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Reduction of dioxin-like toxicity in effluents by additional wastewater treatment and related effects in fish

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ABSTRACT

Efficiency of advanced wastewater treatment technologies to reduce micropollutants which mediate dioxin-like toxicity was investigated. Technologies compared included ozonation, powdered activated carbon and granular activated carbon. In addition to chemical analyses in samples of effluents, surface waters, sediments, and fish, (1) dioxin-like potentials were measured in paired samples of effluents, surface waters, and sediments by use of an *in vitro* biotest (reporter gene assay) and (2) dioxin-like effects were investigated in exposed fish by use of *in vivo* activity of the mixed-function, monooxygenase enzyme, ethoxyresorufin *O*-deethylase (EROD) in liver. All advanced technologies studied, based on degradation or adsorption, significantly reduced dioxin-like potentials in samples and resulted in lesser EROD activity in livers of fish. Results of *in vitro* and *in vivo* biological responses were not clearly related to quantification of targeted analytes by use of instrumental analyses.

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are well-known chlorinated organic compounds ("dioxin-like") some congeners of each class which affect metabolic pathways modulated by binding of ligands to the aryl hydrocarbon receptor (AhR). Along with some non-*ortho*-substituted congeners of polychlorinated biphenyls (PCBs) (Lee et al., 2013), which are dioxin-like compounds, dioxins are classified as persistent organic pollutants (POPs) (WHO, 2010). Exposure to these compounds can lead to hepatotoxic, embryotoxic, teratogenic, immunotoxic, dermal toxic, lethal, and carcinogenic effects (Hilscherova et al., 2000).

Besides PCDDS, PCDFs, and PCBs, a range of other substances, due to their structure, size and conformation, are able to bind to the AhR (Denison and Nagy, 2003; Forrest et al., 2014; Murray et al., 2014). These include chlorinated azobenzenes and azoxybenzenes, several polycyclic aromatic hydrocarbons (PAHs) (Lee et al., 2015) and polychlorinated naphthalenes (PCNs) (Blankenship et al., 2000; Villeneuve et al., 2000b). Furthermore, some chemicals also seem to have the potential to bind to the receptor although it is not confirmed. Among these are polybrominated and chloro-/bromo-analogs of the previously listed substances, alkylated-chlorinated dioxins and furans, chlorinated dibenzothiophenes, chlorinated xanthenes and xanthones, polychlorinated

Abbreviations: AA-EQS, annual average environmental quality standard; AhR, aryl hydrocarbon receptor; BNF, beta-naphthoflavone; dm, dry mass; DMSO, dimethyl sulfoxide; EE_2, 17α-ethinylestradiol; EQS, environmental quality standard; EROD, ethoxyresorufin *O*-deethylase; LOQ, limit of quantification; PAHs, polycyclic aromatic hydrocarbons; PCAs, polychlorinated anisols; PCANs, polychlorinated anthracenes; PCBs, polychlorinated biphenyls; PCDDs, polychlorinated dibenzodioxins; PCDFs, polychlorinated dibenzofurans; PCFLs, polychlorinated fluorenes; PCNs, polychlorinated naphthalenes; PCDTs, polychlorinated diphenylthienes; PE, population equivalent; POPs, persistent organic pollutants; TEQ, toxic equivalents; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; wm, wet mass; WWTP, wastewater treatment plant

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diphenylthienes (PCDTs), anisols (PCAs), anthracenes (PCAN), and fluorenes (PCFL) (Giesy et al., 1994). In addition, ligands that bind with lesser affinities to the AhR, such as indoles, tryptophan-derived products, oxidized carotinoids, heterocyclic amines, and pesticides or drugs like imidazoles and pyridines, have also been reported (Hilscherova et al., 2000).

Dioxin-like effects are mediated by binding of ligands to the cytoplasmic AhR, which, due to translocation to the nucleus of the cell, acts as a transcription factor for genes encoding for proteins as *e.g.* CYP1A1 as one representative of the cytochrome P-450 family (Hilscherova et al., 2000). Enzymes of the CYP1A family are responsible for detoxification of xenobiotic chemicals such as PAHs and PCBs (Andersson and Förlin, 1992; Sanderson et al., 1996; Whyte et al., 2000). In unexposed fish, CYP1A is often not detectable but activities increase after exposure to, for example, PAHs (Stegeman and Lech, 1991). EROD assay measures activity of CYP1A1 on a catalytic level (Whyte et al., 2000).

Due to lipophilicity of these substances, while their concentrations in surface waters are relatively small, they accumulate in sediments and biota, especially in fatty tissues of fishes (WHO, 2010). They reach the environment by air (Sakurai et al., 1998), by run-off from agricultural fields treated with agrochemicals (Masunaga et al., 2001), or by wastewater treatment plants (WWTPs) (Moon et al., 2008).

At the end of the 1980s, the German Federal Government started to implement measures to reduce discharges of dioxin and dioxin-like compounds by implementation of threshold values for exhaust gases of municipal waste incinerators and agriculturally used sludge, and by bans for use of scavengers and production of pentachlorophenol (PCP), PCBs, and some polybrominated flame retardants (Schulz, 1993).

