APPLICATION OF A STEROID RECEPTOR-BASED BATTERY OF CALUX® BIOASSAYS FOR WATER QUALITY CONTROL ANALYSIS

van der Linden SC[1], Heringa M[2], Puijker L[2], van der Burg B[1], Brouwer A[1]
[1]Biodetection Systems BV, Amsterdam, The Netherlands
[2]KIWA Nieuwegein, The Netherlands

Abstract

Monitoring the quality of the aquatic environment by analyzing a (limited) defined set of compounds can impossibly be translated into the biological effects of the mixture. A group of aquatic contaminants that raised concern about possible biological effects are endocrine disrupting compounds (EDC). Research has focussed on compounds that interfere with the binding of natural hormones to nuclear hormone receptors including thyroid hormone, sex hormone, stress hormone, vitamin D3 and retinoic acid receptors. 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®), progesterone (PR CALUX®), glucocorticoid (GR CALUX®) and thyroid (TRβ CALUX®) receptor (agonistic and antagonistic) activity has been applied to extracts of STP effluents (industrial and municipal), surface water and drinking water. The results show that the CALUX® panel can successfully be applied to complex samples such as waste water and surface water extracts. With the CALUX® panel, different types of hormone receptor agonistic activity could be detected in the effluent and surface water samples, but none in the drinking water. No antagonistic activity could be determined in any of the samples.

Introduction

Monitoring of the quality of the aquatic environment is typically based on the chemical analysis of a defined set of compounds. It is impossible to chemically identify all compounds present, nor know the biological effects of the mixture. A group of aquatic contaminants that raised concern about biological effects are endocrine disrupting compounds (EDCs). The main focus of EDC research has been on compounds that interfere with the binding of natural hormones to nuclear hormone receptors, a large family of ligand-dependent transcription factors including thyroid hormone, sex hormone, stress hormone, vitamin D3 and retinoic acid receptors.

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. Historically, EDC research has focussed on two nuclear hormone receptors, namely the estrogen receptor and the androgen receptor. In this study, a panel of CALUX® bioassays[1] is used to screen for five different types of specific receptor (agonistic and antagonistic) activity: estrogen (ERα), androgen (AR), progestagen (PR), glucocorticoid (GR) and thyroid receptor (TRβ).

Materials and Methods

Water samples collection and extraction
Samples were taken from sewage treatment plants (industrial and municipal), surface water and drinking water using glass bottles. Samples were cooled until further processing. One liter of water 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 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), TRβ (triiodothyronine, T3), AR (dihydrotestosterone, DHT), PR (Org2058), 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). EC50 values were calculated by determining the concentration by which 50 percent of maximum activity was reached using the sigmoidal fit equation. 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.

Results and Discussion

The results show that the CALUX® panel can successfully be applied to complex samples as waste water and surface water extracts. Almost all types of hormone receptor agonistic activity could be detected in the effluent and surface water samples, but none in the drinking water (Figure 1). No TRβ CALUX® activity was detected in the water samples. Also, no antagonistic activity was detected in any of the samples (results not shown). Highest GR CALUX® and ER CALUX® activity was detected in the municipal STP effluent (51 ng Dex-eq. per liter and 1.1 ng E2-eq. per liter respectively), but highest PR CALUX® activity was detected in surface water (5.8 ng Org2058-eq. per liter). Presence of one type of receptor activity does not seem to predict the presence of activity for the other types of receptors.

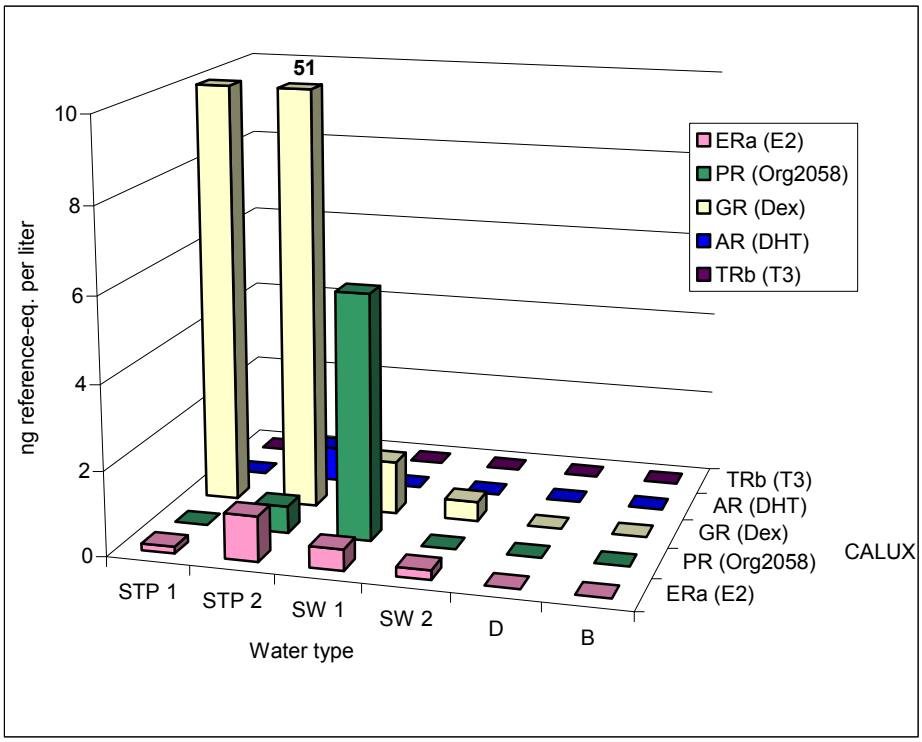

Figure 1. CALUX® profile of different types of water samples. Results are expressed as ng of the reference compound equivalents per litre of water. Samples are STP effluents (STP 1= industrial, STP 2 =municipal), surface water (SW 1,2), drinking water (D) and a procedure blank (B). For visualization purposes, the GR response of sample location 2 (51) is not completely shown.

Of the receptor agonists found, only estrogenic activity is regularly measured in water quality monitoring. The levels of estrogenic activity was in the normal range for Dutch waters, ranging from 0.5-1 ng E2-eq. per liter for STP effluents and 0.16-0.5 ng E2-eq. per liter for surface waters. 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. For the other CALUX® bioassays, no information is currently available about the levels present in the aquatic environment.

In conclusion, the panel of CALUX® bioassays consisting of the ERα, AR, PR, GR and TRβ CALUX® adds currently unknown information about the presence of biologically active compounds in the aquatic environment. Compounds are present that can transactivate the estrogen, androgen, progesteron 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.

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