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Mixture effects in samples of multiple contaminants – An inter-laboratory study with manifold bioassays

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ABSTRACT

Chemicals in the environment occur in mixtures rather than as individual entities. Environmental quality monitoring thus faces the challenge to comprehensively assess a multitude of contaminants and potential adverse effects. Effect-based methods have been suggested as complements to chemical analytical characterisation of complex pollution patterns. The regularly observed discrepancy between chemical and biological assessments of adverse effects due to contaminants in the field may be either due to unidentified contaminants or result from interactions of compounds in mixtures. Here, we present an interlaboratory study where individual compounds and their mixtures were investigated by extensive concentration-effect analysis using 19 different bioassays. The assay panel consisted of 5 whole organism assays measuring apical effects and 14 cell- and organism-based bioassays with more specific effect observations. Twelve organic water pollutants of diverse structure and unique known modes of action were studied individually and as mixtures mirroring exposure scenarios in freshwaters. We compared the observed mixture effects against component-based mixture effect predictions derived from additivity expectations (assumption of non-interaction). Most of the assays detected the mixture response of the active components as predicted even against a background of other inactive contaminants. When none of the mixture components showed any activity by themselves then the mixture also was without effects. The mixture effects observed using apical endpoints fell in the middle of a prediction window defined by the additivity predictions for concentration addition and independent action, reflecting well the diversity of the anticipated modes of action. In one case, an unexpectedly reduced solubility of one of the mixture components led to mixture responses that fell short of the predictions of both additivity mixture models. The majority of the specific cell- and organism-based endpoints produced mixture responses in agreement with the additivity expectation of concentration addition. Exceptionally, expected (additive) mixture response did not occur due to masking effects such as general toxicity from other compounds. Generally, deviations from an additivity expectation could be explained due to experimental factors, specific limitations of the effect endpoint or masking side effects such as cytotoxicity in in vitro assays. The majority of bioassays were able to quantitatively detect the predicted non-interactive, additive combined effect of the specifically bioactive compounds against a background of complex mixture of other chemicals in the sample. This supports the use of a combination of chemical and bioanalytical monitoring tools for the identification of chemicals that drive a specific mixture effect. Furthermore, we

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demonstrated that a panel of bioassays can provide a diverse profile of effect responses to a complex contaminated sample. This could be extended towards representing mixture adverse outcome pathways. Our findings support the ongoing development of bioanalytical tools for (i) compiling comprehensive effect-based batteries for water quality assessment, (ii) designing tailored surveillance methods to safeguard specific water uses, and (iii) devising strategies for effect-based diagnosis of complex contamination.

1. Introduction

The provision of clean water for ecosystems and humans is central for reaching all of the United Nations sustainable development goals (UNEP, http://web.unep.org/post2015/). Faced with a rapidly accelerating increase in chemical innovation, production, consumption and emission, and a growing world population with increasing demands, safeguarding the quality of surface waters has becomes a major challenge (Schwarzenbach et al., 2006). Two complementary approaches have been developed to deal with unwanted chemical contamination. In prospective risk assessment potential environmental risks are assessed by comparing predicted environmental chemical exposure with expected adverse effects based on prior information on compound toxicities and other properties. In monitoring efforts, we seek to screen relevant contaminations in the environment. Both approaches rely strongly on a perspective that focuses on single chemicals, one-by-one, falling short of the reality of contamination of many environmental systems with complex mixtures of chemicals (Loos et al., 2009; Brack et al., 2015; Escher et al., 2013a).

Prospective chemical assessment dealing with mixture exposures and their potential combined effects has progressed considerably (Deneer, 2000; Altenburger and Greco, 2009). A component—based approach which seeks to predict the toxicity of mixtures on the basis of the effects of its components has gained substantial empirical support and is now widely accepted (Kienzler et al., 2016). In routine environmental monitoring, by contrast, exposure-oriented chemical analytical studies and biology-focused investigations are completely separate activities. Under the water framework directive (WFD, 2000) indicators of chemical and ecological quality are regarded as two separate, poorly connected categories. Causal links between chemical exposures and ecological effects are often discussed from a single cause-effect perspective, with a focus on single chemicals, but do not consider the occurrence of multiple chemicals as mixtures, multiple stress factors and their combined effects. The integration of bioassays as effect-based methods in environmental monitoring is intended to bridge this gap, supporting the identification of mixture exposures (Altenburger et al., 2015; Wernersson et al., 2015; Brack et al., 2017).

