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Effect-based methods in combination with state-of-the-art chemical analysis for assessment of water quality as integrated approach

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ABSTRACT

Complex mixtures of chemicals in waste and finally in surface water may pose a risk to the environment and also to human health. This contamination of surface water cannot be addressed with chemical analysis alone. Tools are required to detect and assess these micropollutants which might cause adverse effects. Effect-directed analysis (EDA) with effect-based methods in combination with state-of-the-art chemical analysis can meet this challenge. The present paper summarizes and outlines current experiences with analytical tools and bioassays as integrated approach for assessment of water quality. The need for a holistic and solution-oriented procedure of water quality monitoring is described. To integrate and evaluate existing information about toxicity pathways, which are essential for the EDA approach, the adverse outcome pathway (AOP) concept is useful and recommended. An integration of AOP concept in water quality assessment and further requirements are discussed.

1. Background

1.1. General introduction

Contamination of surface waters with micropollutants is one of the major threats to water quality in Europe (Malaj et al., 2014). Although much is still unknown about the effect of these micropollutants, also called trace organic compounds (TOrC) or contaminants of emerging concern (CEC) on the aquatic environment, there are several indications that they have adverse biological effects in organisms. These effects comprise endocrine disruption, cytotoxicity and/or genotoxicity (Kidd et al., 2007, Liu et al., 2017; Masteling et al., 2016; Neale et al., 2015; König et al., 2017; Zhu et al., 2018). Therefore, the EU implemented the water framework directive (WFD) to achieve and keep a good chemical and ecological status of surface waters until 2027 (EU, 2000/60; EU, 2008/105). To measure the goals and be able to classify water bodies with regard to their chemical status, a list of priority substances (annex X of the WFD) with environmental quality standards was developed (EU COM(2011)876). In 2013 the watch-list was established and updated in 2015 and 2018 to monitor potential candidate compounds to get broader Union wide information to be gathered for the purpose of supporting future prioritization exercises on updating the priority list in annex X (EU, 2013/39; EU, 2015/495, EU, 2018/840). The chemical status of surface water is predicted on the basis of 45 priority substances (EU, 2013/39), but thousands of chemicals in complex mixture are present in the water body. Although the compounds have been prioritized according to a thorough and scientifically sound procedure, the current definition of a good chemical status including the compounds on the watch list covers only a small fraction of actual contamination and extensively ignores mixture risks (Altenburger et al., 2018).

A holistic and solution-oriented monitoring, which at the same time helps to provide links to the ecological status of a water body is needed. Major elements include (1) advanced chemical screening techniques supporting mixture risk assessment and unraveling of source-related patterns in complex mixtures, (2) effect-based monitoring for the detection of groups of chemicals with similar effects and the establishment of toxicity fingerprints, (3) effect-directed analysis of drivers of toxicity and (4) the translation of chemical and toxicological fingerprints into chemical footprints for prioritization of management measures (Brack et al., 2018). A solution-oriented chemical status procedure can bridge the gap between the ecological status monitoring and management in water bodies impacted by toxic stress caused by contaminants of emerging concern (Fig. 1). The procedure implements chemical and effect-based monitoring to reach an effect-directed analysis of toxicity drivers. To date, the combination of chemical and

