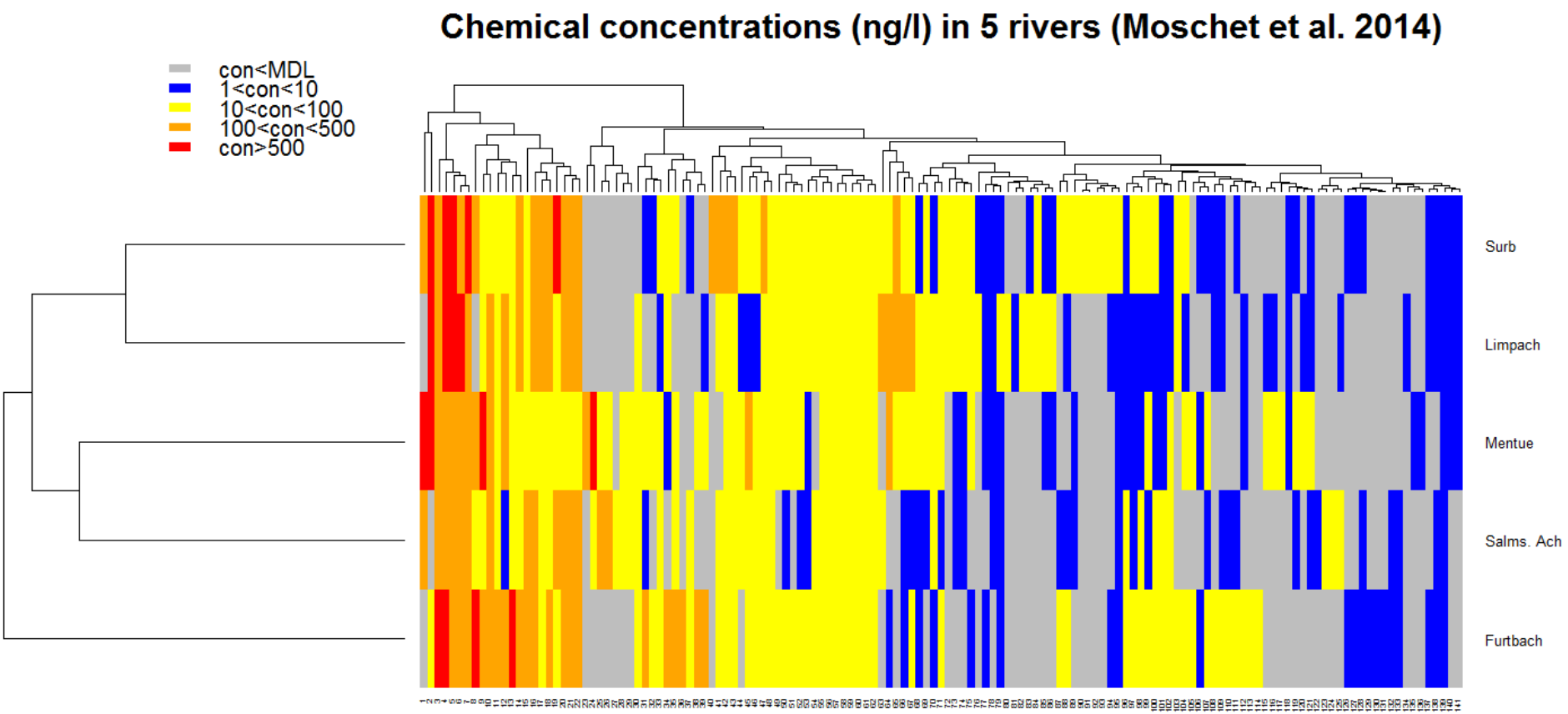

Priority mixtures
Solution-oriented monitoring
Impact of mixtures
Drivers of mixture toxicity

