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

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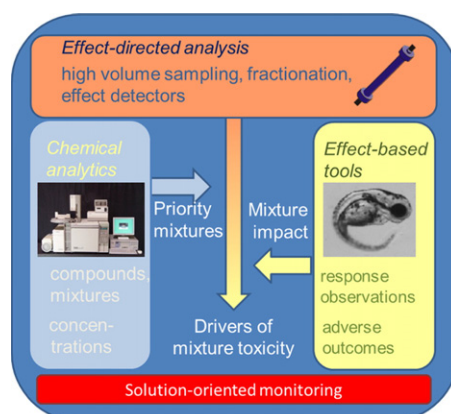
H I G H L I G H T S

- Future water contamination monitoring can address the detection of priority mixtures.
- Effect-based tools will help to assess the impact of mixture on water quality.
- Drivers of mixture toxicity can be identified using effect-directed analysis.

G R A P H I C A L   A B S T R A C T

A B S T R A C T

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 characterise 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 organised 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.

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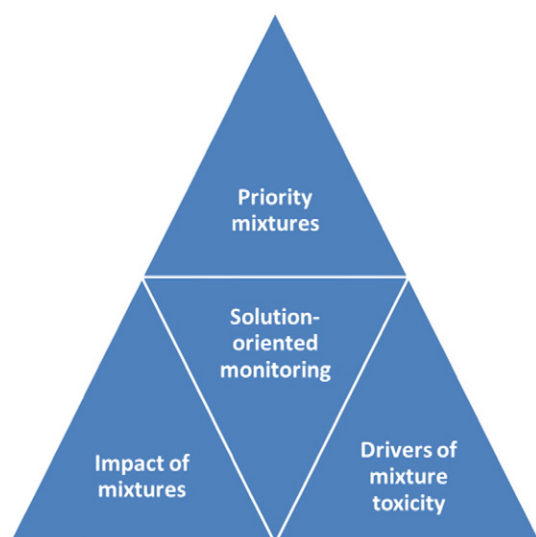
Fig. 1. Challenges to deal with mixtures of pollutants in water quality monitoring and to provide management solutions.

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.

The major questions of combination effects of chemicals (EC COM 252, 2012) with regard to their impact on water quality assessment and the abovementioned 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 et 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

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[a] (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

[a] 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. (2014b)
	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)
Inadequate coverage of environmental mixture components	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)
	Automated workflows for 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 integration of analytical information from GC– and LC–HRMS/MS with prediction tools for retention, MS fragmentation, hydrogen–deuterium exchange and mass spectral and compound databases	Workflow integrating analytical techniques and the use of databases and software in a consensus lines of evidence approach	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 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 unravelling 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, 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)

determine compounds at the very low EQS concentrations set for them in the WFD.