In a ring trial, Carvalho et al. (2014) investigated two mixtures of substances of concern. Using a panel of 35 different bioassays, mixtures with components at their individual environmental quality standard level (EQS) were shown to elucidate effects in several of the assays. These findings and earlier reviews demonstrated that regulatory single-chemical threshold values may not be fit for purpose to protect against mixture exposure (Carvalho et al., 2014; Kortenkamp et al., 2009). Schoenfuss et al. (2015) studied mixtures of pharmaceuticals at environmentally relevant concentrations together with effluent exposures by using various effect biomarkers in fish. The authors interpreted their observations as interactions between contaminants in the mixture, however, without reference to an expected additive effect of the combination.

Case studies of extracted freshwater samples using chemical and bioanalytical analysis have demonstrated that bioassays can provide complementary information for water monitoring. For instance, the pattern of bioassay responses obtained across 22 sites stretching across a major part of the river Danube resembled well those of chemical analytical concentrations of target chemicals (Neale et al., 2015). Further, a comparison of bioassay effects with samples upstream a effluent outlet, downstream and with the effluent itself with measured chemicals and their effects consistently showed an increased impact of effluents from wastewater treatment plants at tributaries of the Rhine (Neale et al., 2017a, 2017b) and river Danube (König et al., 2017). When the combined effects are expressed as the sum of bioanalytical equivalent concentrations for quantified chemicals and are then compared to the actually observed effects in environmental samples the findings can be separated into two groups: First, there are assays indicative of highly specific receptor-mediated effects such as algal photosynthesis inhibition, or binding to the estrogen receptor. In these assays, most of the observed bioactivity can be explained in terms of the detected photosystem II inhibiting herbicides or natural estrogens, respectively. Second, with assays sensitive to more general effects triggered by many different chemicals, such as cytotoxicity and induction of oxidative stress response there is an explanation gap of effects that remain unaccounted for. Thus, it is sometimes difficult to explain observed mixture effects using component-based mixture effect prediction. Potential reasons might be due to compounds that were overlooked in the chemical target analyses (Escher et al., 2013a) or to an inaccurate quantification of bioactive concentrations close or below the analytical detection limit, such as for potent xenoestrogens. Furthermore, our current knowledge of the components' bioactivities in specific assays (Neale et al., 2017a, 2017b) and the validity of common mixture effect concepts under conditions of complex exposure need to be scrutinized (Altenburger et al., 2004).

The objective of this study was to verify the ability of a suite of bioanalytical tools to detect bioactivity of specific compounds in a mixture exposure setting against a background of co-occurring water contaminants. We extend previous work (Busch et al., 2016; Neale et al., 2017a, 2017b) by rigorous investigation of the ability of a panel of bioassays to detect joint bioactivities in a mixture of chemicals with diverse modes of actions (MoAs). To achieve our aims, we (i) defined a bioassay panel comprising assays for detection of different key events and apical endpoints (Altenburger et al., 2015; Neale et al., 2017a, 2017b), and (ii) utilised a component-based mixture prediction approach with best-fit modelling of concentration effect relationships (Scholze et al., 2001, 2014). We designed a mixture of twelve compounds with anticipated non-similar modes of actions in two different mixture ratios with the aim of studying (a) the detectability of combined effects against a background of components presumed to be inactive, (b) the ability to capture relevant bioactivities at mixture compositions that may occur in environmental exposures. Results were assessed by comparing predicted and observed combined effects for each assay and through mapping against the expected occurrence of specific biological effects (key events). By testing the same two mixtures in different bioassay we were able to assess the performance of different bioassays for complex exposure analysis and gained an impression of the usefulness of response data for individual compounds for predicting mixture effects in environmental exposure scenarios.

2. Materials and methods

2.1. Approach

For our round robin mixture effect study we started with single compound testing using 21 different bioassays. The compounds to be characterised by individual concentration-effect relationships were a subset of chemicals of the chemical fingerprinting effort described in Neale et al. (2017a). Components for the mixture testing were selected