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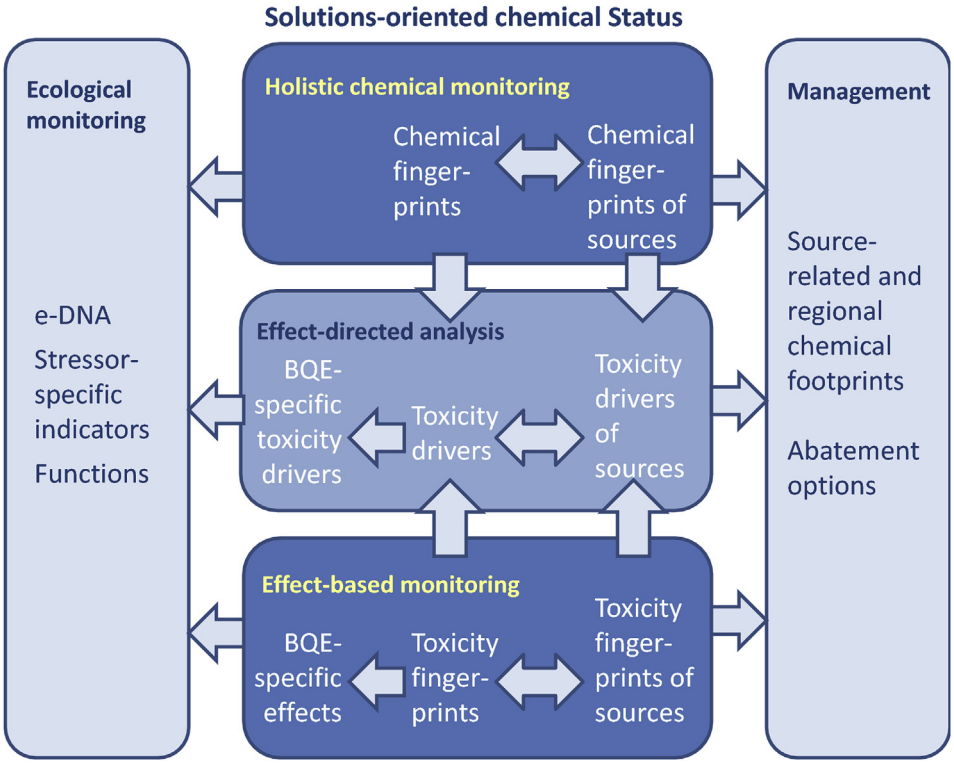

Fig. 1. Holistic and solution-oriented procedure of water quality monitoring (Brack et al., 2018). BQE: Biological Quality Elements.

biological analysis is the only possibility to create a holistic monitoring tool that meets all the mentioned requirements.

This review article presents current methods from the field of effect based as well as instrumental analysis for a holistic evaluation of modern advanced waste water treatment technologies and corresponding water bodies with regard to the water framework directive.

2. Advanced waste water treatment technologies for removal of micropollutants

Contaminants of emerging concern (CECs) can reach surface waters in ecologically relevant concentrations of up to several micrograms per liter (UBA, 2015). Major point sources for CEC are municipal waste water treatment plants (WWTPs). With the conventional set-up of today's municipal WWTPs only biodegradable organic compounds (C removal) and nutrients are eliminated sufficiently. The initial micropollutants concentration can be reduced to a certain degree, however, at the residual low concentrations substances with a high bioactive potential are still a risk for the aquatic environment (Escher et al., 2011; Luo et al., 2014). Among different pharmaceutical agents, endocrine active substances (EAS) build a relevant group in risk assessment, because of their impact on the hormone and reproduction system of organisms in the low ng/L or sub ng/L range (Kidd et al., 2007, 2014). EAS cover different chemical and physical properties, biodegradation behavior and toxic potentials, resulting in a challenging task to analyze, eliminate and assess this substance group in the water cycle.

To reach the goals of the WFD and to ensure a sustainable water management in future especially for highly populated areas and areas with a dominating load of waste water into surface water, new advanced treatment technologies are necessary in WWTPs to reduce the discharge of micropollutants and potential ecological threats.

Potential new technologies, which can be implemented as advanced treatment step for removal of micropollutants in WWTPs, are, for example, ozonation, reverse osmosis or the use of activated carbon, which is also applied in drinking water production (advanced oxidation systems based on microorganisms and/or oxidative enzymes are also applicable but technically still under development). Due to high costs for filtration processes, oxidative and sorption processes were further investigated in the last decades for waste water treatment. Examples for oxidative processes are UV-irradiation, which can either react by direct photolysis or selective bond cleavage, or the addition of ozone, which reacts selective with electron rich moieties or nonspecific by forming radicals in presence of organic matter (Gligorovski et al., 2015; Lee and von Gunten, 2010). In some pilot scale studies, the combination of different reagents to enhance radical formation was investigated like UV/H_2O_2, H_2O_2/O_3 or UV/H_2O_2/O_3 resulting in so called advanced oxidation processes (AOPs) (Bielak et al., 2015; De la Cruz et al., 2013; Salimi et al., 2017). As such combinations are difficult to scale up especially for UV due to high particle load and therewith inefficient reaction rate with the target micropollutant as well as cost aspects, mainly the application of ozone and activated carbon is tested in most of the full-scale investigations today (Audenaert et al., 2014; Mahamuni and Adewuyi, 2010; Margot et al., 2013; Rechenberg, 2015; Sperlich and Gnirß, 2016). The decision to use ozonation or activated carbon adsorption for micropollutant removal is often based on technical reasons, such as the pre-equipment of the wastewater treatment plant or the available space.

