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Detection and elimination of human exposure to environmental hormone disrupting substances.

Estrogen Receptor Binding Assay protocol

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Experimental procedure Estrogen Receptor Binding Assay.

The ERBA results for the pure compounds were obtained in the framework of a different project and are not presented in this report. Hereunder, a general overview of the method is given. A detailed description of the experiments is in preparation, and will be presented for publication in a peer reviewed international journal.

A Yeast culture and cytosol preparation

Yeast cultures were performed according to the protocol described in C.1.4.6. After protein induction, the yeast were pelleted at 1000g and frozen at –196 °C. The frozen yeast pellets were dismembrated using a Berthold Mikrodismembrator and the resulting powder was resuspended in a receptor stabilising buffer (Tris-HCl (50mM), EDTA (1mM), DTT (2mM), sodium orthovanadate (1mM), KCl, (0.5M), glycerol (10%) pH 7.4 supplemented with 1mM PMSF, 1 µg/ml aprotinin, 1µM pepstatin A, 100µM leupeptin, 1 µg/ml chymostatin, all Sigma-Aldrich NV, Bornem, Belgium). After ultracentrifugation (1 hour, 100000 g; Beckmann L5-65) the cytosolic fraction was collected and frozen at –80°C.

B Estrogen Receptor Binding Assay (ERBA) procedure

In this competitive binding assay, different concentrations of compounds and a fixed concentration of labeled 17β-estradiol are incubated with a cytosolic yeast extract containing cloned human estrogen receptors. In this way, the capacity of chemicals or mixtures to displace 17β-estradiol from the estrogen receptors can be studied. When a standard curve of unlabeled estradiol is included in the assay, the estrogenic potency of the samples can be quantified (Figure B.5.1-1).

200µL of yeast cytosol was incubated with a serial dilution (5 x ¼) of the extracted sample or a standard curve of unlabeled 17β-estradiol (5pM-50nM) in the presence of 0.4 pmol radioactive 17β-estradiol. After an overnight incubation at 0°C, the 200 µL were transferred to the coated plates and incubation continued at 37°C for 1 hour. Subsequently, plates were washed and treated with 200 µL of ethanol. The extracted radioactivity was quantitatively transferred to 3.5 mL of UltimaGoldXR (Perkin Elmer LifeSciences, Zaventem Belgium) and measured using a Packard Tricarb liquid scintillation counter (Perkin Elmer LifeSciences, Zaventem België). The measured scintillation data were used to generate a competition graph (see further). by plotting absorbance values versus the dilution factors of the sample or the concentration of unlabeled 17 -estradiol.

C Calculations

Dose response curves for luminescence counts were plotted against the dilution factor of the samples. The best fitting curve was calculated using SigmaPlot for Windows, version 4 (SPSS, Heverlee, Belgium) by a four parameter logistic regression as advocated by Moore et al. (1997), using the Marquardt-Levenberg algorithm according to the formula:

$$\%comp = min + \frac{max - min}{1 + 10^{(\log EC_{50} - x)Hillslope}}$$

where min and max are the minimal (baseline) and maximal absorbance; Hillslope is the slope of the curve at the transition centre and EC50 is the concentration of test compound yielding half maximal effects. The estrogenic equivalent value is defined as the concentration of 17β-estradiol yielding a an effect equal to the effect seen at the sample's EC50.