INTRODUCTION

Concerns have been raised by scientists, media and consumers on certain chemicals which might be disrupting the endocrine systems and wildlife, recently, the European commission adopted criteria to define an Endocrine Active Substance (EAS). Efforts have been done to build an evaluation tool to cover human and environmental safety assessment using in vitro, in vivo and fish-embryo models for early screening purposes. A set of 21 chemicals from various origins and known for their estrogenic like properties in vitro were evaluated with assays currently available for the screening of endocrine active compounds. The collaborative work was focused on the estrogen receptor binding pathways, which is one of the most documented to fuzzy allowing:

- The comparison of currently used human based nuclear receptors (NR) assays from in vitro assays to cellular based in vitro assays (Wilson 2004).
- An interspecies comparison human vs zebrafish (Danio rerio) (Chen 2008).
- The evaluation of the relevance of using the transgenic Erα-GFP fish-embryo model (Chen et al 2008).

The project was inspired from the inter-species studies of (Ding et al., in which they did a mapping of 92 EAS from heterogeneous in vitro data. We have decided to focus on an evaluation of 21 benchmarks compounds performed in a homogeneous in vitro context, supplemented with a non-comprehensive model based on fish embryonic estrogenic assays. Benchmarks from different origins have been selected (drugs in red, natural compounds in green, other chemical industry compounds in purple, and cosmetic ingredients in blue even if the parabens were not only used in the cosmetic industry).

This study was focused on the estrogen receptor alpha (ERα) in the endocrine disruption field. This study was focused on the estrogen receptor alpha (ERα) in the endocrine disruption field.

MATERIAL AND METHODS: Binding and transactivation assays

**Schematic binding assay principle**

Transactivation assay principle for the transactivation assay allows the measurement of the ability of a compound to activate the estrogen receptor (ERα) of the Chorion gene (ChgH), a naturally developed by Vitargent (Chen et al. 2008). Briefly, eGFP is driven by the transcriptional promoter region of the Choriogenin H (ChgH), a naturally developed by Vitargent (Chen et al. 2008).

**List of selected compounds**

- **Panopixyl**
- **Bisphenol A**
- **Bisphenol F**
- **Bisphenol S**
- **Bisphenol D**
- **Bisphenol 3F**
- **Desmethylparaben**
- **Parabens**
- **Aldosterone**
- **Arenes**
- **Cortisol**
- **Dexamethasone**
- **Hydrocortisone**
- **Methylprednisolone**
- **Prednisolone**
- **Prednisone**
- **Prednisolone 21**
- **Steroids**

**Binding assay**

1. Compounds were dissolved in 1% ethanol DMSO and diluted to a working concentration of 0.1% DMSO.
2. Cells were seeded at a density of 5,000 cells per well in 96 well plates.
3. Cells were left for 24h to adhere to the plate.
4. Cells were washed with PBS buffer.
5. Cells were exposed to compounds for 24h at a concentration of 100 nM.
6. Cells were washed with PBS buffer.
7. Cells were treated with an Ethanol:PBS solution mixture (1:4) for 10 min.
8. Cells were washed with PBS buffer.
9. Cells were fixed with 4% paraformaldehyde for 10 min.
10. Cells were washed with PBS buffer.
11. Cells were permeabilized with 0.1% Triton X-100 for 5 min.
12. Cells were washed with PBS buffer.
13. Cells were stained with Alexa Fluor 488-conjugated secondary antibody for 1h.
14. Cells were washed with PBS buffer.
15. Cells were mounted with DAPI mounting medium.
16. Cells were imaged using a confocal microscope.

**Transactivation assay**

1. Compounds were dissolved in 1% ethanol DMSO and diluted to a working concentration of 0.1% DMSO.
2. Cells were seeded at a density of 5,000 cells per well in 96 well plates.
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**RESULTS**

**Results**

- **Transactivation assay**
  - The binding assay evaluates the affinity of a compound towards a 17α-estradiol, displayed a LOEC at 2.9 nM. 71.5% of the tested compound showed an estrogenic effect in the fish embryo model with the highest estrogenic potential followed by 17α-oestraceol, methylparaben, and tetrachlorobisphenol A.
  - The transactivation assay allows the assessment of the ability of a compound to activate the estrogen receptor (ERα) of the Chorion gene (ChgH), a naturally developed by Vitargent (Chen et al. 2008). Briefly, eGFP is driven by the transcriptional promoter region of the Choriogenin H (ChgH), a naturally developed by Vitargent (Chen et al. 2008). The transactivation assay principle allows the evaluation of the estrogenic effects of the substances tested compared to 17α-estradiol.
  - An interspecies comparison human vs zebrafish (Danio rerio) (Chen 2008)
  - The comparison of currently used human based nuclear receptors (NR) bioassays from in tubo assays to cellular based in vitro assays (Wilson 2004).
  - A set of 21 chemicals from various origins and known for their estrogenic like properties in vitro were evaluated with assays currently available for the screening of endocrine active compounds.

**Conclusion**

- **Transactivation assay**
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