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MONITORING OF ENDOCRINE DISRUPTORS IN CZECH WASTE WATERS AND THEIR RECIPIENTS

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Abstract: *In recent years, widespread occurrence of feminized male fish downstream of waste water treatment plants (WWTPs) has received a lot of attention from environmental scientists. This effect seems to be caused by exposure to mixtures of contaminants that interfere with endocrine system of wildlife (Endocrine Disruptors; EDCs). By use of in vitro bioassays we detected very high efficiencies to remove estrogens and androgens (from 80 to more than 99 %) at all tested municipal WWTPs and poor ability to remove waterborne dioxin-like compounds. However, levels of dioxin-like activities present in water were mostly about one order of magnitude lower than the levels of estrogenic activities.*

Keywords: endocrine disruptor, *in vitro* assay, POCIS, waste water

INTRODUCTION

Anti/estrogens, anti/androgens, dioxin-like pollutants and some other groups of compounds are of great concern due to their ability to interfere with normal functioning of the endocrine system in organisms. Waste water treatment plant discharges are one of the main known sources of these compounds and numerous cases of feminized fish downstream of them have been documented (e. g. Gross-Sorokin et al. 2006). In the contrary to chemical analyses, *in vitro* bioassays provide an integrative measure of overall non-specific cytotoxicity as well as specific biological activities of pollutants present in complex environmental matrices and therefore represent suitable tool for EDCs monitoring.

Using battery of *in vitro* bioassays, we evaluated cytotoxicity and endocrine disruptive (ED) potential of (i) influents and effluents from 8 different municipal WWTPs in the Czech Republic (CZ) and (ii) their seasonal variability in influents and effluents from one of the WWTPs. Moreover, Polar Organic Integrative Samplers (POCISes) were deployed in rivers upstream and downstream of 7 domestic WWTPs situated in relatively unpolluted areas of CZ to assess background levels of ED potentials and to evaluate the environmental impacts of the WWTPs.

METHODS

In the first study, influents and effluents of municipal WWTP in Brno Modřice were sampled monthly for whole year. The procedure was described by Jedličková et al. (2010). In the second study, POCISes were deployed several kilometres upstream of the WWTPs discharges (US) and tens of meters downstream (DS) of them for about 3 weeks. Two types of POCISes (Pesticide and Pharmaceutical) were used at each site and processed according to Grabic et al. (2010).

Last study was designed to compare the levels of estrogens in influents and effluents of municipal mechanical-biological WWTPs with different sizes and sources of waste waters. Two WWTPs with capacities up to 10 000 equivalent citizens (EC) receiving only domestic waste waters, 2 WWTPs with capacities up to 100 000 EC receiving domestic waste waters and rain waters and 3 WWTPs with capacities higher than 100 000 EC receiving domestic waste waters, rain waters as well as industrial waste waters were sampled in June 2010 and processed by the same procedure as for the WWTP Brno, Modřice.

Four bioassays were used to determine overall cytotoxicity, anti/estrogenity, anti/androgenity and dioxin-like potentials of sample extracts (Novak et al. 2009; Leskinen et al. 2005). Results were expressed as estrogenic, androgenic or dioxin-like equivalents (EEq, AEq or TEq_{bio}) with respect to standard estrogen (17β-estradiol), androgen (testosterone) or dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin).

RESULTS

In the year-round assessment of waste waters in Brno WWTP, significant estrogenic activity was detected in influents as well as in effluents. The levels of estrogenic potential varied from 3.6 to 108 ng/l EEq in influent and from 0.1 to 3.9 ng/l EEq in effluent, respectively. The efficiency to remove estrogens was high (from 80 to more than 99 %), mostly more than 96 %. Androgenity was detected only in influent samples, whereas dioxin-like activity were relatively constant in both types of samples (from 0.1 to 3.3 ng/l TEq_{bio}). High cytotoxicity of influents was also dramatically decreased in the treatment process.

Very low cytotoxicity was detected for extracts from POCIS exposed in rivers with minor anthropogenic pollution. No extract showed significant antiestrogenic, androgenic nor antiandrogenic activity, while estrogenicity was detected in nearly all samples and differences were evident among US and DS samples. Background levels of the estrogenic potential were roughly estimated to fluctuate around 0.2 ng/l EEq (Arditsoglou, Voutsa 2008). There was about 2 times higher estrogenic potential in the rivers downstream of WWTPs compared to upstream but in two cases, the estrogenic potential was more than 4 times

higher and reached maximal values around 2 ng/l EEq. The levels of dioxin-like activity were near or under the assay detection limit (0.08 ng/POCIS TEQ_{bio}) in most cases. However, their increase in downstream samples was significant at least in extracts of POCIS Pharmaceutical.

No remarkable differences could be seen among estrogenic potentials of WW from 7 different municipal WWTPs (fig. 1). Estrogenic activity was detected in nearly all tested samples in ranges 25 – 63 EEq ng/l for influents and < 0.5 – 2 EEq ng/l for effluents, respectively. The efficiency of estrogen removal by all tested WWTPs was higher than 92 %.

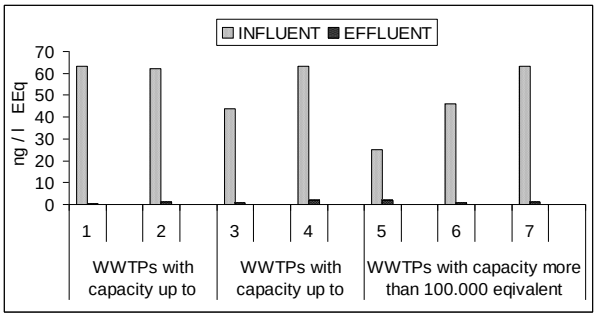

Fig. 1: Estrogenic activities of 7 municipal WWTPs influents and effluents measured by MVLN *in vitro* assay and expressed as ng/l EEq.

DISCUSSION

All evaluated WWTPs were efficient in removing at least 92 % of the estrogenic activity (with exception of one month sample in the year study). This fact as well as the determined EEqs correspond well with data from most other WWTP with activated sludge treatment in Europe (e. g. Korner et al. 2000). Predicted No Effect Concentration (PNEC) in water was determined as 1 ng/l EEq (Young et al. 2004). Theoretically, after dilution of WWTP effluents by recipient rivers PNEC would be hardly exceeded at any location in our studies. However, in unpolluted areas, discharge of single municipal WWTP effluent caused exceedance of EEq PNEC levels in recipient river in 2 out of 7 cases although the contributions of the effluent to the recipient was not higher than 10 and 30 %. Little information is available about dioxin-like activity in water phase, which we have found even in unpolluted rivers. Some recent studies detected dioxin-like activity in dissolved phase of grab samples of municipal waste waters (Dagnino et al. 2010).

CONCLUSIONS

Although *in vitro* bioassays revealed high WWTPs efficiencies for estrogens removal, significant estrogenic activity was detected in all types of

samples. This brought up important questions about other sources of EDCs, e. g. discharge of untreated waters to the recipients due to insufficient capacity of WWTPs. Further research of other sources of EDCs is needed.

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