Estrogenic, androgenic and glucocorticoid-like activity in Dutch waste water using a panel of CALUX bioassays

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Abstract

A group of aquatic contaminants that raised concern about possible biological effects are endocrine disrupting compounds (EDC). Since it is virtually impossible to analyze all individual components responsible, bioassays are used to quantify the presence of compounds based on their biological effects. Current emphasis of EDC research lies on the measurement of estrogenic and androgenic compounds. In this study, a panel of CALUX® bioassays testing for estrogen (ERα CALUX®), androgen (AR CALUX®) and glucocorticoid (GR CALUX®) receptor activity has been applied to extracts of municipal seweage treatment plants effluents. The results show that the CALUX® panel can successfully be applied to complex samples such as waste water effluents and that different types of hormone receptor agonistic activity could be detected.

Introduction

Monitoring of the quality of the aquatic environment is typically based on the chemical analysis of a defined set of compounds. It is virtually impossible to analyse all compounds individually, let alone all metabolites. Therefore, bioassays are used more and more for the detection of compounds with a specific effect, rather than a specific identity. An important application of bioassays in water quality control is the detection of endocrine disrupting compounds (EDCs). The main focus of EDC research has been on compounds that interfere with the estrogen receptor, mimicking or blocking the function of the natural estrogen estradiol. However, more hormone receptors exist that can be influenced by compounds in the environment, either being natural hormones or xenobiotic compounds.

A logical and reliable way of detecting and characterizing these hormonally active compounds in the environment is by quantifying the amount of hormone receptor activation produced by environmental extracts relative to a (natural or synthetic) reference standard. Bioassays like CALUX® can quantify this activity by coupling the normal response to the production of the enzyme luciferase that can be easily measured. Recently, we expanded our panel of CALUX® bioassays[1]. Since effluents are shown to be an important source of endocrine disrupting compounds, we used our bioassay panel to determine the amount of estrogenic (ER), androgenic (AR) and glucocorticoid-like (GR) compounds in influent and effluents.

Materials and Methods

Water samples collection and extraction
Samples were taken from different municipal sewage treatment plants using glass bottles. Samples were frozen until further processing. Of each sample, 500 mL was extracted by liquid-liquid extraction with ethyl acetate. After extraction, samples are taken up into 50 µl of DMSO.

Cell culture
Stably transfected human U2OS-cell lines[1,2] were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium (DF, Gibco) supplemented with 7.5 % fetal calf serum and 200 µg/ml G418.

CALUX® reporter gene assays
CALUX® reporter gene assays were performed as described elsewhere[1,2]. In short, cells were seeded into 96-well plates in medium supplemented with hormone-stripped serum. The next day, the medium was replaced with medium containing the water extracts (0.1 % DMSO). After 24 hours exposure, the medium was removed and the cells were lysed. The substrate luciferin was added to the wells to quantify the amount of luciferase produced by the cells by measuring the amount of light using a luminometer. For reference equivalents calculation, a dose-response curve of the reference compound was included in the analysis. The reference compounds used for the different bioassays were: ERα (estradiol, E2), AR (dihydrotestosterone, DHT), and GR (Dexamethasone, Dex).

Data analysis
Luciferase activity per well was measured as relative light units (RLUs). Fold induction was calculated by dividing the mean value of light units from exposed and non-exposed (solvent control) wells. Luciferase

induction as a percentage of maximum activity (by reference compound) was calculated by setting the highest fold induction at 100%. Dose-response curves were fitted using the sigmoidal fit y = a0 + a1/(1 + exp(-(x-a2)/a3)) in GraphPad Prism (version 4.00 for Windows, GraphPad Software, San Diego, CA), which determines the fitting coefficients by an iterative process minimizing the c2 merit function (least squares criterion). In Graphpad Prism, the relative light unit values of the sample extracts were interpolated in the dose-response curve of the reference compound to calculate the amount of reference equivalents present in the sample.

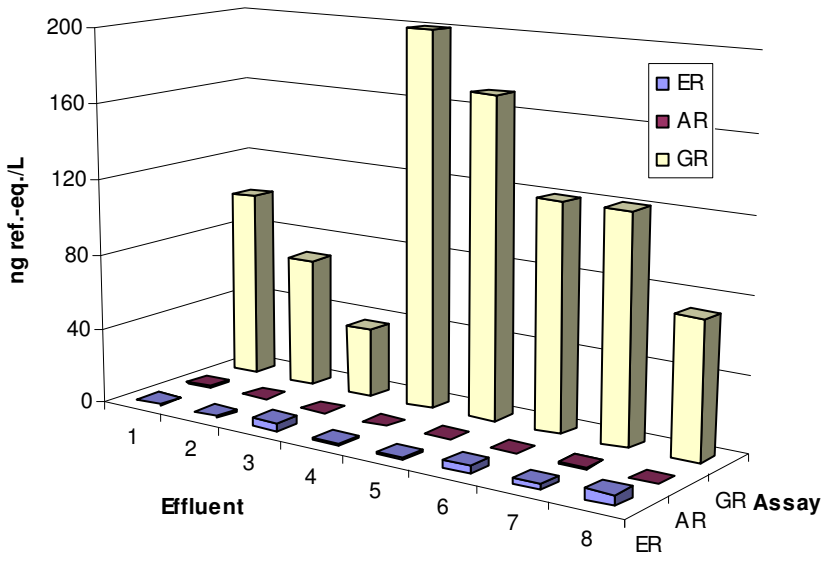

Figure 1. CALUX® profile of the different effluents. Results are expressed as ng of the reference compound equivalents per litre of water.

Results and Discussion

The results as presented above show that the CALUX® panel can successfully be applied for complex environmental samples as waste water effluents. All types of hormone receptor agonistic activity could be detected in the effluents (Figure 1), which means they enter the aquatic environment.

Estrogenic activity is present in all effluents, at levels ranging from 0.5-6 ng E2-eq. per liter which is in the normal range for Dutch effluents. Currently, in the Netherlands a trigger value for estrogenic activity in water control is discussed at a level of 7 ng E2-eq. per liter. Androgenic activity is also detected at quantifiable levels in 5 out of the 8 effluents. This level of androgenic activity is in the same range as reported previously for Dutch municipal effluents[4]. Agonistic glucocorticoid-like activity is detected in all effluents, at levels higher than estrogens and androgens. Compounds with glucocorticoid-like activity have been reported to be present in the aquatic environment at detectable levels[3,4]. The level of glucocorticoid-like activity is higher than reported previously for Dutch municipal effluents[4].

So far, the focus regarding EDC research is on agonistic estrogenic activity, which is regularly measured in water quality monitoring. The panel of CALUX® bioassays utilized here, consisting of the ERα, AR and GR CALUX®, adds more, currently unknown information about the presence of biologically active compounds in the aquatic environment. Compounds are present that can transactivate the estrogen, androgen and glucocorticoid receptor respectively. More information is needed about the levels, identity and removal processes of these nuclear hormone receptor mimics, to asses the possible risk that they might pose to the environment and to human health

References

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