However, in oxidative processes such as ozonation there is no complete mineralization of micropollutants. Instead, micropollutants get transformed into new structures, which might even increase the toxic effects (Knoop et al., 2017; Knopp et al., 2016; Schlüter-Vorberg et al., 2015). However, in most studies no increase in toxicity by formed TPs is documented (Richard et al., 2014). The knowledge about the effects of such transformation products (TPs) on aquatic ecosystems is still limited. In a few cases higher or at least similar toxicological potentials of TPs compared to the parent substance were observed (Chen et al., 2012). Due to uncertainties of toxicity by TPs formed during ozonation, a further biological treatment or sorption by activated carbon is recommended to subsequently remove the produced substances (Hollender et al., 2009; Liu et al., 2017). Due to the fact that oxidized molecules are more polar, they exhibit a higher bioavailability (Hübner et al., 2014). Biological filters are one option to reduce TPs and

were already tested in the Swiss project "Re-treat" and are recommended within the Swiss water protection ordinance (Böhler et al., 2017; Bourgin et al., 2018; GschV 814.201 2016). As TPs potentially exhibit a higher bioavailability, the assimilable organic carbon (AOC) fraction of the dissolved organic carbon (DOC) could be a further important indicator parameter to assess the removal effectivity of a certain post-treatment (van der Kooij, 1992). The AOC analysis method was originally developed to determine the biological regrowth potential of freshwater distribution networks (van der Kooij et al., 1989; van der Kooij, 1992). The correlation of AOC and ozone dose was already observed during ozonation of drinking water, indicating the correlation to formed TPs (Escobar and Randall, 2011; van der Kooij et al., 1989). As the AOC analysis method is based on isolated drinking water specific organisms (*Pseudomonas fluorescens* P17 and *Spirillum* species NOX) the influence of high DOC containing waste water samples remains a challenge. However, in a study by Bourgin et al. (2018) three different biological waste water post-treatment systems were successfully investigated using the AOC method by van der Kooij. They demonstrated the usability of the AOC as parameter for the characterization of a post-treatment in terms of transformation product elimination.

In addition to advanced post-treatment steps a holistic evaluation of the effluent is essential to prove an effective removal of harmful substances. (Bio-)Analytical tools for that task are introduced in the next sections.

