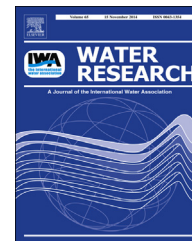




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Biological plausibility as a tool to associate analytical data for micropollutants and effect potentials in wastewater, surface water, and sediments with effects in fishes

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ABSTRACT

Discharge of substances like pesticides, pharmaceuticals, flame retardants, and chelating agents in surface waters has increased over the last decades due to the rising numbers of chemicals used by humans and because many WWTPs do not eliminate these substances entirely. The study, results of which are presented here, focused on associations of (1) concentrations of micropollutants in wastewater treatment plant (WWTP) effluents, surface waters, sediments, and tissues of fishes; (2) results of laboratory biotests indicating potentials for effects in these samples and (3) effects either in feral chub (*Leuciscus cephalus*) from two German rivers (Schussen, Argen) or in brown trout (*Salmo trutta f. fario*) and rainbow trout (*Oncorhynchus mykiss*) exposed in bypass systems to streamwater of these rivers or in cages directly in the rivers. The Schussen and Argen Rivers flow into Lake Constance. The Schussen River is polluted by a great number of chemicals, while the Argen River is less influenced by micropollutants. Pesticides, chelating agents, flame retardants, pharmaceuticals, heavy metals, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) were detected in effluents of a WWTP discharging to the Schussen as well as in surface water, and/or fishes from downstream of the WWTP. Results obtained by biotests conducted in the laboratory (genotoxicity, dioxin-like toxicity, and embryotoxicity) were linked to effects in feral fish collected in the vicinity of the WWTP or in

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fishes exposed in cages or at the bypass systems downstream of the WWTP. Dioxin-like effect potentials detected by reporter gene assays were associated with activation of CYP1A1 enzymes in fishes which are inducible by dioxin-like chemicals. Abundances of several PCBs in tissues of fishes from cages and bypass systems were not associated with these effects but other factors can influence EROD activity. Genotoxic potentials obtained by *in vitro* tests were associated with the presence of micronuclei in erythrocytes of chub from the river. Chemicals potentially responsible for effects on DNA were identified. Embryotoxic effects on zebrafish (*Danio rerio*), investigated in the laboratory, were associated with embryotoxic effects in trout exposed in streamwater bypass systems at the two rivers. In general, responses at all levels of organization were more pronounced in samples from the Schussen than in those from the Argen. These results are consistent with the magnitudes of chemical pollution in these two streams. Plausibility chains to establish causality between exposures and effects and to predict effects in biota in the river from studies in the laboratory are discussed.

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1. Introduction

Pollution of surface waters is caused not only by diffuse sources such as agricultural run-off (Parris, 2011), but also via wastewater treatment plants (WWTPs) and stormwater overflow basins (SOBs) (Batt et al., 2006; Becker et al., 2008; Bueno et al., 2012; Reemtsma et al., 2006). This discharge of substances like pesticides, pharmaceuticals, flame retardants, and chelating agents in surface waters has increased over the last decades due to the rising numbers of chemicals used by humans and since many WWTPs do not eliminate these substances entirely (Fobbe et al., 2006; Gartiser, 1999; Honnen et al., 2001; Kratz et al., 2000). This is true for micropollutants known to act as endocrine disruptors (Boxall et al., 2012; Coors et al., 2003, 2004), but also for chemicals with other mode of actions as e.g. carbamazepine or diclofenac (Ternes, 1998; Tixier et al., 2003). Several possibilities for enhancing efficiency of eliminating pollutants from wastewater have been developed. Among these are treatments with powdered or granular activated carbon, ozonation, ultraviolet light, and reverse osmosis (Gabet-Giraud et al., 2010). For WWTPs, powdered or granular activated carbon and/or ozonation in combination with different types of sand filters are currently the most common advanced wastewater treatment technologies (Margot et al., 2013).

Several investigations on the capacity of activated carbon filters and ozonation to remove residues revealed these techniques to eliminate micropollutants such as chelating agents, pharmaceuticals, pesticides, hormones or synthetic hormonal contraceptives more effectively than traditional wastewater treatment (Hollender et al., 2009; Margot et al., 2013; Snyder et al., 2007; Ternes et al., 2003). Overall rates of elimination vary due to adsorption characteristics or, respectively, the ozone reactivity of the micropollutants (Hollender et al., 2009; Margot et al., 2013; Ternes et al., 2003). Besides the limitations posed by pollutant's physicochemical properties, other limitations occur when applying additional treatment steps.

Competition of micropollutants with organic matter for sites on activated carbon to which to adsorb, leads to the need of an increased amount of activated carbon in the presence of organic matter (Margot et al., 2013). Furthermore, after some time of use, activated carbon is known to be depleted, which results in a reduced capacity to adsorb micropollutants (Matilainen et al., 2006). Depleted activated carbon can be treated as a waste and incinerated (Margot et al., 2013) or regenerated and used again (Maroto-Valer et al., 2006; Matilainen et al., 2006).

Efficiencies of new techniques to reduce micropollutants in the environment are widely accepted. However, little is known about the positive effects for ecosystems related to the large-scale implementation of improved treatments.

Numerous studies were conducted to assess water quality in general and the quality of treated wastewater in particular. Heeb et al. (2012) conducted chemical analyses of river water and WWTP effluent samples whereas Nam et al. (2014) solely performed chemical analyses of WWTP influent and effluent samples. Jarošová et al. (2014) measured estrogenic activity in effluent samples using *in vitro* bioassays. In view to approach ecological aspects, Griffin and Harrahy (2014) conducted fish reproduction assays on effluent samples in the laboratory and the field, the latter with fish caged up- and downstream of a WWTP. Furthermore, they tested for acute and chronic toxicity using fish larvae exposed to different concentrations of effluent samples. In addition, Magdeburg et al. (2014) assessed raw WWTP samples and WWTP samples after treatment with activated carbon, ozonation, and sand filtration from a pilot scale WWTP using laboratory biotests for genotoxicity, and combined them with chemical analyses. A combination of chemical analyses and *in vitro* bioassays was also used by Zounkova et al. (2014) who additionally integrated an *in situ* exposure assay with *Potamopyrgus antipodarum* in their study on sediment and water estrogenicity and toxicity. The combination of chemical analyses, laboratory biotests, and field effects in a test battery had already been established by Triebkorn et al. (2003) who have focused on both, embryotoxicity and endocrine disruption.

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