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1. Introduction

River systems are contaminated by various chemicals, including classical ones such as PCBs, PAHs, pesticides or alkylphenols as well as emerging pollutants such as pharmaceuticals compounds, personal care products (PCPs), steroid hormones and their metabolites. A number of these substances are described as endocrine disrupting compounds (EDCs), through their ability to modulate the synthesis, the secretion, the transport, the excretion or the binding of endogenous hormones [1]. *In vitro* bioassays based on mechanisms of action at receptor level are commonly used as rapid, sensitive and quantitative biological methods to assess the occurrence of EDCs in aquatic systems. While estrogen (ER) and aryl hydrocarbon receptors (AhR) activities are well documented [2], there is still a lack of information on other nuclear receptor-related activities in the environment. Nonetheless, xenobiotic-sensing receptors [e.g. pregnane X receptor, PXR] or steroid receptors [e.g. androgen (AR), glucocorticoid (GR), progestagen (PR) receptors…] have been shown to be activated by environmental ligands like pesticides and pharmaceuticals. Hence, monitoring EDCs by using a panel of complementary bioassays should increase the detection capacity of EDCs and allow a better hazard assessment by establishing toxicity profiles.

A number of emerging compounds are polar and occur mainly in surface water at very low concentration. Their contamination levels are function of the season and can fluctuate with episodic pollution. Until recently, the monitoring of environmental contaminants in water use discrete sampling but it gives an incomplete picture of the contamination level. Thus passive samplers giving an integrate picture in the time were developed (review in [3]). Among them the Polar Organic Compounds Integrative Sampler (POCIS) allow the sampling of pesticides, steroids and pharmaceuticals.

In this study, we report a multi-receptor approach based on a panel of different reporter cell lines (Table 1) to establish toxicity profiles in environmental samples and its combined use with passive sampling (POCIS) for the detection of polar emerging compounds in freshwater systems. By using this approach, we newly report the occurrence of steroid-like activities in the water phase but not in sediments at a river site that is subjected to pharmaceutical industry effluent and where development abnormalities in fish were noted.

2. Materials and methods

In vitro Bioassays:

Receptors	**Cell lines** (principle)	**Reference ligands (EC50)**	**Examples of environmental ligands**	**Ref.**
Estrogen (**ER**)	**MELN** (MCF-7, ERE-LUC)	17β-E2 (0.01 nM)	(Xeno)Estrogens, Pharmaceuticals, PCPs	[4]
Dioxin (**AhR**)	**PLHC-1** (EROD induction)	TCDD (0.1/0.07 nM)	PAHs, PCBs, PCDD/Fs...	[2]
Pregnane (**PXR**)	**HG5LN-hPXR** (GAL4RE-Luc/GAL4-hPXR)	SR12813 (70 nM)	Pesticides, Pharmaceuticals, Steroids, Plasticizers	[5]
Androgen (**AR**)	**MDA-kb2** (MDA-MD-453,MMTV-Luc)	DHT (0.1 nM)	Steroids, Pharmaceuticals	[6]
Glucocorticoid (**GR**)	**MDA-kb2** (MDA-MD-453,MMTV-Luc)	Dexamethasone (100 nM)	Steroids, Pharmaceuticals Vinclozolin	[6]
Mineralocorticoid (**MR**)	**HG5LN-hMR** (GAL4RE-Luc/GAL4-hMR)	Aldosterone (10 nM)	Steroids Pharmaceuticals?	[7]
Progesterone (**PR**)	**HG5LN-hPR** (GAL4RE-Luc/GAL4-hPR)	R5020 (100 nM)	Steroids Pharmaceuticals?	[7]

Table 1: In vitro bioassays based on reporter cell lines used in this study

POCIS property and calibration: In order to calculate time weighted average (TWA) aquatic concentrations of studied compounds, Rs were calculated for several pollutants. To this end, POCIS (pharmaceutical

configuration) were exposed to standard chemicals under controlled conditions in laboratory [8]. In each experiment, the water from the calibration system and the sorbent from the POCIS samplers were regularly analyzed for target analyte concentration in order to evaluate the sampling kinetics of the passive sampler.

Study sites and sample preparation: The studied river is under mixed anthropogenic pressures (i.e. industrial and urban). Three different stations were sampled: the stations A, B and C were located upstream (A), 0.2 km downstream (B) and 1 km downstream (C) from a pharmaceutical industry effluent. Moreover, a municipal sewage treatment plant (1,000 eq-inhabitants) is located between stations B and C. In July 2009, sediments were sampled, sieved, freeze-dried and then 5 g were extracted (ASE) by heptane:acetone mixture (50:50). Extracts were evaporated to dryness and redissolved in 1 mL of MeOH. POCIS were deployed from June to November 2009 (6 sampling campaigns of one month each). After exposure, each POCIS was rinsed with ultrapure water. The sorbent was transferred into glass SPE tube by rinsing with ultrapure water and then dried for 1h. Extraction of organic compounds was done with sequential elution with 10 mL of dichloromethane, 10 mL dichloromethane:methanol (50:50) and 10 mL of methanol. POCIS extracts were evaporated to dryness and redissolved in 200 µL of methanol. Methanol organic extracts were then used for bioassays.

3. Results and discussion

In sediments, weak estrogenic activity (E2-EQ = 0.05-0.3 ng/g), weak persistent dioxin-like activity (TCDD-EQ = 1-7 ng/g) and moderate non-persistent dioxin-like activity (BaP-EQ = 2-10 µg/g) were found at the three sites whereas no AR, (anti)-AR, GR, and PXR activities were found.

In POCIS, summer samples presented weak PAH-like activity (0.58-0.86 µg/g) and strong PXR and ER activities were found at the tree stations, with the highest activities at the upstream station A. Interestingly, very strong GR activities were recorded at site B (Dex-EQ = 61 µg/g) and C (Dex-EQ = 69 µg/g) but none at site A located upstream from the pharmaceutical industry effluent. In addition, anti-MR, anti-PR and AR activity were also found downstream from the effluent hence suggesting a release of polar steroid compounds in the water phase during summer. Samples from the other campaigns are under analyses and results will be presented.

4. Conclusions

The combined use of POCIS and in vitro bioassays allowed detecting GR, AR, anti-MR and anti-PR activities downstream from a pharmaceutical industry. Such response profile strongly resembles that of certain classes of steroid drugs. Further in vitro bioassays are under progress to assess the seasonal fluctuation of these activities. Moreover, chemical analyses on POCIS extracts are under investigation in order to identify the substances responsible for activities.

5. References

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