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Estrogen-, androgen- and aryl hydrocarbon receptor mediated activities in passive and composite samples from municipal waste and surface waters

V. Jálová [a], B. Jarošová [a], L. Bláha [a], J.P. Giesy [b,c,d,e,f], T. Ocelka [g], R. Grabic [h], J. Jurčíková [g], B. Vrana [a], K. Hilscherová [a,]*

[a] Research Centre for Toxic Compounds in the Environment (RECETOX), Masaryk University, Kamenice 753/5, 625 00, Brno, Czech Republic
[b] Dept. of Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[c] State Key Laboratory of Pollution Control and Resources Reuse, School of the Environment, Nanjing University, Nanjing, 210046, PR China
[d] Department of Zoology, Center for Integrative Toxicology, Michigan State University, East Lansing, MI, USA
[e] School of Biological Sciences, University of Hong Kong, Hong Kong, China
[f] Department of Biology and Chemistry, State Key Laboratory for Marine Pollution, City University of Hong Kong, Hong Kong, China
[g] Institute of Public Health Ostrava, National Reference Laboratory for POPs, Ostrava, Czech Republic
[h] University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zatisi 728/II, Vodnany, 389 25 Czech Republic

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ABSTRACT

Passive and composite sampling in combination with *in vitro* bioassays and identification and quantification of individual chemicals were applied to characterize pollution by compounds with several specific modes of action in urban area in the basin of two rivers, with 400,000 inhabitants and a variety of industrial activities. Two types of passive samplers, semipermeable membrane devices (SPMD) for hydrophobic contaminants and polar organic chemical integrative samplers (POCIS) for polar compounds such as pesticides and pharmaceuticals, were used to sample wastewater treatment plant (WWTP) influent and effluent as well as rivers upstream and downstream of the urban complex and the WWTP. Compounds with endocrine disruptive potency were detected in river water and WWTP influent and effluent. Year-round, monthly assessment of waste waters by bioassays documented estrogenic, androgenic and dioxin-like potency as well as cytotoxicity in influent waters of the WWTP and allowed characterization of seasonal variability of these biological potentials in waste waters. The WWTP effectively removed cytotoxic compounds, xenoestrogens and xenoandrogens. There was significant variability in treatment efficiency of dioxin-like potency. The study indicates that the WWTP, despite its up-to-date technology, can contribute endocrine disrupting compounds to the river. Riverine samples exhibited dioxin-like, antiestrogenic and antiandrogenic potencies. The study design enabled characterization of effects of the urban complex and the WWTP on the river. Concentrations of PAHs and contaminants and specific biological potencies sampled by POCIS decreased as a function of distance from the city.

1. Introduction

There is increasing evidence that environmental contaminants have the potential to disrupt endocrine processes. This might result in adverse effects on reproduction, cause certain cancers, and other toxicities related to (sexual) differentiation, growth, and development (Giesy et al., 2000; Miles-Richardson et al., 1999; Sanderson and van den Berg, 2003; Snyder et al., 2000). A variety of pollutants that are found in surface and waste waters, such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polychlorinated dioxins and furans (PCDD/Fs), polycyclic aromatic hydrocarbons (PAHs), alkylphenols, synthetic steroids, pesticides, pharmaceuticals and personal care products (PPCPs), but also natural products such as phytoestrogens, have been shown to elicit endocrine disruptive effects.

Sources of endocrine disrupting compounds (EDCs) are associated with larger urbanized and industrial areas. However, influences of smaller local sources can also be significant, especially where dilution is minimal (Jarosova et al., 2012). EDCs are also released to aquatic environments from both municipal and various industrial waste waters (Garcia-Reyero et al., 2004). Relative contributions of EDCs to surface waters depend on efficacies of sewage treatment systems, which is dependent on both capacity and technology of the wastewater treatment plant (WWTP). Potential risks of adverse effects of effluents from WWTPs to aquatic environments are influenced by volume of effluent, discharge of the receiving river, weather conditions and probably other factors that affect dissipation through dilution and/or degradation (Sumpter, 1995). Wastewater treatment plants receive mixtures of molecules from domestic, agricultural, and/or industrial wastes and

* Corresponding author. Tel.: +420 54949 3256; fax: +420 54949 2840.
E-mail address: hilscherova@recetox.muni.cz (K. Hilscherová).

