TWO YEARS OF ESTROGENIC ACTIVITY ASSESSMENT IN THE SURFACE WATERS IN SAO PAULO STATE, BRAZIL

Martini GA[1], França DD[2], SoaresWA[2], Quináglia GA[2]

[1]IPEN, Av Prof Lineu Prestes, São Paulo, Brazil; [2]CETESB, Av. Frederico Hermann, São Paulo, Brazil

Introduction

The endocrine disrupting chemicals (EDCs) have received extensive attention due to their implications in human and environmental health and its occurrence in waters worldwide. The presence of EDCs can alter the hormonal systems of organisms and are arousing great interest among the scientific community due to their industrial and domestic uses and potential adverse effects[1]. Scientific studies highlight that endocrine active pharmaceuticals can cause adverse effects on aquatic ecosystems also at extremely low levels with consequent reduction of the biodiversity of sensitive aquatic species[2]. In particular, surface waters in Brazil have continuously received several chemicals, which are responsible for causing adverse effects to the aquatic biota. In order to monitor these substances, chemical analyses generally require a priori knowledge about the type of substances to be screened, whereas, for technical and economic reasons, it is not possible to analyze, detect and quantify all substances that are present in the aquatic environment. Chemical monitoring is therefore usually focused on regulated substances that are known to pose a threat to or *via* aquatic environment[2]. Considering this, it is important to assess the presence of EDC in surface water. Among the available methods of investigation, the *in vitro* bioassays are a low cost and rapid screening tool and have been used prior to chemical analyses. According to Carvalho[3] *in vitro* bioassays, as a part of a multi-tiered approach to water quality monitoring, can fill the gap between chemical and ecological assessments for a more holistic characterization of water quality. Considering these features, *in vitro* bioassays have been used to identify and quantify total estrogenic activity (EA) caused by the EDCs, providing a quick response of surface waters quality. The key advantage of monitoring a biological activity is that the overall activity from co-exposure to multiple, bioavailable chemicals with the same mode of action can be assessed[4]. In the present study the bioluminescent yeast estrogen assay (BLYES) was used as an *in vitro* bioassay to assess the estrogenic activity in São Paulo State for two years.

Materials and methods

Thirty-five sites in São Paulo State water bodies were selected for BLYES bioassay evaluation. Sites were chosen widely distributed throughout the State, among sites where protection of aquatic organisms is a concern. This work focuses on the ten sites that showed average estrogenic activity higher than 1.0 EEQ (Fig.1). The ten sites are part of CETESB monitoring programm, being those: Guarapiranga Reservoir (GUAR 00100), Ribeirão Pires (PIRE 02900), Araras River (ARAS 02900), Ribeirão Grande (RGRA 02990), Sapucaí-Guaçu River (SAGU 02100), Piracicaba River (PCAB 02135), São Miguel Arcanjo River (SMIG 02800), Jaguari Reservoir (JAGJ 00200), Jaguari River (JAGR 02100) and Cascata Reservoir (CASC 02050).

