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Mixture effects of organic micropollutants present in water: Towards the development of effect-based water quality trigger values for baseline toxicity

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ABSTRACT

In this study we propose for the first time an approach for the tentative derivation of effect-based water quality trigger values for an apical endpoint, the cytotoxicity measured by the bioluminescence inhibition in *Vibrio fischeri*. The trigger values were derived for the Australian Drinking Water Guideline and the Australian Guideline for Water Recycling as examples, but the algorithm can be adapted to any other set of guideline values. In the first step, a Quantitative Structure–Activity Relationship (QSAR) describing the 50% effect concentrations, EC_{50}, was established using chemicals known to act according to the nonspecific mode of action of baseline toxicity. This QSAR described the effect of most of the chemicals in these guidelines satisfactorily, with the exception of antibiotics, which were more potent than predicted by the baseline toxicity QSAR. The mixture effect of 10–56 guideline chemicals mixed at various fixed concentration ratios (equipotent mixture ratios and ratios of the guideline values) was adequately described by concentration addition model of mixture toxicity. Ten water samples were then analysed and 5–64 regulated chemicals were detected (from a target list of over 200 chemicals). These detected chemicals were mixed in the ratios of concentrations detected and their mixture effect was predicted by concentration addition. Comparing the effect of these designed mixtures with the effect of the water samples, it became evident that less than 1% of effect could be explained by known chemicals, making it imperative to derive effect-based trigger values. The effect-based water quality trigger value, EBT-EC_{50}, was calculated from the mixture effect concentration predicted for concentration-additive mixture effects of all chemicals in a given guideline divided by the sum of the guideline concentrations for individual components, and dividing by an extrapolation factor that accounts for the number of chemicals contained in the guidelines and for model uncertainties. While this concept was established using the example of Australian recycled water, it can be easily adapted to any other set of water quality guidelines for organic micropollutants. The cytotoxicity based trigger value cannot be used in isolation, it must be applied in conjunction with effect-based trigger values targeting critical specific modes of action such as estrogenicity or photosynthesis inhibition.

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1. Introduction

Organic micropollutants are omnipresent in our sewage, aquatic ecosystems and drinking water (Schwarzenbach et al., 2006). Although organic micropollutants occur typically at very low concentrations, they are numerous and can be transformed by biotic and abiotic transformation processes (Escher and Fenner, 2011), creating complex mixtures of unknown composition. There are regulations and water quality guidelines for individual chemicals in different water types available in many countries (for an overview see Escher and Leusch, 2011) and there is some guidance for including mixtures into Fresh and Marine Water Quality (ANZECC/ARMCANZ, 2000) and for the risk assessment of chemicals (USEPA, 2002; EU Council, 2009). Nevertheless there exist no effect-based water quality trigger values relating to simple screening type bioassays for cytotoxicity.

The field of mixture toxicity assessment has matured over the last decade (as reviewed by Kortenkamp et al. (2009)). From designed mixture toxicity studies, we have learnt that even if single chemicals are present below concentrations that cause a visible effect, they may contribute to the mixture effect (Silva et al., 2002; Kortenkamp et al., 2009). There is also ample experimental evidence that the mixture toxicity model of concentration addition (CA), which is strictly only valid for chemicals that have the same mode of toxic action, gives robust and accurate predictions for many multicomponent mixtures. The alternative concept of independent action (IA) holds for chemicals with dissimilar modes of action. For multicomponent mixtures the two mixture models of CA and IA often give fairly similar predicted effects although the subtle differences can be used as a diagnostic tool for mode-of-action analysis (Backhaus et al., 2000, Kortenkamp et al., 2009). Further, mixture effects of chemicals combined in ratios as they were found in environmental samples could be satisfactorily predicted by IA and CA (Altenburger et al., 2004; Junghans et al., 2006). Therefore it has been proposed to apply CA as a precautionary first tier in environmental risk assessment of mixtures (Posthuma et al., 2008; Backhaus and Faust, 2012).