Fig 2



Heatmap No.	Compound Name				
1	Dicamba	4	Metamitron	8	Propyzamide
2	Prosulfocarb	5	S-Metolachlor	9	Diethyltoluamide (DEET)
3	Chloridazon-desphenyl	6	Metamitron-Desamino	10	Chloridazon-methyl-desphenyl
		7	Terbutylazine	11	Azoxystrobin

12 Propamocarb
13 Metazachlor-ESA
14 Isoproturon
15 Metalaxyl-M
16 Metolachlor-OXA
17 Flufenacet
18 Ethofumesate
19 Chloridazon
20 Metolachlor-ESA
21 2-methyl-4-chlorophenoxyacetic acid (MCPA)
22 Mecoprop-P
23 Asulam
24 5-Chloro-2-methyl-4-isothiazolin-3-on (CMI)
25 Piperonyl butoxide
26 Carbetamide
27 N-(2,4-Dimethylphenyl)formamide
28 Diuron-desmonomethyl (DCPMU)
29 Simazine
30 Carbofuran
31 Metazachlor
32 Napropamide
33 Pethoxamid
34 Cycloxydim
35 Linuron
36 Propachlor-OXA
37 Pyrimethanil
38 Propachlor-ESA
39 Propachlor
40 Trinexapac acid
41 Cyprodinil
42 Azoxystrobin free acid
43 Atrazine
44 Foramsulfuron
45 N,N-Dimethyl-N'-phenylsulphamide (DMSA)
46 Dimethomorph
47 Sulcotrione
48 2,4-dichlorophenoxyacetic acid
49 Pymetrozine
50 Fluazifop free acid
51 Propiconazole
52 Tebuconazole
53 Diazinon
54 Mesotrione
55 Atrazine-2-Hydroxy
56 Desethylatrazine
57 2,6-Dichlorobenzamide
58 Diuron
59 Nicosulfuron
60 Desethylterbuthylazine
61 Acetochlor-, Alachlor-OXA
62 Carbendazim
63 4-(4-chloro-2-methylphenoxy)butanoic acid (MCPB)
64 Pencycuron
65 Lenacil
66 Metribuzin
67 Cyproconazole
68 Prothioconazole desethio
69 Metribuzin-Desamino (DA)
70 Ioxynil
71 Flufenacet-ESA
72 Fluroxypyr
73 Tembotrione
74 Thiacloprid
75 Epoxiconazole
76 Pyraclostrobin
77 Mesosulfuron-methyl
78 Thiacloprid_amide
79 Dimethenamid
80 Metrafenone
81 Dimethe mid-OXA
82 Kresoxim-methyl
83 Fenpropimorph
84 Dimethenamid-ESA
85 Spiroxamine
86 Bromoxynil
87 Fenhexamid
88 Boscalid
89 Imazamox
90 Difenoconazole
91 Flusilazole
92 Iprovalicarb
93 Metosulam
94 Fenpropidin
95 Fipronil
96 Thiamethoxam
97 Pirimicarb
98 Terbutylazine-2-hydroxy
99 Atrazine-desethyl-2-hydroxy
100 Terbutylazin-desethyl-2-hydroxy
101 Bifenox Acid
102 Dimethoate
103 Dimethachlor ESA
104 Fenamidone
105 Fludioxonil
106 Imidacloprid
107 3,5-dibromo-4-hydroxybenzoeacid
108 Isoproturon-monodemethyl
109 Metolachlor-Morpholinon
110 Tebufenozide
111 Terbutryn
112 Mandipropamid
113 Methomyl
114 Imidacloprid desnitro
115 Mefenpyr-Diethyl
116 Chlorotoluron
117 Benthiavalicarb-isopropyl
118 Monolinuron
119 Trifloxystrobin
120 Fluoxastrobin
121 Triflusulfuron methyl
122 Methoxyfenozid
123 Cyromazine
124 Dichlorprop
125 Myclobutanil
126 Mepanipyrim