3. Analytical tools for water quality analysis

3.1. Target analysis

Known compounds and their fate during a specific treatment technology can be analyzed by chemical target analysis. Especially regulatory requirements are, up to now, based on specific indicator parameters. In the European Union the Water Framework Directive (WFD) was set in 2000 to assess to status of all surface water bodies in the EU (EU, 2000/60). To fulfill the requirements of the EU WFD, environmental quality standards (EQS) for compounds listed in the annex X have to be met (EU, 2008/105). For various compounds sensitive analytical methods have been developed, but still the detection of the estrogenic compounds estrone (E1), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) regarding the requirements of the EU WFD is a difficult task with state-of-the-art analytical procedures, especially for EE2 (Itzel et al., 2018,2019; Locatelli et al., 2016). In a report by the Joint Research Council (JRC), literature was screened to find a suitable method reaching the required limits of detection (LODs) within the EQS of 35 pg/L for EE2 and 0.4 ng/L for E1 and E2 (EU, 2018/840; Loos, 2015). In this report, it was mentioned that an enrichment step by solid phase extraction (SPE) seems to be necessary in general to be able to reach the required LODs (Loos, 2015). In a current JRC report besides SPE-LC-MS/MS, different sample enrichment methods such as liquid/liquid extraction in combination with GC-MS/MS were demonstrated to reach a LOD of 30 pg/L for EE2 in some laboratories in the EU (Loos et al., 2018). The difficulty in the analysis of EE2 in the low pg-range lies in the separation of the matrix during sample preparation (Locatelli et al., 2016). Numerous studies were able to demonstrate that the sensitivity could be significantly increased by an appropriate matrix separation following SPE (Huang and Sedlak, 2001; Schlusener and Bester, 2005; Ternes et al., 1999; Williams et al., 2012). Since modern analytical systems such as LC-MS/MS or GC-MS/MS in principle are able to detect the required low concentrations, current research is focused on appropriate matrix separation through various clean-up procedures followed by SPE. However, to be able to quantify and assess EE2 at a concentration of 35 pg/L, the required LOD is consequently 1/3 of the EQS resulting in 10 pg/L (2009/90/EC). In terms of ozonation transformation products, there are also some known toxic TPs like bromate or N-Nitrosodimethylamine (NDMA) (Lee et al., 2007). These known compounds are good parameters to evaluate a certain treatment. In a study by Böhler et al. (2017) it was shown that NDMA was reduced by a biological sand filtration by 65%, showing that such post-treatments are a useful removal technique. However, such an evaluation by target compounds is of a certain risk due to uncertainties by unknown compounds and TPs, which might have similar toxic effects, but are not analyzed yet.

3.2. Non-target analysis

There are more than 100.000 substances registered under EU REACH and many of them are detectable as target compounds at very low concentrations in the water cycle when using modern analytical devices (European Chemical Agency, 2018). To be able to measure relevant biological effects, there is the need to understand how these different compounds are able to interact with organisms. As this information is not known in most cases, unspecific non-target analysis can be applied to detect as many features as possible. Features are peak-shaped signals, which are defined by their accurate mass (m/z) and retention time (RT) and meet the selected criteria for peak detection (e.g. intensity threshold). Thus, transformation products formed during oxidative waste water treatment can be detected without reference standards. The relevance of the TPs or the whole treated sample can only be investigated by combining non-target screening approaches with effect-based methods for correlation with toxic effects (Schmidt, 2018). This approach was used in several studies (Ihara et al., 2014; Itzel et al., 2018,2019; Muschket et al., 2018; Weiss et al., 2011). But even with classical non-target approaches, only substances in a so called "analytical window" can be detected with the respective screening method. Besides multidimensional LC to create a higher peak capacity and broader selectivity, it may be necessary to develop couplings of GC and LC in order to detect more substances by instrumental methods if used in combination with high-resolution mass spectrometry (HRMS) (Leonhardt et al., 2015). At present, there is still a considerable need for research in this area as such couplings have not yet been established in this context. So, it is important to note that the "analytical window" during non-target analysis has to be selected according to the respective research question.

As example, it has become obvious that there is a high variation of compounds, which are relevant for antagonistic endocrine effects, like PCBs (Kortenkamp et al., 2014; Li et al., 2013), insecticides (Orton et al., 2011, 2012), flame retardants (Zhang et al., 2014; Itzel et al., 2018,2019) or anti-inflammatory drugs such as diclofenac (Ezechiáš et al., 2016). It is necessary to combine different analytical methods like gas chromatography (GC) and liquid chromatography (LC) to discriminate as many compounds as possible by the choice of the method (Fig. 2).

Besides the exclusion of compounds by the choice of GC or LC, there

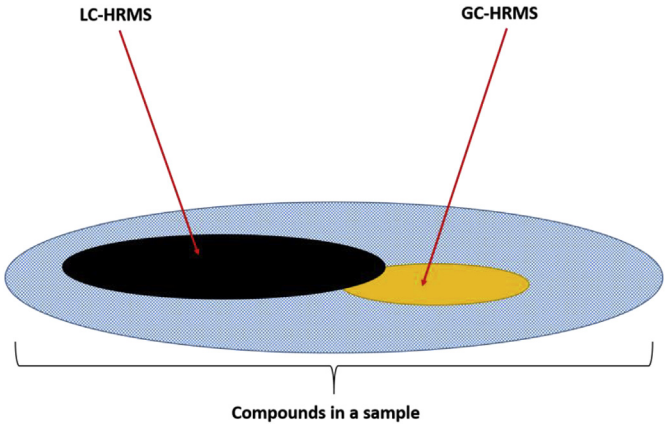

Fig. 2. Analytical window using LC- and GC-HRMS non-target screening methods.

