

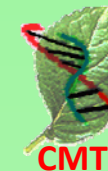


***In vitro* methods for determination of endocrine disrupting effects**

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Overview

- Samples tested for endocrine disrupting effects
- *In vitro* methods:
 - Receptor induced Luciferase reporter gene assay
 - Estrogen receptor (ER)
 - Androgen receptor (AR)
 - Aryl Hydrocarbon Receptor (AhR)
 - Proliferation assay
 - T-Screen
 - Steroid hormone synthesis
 - Aromatase assay
 - EDC induced
 - Gene expression (Real Time PCR)
 - Genotyping (Real Time PCR)





Samples tested for endocrine disrupting effects

- Single endocrine disrupting compounds (EDCs):
 - Plastic components
 - Phenols and phthalates
 - Persistent organic pollutants (POPs):
 - polychlorinated biphenyls (PCBs), organochlorine pesticides, dioxins, brominated flame retardants, perfluorinated compounds (PFCs)
- Mixtures of single EDCs
- Human serum and breast milk
- Waste water





In vitro Methods

- Receptor induced Luciferase reporter gene assay
- Proliferation assay
- Aromatase assay

- Real Time PCR

Principle:

- Samples are tested alone to determine the potential of the sample to directly affect the function of the receptor / enzyme
- Samples are tested with the natural ligand to determine the potential of the sample to compete with endogenous hormones / ligands

Real time PCR is based on the polymerase chain reaction (PCR), which is used to amplify and simultaneously quantify a targeted DNA molecule.





