

# Oestrogenic activity using a recombinant yeast screen assay (RCBA) in South African laboratory water sources

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## Abstract

Many chemicals released into the environment are believed to disrupt normal endocrine functions in humans and animals. These endocrine disrupting chemicals (EDCs) affect reproductive health and development. A major group of EDCs that could be responsible for reproductive effects are those that mimic natural oestrogens, known as xeno-oestrogens. A number of in vivo and in vitro screening strategies are being developed to identify and classify xeno-oestrogens, in order to determine whether they pose a health risk to humans and animals. It is also important to be able to apply the assays to environmental samples for monitoring purposes. In South Africa information on the levels of EDCs in water is limited. While establishing the recombinant yeast screen bioassay (RCBA) using the yeast strain *Saccharomyces cerevisiae* for oestrogenic activity, problems were experienced with contamination. Four South African laboratory water sources were assessed. From the results it was clear that the water used in the preparation of the medium for the assay was the source of oestrogenic contamination. Care should be taken to eliminate all possible sources of contamination in the test procedures to eliminate the reporting of false positive results. The fact that South African laboratory and surface waters tested positive for oestrogenic activity has far reaching implications regarding reproductive and general health.

**Keywords:** endocrine disrupting chemicals, water, yeast screen, oestrogenic activity.

## Introduction

A number of chemicals released into the environment are believed to disrupt normal endocrine function in humans and animals (Colborn et al., 1993; Toppari et al., 1996). These endocrine disrupting chemicals (EDCs) have various endocrine and reproductive effects, believed to be due to their:

- Mimicking effects of endogenous hormones such as oestrogens and androgen
- Antagonizing the effects of normal, endogenous hormones
- Altering the pattern of synthesis and metabolism of natural hormones
- Modifying hormone receptor levels (Soto et al., 1995).

Documented effects on fish, wildlife and evidence from human epidemiology and experimental toxicology have led to an emerging hypothesis that chemicals may be affecting reproduction and development (Krimsky, 2000; Parrott et al. 2001). One major group of EDCs that could be responsible for disruptive reproductive effects, are those that mimic natural oestrogens.

Currently attention is being devoted to the development of in vivo and in vitro screening strategies to identify and classify xeno-oestrogens, in order to determine whether such chemicals pose a hazard to human health (Beresford et al., 2000). The in vitro assays range from simple competitive binding assays, relying solely on the chemical's ability to bind to the oestrogen receptor (Jobling et al., 1995; Shelby et al., 1996), to more complex systems where the chemical binds to and activates the receptor. These latter assay systems include the proliferation of the hu-

man breast cancer cell line (MCF-7) (Soto et al., 1995; Soto et al., 1994), vitellogenin gene expression in hepatocyte cultures (Jobling and Sumpter, 1993), and yeast-based assays expressing either rainbow trout (Petit et al., 1997) or human oestrogen receptors (Routledge and Sumpter, 1996). The oestrogen receptor (ER)-mediated chemical activated luciferase gene expression (ER-CALUX) assay uses T47-D human breast adenocarcinoma cells expressing endogenous ER  $\alpha$  and  $\beta$ , which are stably transfected with an oestrogen-responsive luciferase reporter gene (Legler et al., 1999) and the MVLN cells (Gray et al., 2002).

In general, yeast test systems rely on yeast constructs expressing an (human) oestrogen receptor, which upon binding of suited substrates acts as a transcriptional enhancer for oestrogen-responsive DNA element-controlled reporter genes, in most cases bacterial  $\beta$ -galactosidase. The activity of this enzyme can be determined photometrically by using a chromogenic substrate and may thus serve as a measure for the oestrogenic potency of the sample under investigation (Rehmann et al., 1999).

Short-term assays need to be standardized and validated and applied to identify EDCs and to determine the relative potency for hormonal responses. From the risk assessment point of view it would be very important to apply such tests for environmental monitoring to identify emitters, actual environmental loads and environmental reservoirs of xeno-oestrogenic compounds (Rehmann et al., 1999).

In South Africa the environment in rural and urbanized areas is often contaminated with a complex mixture of toxic compounds originating from industries, agriculture and private households. Many of these pollutants end up in surface waters, such as dams, rivers and eventually the sea (Aneck-Hahn, 2003; De Jager et al., 2002). Toxic contaminants may disturb the biological equilibrium of aquatic ecosystems and be harmful to humans, if they are transmitted to human food or drinking water. In many rural areas in South Africa the only access

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