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 and Han, 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 summarises 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., 2014a) demonstrated that more comprehensive analytical compound screening may substantially alter the assessment of surface water quality. In the study of Moschet et al. (2014a), 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
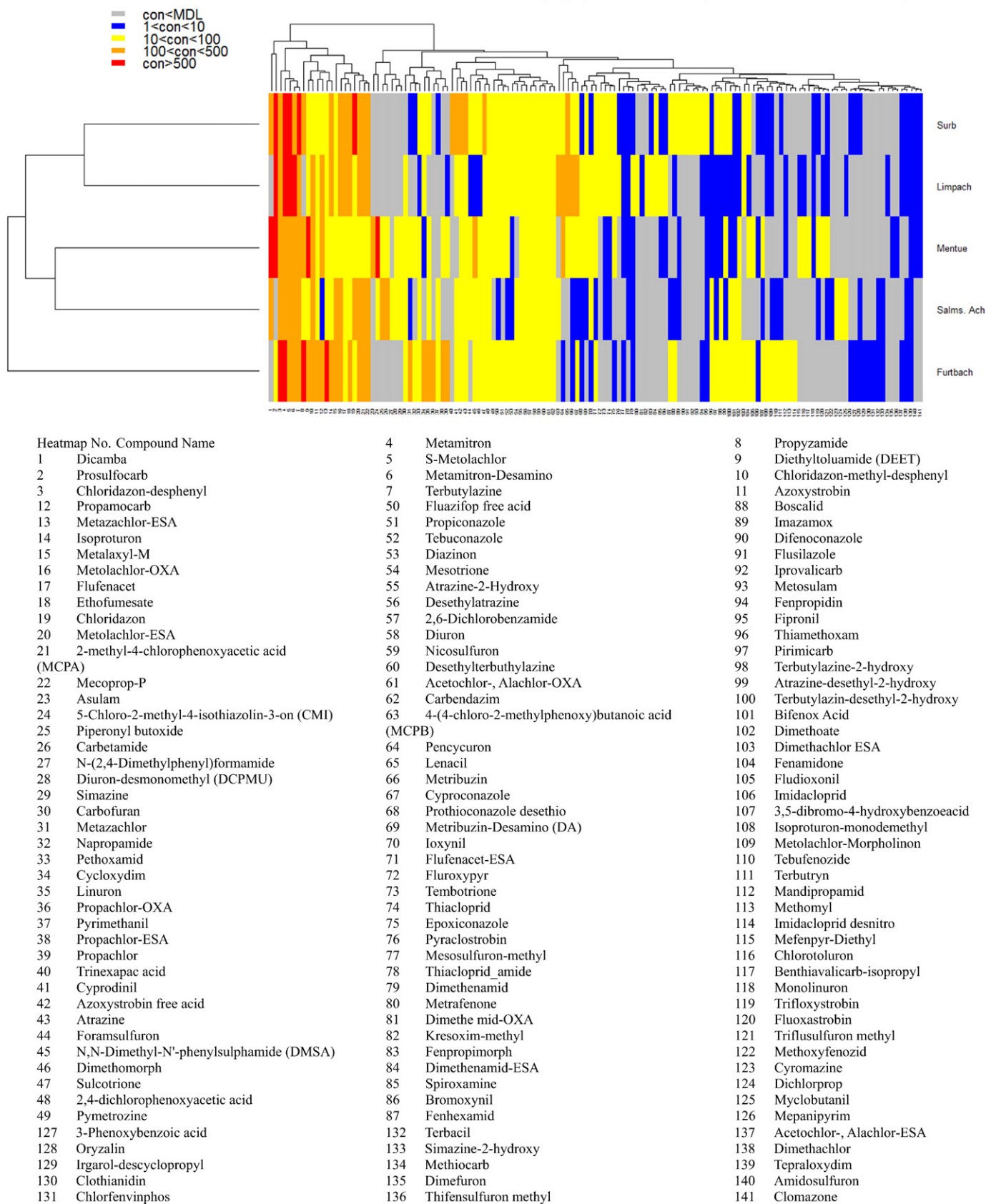
Fig. 2. Heatmap of concentrations for 141 chemicals reported in Moschet et al. (2014a) in five rivers, clustered to identify occurring mixture patterns. MDL = minimum detection limit.

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 mixture 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.

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 Fig. 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., 2014a), 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.

Efforts such as those from Malaj et al. (2014) and Moschet et al. (2014a) 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
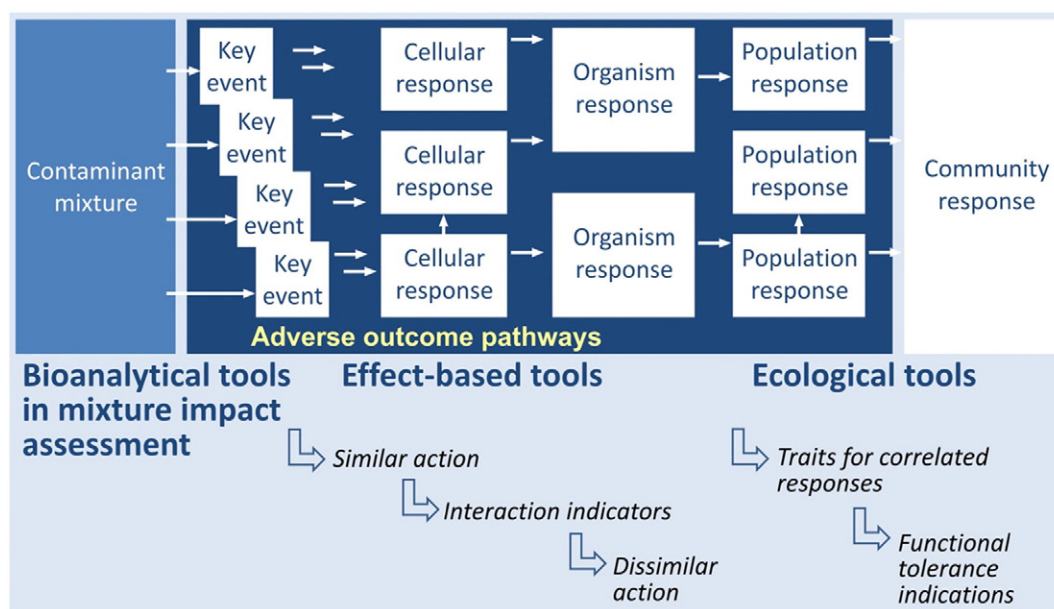

Fig. 3. Conceptual framework for bioanalytical tools illustrating their place in an adverse outcome pathway network elucidated by mixture exposure and indicating the potential roles of bioanalytical tools in mixture impact assessment.

