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Toxicity of the micropollutants Bisphenol A, Ciprofloxacin, Metoprolol and Sulfamethoxazole in water samples before and after the oxidative treatment

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ABSTRACT

The amount of organic micropollutants detected in surface waters increases steadily. Common waste water treatment plants are not built to remove these substances. Thus there is a need for new technologies. A promising technology is the use of advanced oxidation processes through which organic micropollutants can be removed from waste water. However, the formation of oxidation by-products is likely and needs to be investigated since the by-products not only differ from their parent compounds in regard to their chemical and physical properties but they can also differ in toxicity. Therefore this study was designed to combine chemical and toxicological analyses of the advanced oxidation (O_3 [5 mg/L] or UV/H_2O_2 [Hg-LP lamp; 15 W; 1 g/L H_2O_2]) of waste water treatment plant effluents and pure water. Effluent samples from conventional activated sludge waste water treatment (mechanical treatment, activated sludge basin, and primary as well as secondary treatment steps) and high-purity deionized water (pure water) were spiked with Bisphenol A, Ciprofloxacin, Metoprolol or Sulfamethoxazole and treated with O_3 or UV/H_2O_2. For the toxicological analyses mammalian cells (CHO-9, T47D) were exposed to the water samples for 24 h and were tested for cytotoxicity (MTT Test), genotoxicity (Alkaline Comet Assay) and estrogenicity (ER Calux®). The results indicate that the oxidative treatment (O_3 or UV/H_2O_2) of Bisphenol A, Metoprolol, Sulfamethoxazole or Ciprofloxacin in waste water did not result in toxic oxidation by-products, whereas the UV/H_2O_2 treatment of Bisphenol A and Ciprofloxacin in pure water resulted in by-products with cytotoxic but no estrogenic effects after 60 min.

Introduction

Increasing numbers of organic micropollutants like pharmaceuticals and personal care products are detected in surface waters (Fick et al., 2009; Hirsch et al., 1999; Tambosi et al., 2010; Vieno et al., 2007). These micropollutants are not removed by the conventional activated sludge treatment and are thus reaching surface waters where they are found at concentration levels of up to several µg/L (Bergmann et al., 2011; Heberer et al., 2002; Korner et al., 2000; Maurer et al., 2007; Reungoat et al., 2010; Tambosi et al., 2010). The occurrence of chemical substances in surface waters has been linked to biological effects, such as estrogenic, mutagenic or genotoxic effects, in aquatic organisms (Desbrow et al., 1998; Korner et al., 2001; Liney et al., 2006; Schrank et al., 2009; Tambosi et al., 2010). This in fact is in contrast to the Water Framework Directive which requires a "good chemical and biological status" of all water bodies until 2015 (EU WFD, 2000). Therefore it is necessary to apply additional treatment technologies to remove remaining micropollutants from WWTP effluents. Advanced oxidation processes (AOPs) have been described as a useful tool for the removal of micropollutants from waste water (Andreozzi et al., 1999; Ikehata et al., 2008; Misik et al., 2011; Tuerk et al., 2010) and in the last years the first WWTP were upgraded with a full scale ozonation in Germany and Switzerland (Hollender et al., 2009; MKUNLV, 2011). Various AOP methods are described and the treatment with O_3 and UV/H_2O_2 are the two most effective techniques also in regard to removing toxic effects of micropollutants (Ikehata et al., 2008; Ormad et al., 2008; Stalter et al., 2011; Ternes et al., 2003; Tuerk et al., 2010).

Despite the benefits of AOP a few studies were able to show that AOPs like ozonation or photo-Fenton reactions result in the formation of toxic by-products (Dirany et al., 2011; Garcia-Käufer et al.,

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2012; Larcher et al., 2012; Li et al., 2013; Schrank et al., 2009; Stalter et al., 2010) which can be overcome by the use of e.g. sand filtration or activated carbon after the oxidative treatment (Abegglen, 2009; Stalter et al., 2010, 2011).

The compounds tested in this study were Metoprolol (β-adrenergic receptor antagonist), Sulfamethoxazole, Ciprofloxacin (antibiotics), and Bisphenol A which is used in polymeric resins. These compounds have been chosen as representatives for the classes of Sulfonamide antibiotics, β-blocker and Fluoroquinolones and because of their constant occurrence and their reported concentrations in the environment up to several μg/L (Giger et al., 2003; MKUNLV, 2011; vom Eyser et al., 2013). In addition, Bisphenol A is suspected to have an influence on human health and therefore has been added to the Community Rolling Action Plan by the ECHA in 2012 (ECHA, 2012). Currently there are no environmental quality standards (EQS) described in any guideline, however, the Oekotoxzentrum proposed acute and chronic EQS for Metoprolol (acute: 76 μg/L; chronic 64 μg/l), Sulfamethoxazole (acute: 2.7 μg/L; chronic 0.6 μg/l) and Bisphenol A (acute: not proposed; chronic 1.5 μg/l) (EAWAG, 2013).

The annual prescriptions of Ciprofloxacin add up to 33,000 kg leading to a widespread distribution of this compound in surface waters (Bergmann et al., 2011; Schwabe, 2010). The degradation mechanisms and pathways of Ciprofloxacin are well described (de Witte, 2012). Ciprofloxacin is still of particular concern since genotoxic and mutagenic effects have been reported (Hartmann et al., 1999; Hu et al., 2007; Kümmerer et al., 2000; Yim et al., 2011). The general toxicity of Ciprofloxacin has been investigated by Gürbay et al. (2005) resulting in an IC_{50} value (half maximal inhibitory concentration) of 100 mg/L. Bacterial tests with *Vibrio fischeri* revealed an EC_{50} value (half maximal effective concentration) greater than 5.9 mg/L (Hernando et al., 2007).

