

New: REA bioassay for analysis of estrogenic activity in the water cycle

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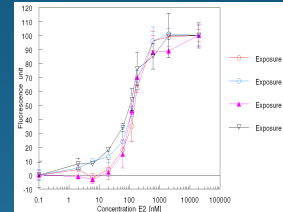


Abstract

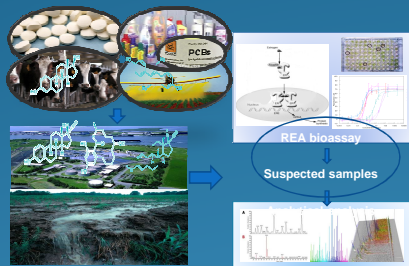
Estrogenic endocrine disrupting compounds (eEDCs) can enter surface water bodies due to incomplete removals from wastewater treatment systems or by run-off from agriculture areas. Since broad spectrum chemical analytical methods for EDCs are expensive and laborious, screening bioassays that are able to detect estrogenic activity can be applied for prior selection of samples. In this study, the RIKILT yeast Estrogen bioAssay (REA), which has been developed for detection of estrogenic compounds in animal urine and feed, has been validated for water samples. The REA is sensitive, specific and stable for estrogenic analyses in water samples. Estrogenic activity is stable for at least 4 weeks when samples are stored at 4°C.

Results

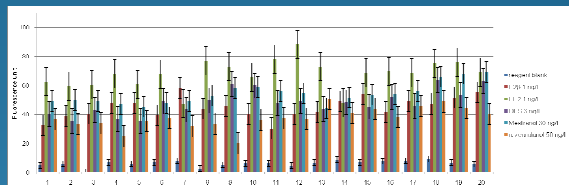
Reproducibility of the dose-response curves of relative fluorescence responses from different experiments was good. Both curves and slopes show good similarity. Decision limit CC α and detection limit CC β were determined for all hormones. No false negatives and cytotoxicity were observed.



Dose response curves of REA after 24 hours exposure to E2.



Products and sources of eEDC, and methodology of this study



Mean fluorescence responses in REA of validation experiment with 20 blanks and 20x5 spiked water samples.

Methods

Water samples are sand filtered, extracted with Oasis-HLB SPE columns and concentrated with a sample concentrator at 55°C under nitrogen flow. Final extracts are dissolved in DMSO and stored at 4°C.

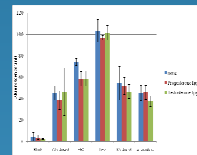
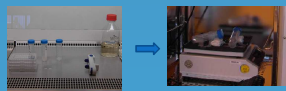


Water extraction.

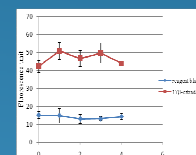


Yeast is inoculated one day before exposure. Inoculated yeast is exposed to the extracts in 96-well plates and incubated at 30°C for 22 hours. Fluorescence at t=0 and t=22h are measured in TECAN Spectrofluor+ platereader (excitation 485 nm and emission 530 nm).

REA bioassay performance.



Specificity and interference with androgenic hormones



Stability of E2 spiked water samples stored at 4°C

The REA response was not interfered by large amounts of progesterone or testosterone (1000 ng/L). The stability of E2 in water stored at 4°C appeared to be good, since variation is less than 10% in 4 weeks.

There are three effluent samples from Amsterdam Westpoort, Amstelveen and Den Helder collected from total 12 Waste Water Treatment Plants (WWTP) are suspected as content estrogenic activities of 1, 11 and 4 ng E2 equivalent per L (EEQ/L), respectively. Limit of quantification (LOQ) was defined as CC α and is calculated from a fitted EE2 dose-response curve as 0,3 ng EEQ/L. Cytotoxicity is observed in most of extracts which leads to increased LOQ. No estrogen conjugation is detected in these WWTP effluents.

Conclusions

- Low detection levels of E2 and EE2 (1ng/L), DES (3ng/L), mestranol (30 ng/L) and α -zearalanol (50 ng/L) in surface water and effluent
- Specific and sensitive for estrogenic substances
- Storage of water samples up to 4 weeks at 4°C
- REA assay is sensitive for cytotoxicity from WWTP effluent samples

Acknowledgments

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Reference

Bovee, TFH, HH Heskamp, ADR Hamers, RLAP Hoogenboom and MWF Nielen (2005). *Analytica Chimica Acta*, 529, 57-64.