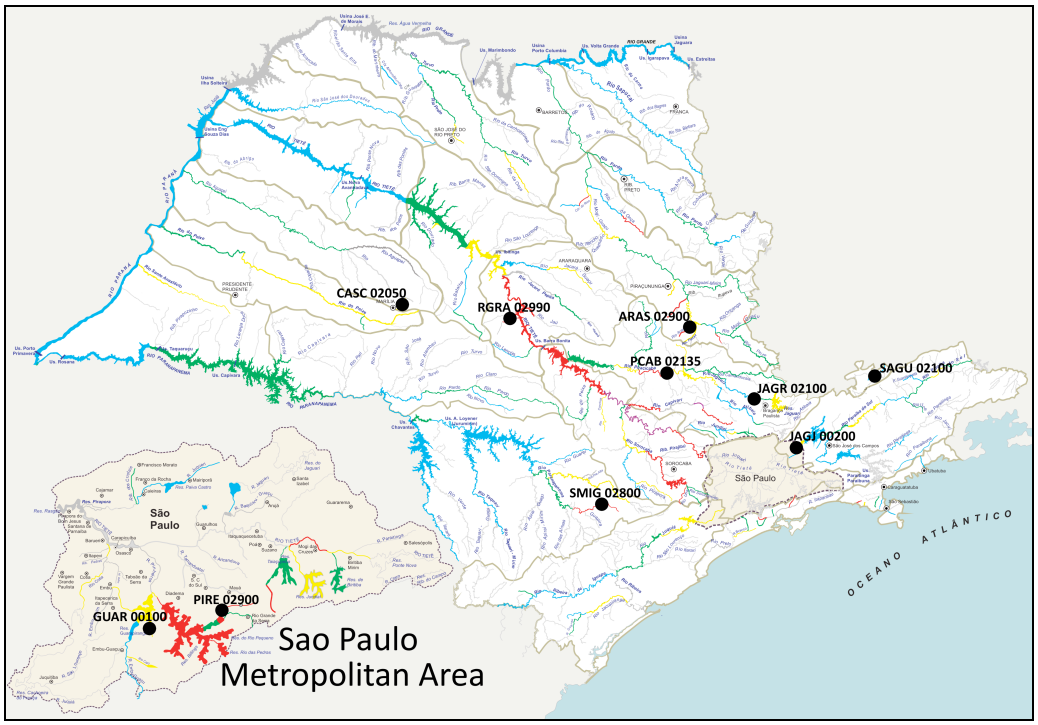

Figure 1 – Sao Paulo State map showing the ten sampled sites

The water samples were collected according to ANA[5] in 1L amber glass clean bottles protected from light and transported to the laboratory on ice. Sites were surveyed every two months for two years (2013-2014). The samples were then extracted within 7 days. Solid phase extraction was performed using 500 mg HLB Oasis cartridges (Waters, Milford, USA). Briefly, conditioning of the cartridges was performed with 6 mL of methanol. After the sample extraction, cartridge was dried under a nitrogen stream and then eluted using 6 mL of methanol. The eluates were collected in glass vials and were concentrated to final volume of 0.5 mL using a automated concentrator (Genevac Ez-2). Extracts of water samples were measured using a *lux*-based *Saccharomyces cerevisiae in vitro* bioassay, BLYES assay (bioluminescent yeast estrogen screen). This technique screens for compounds that bind to the estrogen receptor. A constitutively bioluminescent yeast strain (BLYR) was employed for measuring toxicity, as described by Sanseverino *et al*.[6] Succinctly when an estrogenic compound crosses the yeast cell membrane, the compound binds to a human estrogen receptor. This complex binds to an estrogen responsive element (ERE) activating the transcription of *luxA* and *luxB* which produces the luciferase enzyme. The luciferase hits the aldehyde substrate resulting in light emission. BLYES and BLYR strains were grown overnight at 30 ºC and 200 rpm shaking, until they reach OD_{600} 1.0. Samples were tested in 9 different concentrations and then pipetted into microplates along with the yeast strains. Positive and negative controls were used for quality assurance. Bioluminescence was measured on a luminometer (Perkin Elmer Victor X3), readings were plotted on a log–log scale graph, generating a dose–response curve, using SigmaPlot® software. Results were expressed as EEQ (estrogen equivalent in ng L^{-1}) using 17β-estradiol (E2) as a reference substance.

Results and discussion

All extracts of the ten river waters induced significant estrogenic activity. Mean values of estrogenic activity higher than 1.0 EEQ (on a two-year period) were present in 8 surface water samples (Fig. 2). Limit of quantification for the BLYES method was 0.1 EEQ.

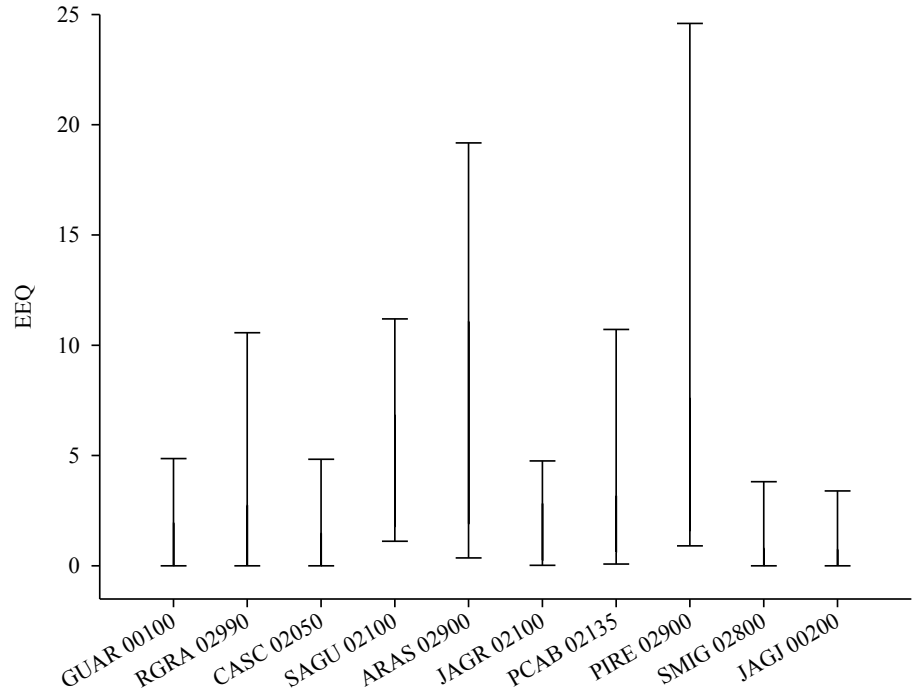

Figure 2 – Average estrogenic activity (EEQ) in ten sites in two years (2013-2014) of assessment.