In vitro cell-based bioassays have been widely and successfully applied for water quality monitoring, benchmarking of water quality and assessment of treatment technologies in a research context (Escher and Leusch, 2011) but they have not been used for regulatory purposes. The bioluminescence inhibition assay with *Vibrio fischeri* and other related bioluminescent bacterial assays have been used for many years to assess water quality (Johnson, 2005; ISO11348-1 2007) due to their ease of operation, rapidity and high sensitivity to organic chemicals and because their effect concentrations are highly correlated to other aquatic toxicity endpoints (e.g., Kaiser, 1993; 1998). The bioluminescence inhibition assay with *V. fischeri* has also been widely used to test mixture toxicity hypotheses (Altenburger et al., 2000; Backhaus et al., 2000) and to develop Quantitative Structure–Activity Relationships (QSARs) for the prediction of effect concentrations of untested chemicals using the octanol–water partition coefficient of the chemicals (selected examples are (Cronin and Schultz, 1997; Zhao et al., 1998; Vighi et al., 2009)).

Effect-based trigger values provide the opportunity to integrate mixtures into water quality assessment. Trigger values are numerical values that indicate an acceptable risk to the environment or human health provided they are not exceeded. The classical approach to setting effect-based trigger values would relate the outcomes of *in vitro* bioassays directly to adverse health outcomes but *in vitro* to *in-vivo* extrapolations have many limitations. Therefore we propose as an alternative approach to translate existing individual chemical based water quality guideline values directly to effect-based trigger values (Fig. 1).

In a first step we tested if chemicals typically encountered in water samples will fit QSAR models developed with known baseline toxicants (Section 3.1) and if the mixture effect of large numbers of chemicals commonly occurring in water, mixed in equipotent concentration ratios (Section 3.2) and in water quality guideline concentrations ratios (Section 3.3), can be predicted by the CA model of mixture toxicity. From these models we computed tentative effect-based trigger values (Section 3.4). We then validated the proposed approach using a diverse set of water samples, where we assessed both the effect with the bioluminescence inhibition assay with *V. fischeri* and quantified 269 chemicals analytically (Section 3.5). We mixed the detected chemicals in their encountered concentration ratios and called them "iceberg mixtures" (strictly speaking they should be called "tip-of-the-iceberg mixtures") as they constitute the known chemicals (tip of the iceberg) among the unknown complex mixture of chemicals in environmental samples (immersed part of the iceberg) together causing the observed mixture effect in an environmental water sample. The iceberg mixtures were tested for compliance with mixture toxicity predictions (Section 3.6) and it was independently assessed how much of the measured effect can be explained by the analytically quantified chemicals (Section 3.7).

As a case study we used water quality data and guideline values from Australia but the concepts are generic and can be

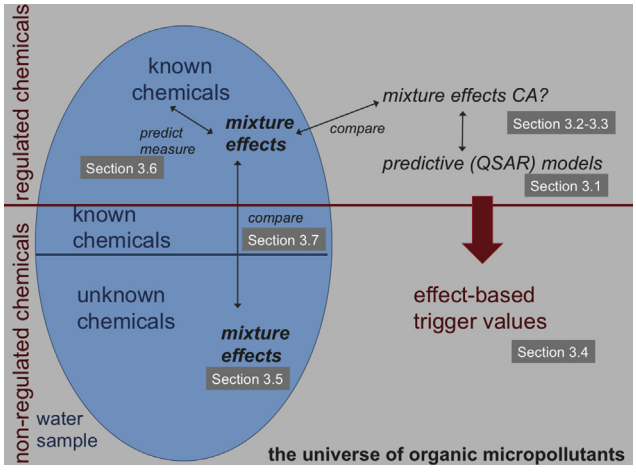

Fig. 1 – Approach taken in this paper to evaluate the contribution of known and unknown chemicals in a water sample and to derive effect-based trigger values, with paper sections where the different points will be addressed.