Luciferase reporter gene assay

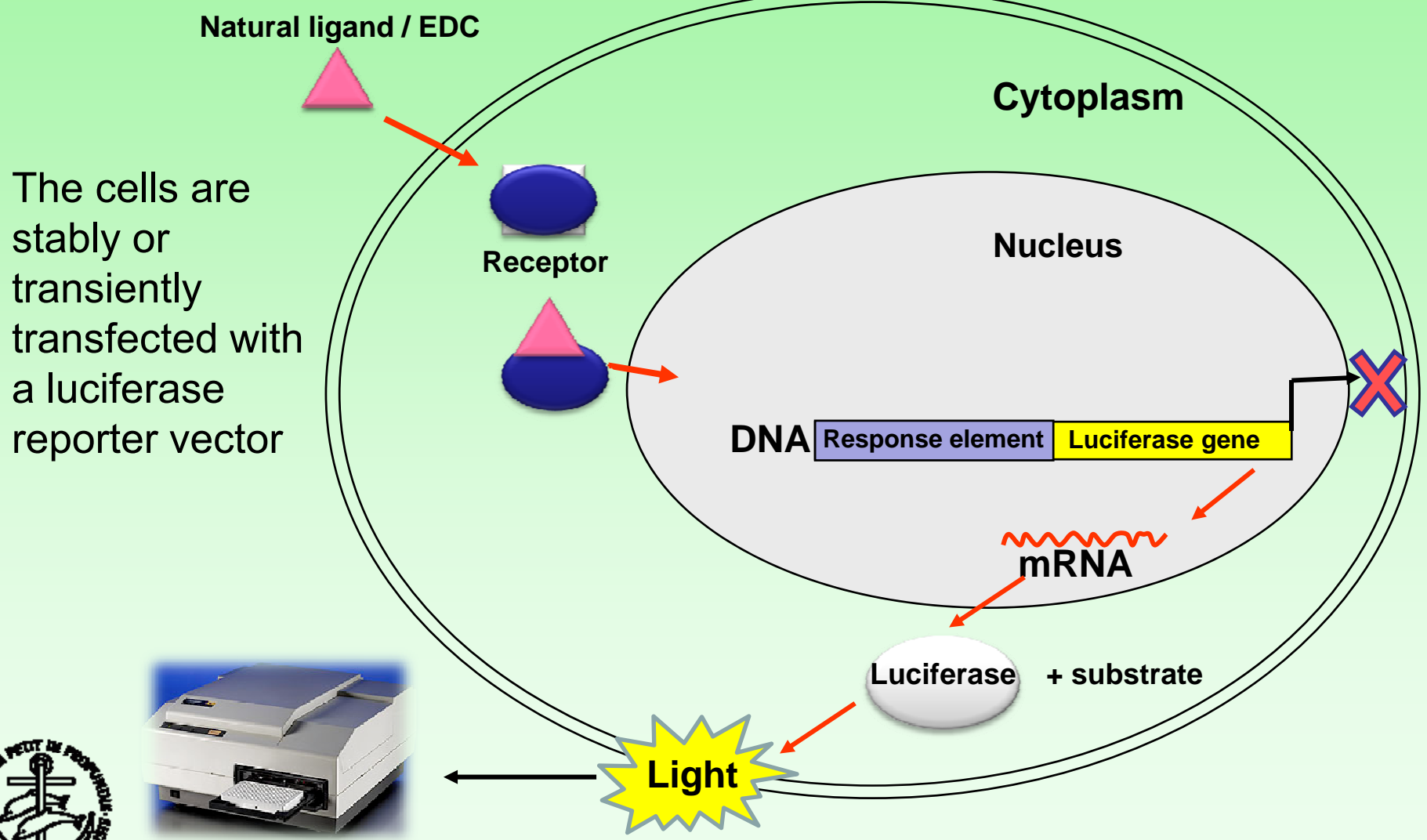
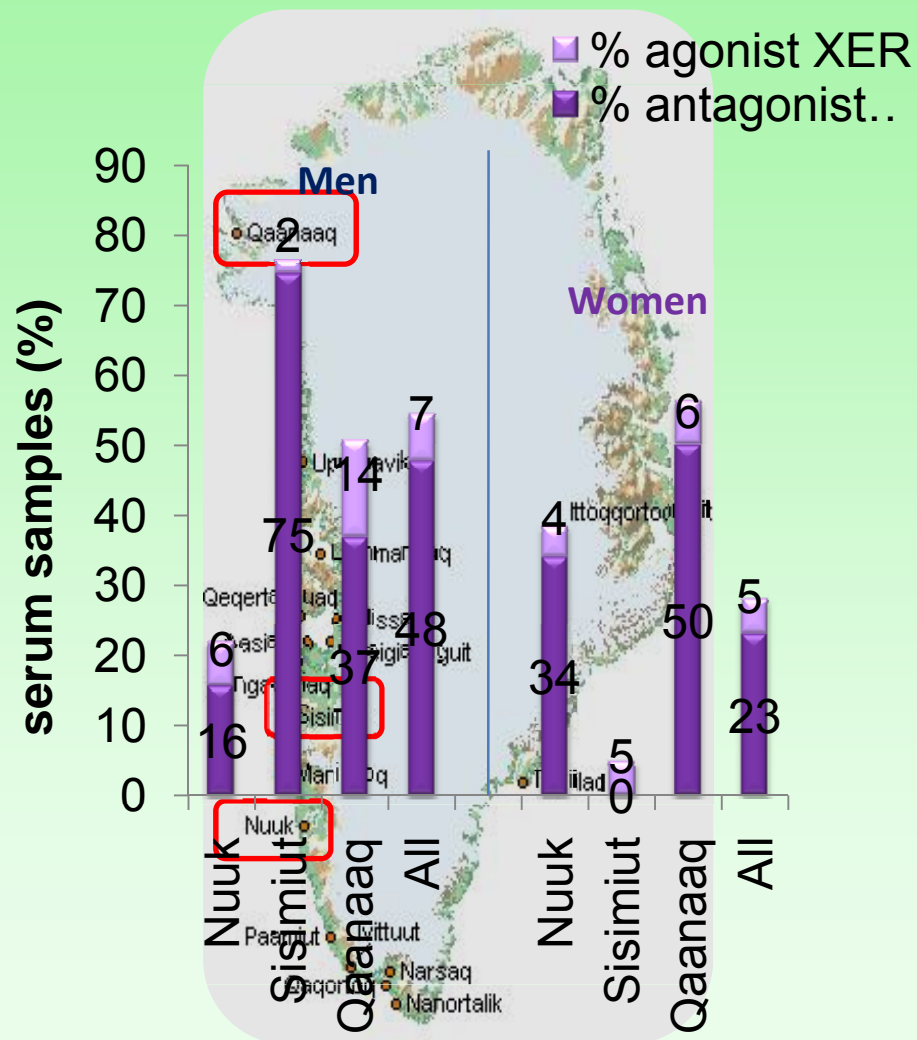
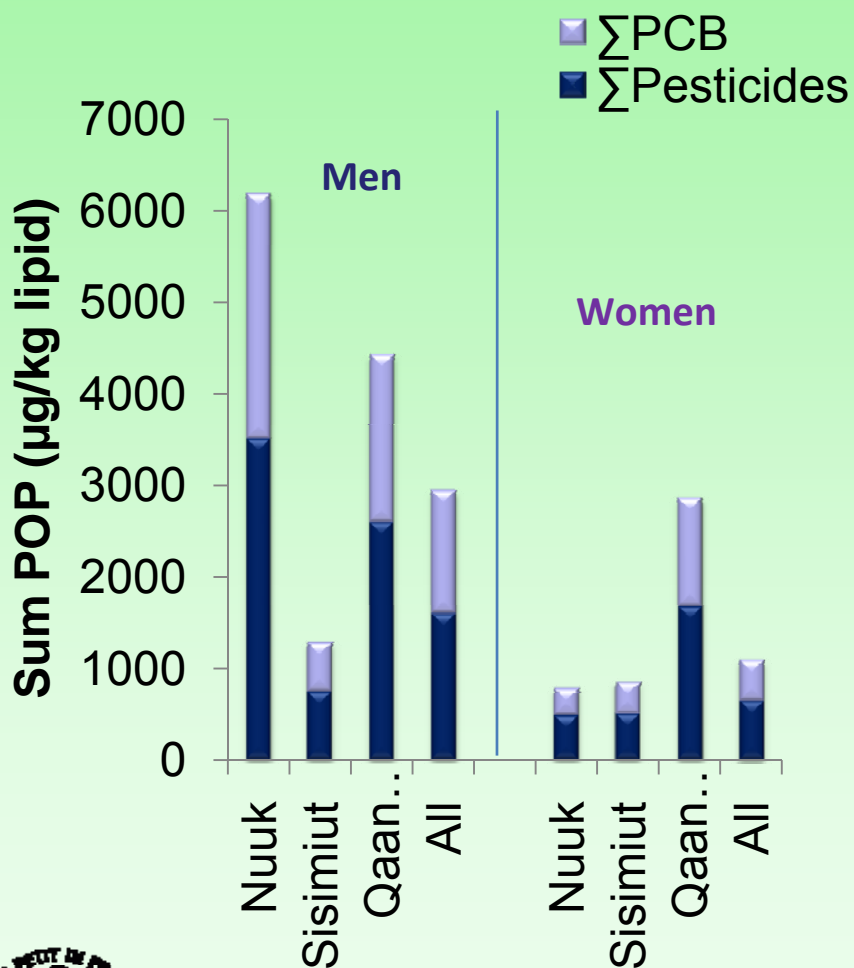
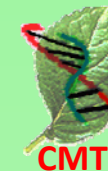


