Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Methodology for profiling anti-androgen mixtures in river water using multiple passive samplers and bioassay-directed analyses

*Camilla Liscio, Alaa Abdul-Sada, Raghad Al-Salhi, Michael H. Ramsey, Elizabeth M. Hill**

University of Sussex, School of Life Sciences, BN1 9QG Brighton, UK

ARTICLE INFO

Article history:
Received 1 September 2013
Received in revised form
18 February 2014
Accepted 17 March 2014
Available online 27 March 2014

Keywords:
Passive sampling
Anti-androgens
River water
Bioassay-directed analysis
Endocrine disrupting chemicals (EDCs)
Wastewater effluent

ABSTRACT

The identification of endocrine disrupting chemicals in surface waters is challenging as they comprise a variety of structures which are often present at nanomolar concentrations and are temporally highly variable. Hence, a holistic passive sampling approach can be an efficient technique to overcome these limitations. In this study, a combination of 4 different passive samplers used for sampling polar (POCIS A_{pharm} and POCIS $B_{pesticide}$) and apolar compounds (LDPE low density polyethylene membranes, and silicone strips) were used to profile anti-androgenic activity present in river water contaminated by a wastewater effluent. Extracts of passive samplers were analysed using HPLC fractionation in combination with an *in vitro* androgen receptor antagonist screen (YAS). Anti-androgenic activity was detected in extracts from silicone strips and POCIS A/B at (mean ± SD) 1.1 ± 0.1 and 0.55 ± 0.06 mg flutamide standard equivalents/sampler respectively, but was not detected in LDPE sampler extracts. POCIS samplers revealed higher selectivity for more polar anti-androgenic HPLC fractions compared with silicone strips. Over 31 contaminants were identified which showed inhibition of YAS activity and were potential anti-androgens, and these included fungicides, germicides, flame retardants and pharmaceuticals. This study reveals that passive sampling, using a combination of POCIS A and silicone samplers, is a promising tool for screening complex mixture of anti-androgenic contaminants present in surface waters, with the potential to identify new and emerging structures with endocrine disrupting activity.

1. Introduction

Contamination of natural waters is a major concern in many parts of the world, and there is a limited understanding of the toxicological consequences of pollution of surface waters through discharges of wastewater effluents. Many emerging contaminants originate from human use, and are still present in treated effluents from wastewater treatment plants (WWTPs). Aquatic monitoring is an on-going challenge and a key issue is to identify the most important biologically active compounds currently not covered by existing water-quality regulations, and which have the potential to cause

* *Corresponding author*. Tel.: +44 1273678382; fax: +44 1273877586.
E-mail address: e.m.hill@sussex.ac.uk (E.M. Hill).

http://dx.doi.org/10.1016/j.watres.2014.03.039
0043-1354/

deleterious health effects to aquatic biota (Snyder and Benotti, 2010; Soffker and Tyler, 2012). Amongst emerging pollutants, Endocrine Disrupting Compounds (EDCs) appear to be particularly prevalent in the aquatic environment, and some aquatic animals are highly susceptible to their effects as they can be continually exposed to these contaminants through discharges of WWTP effluents, and these exposures can be life-long (Jobling et al., 2006; Liney et al., 2006). Future concentrations of EDCs may increase in certain river catchments due to climate change resulting in changes in hydrology and high demands on limited water resources.

