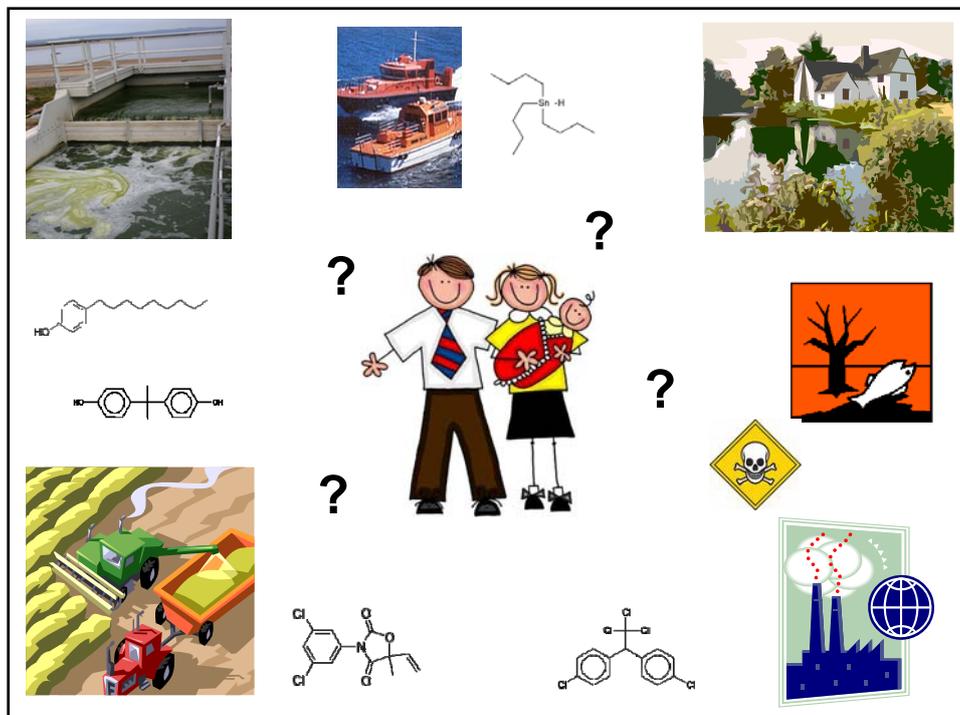




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## Yeast-cell-based Bioassays for Detection of Endocrine Disrupting Activity in the Environment

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## Bioassays for evaluation of the risks of chemicals

- Based on living cells
  - mammalian
  - eukaryotic/prokaryotic
- Cells + chemical / environmental sample => response
  - cell proliferation / growth prevention
  - production of a certain metabolite
  - expression / activation of a reporter gene product
- Evaluation of possible risks of chemicals
  - toxicity, carcinogenicity
  - hormonal activity
  - calculation of LD, EC or IC



## Endocrine disrupting compounds: What are they?

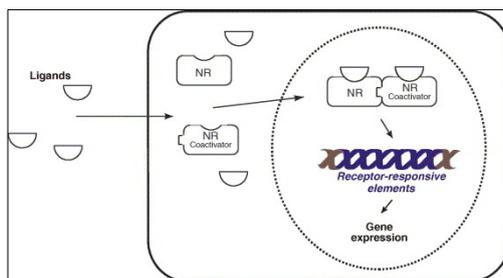
- "An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the **endocrine system** and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations."

The International Programme on Chemical Safety 2002

- Endocrine disruptors are able to **mimic** or **antagonize** the natural hormones and their action



## Endocrine disrupting compounds: Mechanism of action

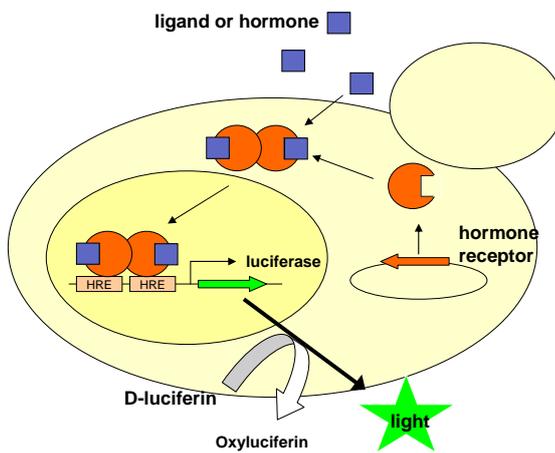


- Nuclear Receptors (NR)
  - Estrogen, testosterone, progesterone
  - Thyroid hormone, vitamins D and A, some fatty acids
  - Growth, differentiation, development, metabolism, homeostasis...