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 (Carvalho et al., 2014, Escher et al. 2014). Subsequently 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 not only 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 organism fitness measures are observed.

Effect-based tools summarise all the various cell- or organism-based bioassays that typically are performed in the lab to characterise 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 levels we aim to improve our understanding of potential biases in the existing effect detection tools.

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	Oestrogen/anti-oestrogen	–	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)
Cellular responses	Isolated enzyme	Acetylcholine–esterase activity	Neurotoxicity	EDA detector	Holth and Tollefsen (2012)
	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), Reifferscheid 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	Oestrogenic cyp 19a1b-GFP activation	Oestrogen/anti-oestrogen	Validation of cellular response indication; EDA detector	Brion et al. (2012), Fetter et al. (2014)
	Medaka embryo	Oestrogenic choriogenin-GFP activation	Oestrogen/anti-oestrogen	–	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	–	Schmidt et al. (2012)
	Abramis abramis	Histopathology	Organ toxicity	In situ effects	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.

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).

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.

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,
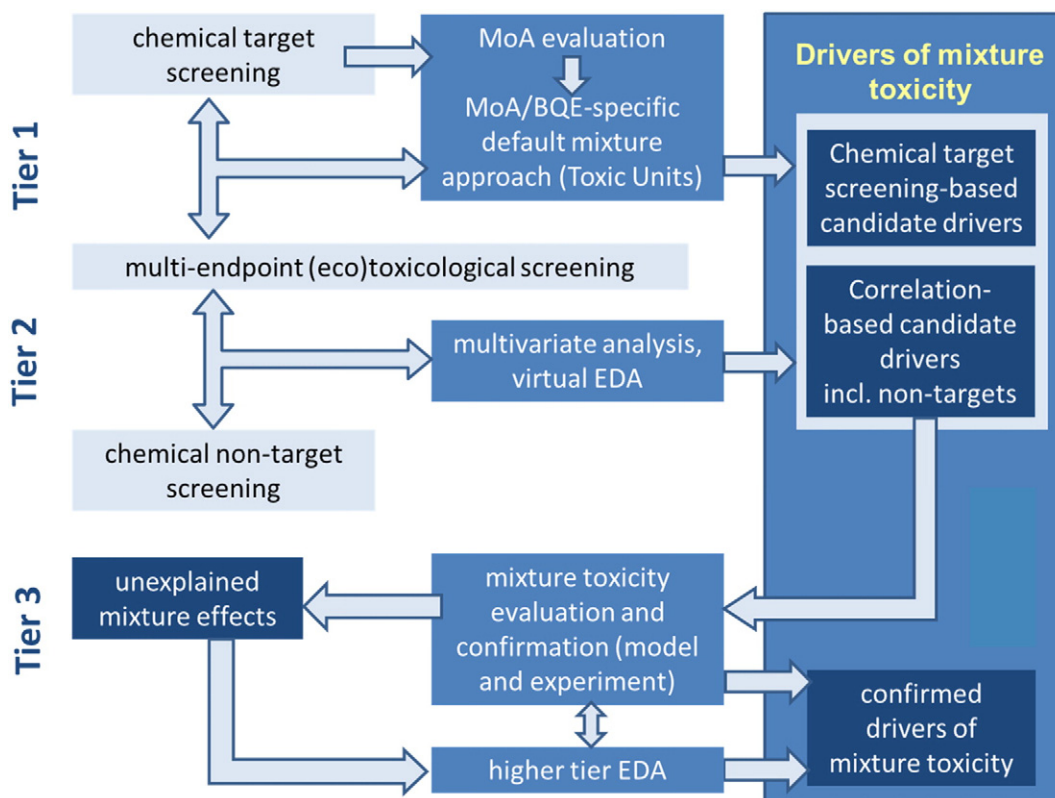

Fig. 4. Principles of a tiered effect-directed analysis (EDA).

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 Fig. 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 preselecting 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.

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. For example, 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 shall facilitate 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., 2015).

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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