Figure designed by Mandana Ghisari



POP exposure and Xeno-estrogenic activities

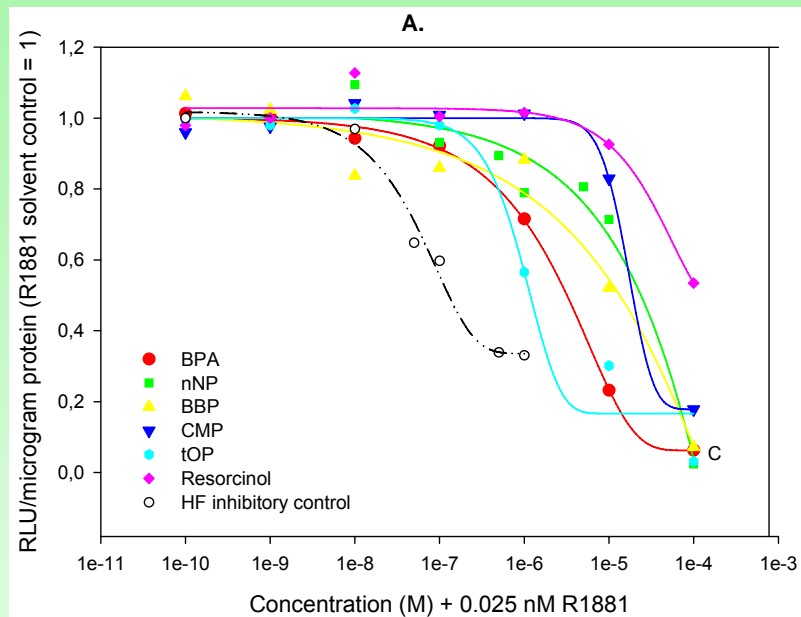
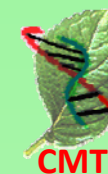


POP exposure: Men > women

Predominantly antagonising effect

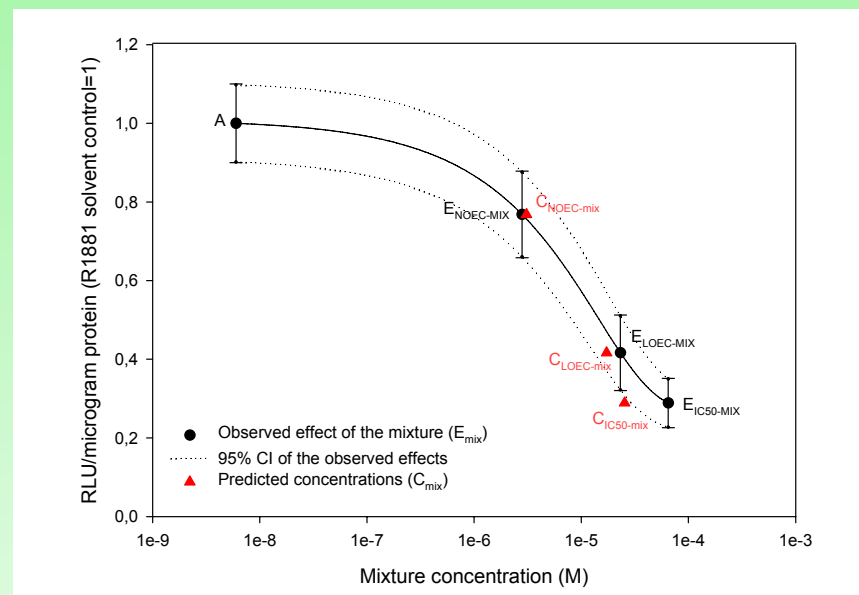


Effects of plastic components on the Androgen Receptor function



Concentration-response curves for 6 plastic components

(BPA: bisphenol A; nNP: 4-*n*-nonylphenol; BBP: benzyl butyl phthalate; CMP: 4-chloro-3-methylphenol; tOP: 4-tert-octylphenol; HF: hydroxyflutamide).

