

Endocrine Disruption Screens

ER and AR Transactivation Assays and Binding Assays



CeeTox's *in vitro* screens identify endocrine disrupting chemicals (EDC's) and support the 3Rs by reducing the need to test on animals

What is the challenge?

There is growing concern about the potential of some chemicals to disrupt the human endocrine system. Endocrine disruptive chemicals (EDCs) are thought to be associated with an increase in testicular cancer, regional declines in sperm counts, altered sex ratios in wildlife populations, as well as an increase in the incidence of breast cancer, endometriosis, and accelerated puberty in females.

Although not yet required, future implementations of the EU program Registration, Evaluation and Authorisation of Chemicals (REACH) and the US-Environmental Protection Agency's Endocrine Disruption Screening Program (EDSP) will entail *in vitro* testing and labeling of substances that have the potential to interact with endocrine systems.

How does CeeTox support you?

CeeTox can greatly reduce the use of animals in testing with our *in vitro* approach to identify and classify EDCs. Endocrine screening provides early analysis of potential hazards and supports a weight-of-evidence approach to identify priority chemicals. Our resulting data will be key to your design of a chemical-specific intelligent testing (IT) strategy for REACH. And CeeTox's unit cost is much lower cost than traditional, *in vivo* methods, so your budget goes farther.



CeeTox Expertise –

Since 2004, CeeTox has provided ER and AR binding and pre-screens for transcriptional activation to the US-EPA and chemical and pharmaceutical firms. We have completed pre-validation studies of over 170 chemicals, including many known and suspected EDCs. Our approach and data are undergoing validation and review by the National Toxicology Program's Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), which coordinates the activities that ICCVAM requires to evaluate and make recommendations regarding new, revised, and alternative test methods.



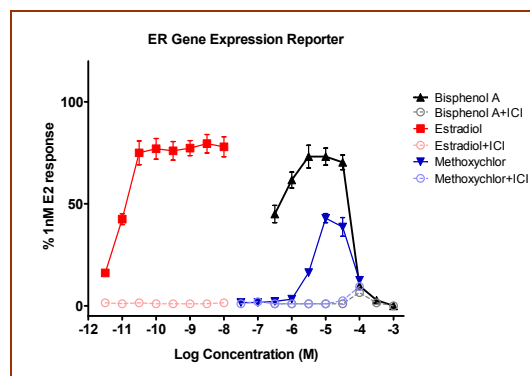
A Two-Tiered Approach to Assess Endocrine Disruption-

CeeTox performs low cost *in vitro* transcriptional activation and binding assays to prioritize the agonist/antagonist activity in chemical test articles. We include a wide range of exposures up to the highest concentration soluble in the media as well as a high number of replicates. We use a unique and comprehensive array of controls to accurately confirm receptor-specific agonism or antagonism.

Tier A: Initial test using Transactivation assays:

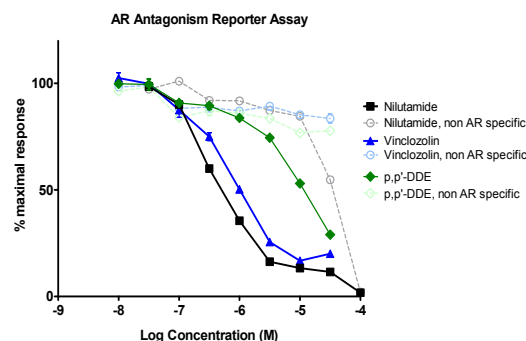
- Chemicals are tested for potential to elicit estrogen receptor (ER) and androgen receptor (AR) mediated activity.
- Both agonistic and antagonistic activity is assessed using a cell-based transcriptional activation (TA) model consisting of T47D-KBluc cells (ER) and MDA-kb2 cells (AR).
- Solubility and Cytotoxicity tests are also performed on all chemicals for proper interpretation of results.
- Relative Potency ranking can be ascertained.