Thus far, the identification of EDCs in aquatic environments has been mostly focused on estrogenic compounds, but a recent UK survey study has revealed that the majority of the investigated WWTP effluents contained anti-androgenic (AA) as well as estrogenic activity. In addition, the observed feminisation of wild fish (roach, *Rutilus rutilus*) in downstream waters was correlated with exposure to both AA activity and estrogen levels or with AA activity alone (Jobling et al., 2009). Reports of AA activity in sediments, water and fish of European rivers have already been described suggesting their presence in the aquatic environment could be widespread (Hill et al., 2010; Urbatzka et al., 2007; Weiss et al., 2009), however in many cases the identities of AA structures still remain to be elucidated. Anti-androgens can bind to the androgen receptor (AR), but are unable to activate it (AR antagonism). The structures of chemicals containing androgen receptor antagonist properties can be extremely diverse (Rostkowski et al., 2011; Vinggaard et al., 2008) and it is therefore important to use methods which do not make any assumptions as to the nature of the chemicals involved. However, the identification of biologically active compounds in surface waters or treated effluents can be problematic, since they are present at ultra-trace levels (often 1–100 ng/L) and encompass a variety of chemical classes differing significantly in physical–chemical properties. Thus their identification may require sensitive analytical techniques, intensive sampling programs and large sample volumes (Focazio et al., 2008; Schultz et al., 2010). To overcome these limitations, the use of a holistic passive sampling approach to screen for AA contaminants in surface waters could be an efficient alternative to grab sampling. The use of a combination of different passive samplers would allow sampling of a wide range of chemical polarities with a significant pre-concentration of contaminants from surface waters (Mills et al., 2011; Tapie et al., 2011). Moreover, passive samplers can provide an integrative sample of mixtures of environmental contaminants over an exposure period and permit the sequestration of residues from episodic events that are not always detected with grab sampling. Currently available passive sampling devices are only able to efficiently sample a limited polarity range. Since AA compounds in effluents are a complex mixture of hydrophilic and lipophilic chemicals (Rostkowski et al., 2011), a combination of different passive samplers covering the broadest range of log K_{ow} (Vrana et al., 2005) must be used to guarantee an efficient sampling of the whole array of anti-androgens that are potentially present in the aquatic environment.

In this study, 4 different passive samplers were investigated for their ability to sample AA activity present in contaminated surface waters. Two types of Polar Organic Chemical Integrative Samplers (POCIS; POCIS A designed for pharmaceuticals and POCIS B for pesticides) were used for covering the polar log K_{ow} range, whilst silicone strips and low density polyethylene (LDPE) membranes were selected for sampling any apolar components contributing to AA activity. The POCIS samplers contain a sorbent phase sandwiched between two microporous polyethersulphone (PES) membranes. Chemicals diffuse from the water and adsorb onto the sorbent phase (i.e. OASIS HLB for POCIS A or a triphasic mixture for POCIS B) from which they can be extracted after deployment. The use of POCIS to investigate the presence of phenolic estrogens as well as a variety of pharmaceuticals in rivers or effluents is well established (Liscio et al., 2009; Morin et al., 2012; Rujiralai et al., 2011; Vallejo et al., 2013). LDPE and silicone are single phase samplers which allow the uptake of hydrophobic chemicals, where the driving force for analyte uptake by the sampler is the chemical activity gradient between the polymer and the sampled medium (Rusina et al., 2010b). Single phase LDPE and silicone material have largely replaced traditional semi-permeable membrane devices, and are widely used as passive sampling devices for assessing non-polar organic compounds (log $K_{ow} \geq 4$) in aquatic environments including chlorinated EDCs (Allan et al., 2009; Sacks and Lohmann, 2011).

Three questions were investigated in this study: a) Are there differences between the concentrations of AA activity sampled by the POCIS and single membrane passive sampling devices? b) How do the profiles of AA activity sampled by the different devices differ from each other and from a representative profile of AA activity present in grab samples of the water phase taken during the deployment period? c) Which of the passive sampling devices, or combinations thereof, are most suitable to screen the variety of contaminants with potential AA activity that are present in effluent-contaminated water?

In this study, four canisters, each of them containing all the four different sampling devices, were deployed for two weeks in river water 200 m downstream a domestic sewage effluent. Organic chemicals in extracts obtained from the passive samplers were analysed by a yeast recombinant androgen receptor transcription screen (YAS) to investigate the sampled amount of AA activity in each sampler type. The profiles and identification of some structures of potential anti-androgens were investigated using a bioassay-directed fractionation approach. Extracts of passive samplers were fractionated by HPLC and the fractions analysed by YAS. Contaminants present in fractions containing AA activity were identified by mass spectrometry techniques (GC–MS or LC-QTOFMS). Where available, commercial standards of putatively identified contaminants were tested for AA activity in YAS and used to confirm structural identity by comparison with retention time and mass spectral data.

2. Material and methods

2.1. Passive sampling devices

POCIS samplers were obtained from Environmental Sampling Technologies Inc, St. Joseph, USA. POCIS A contained 200 mg