NEUROSPHERES FOR SPECIES-SPECIFIC, MEDIUM-THROUGHPUT ANALYSES OF DEVELOPMENTAL NEUROTOXICITY (DNT)

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Paris, November 5th 2018
Developmental Neurotoxicity (DNT)

OECD TG 426
U.S. EPA OPPTS 870.6300

- Duration: approx. 1 year
- Costs: approx. € 1 Mio
- Animals: approx. 1.400
- Uncertainties in their methodology, evaluation, and regulation
- Species-specificities


Fritsche et al. Consensus statement on the need for innovation, transition and implementation of developmental neurotoxicity (DNT) testing for regulatory purposes. 2018 TAAP 354:3-6.

Tsuji & Crofton Developmental neurotoxicity guideline study: Issues with methodology, evaluation and regulation Cong Anomal 2012
Studying Species-Specificities in DNT

Primary Neural Progenitor Cells (NPCs) for Species-specific evaluations

Culture as 3D Neurospheres

- Molecular equipment
- Cellular function
- Responses to compounds
The 'Neurosphere Assay'

BrdU = Bromodesoxyuridine

Migration

Assessment of Viability

Differentiation

Day 0

Day 3

Proliferation
Genomic Analyses of Primary NPC from Different Species

Microarray analyses > 2-fold, p<0.01:

Masjosthusmann et al. TAAP 2018
Cytoscape plugin ClueGO v2.2.5
Bindea et al., Bioinformatics, 2009

Overrepresentation Analysis of hNPC

Masjosthusmann et al. TAAP 2018
Overrepresentation Analyses

Species Comparison

<table>
<thead>
<tr>
<th></th>
<th># of Processes (% Human)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human-Mouse</td>
<td>122 (78)</td>
</tr>
<tr>
<td>Human-Rat</td>
<td>98 (63)</td>
</tr>
<tr>
<td>All Species</td>
<td>90 (58)</td>
</tr>
</tbody>
</table>

Masjosthusmann et al. TAAP 2018

Cytoscape plugin ClueGO v2.2.5, Bindea et al., Bioinformatics, 2009
# Test Compounds

<table>
<thead>
<tr>
<th>Positive Compounds</th>
<th>Test compounds</th>
<th>Negative Compounds</th>
<th>Test compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staurosporin</td>
<td></td>
<td>Ibuprofen</td>
<td></td>
</tr>
<tr>
<td>Retinoic Acid</td>
<td></td>
<td>Acetaminophen</td>
<td></td>
</tr>
<tr>
<td>Methylmercurychloride</td>
<td></td>
<td>Captopril</td>
<td></td>
</tr>
<tr>
<td>Sodium Arsenite</td>
<td></td>
<td>Penicillin G sodium</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td></td>
<td>Sodium glutamate</td>
<td></td>
</tr>
<tr>
<td>Dichlorodiphenyltrichloroethan</td>
<td></td>
<td>Diethyleneglycol</td>
<td></td>
</tr>
<tr>
<td>Tetrabromobisphenol A</td>
<td></td>
<td>Mannitol</td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td></td>
<td>Saccharin</td>
<td></td>
</tr>
<tr>
<td>Dibutyl phthalate</td>
<td></td>
<td>Sorbitol</td>
<td></td>
</tr>
<tr>
<td>Perfluorooctanoic acid</td>
<td></td>
<td>Warfarin</td>
<td></td>
</tr>
<tr>
<td>Butyl benzyl phthalate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methylazoxymethanol acetate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parathion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium Valproate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamin hydrochlorid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium Fluoride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dichlorodiphenyl dichloroethene</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TH-dependent Oligodendrogeneration

Oligodendrocyte Formation

<table>
<thead>
<tr>
<th>Condition</th>
<th>WT mNPC</th>
<th>THR α KO mNPC</th>
<th>THR β KO mNPC</th>
<th>hNPC</th>
<th>rNPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3 nM T3</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
</tbody>
</table>

Oligodendrocyte Maturation

<table>
<thead>
<tr>
<th>Condition</th>
<th>MOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.0</td>
</tr>
<tr>
<td>3 nM T3</td>
<td>5.0</td>
</tr>
</tbody>
</table>

MOG = Myelin Oligodendrocyte Glycoprotein; MBP = Myelin Basic Protein

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Dach et al. Sci Rep 2017
Human Oligodendrocyte Maturation Quotient: Test for human TH disruption

Oligodendrocyte maturation quotient: $Q_M = \frac{MBP \text{ or } Mog \text{ expression}}{\% \text{ oligodendrocytes}}$

Singh et al. JOC 2016

Dach et al. Sci Rep 2017
Adverse Outcome Pathways

Putative AOP: Reduced binding of TH to NPC THR causes DNT

Chemical

Molecular interaction
- Decreased TH transport across membranes
- Inhibition of cellular TH metabolism
- Blockade of TR binding site for TH

Molecular response

Cellular response
- Oligodendrocyte formation & maturation
- Oligodendrocyte maturation

Cellular response

Organ response
- Reduced myelin
- Alterations in the white matter

Organism response
- Mental retardation

References:

Dach, Klose et al. in preparation
OECD-EFSA/DK-EPA projects: DNT in vitro Testing Battery

- UKN1 hiPSC → NPC
- NPC1 - NPC proliferation
- ReNCX - NPC proliferation
- ReNCX - NPC apoptosis
- UKN2 MINC assay
- NCC migration
- NPC2 - NPC migration
- NPC2/3 - Neuronal migration
- NPC4/UKN4 - Neuronal morphol (early&late)
- Neuronal network formation
- NPC3 – NPC Neurons
- UKN4 – Dopa-ergic differentiation
- NPC5/6 – NPC (TH) Oligodendrocyt
- Neuronal network formation
- Neuronal maturation
- Neuronal subtype differentiation
- Neuronal network formation

from: Fritsche OECD 2016
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VIP/VIP+ – Validation of innovative products

European Food Safety Authority

CERST NRW

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