Several transformation products such as nitro-aromatic compounds or sulfanimalide, an active sulphonamide, for Sulfamethoxazole (SMX) are described in the literature (Abellan et al., 2008; Lam and Marbury, 2005; Yargeau et al., 2008). Studies on the toxicity of Sulfamethoxazole showed, that the EC_{50} values vary (μg/L to mg/L) between the test and organism used (Baran et al., 2006; Dirany et al., 2011; Escher et al., 2005; MKUNLV, 2011; Wammer et al., 2006). Yargeau et al. (2008) were also able to show that a mixture of several transformation products of SMX had an effect on human hepatocellular carcinoma cells (Yargeau et al., 2008).

Metoprolol is the most frequently prescribed β-blocker in Germany (Bergmann et al., 2011). Benner and Ternes (2009) were able to show the formation of transformation products (aldehyde moieties) after ozonation of Metoprolol. EC_{50} values of 31 mg/L (van den Brandhoff and Montforts, 2010), 63.9 mg/L (Huggett et al., 2002), or 200 mg/L (Hernando et al., 2004), as well as a lowest observed effect concentration of 12 mg/L in *Daphnia magna* (Dzialowski et al., 2006) have been published. Cheong et al. (2008) reported an IC_{50} value of 2.74 g/L for Metoprolol in human ocular cell lines.

Bisphenol A (BPA) is a suspected anthropogenic endocrine disruptor. Although it is less estrogenic toward aquatic organisms than natural hormones, BPA has been reported to be able to induce feminization phenomena in various species of animals at high concentrations (Metcalfe et al., 2001). The oxidation of BPA resulted in the formation of several by-products of which some contained additional hydroxyl-groups. Other products were formed by a ring opening of the original compound (Deborde et al., 2008; Lee et al., 2004). Its toxicity has been studied in regard to its endocrine disrupting effects. EC_{50} values range from 10 μg/L to 253 μg/L depending on the test system and the exposure time (Wu et al., 2011; Zhang et al., 2010). Golub et al. (2010) reviewed the available literature on Bisphenol A concerning its toxicity and concluded that Bisphenol A mainly affects the offspring viability, sex differentiation, immune hypersensitivity and gender differentiated morphology thus affecting the endocrine system when prenatally exposed (Golub et al., 2010). However, Umar et al. (2012) conclude that knowledge about the environmental fate of BPA is mainly based on chemical analyses and not biological effects. Studies of the toxicological effects of the isolated transformation products should therefore be conducted (Umar et al., 2012).

Despite all these results the risk assessment of micropollutants is currently mainly based on the chemical identification of the single compounds, but it is not considering possible biological effects of mixtures in complex matrices (Escher and Leusch, 2011).

The objective of this study was to investigate micropollutants (Bisphenol A, Ciprofloxacin, Metoprolol and Sulfamethoxazole) and their oxidation by-products in waste water treatment plant effluents and pure water by chemical and toxicological *in vitro* analyses using concentrations comparable to those detected in surface waters.

Materials and methods

Chemicals

High-purity deionized water (pure water) was produced by an Elix 10 – Milli-Q Plus water purification system (Millipore, Eschborn, Germany). All chemicals (if not otherwise stated) and the enzyme catalase were purchased from Sigma–Aldrich (Taufkirchen, Germany) in highest quality (≥98%). 10 mg of the respective reference compound was dissolved in 20 mL water:acetonitrile (50:50, v/v) to prepare a stock solution with a concentration of 0.5 g/L. The calibration standards were dissolved in deionized water.

The used effluents of the WWTP Duisburg – Vierlinden still contained organic micropollutants. The concentrations of the four substances were in the range of 50 ng/L for Bisphenol A, 100 ng/L for Ciprofloxacin, 600 ng/L for Metoprolol and 700 ng/L for Sulfamethoxazole. The DOC (dissolved organic carbon) was between 6 and 8 mg/L and the pH ranged from 7 to 8.5. The used methods for quantification are described in the section “chemical analysis”.

AOP equipment

The different oxidation experiments were carried out in laboratory scale using a 15 W Hg-LP UV lamp (Heraeus Noblelight, Hanau, D) or a COM-CD-HF 2 ozone generator (Anseros, Tübingen, D).

UV oxidation was investigated with and without the addition of hydrogen peroxide. The laboratory scale plant (volume of 1 L) was filled with spiked and unspiked samples (pure water and WWTP effluent). All experiments were carried out at a constant temperature (Lauda-Thermostat, Lauda, Königshofen, D). The water was pumped through the laboratory scale plant by a flexible tube pump (Multifix constant M 838, Alfred Schwinherr KG, Schwäbisch-Gmünd, D). Time controlled sampling was performed.

For ozonation experiments ozone water was produced by conveying ozone gas through ice-bath-cooled pure water. The ozone was produced using technical oxygen and an ozone generator. The experiments were performed in batch-mode by adding high concentrated ozone water to spiked and original water samples (pure water and waste water treatment plant effluent). The concentration of the ozone in the water was determined by measuring the absorption using an UV VIS spectrometer (SPECORD® PC 200, Analytik Jena AG, Jena, D) at 260 nm with a molar absorption coefficient of 3300 [mol^{-1} cm^{-1}].

Chemical analysis

Samples were analyzed with an LC 20 HPLC system (Shimadzu, Duisburg, D), which was coupled to a 3200 Q Trap system (AB