Environment International 99 (2017) 120–130

Contents lists available at ScienceDirect

Environment International

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

Review article

Analysis of the sensitivity of *in vitro* bioassays for androgenic, progestagenic, glucocorticoid, thyroid and estrogenic activity: Suitability for drinking and environmental waters

Frederic D.L. Leusch [a,*], Peta A. Neale [a], Armelle Hebert [b], Marco Scheurer [c], Merijn C.M. Schriks [d]

[a] Australian Rivers Institute, Griffith School of Environment, Griffith University, Gold Coast, Qld, 4222, Australia
[b] Veolia Research & Innovation, Paris, France
[c] DVGW – Technologiezentrum Wasser, Karlsruhe, Germany
[d] KWR Watercycle Research Institute, Nieuwegein, The Netherlands

ARTICLE INFO

Article history:
Received 13 August 2016
Received in revised form 13 December 2016
Accepted 15 December 2016
Available online 22 December 2016

Keywords:
Androgenic
Progestagenic
Glucocorticoid
Thyroid
Estrogenic
Sensitivity

ABSTRACT

The presence of endocrine disrupting chemicals in the aquatic environment poses a risk for ecosystem health. Consequently there is a need for sensitive tools, such as *in vitro* bioassays, to monitor endocrine activity in environmental waters. The aim of the current study was to assess whether current *in vitro* bioassays are suitable to detect endocrine activity in a range of water types. The reviewed assays included androgenic ($n = 11$), progestagenic ($n = 6$), glucocorticoid ($n = 5$), thyroid ($n = 5$) and estrogenic ($n = 8$) activity in both agonist and antagonist mode. Existing *in vitro* bioassay data were re-evaluated to determine assay sensitivity, with the calculated method detection limit compared with measured hormonal activity in treated wastewater, surface water and drinking water to quantify whether the studied assays were sufficiently sensitive for environmental samples. With typical sample enrichment, current *in vitro* bioassays are sufficiently sensitive to detect androgenic activity in treated wastewater and surface water, with anti-androgenic activity able to be detected in most environmental waters. Similarly, with sufficient enrichment, the studied mammalian assays are able to detect estrogenic activity even in drinking water samples. Fewer studies have focused on progestagenic and glucocorticoid activity, but some of the reviewed bioassays are suitable for detecting activity in treated wastewater and surface water. Even less is known about (anti)thyroid activity, but the available data suggests that the more sensitive reviewed bioassays are still unlikely to detect this type of activity in environmental waters. The findings of this review can help provide guidance on *in vitro* bioassay selection and required sample enrichment for optimised detection of endocrine activity in environmental waters.

Contents

Abbreviations: AH, amiodarone hydrochloride; AHEQ, amiodarone hydrochloride equivalents; DF, dilution factor; DHT, dihydrotestosterone; DHTEQ, dihydrotestosterone equivalents; DOC, dissolved organic carbon; DPH, diphenylhydantoin; Dexa, dexamethasone; DexaEQ, dexamethasone equivalents; E2, 17β-estradiol; EEQ, 17β-estradiol equivalents; EC, effect concentration; EF, enrichment factor; FLU, flutamide; FLUEQ, flutamide equivalents; FulvestrantEQ, fulvestrant equivalents; Levo, levonorgestrel; LevoEQ, levonorgestrel equivalents; MDL, method detection limit; MifEQ, mifepristone equivalents; OHFLU, hydroxyflutamide; OHFLUEQ, hydroxyflutamide equivalents; OHTMX, hydroxytamoxifen; ORG2058, 16α-ethyl-21-hydroxy-19-nor-pregn-4-ene-3,20-dione; P4, progesterone; P4EQ, progesterone equivalents; PMG, promegestone; PMGEQ, promegestone equivalents; REP, relative effect potency; RU5020, promegestone; SPE, solid phase extraction; T3, triiodothyronine; T3EQ, triiodothyronine equivalents; TMX, tamoxifen; TMXEQ, tamoxifen equivalents; YAS, yeast androgen screen; YES, yeast estrogen screen.