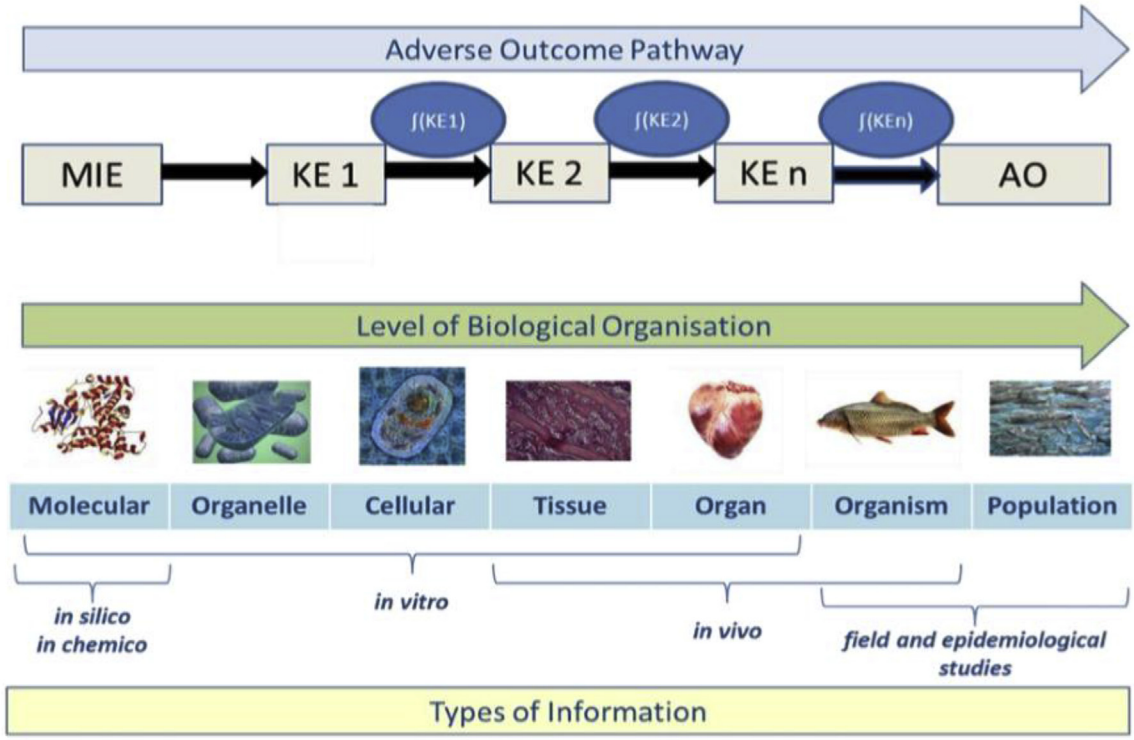

Fig. 3. An Adverse Outcome Pathway (AOP) allows for the mapping, organization and integration of various types of information, ranging from *in silico* and *in chemico* data to field study data, around the Molecular Initiated Event (MIE), Key Events (KEs) and the Adverse Outcome (AO).

are still undetected compounds illustrated by the blue area in Fig. 2. These are "gap" compounds which are highly polar and hardly amendable to GC or commonly applied reversed phase LC. In this area persistent and mobile organic chemicals tend to fall, which are passing through water treatment processes as well (Reemtsma et al., 2016).

To directly use information of un-identified features from non-target screening, trend analysis by *m/z* plots without known compound information can be used to prioritize detected features (Jekel, 2016). Therewith, it is possible to evaluate treatment technologies by a broad range of detected compounds. For example, it would be possible to detect formed transformation products after ozonation and their removal by subsequent post-treatment steps (Schmidt et al., 2014).