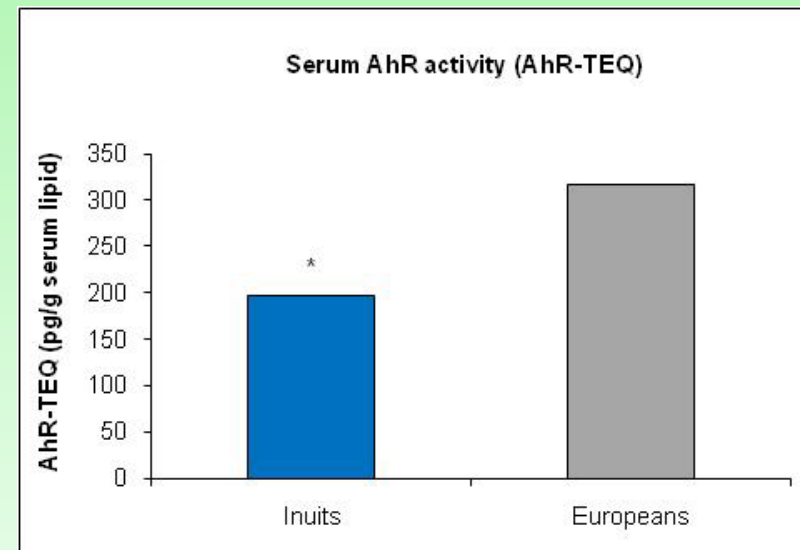
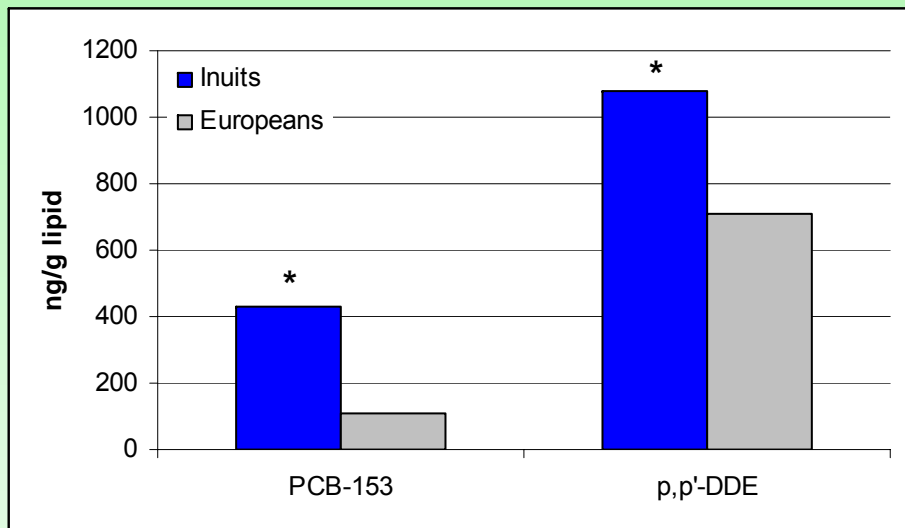


The observed and the predicted AR antagonistic effects of the mixture of 6 plastic components. Additive effects were observed at all levels.