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thus waste waters can contain mixtures of many of the above listed pollutants and their degradation products (Alvarez et al., 2005). Despite intensive removal of xenobiotics by municipal WWTPs, which can range from 88 to >99% and 96 to >99% for xenoestrogens and xenoandrogens, respectively (Korner et al., 2000; Leusch et al., 2010; Murk et al., 2002; Svenson and Allard, 2004), they often do not remove all chemicals from the effluent. Moreover, during treatment some contaminants can be deconjugated to their more biologically active forms (Desbrow et al., 1998). Thus, most effluents still contain complex mixtures of molecules, including transformation products formed during treatment.

Adverse effects on endocrine function and/or reproductive health associated with exposure to effluents from WWTPs, which can persist several kilometers from the point of effluent entry (Harries et al., 1996), have been demonstrated in wild fish populations (Jobling et al., 1998) or fishes caged downstream from WWTPs (Snyder et al., 2004). Several studies combining the use of chemical analyses and *in vitro* assays have revealed steroid estrogens as the most potent endocrine disruptors in WWTP effluents with thresholds for adverse effects of a few ng/L (Korner et al., 2000; Matsui et al., 2000; Nakada et al., 2004; Routledge et al., 1998; Snyder et al., 2000). However, other EDCs can be effective in various landuse conditions (Sole et al., 2000) and special consideration should be paid to mixtures of pollutants. Also, more information is needed to assess the potential contribution from other sources than just the WWTPs.

Selection of an appropriate sampling approach is crucial to determining the presence of contaminants and assessment of their potential for effects on aquatic environment. Traditional grab samples represent the immediate situation, thus only those contaminants present at the time of sampling are characterized. Episodic events such as spills or stormwater runoff can be missed since contaminants can dissipate prior to the next sampling (Alvarez et al., 2005; Huckins et al., 1990, 1993). A more representative way to sample, that represents an integrated estimate of the time-averaged exposure is composite samples collected over time. But, even this type of extensive sampling represents isolated conditions over relatively short durations. This sort of intensive sampling program is resource-intensive, requiring sampling staff and/or special equipment, which cannot be easily employed at many sites, especially at locations where equipment might be at risk to vandalism.

An alternative protocol is passive sampling, which enables estimation of time-weighted concentrations of contaminants and sequesters residues from episodic events commonly not detected by use of intermitent grab sampling. Passive sampling requires minimal resources of both personnel and equipment. Passive samplers have no moving parts to fail and require no electricity to function. They can be placed out of sight to avoid vandalism. Passive sampling can be used in situations of variable water conditions and because they concentrate residues from water they can enable detection of ultra-trace, yet toxicologically relevant concentrations of contaminant mixtures over extended durations (Alvarez et al., 2004). Other advantages include relatively simple, single deployment as compared to collecting and processing multiple water samples, greater mass of chemical residues sequestered, and the ability to detect chemicals which dissipate quickly (Alvarez et al., 2005; Huckins et al., 1990). Passive sampling also eliminates the need for some tedious and time-consuming cleanup steps associated with other types of sample collection.