The dataset at right demonstrates the agonist, or estrogen, receptor activity of Bisphenol A, recently a substance of concern in the US news for its presence in baby bottles and other hard plastic products.



Tier B: Chemicals shown to be positive are carried into Binding assays:

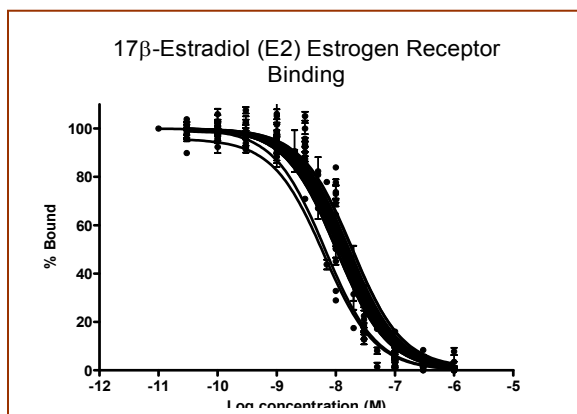
- Receptor binding assays can confirm the chemical's ability to specifically bind to ER- α , ER- β or AR.
- Competitive receptor binding assays use a human recombinant receptor in combination with polarographic fluorescence detection.
- Solubility in the assay buffer and tests for fluorescent interference are also performed on all chemicals.
- Relative Binding Affinity calculations available.



The dataset at left demonstrates the antagonist, or androgen, receptor activity of the fungicide Vinclozolin, a known EDC.

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The dataset above demonstrates ER-β receptor binding curves from a CeeTox Tier B screen.

New Disruption Screens In Development

Our R&D team is developing a screen to assess steroidogenesis disruption, or the body's production of male and female steroid sex hormones, through a cell-based assay that detects chemicals that inhibit enzymes responsible for steroid synthesis and also potential inducers.

Also in development are *in vitro* screens to assess disruption to thyroid and progesterone systems. Any of these screens can be developed to the sponsor's test articles on request.



CeeTox remains committed to supporting the 3Rs with *in vitro* assays with *in vivo* relevance that reduce the need for testing on animals.

About Endocrine Disruption

Endocrine Disruptive Chemicals (EDCs) differ in origin, size, potency, chemical lifecycle, amount, and effects. They are often ingredients in pesticides, plastics, cosmetics, electrical insulators, and other consumer products or in their packaging.

EDCs have been associated with reproductive and developmental problems in wildlife and laboratory animals. Many of the indicted chemicals exhibit distinct effects in different species and organs, and at different developmental stages. Quantity, potency, chemical-to-chemical interactions, and inappropriate timing (such as exposure to developing fetuses, infants, and children whose growing bodies depend on endocrine signals) are important factors that dictate the type and degree of known effects, which include cancer, deformities, and reproductive problems.



The normal signaling of natural hormones can be disrupted in many ways. For example, EDCs can bind to hormone receptors where they mimic, block, or otherwise influence normal actions and alter the ways natural hormones are made. An

EDC chemical may demonstrate more than one action. Effects may be cumulative. Some may interfere in only one way; others can interact in multiple ways. EDCs can alter steroidogenesis, the body's production of male and female steroid sex hormones, causing opposite sex attributes and malformed organs. Known EDCs include p,p'-DDE, a derivative of the pesticide DDT, the fungicide vinclozolin, and the growth steroid trenbolone.

Literature Cited:

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NIH Publication No: 03-4504 Current Status of Test Methods for Detecting Endocrine Disruptors: In Vitro Estrogen Receptor Binding Assays Background Review Document. Prepared for The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) October 2002 National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health, U.S. Public Health Service, Department of Health and Human Services

Bolger et al. (1998) Rapid Screening of Environmental Chemicals for Estrogen Receptor. *Environ. Health Perspect.* 106:551-7.

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In vitro models to predict toxicity