* Corresponding author.
E-mail address: f.leusch@griffith.edu.au (F.D.L. Leusch).

http://dx.doi.org/10.1016/j.envint.2016.12.014
0160-4120/

1. Introduction

There is increasing concern about the presence of endocrine disrupting chemicals in the environment due to their potential effects on both human and ecosystem health (Bergman et al., 2013). Environmental waters in particular can be impacted by endocrine disrupting chemicals, with both point sources, such as wastewater effluent, and diffuse sources, such as agriculture and industry, contributing to the chemical load (Vethaak et al., 2005). In addition to natural and synthetic hormones, a wide range of environmental chemicals, including industrial compounds, pesticides and UV filters, have been identified as known or suspected endocrine disrupting chemicals (Bergman et al., 2012). As a result of the wide range of potential endocrine disrupting chemicals and the fact that they will be present in water as a complex mixture of contaminants, chemical analysis alone is insufficient to monitor endocrine disrupting chemicals. Instead, *in vitro* bioassays indicative of hormonal activity, including androgenic, progestagenic, glucocorticoid, thyroid and estrogenic activity, can be applied to assess endocrine activity in environmental waters. Such assays have been applied widely to wastewater effluent and surface water (*e.g.* Bain et al., 2014; Schiliro et al., 2012; Scott et al., 2014; Thomas et al., 2002), with less focus on cleaner water sources, such as advanced treated or drinking water (*e.g.* Brand et al., 2013; Conley et al., in press). Much of the literature concentrates on the detection of agonistic activity in water, but some environmental contaminants can act as antagonists, which, if present in a sample, can reduce the agonistic response *in vitro* (Ihara et al., 2014), emphasising the importance of evaluating both agonism and antagonism in environmental samples.

As many endocrine disrupting chemicals are present in the aquatic environment at low concentrations, there is a need for sensitive methods to detect both endocrine agonists and antagonists at environmentally relevant levels. In addition to the inherent sensitivity of the assay, factors such as sample enrichment, typically by solid phase extraction (SPE), and sample dilution in the assay, which is dependent on assay solvent tolerance, will affect the overall assay sensitivity. The aim of the current study was to review the sensitivity of a range of specific hormonal activity *in vitro* bioassays to determine whether they are suitable to detect endocrine activity in environmental water samples. The reviewed bioassays focus on androgenic, progestagenic, glucocorticoid, thyroid and estrogenic activity in both agonist and antagonist mode and include a range of assay types, such as receptor binding assays, yeast reporter gene assays, mammalian reporter gene assays and cell proliferation assays. Between 5 and 11 assays were selected for each endpoint, with all assays identified as in at least moderate use for water quality testing (Global Water Research Coalition, 2006, 2012). A literature search in ScienceDirect (31st March 2016) was conducted to determine the number of studies that mentioned each assay specifically (Fig. 1). While this approach may not capture all publications, it does reveal that the different endpoints have received different levels of attention, with the majority of work focusing on estrogenic activity, followed by androgenic activity. In contrast, progestagenic, glucocorticoid and thyroid activity have received much less attention. However, these hormonal systems still play an important role for maintenance of sexual development, growth and homeostasis, thus there is still a need for sensitive methods to detect these less studied endpoints in environmental waters.

2. Experimental approach

The reviewed assays included 11 (anti)androgenic assays, 6 (anti)progestagenic assays, 5 (anti) glucocorticoid assays, 5 (anti)thyroid assays and 8 (anti)estrogenic assays. Assay sensitivity was determined by re-evaluating existing reference compound concentration-effect curves from the literature in order to determine the concentration causing 10% effect (EC_{10}) for each assay. The EC_{10} was selected as the assay limit of detection based on Escher et al. (2014). Ideally, an assay reference compound should be a potent chemical that is related to the mode of action of the bioassay and also potentially present in environmental water samples (Escher and Leusch, 2012). For some of the more recent assays, such the GeneBLAzer assays, limited reference compound data were available in the literature, thus the concentration-effect curves were run for the current study using the GeneBLAzer experimental protocols. Method detection limits (MDL) for each assay were calculated using the re-evaluated EC_{10} value, the typical dilution factor (DF) in the assay and a sample enrichment factor (EF) of 1000, which is a common yield from SPE (Eq. 1). The assay DF depends on the sensitivity of the assay to solvent and was typically between 100 and 1000.

$$\mathrm{MDL} = \frac{\mathrm{EC_{10}} \cdot \mathrm{DF}}{\mathrm{EF}} \quad (1)$$