Effects of serum samples on the Aryl hydrocarbon Receptor function

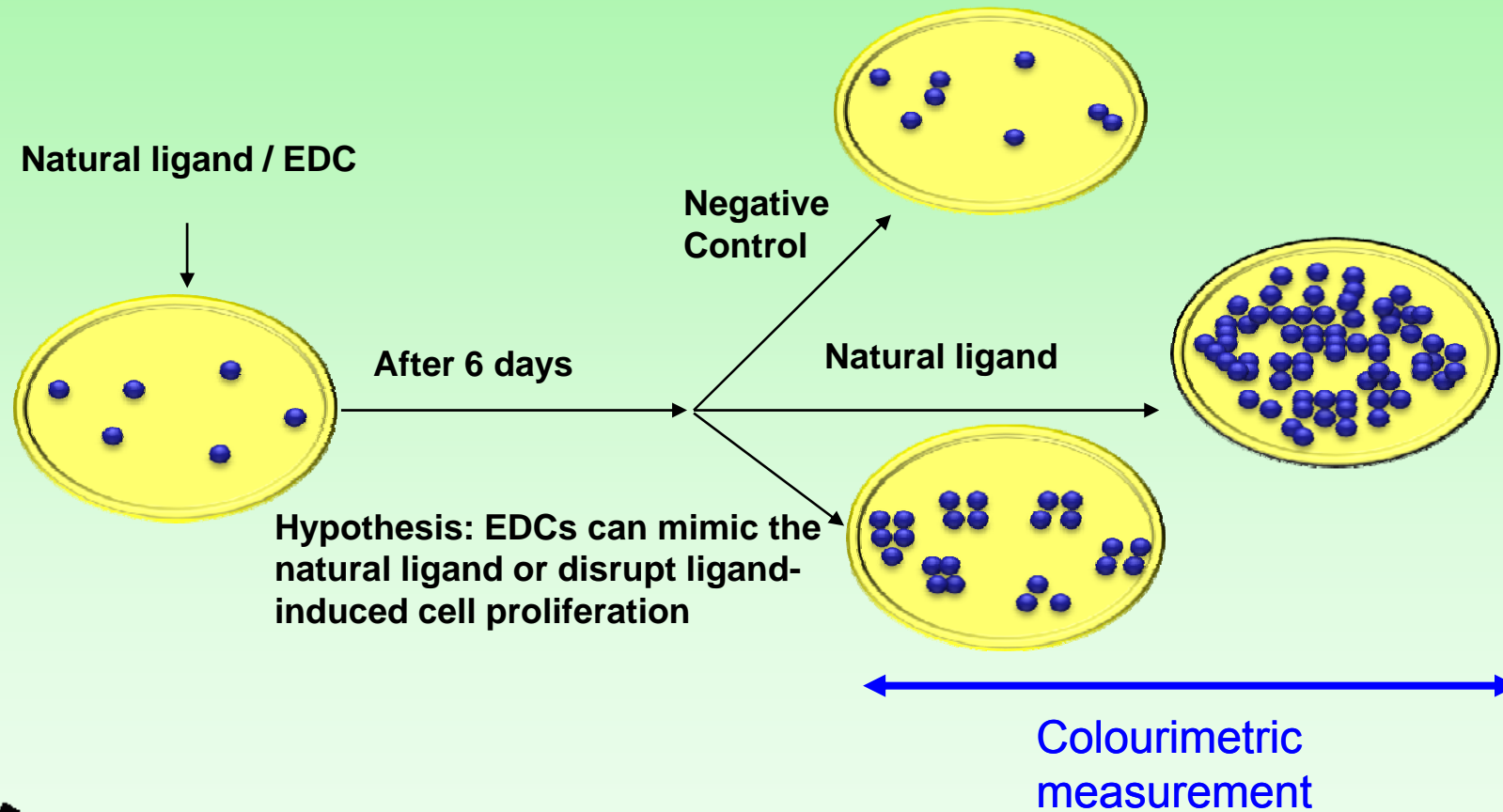


*: p < 0.05 for Inuit vs Europeans



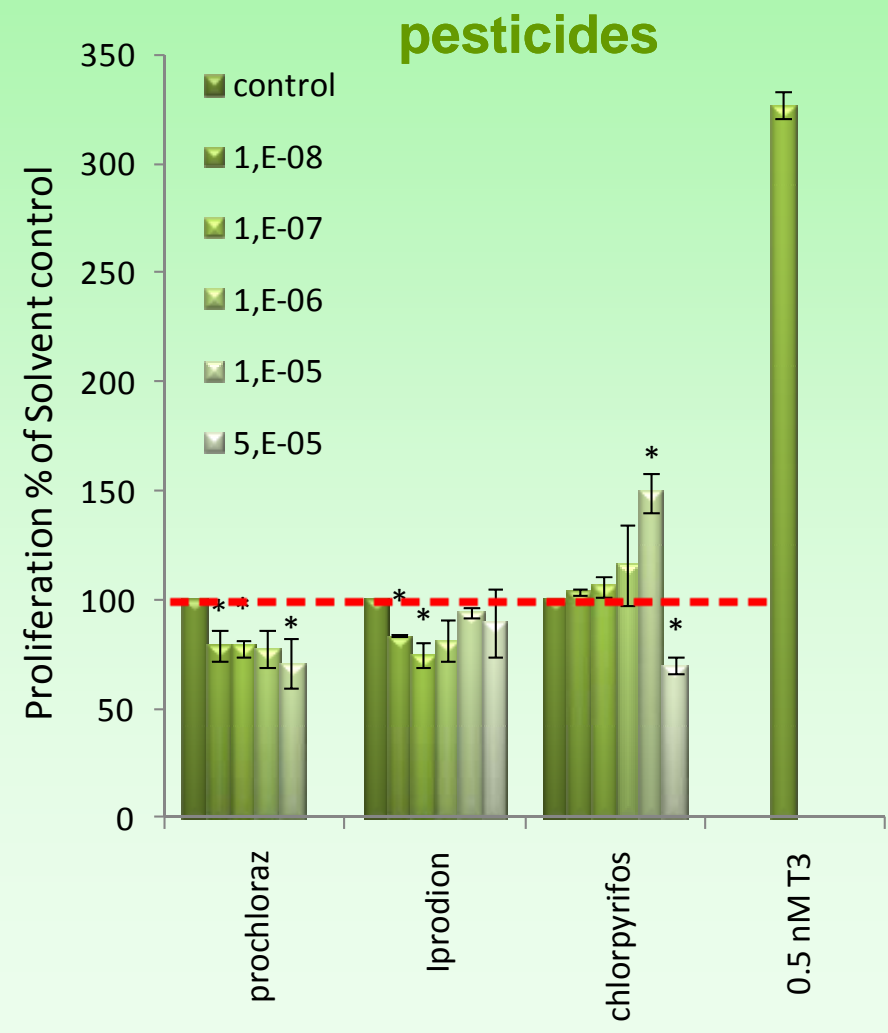
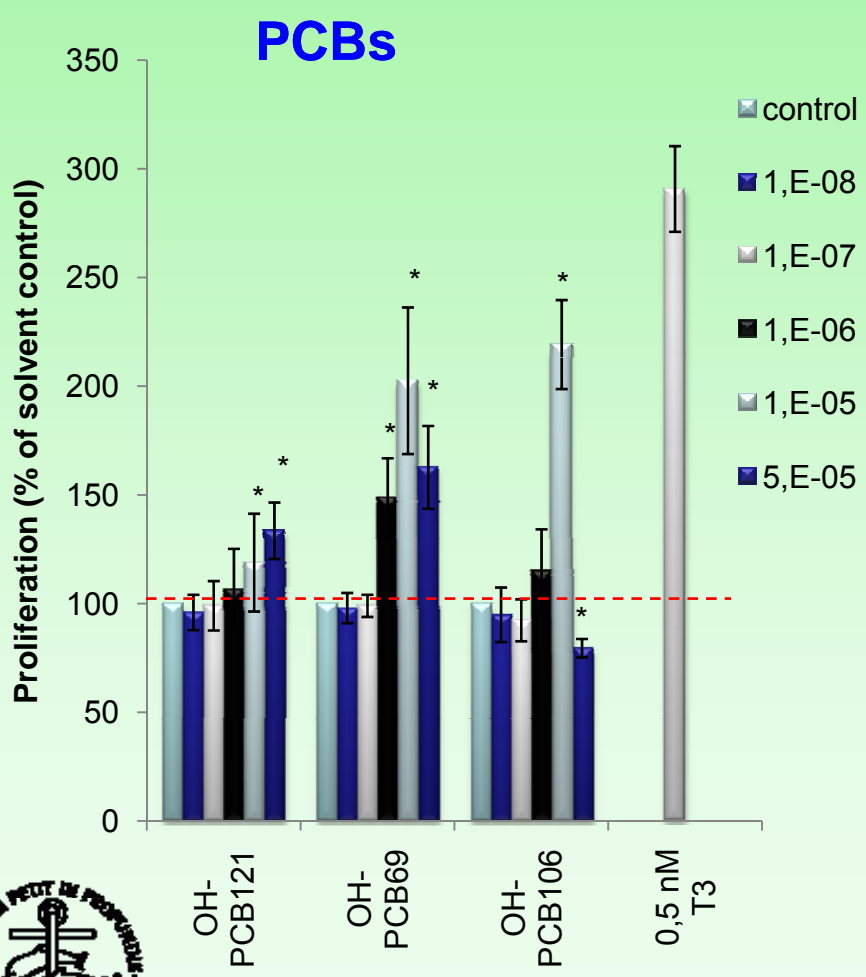


