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Removal of novel antiandrogens identified in biological effluents of domestic wastewater by activated carbon

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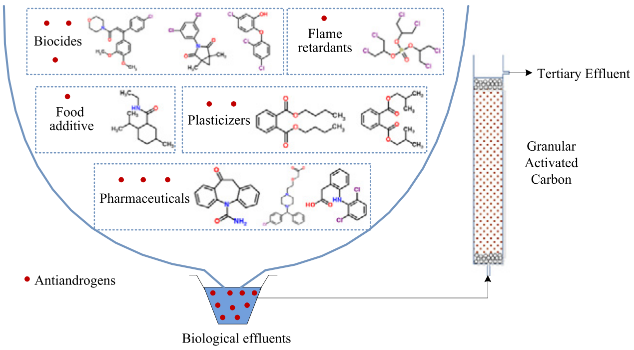
HIGHLIGHTS

- Ten potential antiandrogens were identified in the biological effluents.
- They included biocides, food additives, flame retardants, pharmaceuticals.
- The potential antiandrogenic properties of three compounds were first reported.
- Eight of the identified antiandrogens can be effectively removed by activated carbon.

GRAPHICAL ABSTRACT



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ABSTRACT

Environmental antiandrogenic (AA) contaminants in effluents from wastewater treatment plants have the potential for negative impacts on wildlife and human health. The aim of our study was to identify chemical contaminants with likely AA activity in the biological effluents and evaluate the removal of these antiandrogens (AAs) during advanced treatment comprising adsorption onto granular activated carbon (GAC). In this study, profiling of AA contaminants in biological effluents and tertiary effluents was conducted using effect-directed analysis (EDA) including high performance liquid chromatography (HPLC) fractionation, a recombinant yeast screen containing androgen receptor (YAS), in combination with mass spectrometry analyses. Analysis of a wastewater secondary effluent from a membrane bioreactor revealed complex profiles of AA activity comprising 14 HPLC fractions and simpler profiles of GAC effluents with only 2 to 4 moderately polar HPLC fractions depending on GAC treatment conditions. Gas chromatography–mass spectrometry and ultra-high performance liquid chromatography-nanospray mass spectrometry analyses of AA fractions in the secondary effluent resulted in detection of over 10 chemical contaminants, which showed inhibition of YAS activity and were potential AAs. The putative AAs included biocides, food additives, flame retardants, pharmaceuticals and industrial contaminants. To our knowledge, it is the first time that the AA properties of *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (WS3), cetirizine, and oxcarbazepine are reported. The EDA used in this study was proven to be a powerful tool to identify novel chemical structures with AA activity in the complex aquatic

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environment. The adsorption process to GAC of all the identified antiandrogens, except WS3 and triclosan, fit well with the pseudo-second order kinetics models. Adsorption to GAC could further remove most of the AAs identified in the biological effluents with high efficiencies.

1. Introduction

There is growing concern on the widespread release of endocrine disrupting chemicals (EDCs) because they are bioactive at low, environmentally relevant levels and have the potential to cause potent long-term effects on aquatic organisms even at ng/L concentrations (EEA, 2012, Bergman et al., 2012). In aquatic ecosystems, much of the EDC research has concentrated on estrogenic compounds and only in recent years has disruption of other crucial hormonal pathways such as androgen receptor antagonism received attention in wastewater effluent and receiving waters.

Reports of antiandrogens (AAs) or antiandrogenic (AA) activity in sludge (Ma et al., 2013), sediments (Zhao et al., 2011), water (Shi et al., 2009, Jobling et al., 2009, Urbatzka et al., 2007), freshwater fish (Hill et al., 2010) and marine organism (Misaki et al., 2015) have already been described suggesting their presence in the aquatic environment could be widespread. Triclosan, a significant AA contaminant, contributed AA activity of 0.12 to 2.2 μg/L flutamide equivalent in the effluents of wastewater treatment plants (Deblonde et al., 2011). AAs could cause demasculinization effects, e.g. malformations of male reproductive organs (Foster, 2006), and depression of androgen production (Blystone et al., 2007). They have been associated with reproductive dysfunction in fish (Jobling et al., 2009) and humans (Skakkebaek et al., 2001).

A major source of EDCs, including AAs, into the aquatic environment is via discharges of domestic wastewater, despite the partial removal of EDCs during the biological treatment due to biodegradation and sorption. AAs including octocrylene, ethylhexyl methoxycinnamate, triclosan and bisphenol A were removed by <50% to >80% during biological treatment of three systems (aerobic, anaerobic, and combined anaerobic and aerobic) (Leal et al., 2010). Therefore, a number of physical-chemical tertiary treatment processes have been investigated for EDCs removal in wastewater streams (Liu et al., 2009). These include physical means e.g. absorption by activated carbon and rejection by membrane (Snyder et al., 2007), and chemical advanced oxidation, e.g. O_3, Cl_2 and UV/H_2O_2 (Hu et al., 2003, Chen et al., 2007, Zhang et al., 2008), but studies seldom focus on AAs and AA activity (Stalter et al., 2011). Adsorption onto activated carbon is very effective technique to reduce the load of micropollutants from wastewaters at acceptable cost (Joss et al., 2008). To evaluate the efficiency of advanced treatment processes for the removal of AAs, it is essential to identify these substances.