- Endocrine disrupting compounds
  - agonistic / antagonistic
  - PCBs, phtalates, DDT, organotins, some plasticizers, pesticides and herbicides
  - Developmental disorders, defects in reproduction, cancers...



## Yeast-cell Bioreporters



### ■ Yeast

#### *Saccharomyces cerevisiae*

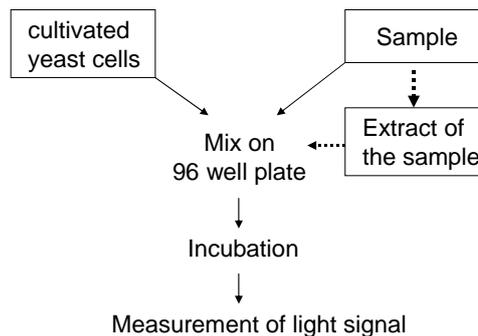
- easy to cultivate and manipulate
- tolerant to the toxic effects of many sample matrices
- no natural nuclear hormone receptors
- mammalian receptors functional



## Yeast-cell-based Bioassays – Principle and present strains

### ■ Sample

- water samples
- compost samples
- soil or sediment
- sludge from water treatment plants
- etc

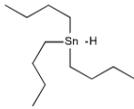


### Receptors:

- human estrogen receptors  $\alpha$  and  $\beta$ : estrogenic activity (Leskinen et. al 2005)
- human androgen receptor: androgenic activity (Michellini et. al 2005)
- human aryl hydrocarbon receptor: dioxin-like activity (unpublished)



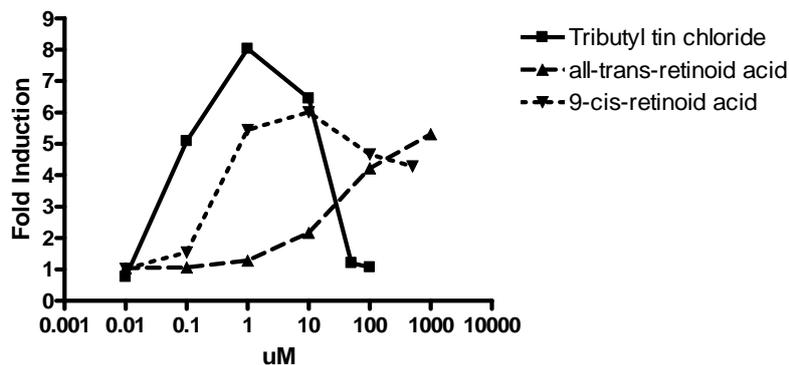
## Yeast-cell-based bioassays – Organotin reporter strain



- Tributyl tin (TBT): a biocide
  - Used in anti-foulant paints and in wood preservation
  - Use was restricted in EU 2003, and completely banned 2008
  - Persistent: accumulates in sediments in port areas and busy waterways
- Binds in the nuclear receptor Retinoid X Receptor (RXR)
  - RXR controls many genes involved in growth and differentiation
  - RXR can dimerize with other nuclear receptors
- TBT causes: imposex in marine species, defects in immune system, induces adipogenesis (obesogen), defects in bone cell differentiation



## Yeast-cell-based bioassays – Organotin reporter strain



- RXR is functional in *S. cerevisiae*: detectable signal with TBT
- Samples from the Baltic Sea at coast of Finland (Peltonen&Helminen 2007)
- Bioavailability of TBT in sediments?



## Conclusion: Possibilities and Drawbacks

### ■ Possibilities

- Rapid, sensitive and cost-efficient method to measure hormonal activity in environmental samples
- Yeast is tolerant to toxicity of many sample matrices
- Toxicity can be corrected using a constitutively luminescent control strain
- Can be used to estimate bioavailability of compounds in sample

### ■ Drawbacks

- Still requires cultivation of yeast
- Some samples are toxic to yeast, eg. composts
- Some heavy sample matrices can block the light, like sediments
- Does the assay mimic real bioavailability situations?
- Extraction of samples: can bioavailability be estimated anymore?
- Compound specificity



## Learning expectations in RACS-course

### ■ Bioavailability

- Principles: What is bioavailability?
- How is bioavailability studied?

### ■ The behaviour of xenobiotic compounds in environment

- absorption and desorption in soil and sediment
- chemical reactions, breaking of compounds = "life cycle"
- leaching of compounds from contamination sites
- remediation possibilities at contaminated sites

### ■ Risk assessment

- toxicity tests
- how to apply toxicity test results to human risk estimation?

### ■ Legislation

- REACH

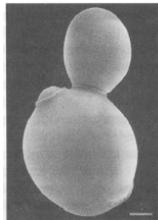


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**Thank you for your attention!**



Any questions?