The aquatic environment receives most of the pollutants released into the environment through effluents from wastewater treatment plants, which leads to many examples of endocrine disruption in aquatic species[7]. Estrogenic activity from 1.0 EEQ up is already a concern for protection of aquatic biota according to published researches [8-11]. The average estrogenic activity in the sampled sites in this study ranged from 0.79 – 6.77 EEQ. Within two years of monitoring, the highest mean values were found in Araras River and Ribeirão Pires, with estrogenic activity reaching 26.6 EEQ in one sample. The *in vitro* estrogenic activity of São Paulo State river waters were similar to related studies that were carried out in European countries and Japan, where the estrogenic activities ranged from 0.30 – 4.50 ng L^{-1} in France, 0.3 – 7.0 ng L^{-1} in Switzerland and 0.7 – 4.01 ng L^{-1} in Japan[12-14]. Estrogenic activity in surface waters from Netherlands, measured by the ER-CALUX bioassay was <0.5 – 0.61 EEQ in a study by Vethaak[15]. According to our findings, the monitoring of surface waters deserves special attention and needs efforts to become an important matter to assure the protection of aquatic biota.

Acknowledgements

The authors would like to thank CNPq for the financial support 141973/2014-5.

References

1. Deblonde, T., Cossu-Leguille, C., & Hartemann, P. (2011). Emerging pollutants in wastewater: a review of the literature. International journal of hygiene and environmental health, 214(6), 442-448.

2. Kunz, P. Y., Kienle, C., Carere, M., Homazava, N., & Kase, R. (2014). In vitro bioassays to screen for endocrine active pharmaceuticals in surface and waste waters. *Journal of pharmaceutical and biomedical analysis*.

3. Carvalho, R. N., Arukwe, A., Ait-Aissa, S., Bado-Nilles, A., Balzamo, S., Baun, A., ... & Lettieri, T. (2014). Mixtures of chemical pollutants at European legislation safety concentrations: how safe are they?. *Toxicological Sciences*, *141*(1), 218-233.

4. Silva, C. P., Otero, M., & Esteves, V. (2012). Processes for the elimination of estrogenic steroid hormones from water: a review. *Environmental Pollution*,*165*, 38-58.

5. ANA - Guia nacional de coleta e preservação de amostras: água, sedimento, comunidades aquáticas e efluentes líquidos / Companhia Ambiental do Estado de São Paulo; Carlos Jesus Brandão *et al*. (2011). São Paulo: CETESB; Brasília: ANA.

6. Sanseverino, J., Gupta, R. K., Layton, A. C., Patterson, S. S., Ripp, S. A., Saidak, L., ... & Sayler, G. S. (2005). Use of Saccharomyces cerevisiae BLYES expressing bacterial bioluminescence for rapid, sensitive detection of estrogenic compounds. *Applied and environmental microbiology*, 71(8), 4455-4460.

7. Sumpter, J. P. (2005). Endocrine disrupters in the aquatic environment: an overview. Acta hydrochimica et hydrobiologica, 33(1), 9-16.

8. Baldwin, W. S., Graham, S. E., Shea, D., & LeBlanc, G. A. (1997). Metabolic androgenization of female Daphnia magna by the xenoestrogen 4-nonylphenol. *Environmental Toxicology and Chemistry*, 16(9), 1905-1911.

9. Hayes, T., Haston, K., Tsui, M., Hoang, A., Haeffele, C., & Vonk, A. (2002). Herbicides: feminization of male frogs in the wild. *Nature*, *419*(6910), 895-896.

10. Fenner-Crisp, P. A., Maciorowski, A. F., & Timm, G. E. (2000). The endocrine disruptor screening program developed by the US Environmental Protection Agency. *Ecotoxicology*, 9(1-2), 85-91.

11. Legler, J., Zeinstra, L. M., Schuitemaker, F., Lanser, P. H., Bogerd, J., Brouwer, A., ... & van der Burg, B. (2002). Comparison of in vivo and in vitro reporter gene assays for short-term screening of estrogenic activity.*Environmental science & technology*, 36(20), 4410-4415.

12. Cargouet, M., Perdiz, D., Mouatassim-Souali, A., Tamisier-Karolak, S., & Levi, Y. (2004). Assessment of river contamination by estrogenic compounds in Paris area (France). *Science of the Total Environment*, 324(1), 55-66.

13. Vermeirssen, E. L., Burki, R., Joris, C., Peter, A., Segner, H., Suter, M. J. E., & Burkhardt□Holm, P. (2005). Characterization of the estrogenicity of Swiss midland rivers using a recombinant yeast bioassay and plasma vitellogenin concentrations in feral male brown trout. *Environmental Toxicology and Chemistry*, 24(9), 2226-2233.

14. Hashimoto, S., Horiuchi, A., Yoshimoto, T., Nakao, M., Omura, H., Kato, Y., ... & Giesy, J. P. (2005). Horizontal and vertical distribution of estrogenic activities in sediments and waters from Tokyo Bay, Japan. *Archives of Environmental Contamination and Toxicology*, 48(2), 209-216.

15. Vethaak, A. D., Lahr, J., Schrap, S. M., Belfroid, A. C., Rijs, G. B., Gerritsen, A., ... & de Voogt, P. (2005). An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of The Netherlands. *Chemosphere*, 59(4), 511-524.