Semipermeable membrane devices (SPMDs) have been developed as *in situ*, integrating passive samplers for monitoring of trace-level, waterborne hydrophobic contaminants (Huckins et al., 1993) and have been used for effective sampling of multiple classes of chemicals, including PAHs, PCBs, OCPs, PCDD/Fs, alkylated phenols, moderately polar organophosphate insecticides, pyrethroid insecticides, neutral organometallic compounds, and certain heterocyclic aromatic compounds (Petty et al., 2000a). Since SPMDs can mimic accumulation by aquatic organisms that can bioconcentrate trace amounts of organic contaminants, SPMDs measure not only the presence, but also the bioavailability and bioconcentration potential of organic contaminants (Huckins et al., 1990; Petty et al., 2000b). Polar Organic Chemical Integrative Samplers (POCIS) sequester waterborne hydrophilic contaminants, such as polar pesticides, pharmaceuticals, ingredients from personal care and consumer products, natural and synthetic hormones (Alvarez et al., 2004, 2005; Petty et al., 2004). Depending on the sorbent used, POCIS can be modified for sampling of general hydrophilic contaminants or pharmaceuticals (Alvarez et al., 2005).

The aim of this study was characterization of the influence of the industrialized urban region of Brno, Czech Republic and its associated municipal WWTP on contamination of the Svratka and Svitava rivers by compounds with endocrine disruptive potency by joint use of bioassays, two types of passive samplers and identification and quantification of selected organic chemicals. One goal was to assess the year-round variability in endocrine disruptive potency of WWTP influent and effluent water and thus treatment efficiency for EDCs by collecting composite samples monthly. The second major goal was to determine the relative magnitude of contributions of the urban area and the WWTP on contamination of these two urban rivers by endocrine disruptive compounds that can modulate the arylhydrocarbon (AhR), estrogen (ER) and androgen (AR) receptors. A battery of *in vitro* bioassays was used to assess potencies of agonists of these three receptors. Two types of passive samplers, POCIS and SPMD, were used to collect integrated samples of hydrophobic and hydrophilic compounds and assess their potencies to interfere with the three receptors signalling.

2. Materials and methods

2.1. Sampling design

Samples were collected from the region around Brno, the second largest metropolitan district of the Czech Republic in Central Europe. The metropolitan region of Brno with more than 400,000 inhabitants is spread through the basin formed by the Svratka and Svitava Rivers. The city has a central wastewater treatment plant and a variety of industrial activities. The municipal WWTP treats wastewater conveyed by a system of sanitary sewers from the city of Brno and increasingly also by a system of pumping stations from its surroundings. The WWTP was recently reconstructed and enhanced to a capacity of 513,000 population equivalent with permissible volume of discharged wastewater of 4222 L/s. Waste water is subjected to primary (mechanical) treatment followed by biological stage of activation with pre-denitrification and anaerobic phosphorus removal (system of circulatory activation with change of anaerobic, anoxic and aerated zones). Excess activated sludge is then anaerobically stabilized (Brněnské vodárny a kanalizace, 2010; Ministry of the Environment, 2010).

The influent and effluent of the WWTP were sampled monthly from May 2007 until April 2008. In addition, SPMD and POCIS passive samplers were placed in the influent (*site 5*) and effluent (*site 6*) of the WWTP and at seven sites in the Svratka, Svitava and Bobrava Rivers at locations upstream and downstream of Brno and downstream of the WWTP effluent (Fig. 1). Passive samplers were deployed for 23 days and collected during October 2007. Sampling locations in the Svratka River were: *Kninicky* (*site 1*) upstream of the city of Brno (downstream of the dam of Brno reservoir) and a site downstream of Brno upstream of the confluence with the Svitava River (*Svratka before confluence*, *site 2*). Locations monitored in the Svitava River included *Bilovice* and *Svitavou* (*site 3*), a small town upstream of Brno, and another site downstream of Brno upstream of the confluence with the Svratka River (*Svitava before confluence*, *site 4*). Another sampling site was selected in the *Bobrava River* (*site 9*), which is a tributary affected mostly by agriculture that flows into the Svratka River downstream of the WWTP. Downstream of the WWTP and the confluence of the Bobrava and Svratka rivers samples were collected near a small town *Rajhradice (site 7)* and at *Zidlochovice* (*site 8*, approximately 20 km downstream from Brno).