

Utilization of a novel chemical fractionation technique and the H295R assay to assess effects of Upper Danube River sediments on steroidogenesis.



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Introduction

Previous investigations of Upper Danube River sediments revealed acute and specific toxic potentials associated with certain local sediments and this toxic potential could have an effect on fish populations within the River. One class of chemicals of concern that had been identified as part of previous sampling efforts in both, water and sediment is endocrine disrupting chemicals. High endocrine effects in both the YES and H295R assay have been measured in previous studies in Upper Danube River sediment extracts. In an effort to identify the causative agents, in this study, the concept of Effect-directed analysis was utilized to characterize four sediment extracts from the Upper Danube River for their ability to disrupt steroidogenesis pathways in the H295R assay. Sediments were first screened for cytotoxicity and effects on the production of testosterone (T) and 17 β -estradiol (E2) *in vitro* by use of H295R cells. To assess which group of chemicals within the sediment sample caused the original effects on steroidogenesis, sediments that showed a potential to affect steroidogenesis were fractionated using a novel fractionation technique. This technique fractionates the sediment extracts into 18 fractions based on polarity, planarity, and the size of the aromatic ring system.

Objectives

1. Assess the toxicity of raw sediment extracts from four locations along the Upper Danube River using the *H295R* Assay
2. Evaluate which groups of chemicals caused the measured toxicities using new chemical fractionation techniques that separate the raw sediment extracts into 18 different chemical fractions.
3. Analyze all 18 chemical fractions using the H295R Assay.

Methods

Sampling and extraction

- Sediments were sampled (top 5cm) at four locations along the Upper Danube River using a Van Veen grabber in January 2006 and 2004 (Figure 1)
- Samples were extracted and fractionated into different chemical groups using a new technique by Varel et al., 2008 that uses 3 HPLC columns and separates the sample into 18 fractions according to their polarity, planarity and the size of their aromatic system
- Crude sediment extracts and all 18 fractions were analyzed for their toxicity using the H295R Assay

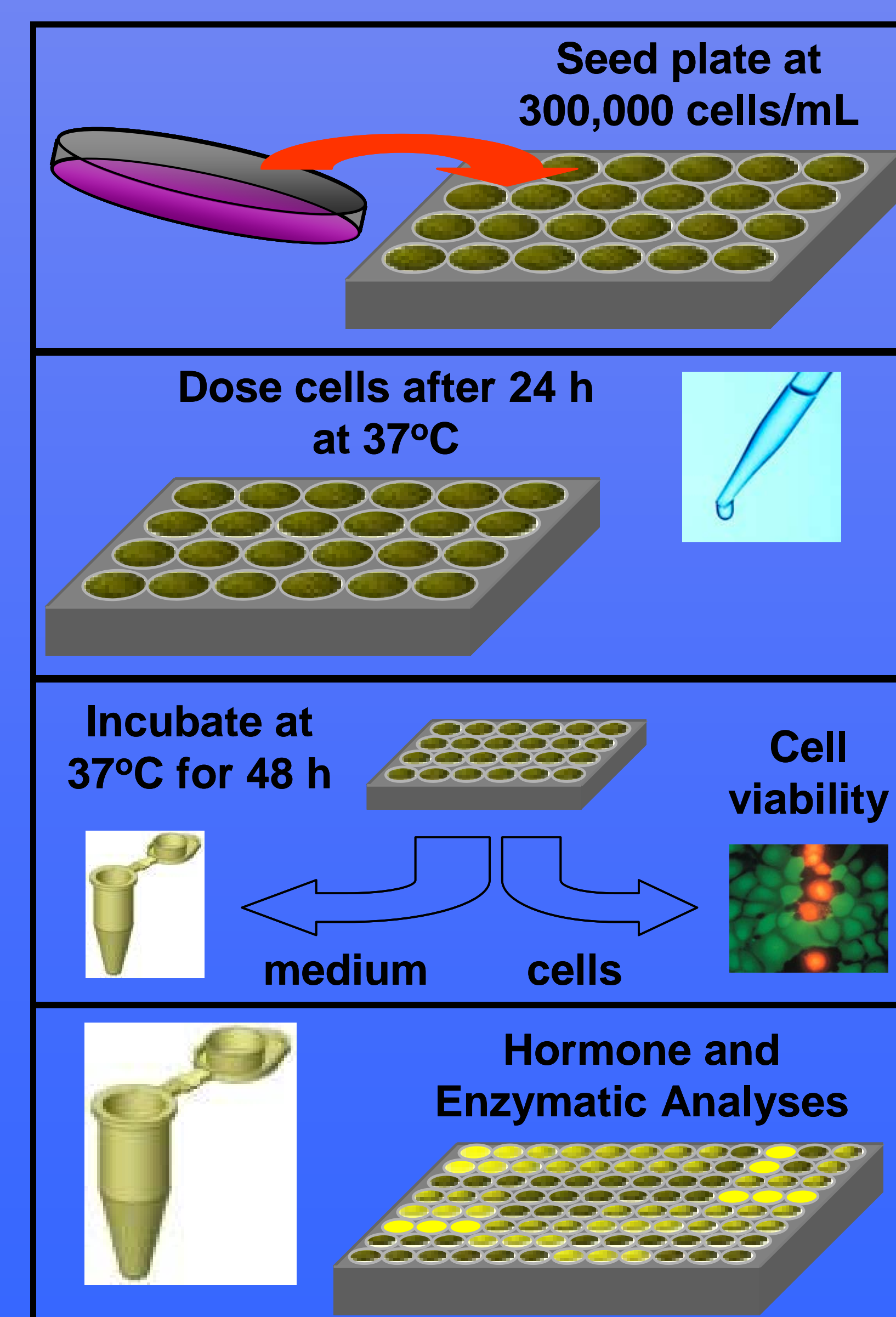


Figure 2 Dose response of four sediment extracts analyzed with the *H295R* Assay. * indicates significant difference from control