4. Effect-based methods for assessment of water quality

Although modern instrumental analysis has been advanced with excellent sensitivity and precision for monitoring of targeted compounds, this approach provides little information on the potential adverse biological effects of complex mixtures under field conditions. Therefore, activity assays that can quantify overall toxic potential of complex mixtures, including cumulative effects, and potency of the compound would be essential to evaluate total environmentally active potentials. In the past decades, *in vitro* and *in vivo* bioassays were developed. The rapid response and reduction of instrument requirements of cell-based bioassays make them an attractive alternative for environmental monitoring. The *in vitro* approach for detection of endocrine active compounds is under discussion to be implemented into the EU WFD (Könemann et al., 2018).

All bioassays have in common that they measure effects in cells or organisms caused by the surrounding conditions. However, the application of bioassays can be very variable. When choosing bioassays for detection of biological effects, a focused research question is essential to prepare an appropriate test design consisting of specific biotests or a complex test battery, *in vitro* or *in vivo* tests, sample preparation techniques etc. Different bioassays measure different toxicological endpoints including cytotoxicity, genotoxicity, endocrine disruption and stress response. Usually those endpoints can be measured both *in vitro* and *in vivo* and with different test organisms, providing specific advantages. *In vitro* investigations are able to explain mechanistic and physiological correlations between CEC and their biological activity, whereas *in vivo* studies provide a closer comprehension of actual ecological and health impacts. Although bioassays give overall effects, some tests can specifically measure initial events affecting toxicity, i.e. receptor binding. Basically, receptor binding assays (or reporter gene assays) can detect much lower substance concentrations than whole cell bioassays, but also the regarded endpoint and used cells determine the detection limits. In water analysis, the detection limit of bioassays can be improved by enrichment techniques similar to those in analytical chemistry. However, enrichment and clean-up methods do not only eliminate undesired matrix effects, but obviously change the original sample. Here again, the research question is important: should substance groups be analyzed using an enrichment step to increase selectivity and detection limit, or should the original sample be analyzed for toxic effects in order to carry out an analysis of the entire water sample? Endocrine disrupting effects can be effectively analyzed with reporter gene assays, based on human cell lines (e.g. CALUX assay) or yeast cells (yeast screen assay). Recently, an ISO guideline was released, providing standard protocols for the determination of estrogenic potentials of water samples (Hettwer et al., 2018; ISO, 19040:2018). Investigations at WWTPs confirm the applicability of these tests for waste water analysis and the assessment of treatment efficiency at different process steps (Gehrmann et al., 2018; Itzel et al., 2017). It was shown in different studies, that EAS and their effects, especially estrogens, in waste water are effectively reduced by ozonation and activated carbon filtration (Gehrmann et al., 2018; Stalter et al., 2011). The above mentioned formation of transformation products after ozonation is a minor issue in bioassay, because unknown endocrine active transformation products are detected the same way as the parent substances based on their biological effects.

In the European projects DEMEAU and SOLUTIONS (EU FP7

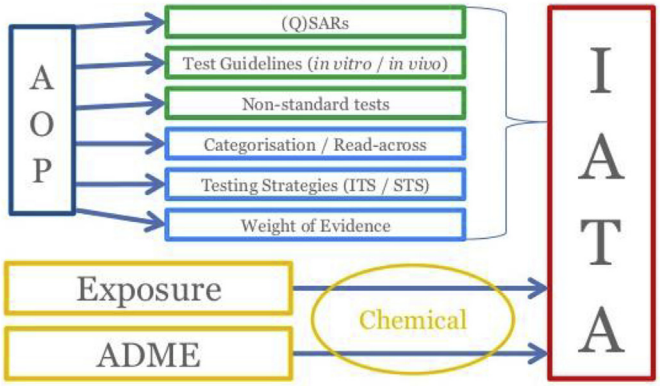

Fig. 4. Integrated Approach to Testing and Assessment (IATA) via AOP information, exposure and metabolisms (ADME). (EC, 2016).