In the project SchussenAktiv*plus*, efficiencies of additional wastewater treatment stages for reduction of dioxin-like potency were investigated in two wastewater treatment plants discharging to the Schussen River, a major tributary of Lake Constance in southern Germany. At the first WWTP (Eriskirch), a small-scale system (model system) of advanced treatment including ozonation, sand filtration, and granular activated carbon was employed. In autumn 2013, the second WWTP (Langwiese), which had been investigated previously, was upgraded by use of powdered activated carbon. Currently, ozonation and activated carbon, which are the most common advanced treatments at WWTPs (Margot et al., 2013), have been shown to significantly reduce concentrations of substances like pharmaceuticals, pesticides, chelating agents, hormones, or synthetic hormonal contraceptives more efficiently than traditional treatments (Coors et al., 2004; Furuichi et al., 2006; Gulkowska et al., 2008; Hollender et al., 2009; Jálová et al., 2013; Jarošová et al., 2014a; Margot et al., 2013; Snyder et al., 2007; Ternes et al., 2003).

Here, comparisons were made among approaches to determine whether additional wastewater treatment can reduce dioxin-like potentials of effluents. Concentrations of known dioxin-like chemicals in wastewater effluents and surface waters, sediments, and fish were measured. Integrated concentrations of all dioxin-like potentials including non-target compounds that might be in mixtures were measured by use of an *in vitro*, trans-activation, reporter gene assay based on rat hepato-carcinoma cells (H4IIE-*luc*). This assay has proven to be suitable for analyses of AhR-active compounds (Eichbaum et al., 2014; Hilscherov,á et al., 2010; Janošek et al., 2006; Larsson et al., 2014). Effects in fish were analyzed by EROD assay which measures activity of the enzyme CYP1A1 and is commonly used as a biomarker of exposure to dioxin-like substances (Whyte et al., 2000). The combination of testing for potential dioxin-like toxicity in surface water, sediment, and effluent and the determination of occurred effects in fish facilitates a complementary and comprising assessment of the load situation and the effectiveness of the additional treatment technologies due to cross connections between the results. Different approaches were made to assess the situation directly at the WWTPs and further down in the associated river.

The present study tested the following hypotheses: 1) dioxin-like potency is lesser in samples of effluent, surface water, and sediment if effluent was treated with additional wastewater treatment stages (ozon and activated carbon) and 2) effects in fish due to dioxin-like toxicity are lesser after additional wastewater treatment.

2. Materials and methods

2.1. Ethical statements

Studies were conducted in strict accordance with German laws regulating use of live animals. Permission was given by the animal welfare authority of the Regional Council Tübingen (*Regierungspräsidium Tübingen*). Permit numbers: ZO 1/09 and ZP 1/12 for brown trout (*Salmo trutta* f. *fario*) and rainbow trout (*Oncorhynchus mykiss*). Fish were anaesthetized with MS-222 (tricaine mesylate). Cell lines were specified in materials and methods.

2.2. Location and description of WWTPs, semi-field bypass systems, and field sites

Locations of the two WWTPs, Eriskirch and Langwiese, and the bypass systems and field sites at the Schussen and the Argen Rivers are shown in Fig. 1.

The Eriskirch WWTP (coordinates: N47°37′11.7″, E9°31′55.5″) serves 40,000 population equivalents (PEs). At this medium-sized WWTP, a model installation was installed to test treatment with ozonation, granulated activated carbon, and sand filters. Effluent from the model installation is not released into the Schussen River. Two aquaria for fish exposure were installed at the Eriskirch WWTP. Each aquarium had a volume of 250 L and the velocity was 0.04 L/s. In each aquarium, 13 rainbow trout were exposed and the size of the fish at sampling time was: 1) in winter 2012/2013 18.58 ± 1.38 cm (regular effluent) and 19.53 ± 1.04 cm (effluent from the model installation), 2) in winter 2013/2014 18.0 ± 3.11 cm (regular effluent) and 19.57 ± 1.83 cm (effluent from the model installation). In winter 2012/2013 one trout died in the aquarium with the model effluent and in winter 2013/2014 one trout in the aquarium with the regular effluent. One aquarium was supplied with water from the regular final effluent after sand filter/flocculation (tertiary treatment stage) while a second aquarium was supplied with water from the model installation employing advanced technologies (fourth treatment stage).

The Langwiese WWTP (coordinates: N47° 44′ 53.22″, E9° 34′ 35.49″) has 170,000 PEs and in September 2013 was upgraded to include powdered activated carbon filtration. At the Langwiese WWTP, fish were exposed in cages located in the Schussen River. Each cage had a size of $100 \times 50 \times 50$ cm (length, width, height). On the top of the cages was a folding aperture and on each site one plastic pipe for swimming. The cages were fixed with chains at trees or dowels. In each cage 22 rainbow trout were exposed. The size of the fish at sampling time was: 1) in winter 2012/2013 14.48 ± 1.18 cm (upstream of the effluent) and 14.51 ± 1.07 cm (downstream of the effluent), 2) in winter 2013/2014 15.26 ± 2.09 cm (upstream of the effluent) 15.15 ± 1.63 cm (downstream of the effluent). No mortalities occurred. One cage was placed 200 m upstream of the effluent of the Langwiese WWTP (coordinates: N47°44′51.2″, E9°34′16.6″) and a second cage was placed next to the effluent (coordinates: N47°44′45.3″, E9°34′ 11.0″) to ensure a mixture between effluent and river water (at