The identification of AA chemicals in the wastewater treatment plant (WWTP) effluents is challenging. The structures of AAs are extremely diverse (Vinggaard et al., 2008). Chemicals having androgen receptor antagonist properties include many different kinds of environmental contaminants, such as pesticides, UV-filters, preservative, and industrial contaminants and are often present in WWTPs effluents at ng to μg per liter concentrations (Hotchkiss et al., 2008). However, effect-directed analysis approaches are powerful tools for determining the causes of biological effects in complex environmental samples (Grung et al., 2007) and thus far, have been applied to determine the structures of AA chemicals in the aquatic environment (Hill et al., 2010, Thomas et al., 2009, Rostkowski et al., 2011, Liscio et al., 2014). Thomas et al. (2009) identified petrogenic naphthenic acids, polycyclic aromatic hydrocarbons (PAHs) and alkylphenols as responsible for antagonizing the androgen receptors in the produced water from offshore oil production platforms. Hill et al. (2010) initially found dichlorophene and di(chloromethyl)anthracene accounted for a small proportion of the total AA activity present in fish bile exposed to WWTPs effluents. After the improvement of the method, a comprehensive array of AA contaminants was revealed, and chlorophene together with triclosan accounted for 51% of the total antiandrogenic activity in bile of fish exposed to wastewater effluents (Rostkowski et al., 2011). Using effect-directed analysis, over 31 chemical structures with potential AA activity were identified in surface waters including fungicides, germicides, flame retardants and pharmaceuticals (Liscio et al., 2014). However, almost all these studies were limited to Europe, and the identification of AA contaminants in the aquatic environment of other countries and regions has been barely reported. As there are various AA substances, for example human and veterinary pharmaceutical products (Vinggaard et al., 2008), the profiles of AAs existing in the aquatic environment might be very different from site to site and thus it is critical to identify the profiles of AAs present in WWTPs effluents of other countries such as China.

The aim of this study was to identify AA contaminants in a biologically treated domestic wastewater and evaluate the removal of these contaminants during treatment with activated carbon, a tertiary treatment known to be effective in removing a wide range of chemical contaminants (Dias et al., 2007). In this study, three questions were investigated: a) What is the influence of GAC on the profiles of AA activity in the tertiary effluents? b) Is effect-directed analysis suitable to screen the novel AA activity contributors that are present in contaminated water? c) Are the AA chemicals in the secondary effluents removed when treated by GAC? Profiles of AA contaminants in biological effluents and tertiary effluents were conducted using HPLC fractionation in combination with yeast androgen receptor transcription screens. The AA chemical contaminants in androgen receptor active fractions were identified by gas chromatography–mass spectrometry (GC–MS) and ultra-high performance liquid chromatography-nanospray mass spectrometry (uHPLC-MS) analyses.

2. Materials and methods

2.1. Materials

Stable isotope internal standards (ISs) [2,3,16,16-2H4] estrone (E1-d4, >98% D atom) was purchases from Cambridge Isotope Laboratories Inc. (MA, USA) and 13C12-triclosan (13C-triclosan, 99% 13C) was obtained from LGC Standard (Teddington, UK). All other standards and media reagents were >97.5% purity and purchased from Sigma-Aldrich, Gillingham, UK. Oasis HLB 200 mg SPE cartridges and Sep-Pak® purification cartridges were from Waters, USA. HPLC grade solvents were obtained from Rathburn Chemicals, Scotland, UK.

2.2. GAC batch tests and continuous GAC column experiments

The nutshell based granular activated carbon (GAC 785-2, Zhejiang Suichang Carbon-Industrial Co., Ltd., China) was used in this study, with specific surface area of 1000 ± 50 Brunner-Emmet-Teller (BET) m^2/g, apparent density of 0.45–0.58 g/cm^3 and iodine number of 800 ± 50 mg/g. Prior to use in experiments, the GAC was rinsed with ultra-pure water to remove fines and dried at 105 °C in the oven. The scheme of the experimental setup for testing the effect of GAC treatment can be found in our previous work (Ma et al., 2016). The total bed volume (BV) in the column was 753.6 mL. The GAC column was first carried out at different contact time by variable flow velocities, from 0.5 min to 15 min, to get the proper contact time. Then the GAC column was continuous operated in up-flow mode at a contact time of 15 min.