Thyroid hormone- like effects of EDCs in GH3-Proliferation assay



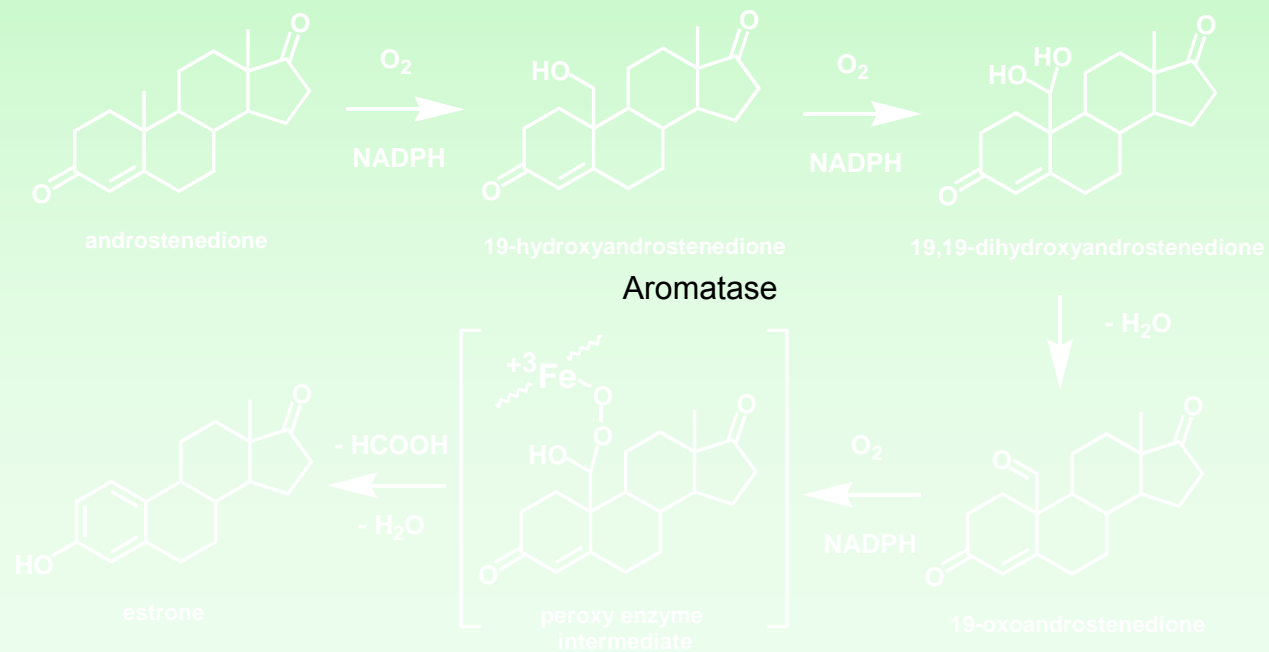
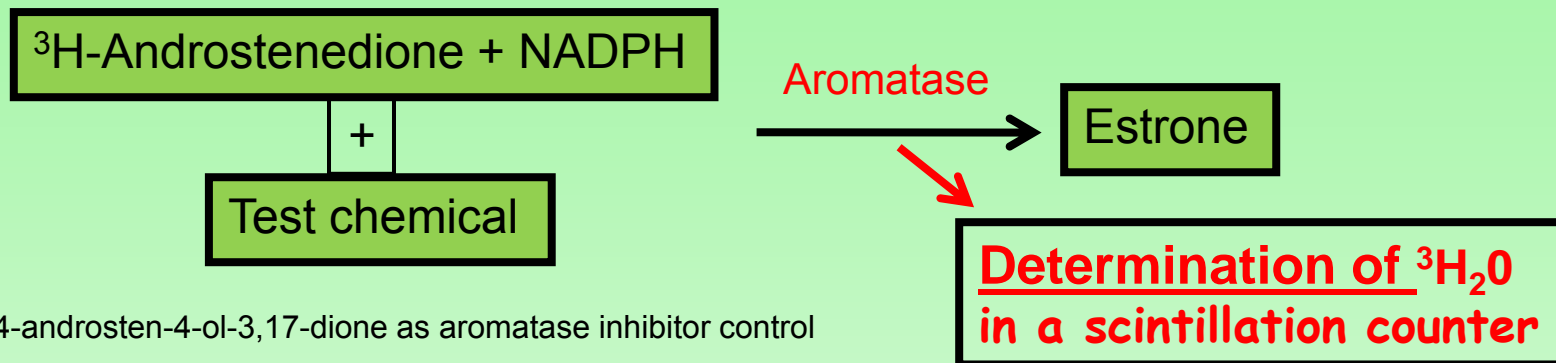


