Predicting developmental toxicity of pyrethroid insecticides \textit{in vitro} using human induced pluripotent stem cells

ASCCT-ESTIV webinar, 24\textsuperscript{th} March, 2023

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Developmental toxicity

Human prenatal development:

Agent orange, herbicide contains TCDD

Thalidomide, 1960s
Animals used in the EU in 2019 for developmental toxicity testing

Source: ALURES Statistical EU Database on the use of animals for scientific purposes
Developmental toxicity

*In vitro and ex vivo approaches*
Human-induced Pluripotent Stem Cells (hiPSC)
A model for embryonic development

Embryoid Bodies (EBs):

- EBs can differentiate into most cell types of the body including cardiomyocytes

Blastocyst
Fetus
Somatic cells from human adult

Embryoid bodies are aggregates of hiPSC that mimic the blastocyst
Methods

Development of the **PluriBeat** and **PluriLum** assays based on 3D embryoid bodies made from hIPSC

hPSC line: BIONi010-C

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**LAUSCHKE K ET AL. A novel human pluripotent stem cell based assay to predict developmental toxicity. Arch.Toxicol 94(11), 3831, 2020**

**LAUSCHKE K, TRESCHOW AF ET AL. Creating a human NKX2.5 reporter stem cell line for developmental toxicity testing. Arch Toxicol 95, 1659, 2021**
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Methods

Characterizing the PluriBeat assay

**a**

![Graph showing expression over days](Image)

<table>
<thead>
<tr>
<th>Marker Genes</th>
<th>Pluripotency States</th>
</tr>
</thead>
<tbody>
<tr>
<td>T, ISL1, MESP1, NKX2.5, TNNT2, MYH6, MYH7</td>
<td>Early cardiomyocyte</td>
</tr>
<tr>
<td>OCT3/4, NANOG, SOX2</td>
<td>Late cardiomyocyte</td>
</tr>
</tbody>
</table>

**b**

![Graph showing expression over days](Image)

**c**

![Images showing DAPI and cTnT localization](Images)

**d**

![Images showing Nkx2.5 and merge](Images)

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**hiPSC line:** BIONi010-C

80 µL compound or extract added day 1, 2, 3 & 6

5x10^3 cells/well

```
D0              D1
D2              D3              D6             D7
medium:         
D-1             DO              TS             Wnt             TS

Day:
-1              0               1               2               3               6               7
exposure:

80 µL compound or extract added day 1, 2, 3 & 6
```

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Project aim

- Three of the most often detected pyrethroids in Danish foods and the common metabolite were selected

- Pyrethroids are known neurotoxicants, acting primarily by interfering with voltage-gated sodium channels

- Evaluate the potential developmental toxicity of pyrethroids and the common metabolite using a stem cell based assay
Results

Cytotoxicity

Reference compounds

Luminescence (PluriLum)

Beat Score (PluriBeat)
Take home messages and future perspectives

Pyrethroids negatively affected cardiomyocyte differentiation in the PluriLum assay, but not the metabolite

PluriLum is more sensitive compared to the PluriBeat assay for the pyrethroids

We need to expand our database and test more chemicals

Human exposure levels are predicted to be much lower than the tested range
  • Mixture effect?
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