DEMEAU, SOLUTIONS), the most relevant modes of action and corresponding toxicological endpoints for application in drinking water and its sources were selected based on bioactivity of chemicals present in different types of water samples. For the measurement of bioactive potentials available high-throughput bioassays as indicated by a large inter-laboratory study using 103 different *in vitro* bioassays studying a broad range of toxicity pathways, were applied (Escher et al., 2014). This study demonstrated that the most responsive toxicity pathways were related to xenobiotic metabolism, modulation of hormone systems, reactivity and adaptive stress responses.

To integrate and evaluate existing information about toxicity pathways of substances in a structured way, the adverse outcome pathway (AOP) concept is recommended by the European Commission (EC, 2016). An AOP is a logical sequence of key events (KEs) triggered by chemical exposure and occurring at the molecular, cellular, organ, whole organism or population level (Fig. 3). These KEs are causally linked to the adverse outcome (AO) under consideration, and are measurable (e.g. by bioassays).

All information of the AOP will finally result in an Integrated Approach to Testing and Assessment (IATA) (Fig. 4). IATA can include a combination of methods and can be informed by integrating results from one or many methodological approaches [(Q)SAR, read-across, in chemico, *in vitro*, ex vivo, *in vivo*] or omic technologies (e.g. toxicogenomics). An OECD guidance document on the use of the Adverse Outcome Pathways in developing IATA was established in 2016 (EC, 2016).

Besides classical effect based bioassays, which are modified to give a response to specific substance classes, microbial test systems could be helpful in evaluation of new waste water treatment technologies as well. As mentioned in the previous subdivisions, ozonation seems to be the technology of choice in many cases. However, because of formed TPs during the oxidative treatment, an integrated approach with effect-directed analysis (EDA) is necessary.

5. Integrated approach of NTS and EDA

As described above, non-target screening (NTS) has been suggested as a possible option to identify yet unknown chemical constituents in complex aqueous mixtures. However, NTS suffers from the same challenges in extraction/concentration as *in vitro* bioassay approaches since the analytical window is already specified in certain limits. In addition, NTS generally requires far more expensive instrumentation (use of high resolution mass spectrometer – HRMS) with an even greater level of expertise needed to operate the instruments and interpret the resulting data. With high-resolution data, the first major challenge is to select and focus on relevant signals. Since thousands of signals are detected and manual interpretation is unavoidable, tools to filter relevant signals are very important. Therefore, methods in the sample preparation as well as particularly the development of intelligent software solutions are helpful. Furthermore, iterative approaches whereby bioassay data is used in combination with chemical fractionation to better separate portions of complex chemical mixtures for further characterization offer tremendous promise (Brack et al., 2016; Desbrow et al., 1998; Itzel et al., 2018,2019; Muschket et al., 2018). Effect-directed analysis (EDA) is an approach connecting effect-based analysis and chemical analysis of whole samples and fractions of samples to identify those fractions, or even compounds, of a sample, which cause specific bioactivities. Thus, when positive bioactivity is detected, the sample can be fractionated by polarity or molecular weight, then each fraction re-evaluated using the particular bioassay and instrumental analysis. In this way, a much narrower field of potential substances can be isolated and better evaluated for possible identification (Altenburger et al., 2015; Brack, 2003, 2011; Brack et al., 2007, 2016; Burgess et al., 2013; Jia et al., 2016; Muschket et al., 2018; Schmidt et al., 2014; Tousova et al., 2017).

There is significant scientific interest in developing a framework to enable the use of *in vitro* bioassays to assess not just the quality, but the safety of drinking water. For example, the BRAVE initiative (http://www.bravebioassays.info) is supported by various water research providers (Water Environment and Reuse Foundation, Global Water Research Coalition, Australian Water Recycling Centre of Excellence, Water Research Australia, PUB Singapore) to develop a research map towards this goal. The think-tank has identified four main areas to focus efforts on, including 1) the identification of adverse effect endpoints relevant to water consumption, 2) the translation of *in vitro* responses to *in vivo* effects, 3) the conversion of *in vitro* concentration to *in vivo* exposure dose, and 4) the adaptation of mixture modelling to support each of these steps.