Effect of PCBs and pesticides on GH3 cell proliferation





Aromatase assay



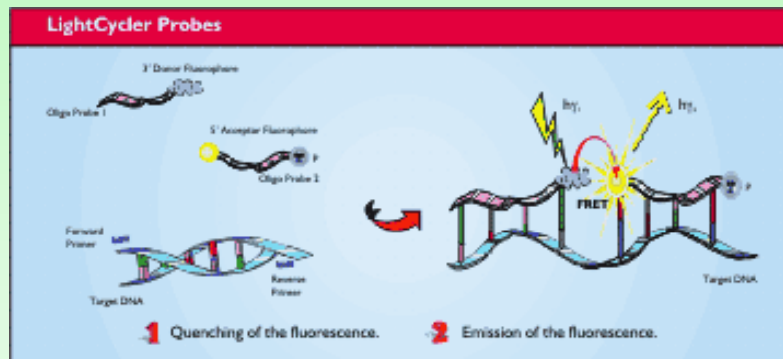


Real Time – PCR

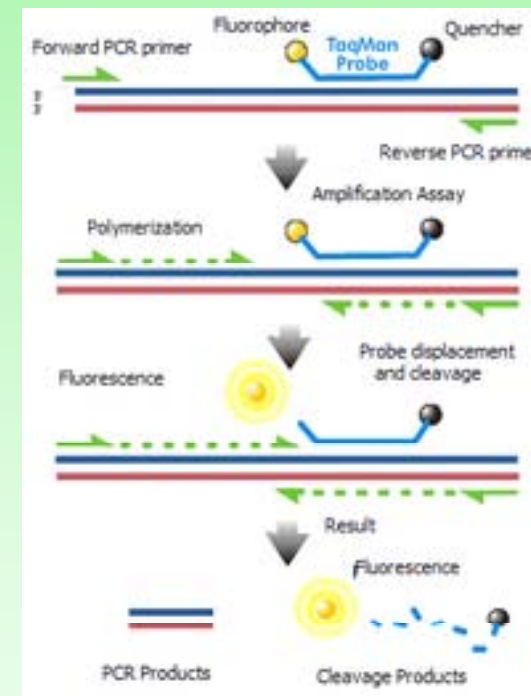


The use of sequence-specific DNA-based probes and the generation of a fluorescent signal allows real time PCR to be used for:

- **Quantification**
- **Genotyping**



Lightcycler probes consists of a donor probe and an acceptor probe.



The Taqman probes are dual labeled hydrolysis probes with a reporter dye and a quencher dye.