127 3-Phenoxybenzoic acid
128 Oryzalin
129 Irgarol-descyclopropyl
130 Clothianidin
131 Chlorfenvinphos
132 Terbacil
133 Simazine-2-hydroxy
134 Methiocarb
135 Dimefuron
136 Thifensulfuron methyl
137 Acetochlor-, Alachlor-ESA
138 Dimethachlor
139 Tepraloxydim
140 Amidosulfuron
141 Clomazone

1
2

Fig 3

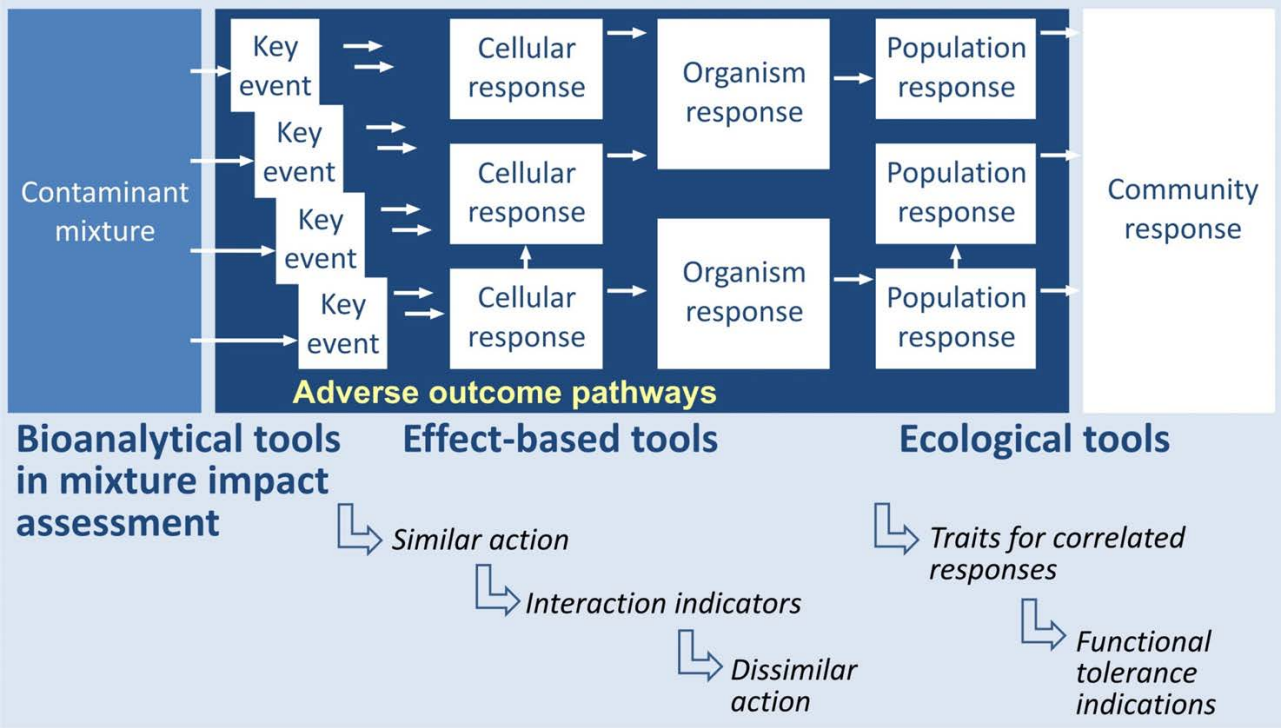
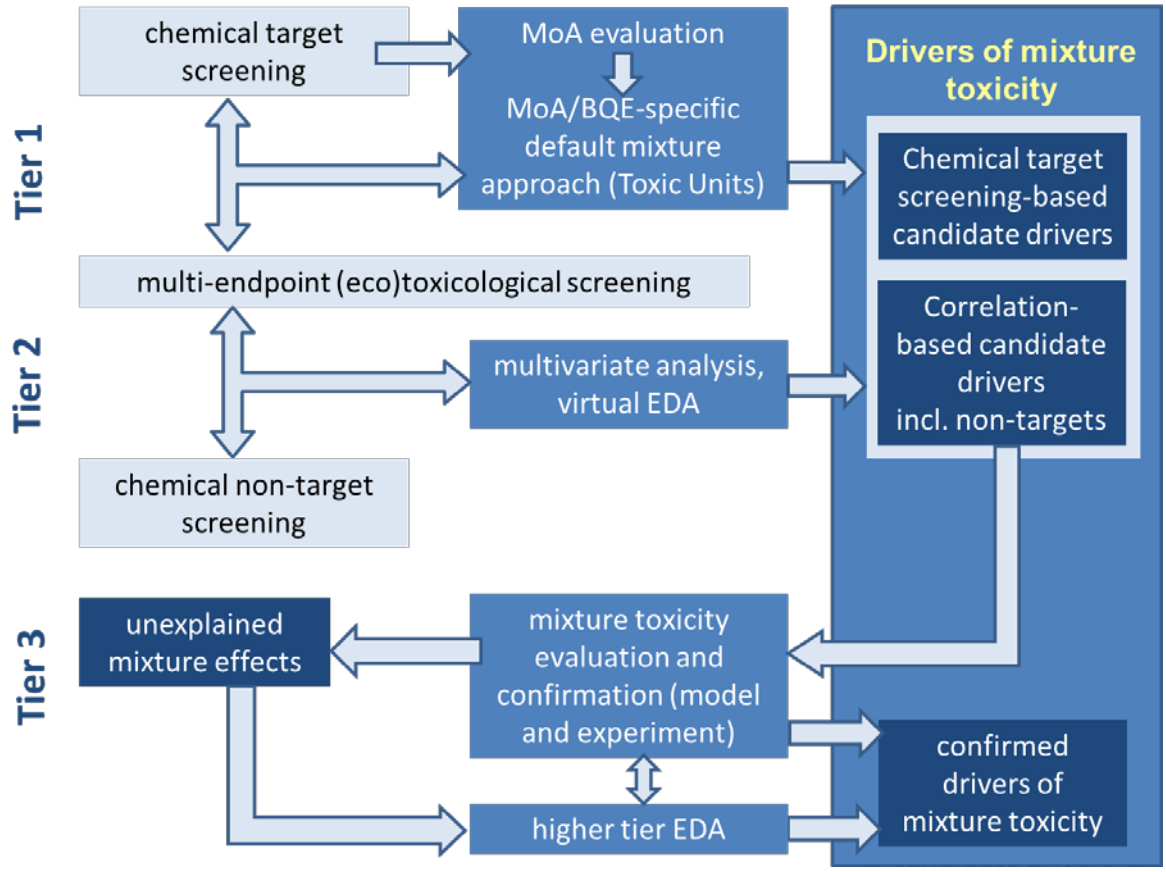

Contaminant mixture
Key event
Key event
Key event
Key event
Cellular response
Cellular response
Cellular response
Organism response
Organism response
Population response
Population response
Population response
Community response
Adverse outcome pathways
Bioanalytical tools in mixture impact assessment
Effect-based tools
Ecological tools
Similar action
Interaction indicators
Dissimilar action
Traits for correlated responses
Functional tolerance indications

Fig 4


Tier 1
Tier 2
Tier 3
chemical target screening
MoA evaluation
MoA/BQE-specific default mixture approach (Toxic Units)
multi-endpoint (eco)toxicological screening
multivariate analysis, virtual EDA
chemical non-target screening
unexplained mixture effects
mixture toxicity evaluation and confirmation (model and experiment)
higher tier EDA
Drivers of mixture toxicity
Chemical target screening-based candidate drivers
Correlation-based candidate drivers incl. non-targets
confirmed drivers of mixture toxicity