While some progress has already been made in some of these areas (e.g., Benigni, 2012; Combes, 2012; Escher et al., 2014, Punt et al., 2013; Sonneveld et al., 2011; Wetmore, 2015), *in vitro* bioassays alone are not capable of assessing the safety of water. But that should not negate the fact that *in vitro* bioassays can expand our analytical universe further into the unknown, thereby providing a more comprehensive evaluation of water quality and more rapidly identifying substances which may pose a risk to public health.

6. Outlook

While *in vivo* bioassays are already applied in surface and wastewater regulations (e.g., the German waste water ordinance (“Deutsche Abwasserverordnung”), US EPA's Whole Effluent Toxicity (WET) testing requirements, and Direct Toxicity Assessment (DTA) in the Australia-New Zealand Guidelines for Fresh and Marine Water Quality), to the authors' knowledge there are no current regulatory applications of *in vitro* bioassays for water quality assessment. However, numerous expert panels have suggested that *in vitro* bioassays should be employed for monitoring of water quality (EPHC/NHMRC/NRMMC 2008; Drewes et al., 2018; WHO, 2017). In fact, a recent expert panel report from the State of California, USA, has indicated that in their expert opinion, bioassays including the estrogen receptor (ER) and aryl hydrocarbon receptor (AhR) already are sufficiently developed for immediate implementation as monitoring tools for recycled water (Drewes et al., 2018).

The California expert panel stated that, “The Panel recommends that the Estrogen Receptor alpha (ER-α) and the Aryl hydrocarbon Receptor (AhR) bioassays be used to respectively assess estrogenic and dioxin-like biological activities in recycled water.” Moreover, the panel further stated that those two bioassays were recommended because “each have clear adverse outcome pathways (AOP) that allow specific molecular responses to be adequately standardized for screening recycled water quality at potable reuse projects.” However, the panel recognized that a process to respond to positive results was not yet “sufficiently mature” and thus recommended that implementation should only cover monitoring/data collection at this point in time.

Beyond the California expert panel, other step-wise frameworks have been suggested to help water utilities understand how to apply and use the results in bioanalytical tools in water quality monitoring. All reasonable suggestions so far recommend the use of *in vitro* bioassays to detect unexpected contaminants in addition to available and emerging chemical methods, and not as stand-alone parameters with hard numerical determinants of quality. In addition, the application of *in vitro* bioassays also provides a more comprehensive view of the chemical mixture biological activity, which is not accounted for analytical monitoring. The proposed effects-based trigger values provide a way to anchor bioassay responses to a chemical context, and thus determine when bioassay results warrant further investigation by chemical, technical or operational means (Carere, 2018; Snyder and Leusch, 2018).

Taken together, *in vitro* bioassays can guide both targeted and non-targeted instrumental analyses in a process called effect-directed analysis to identify and prioritize contaminants. While these tools alone are not yet appropriate to determine whether water is “safe” or “unsafe”, the historical use of animal models to screen the complex mixtures of chemicals in drinking water is vastly infeasible and, as is increasingly recognized, of limited applicability to human health outcomes. Thus, the application of bioanalytical tools for water quality screening is a step forward to increase our understanding of the risks associated with mixtures of chemicals in water.

7. Conclusion

To date, the only possibility to create a holistic monitoring tool that meets all requirements is the combination of chemical and biological analysis.

The advanced waste water treatment enables a reduction of micropollutant inputs and thus contributes to the protection of our valuable water resources. This point source approach is an important building block in achieving a good chemical and biological status of our water bodies.

However, the investigations of different WWTPs, which were equipped with an advanced wastewater treatment for micropollutant removal, show that not all substances can be eliminated and unknown transformation products can be released.

Therefore, an integrated approach using chemical and effect-based methods is necessary for evaluation of the water quality.

To evaluate the biological activity of post-treatments or similar applications, for example for river sediments, the combination of biological (AOC) and non-target chemical screening has been shown to be a powerful tool.

To integrate and evaluate existing information about toxicity pathways of substances in a structured way, the Adverse Outcome Pathway (AOP) concept is recommended.

Information obtained from different bioassays are combined in an AOP.

The combination of all available information (AOP, exposure information, metabolism etc.) results in an integrated approach to testing and assessment (IATA). This approach is supported by the European Environmental Agency and will be integrated in regulatory processes in future.

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