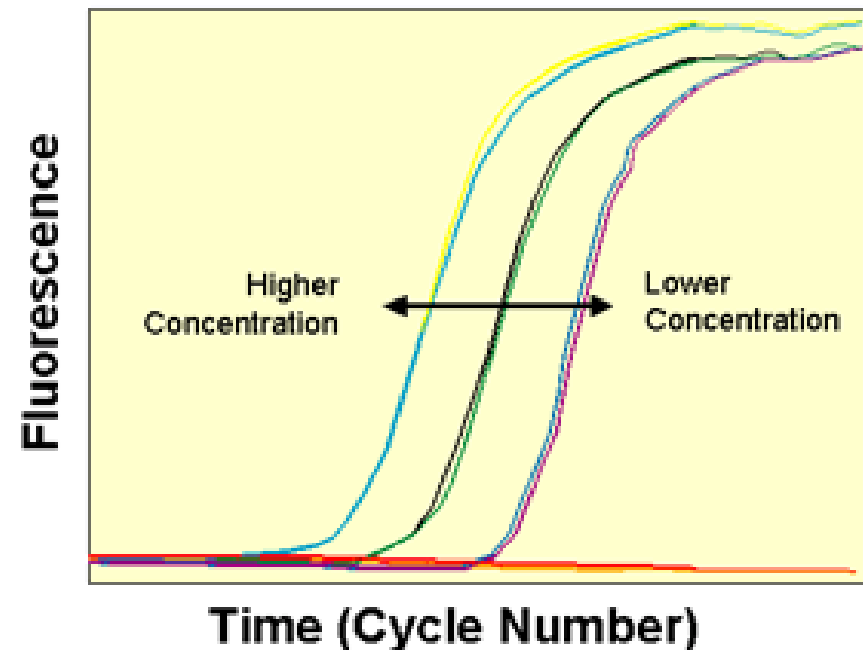


Real Time – PCR

Quantification of gene expression

- The amount of DNA can be measured at each PCR cycle.
- Absolute quantification is possible by comparing an unknown sample to a standard curve.
- Results can be normalized to a "housekeeping" target, i.e. a gene that is stably expressed and not affected by the test compounds.

Real-Time Monitoring of PCR

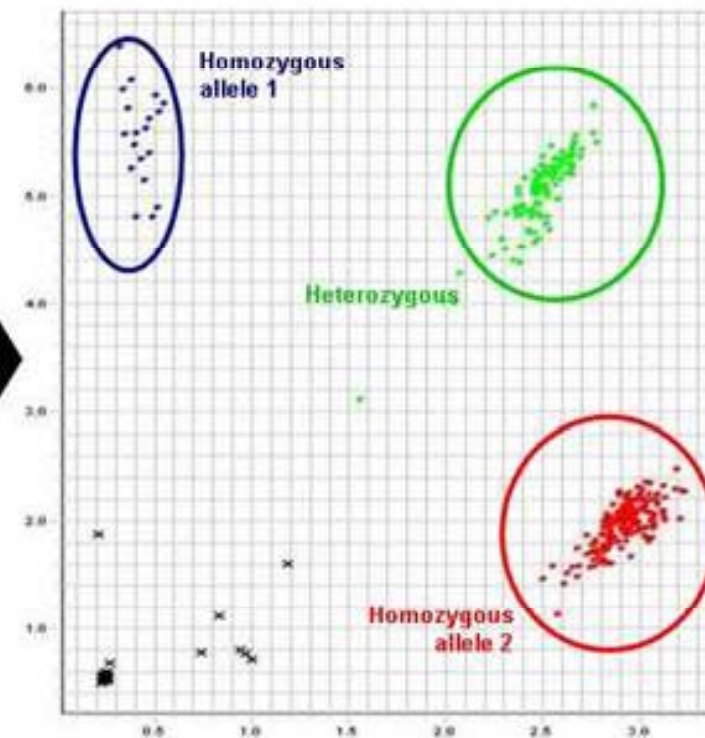
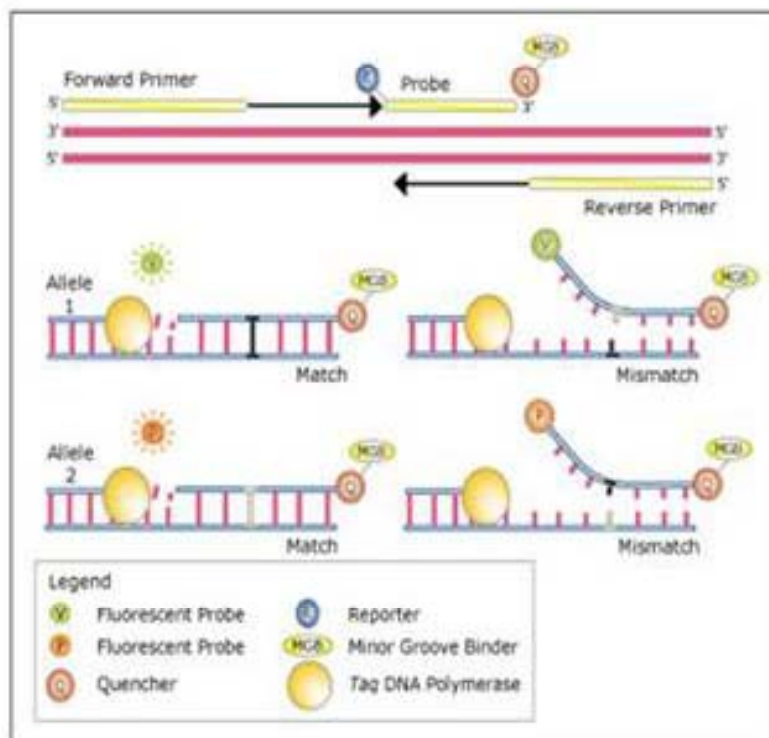




Real Time – PCR Genotyping



- Single nucleotide polymorphism (SNPs) result in different probe stabilities and this can be determined by:
 - Changes in melting temperatures at the end of the PCR (Light cycler).
 - Differences in fluorescent reporter dye signals for the different alleles (TaqMan ABI PRISM 7000 sequence detector)





Thank you for your attention.



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