Zebrafish as a tool to study mechanisms of developmental toxicology of environmental chemicals

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IVM Institute for Environmental Studies
Overview

- Introduce zebrafish as model organism
- Zebrafish embryo toxicity test (ZFET)
- Expanding ZFET
  - Angiogenesis
  - Neurodevelopment
  - Energy metabolism
  - EDA

Zebrafish are a valuable system to study mechanisms of developmental toxicology of environmental chemicals
The Zebrafish (Danio Rerio)

- Freshwater fish
- Native to southeastern Himalaya region
- Inhabits streams, canals, ponds, and slow moving water bodies, including rice fields
- Length of the adults 3-4 cm
Zebrafish are much more then a “tool”

- One female can lay 200-500 eggs per week
- Egg-diameter is around 1 mm
- Clear chorion allows to monitor the early development
- Quick development (hatch at day 3)
- Survive in 96 well plates till day 5
- Till the end of the larva stage they are no „animals“ (replace animal tests)
- Complex organism

1 hour
Embryo (2 days)
Larva (3 days)
But we have many “tools” to work with

- Gene Knock outs
- In situ hybridization
- Mutagenic zebrafish
- Transcriptomics/
  Metabolomics
- Transgenic zebrafish
Zebrafish embryo toxicity test (ZFET)
**ZFET (Zebrafish embryo toxicity – test)**

<table>
<thead>
<tr>
<th>Toxicological endpoints</th>
<th>Exposure time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethal*</td>
<td>8</td>
</tr>
<tr>
<td>Coagulation</td>
<td>•</td>
</tr>
<tr>
<td>Tail not detached</td>
<td>•</td>
</tr>
<tr>
<td>No somites</td>
<td>•</td>
</tr>
<tr>
<td>No heart-beat</td>
<td>•</td>
</tr>
<tr>
<td>Sublethal/Development</td>
<td></td>
</tr>
<tr>
<td>Completion of gastrula</td>
<td>•</td>
</tr>
<tr>
<td>Formation of somites</td>
<td>•</td>
</tr>
<tr>
<td>Development of eyes</td>
<td>•</td>
</tr>
<tr>
<td>Spontaneous movement</td>
<td>•</td>
</tr>
<tr>
<td>Heart beat</td>
<td>•</td>
</tr>
<tr>
<td>Pigmentary</td>
<td>•</td>
</tr>
<tr>
<td>Oedema</td>
<td>•</td>
</tr>
<tr>
<td>Teratogenic</td>
<td></td>
</tr>
<tr>
<td>Malformation of head</td>
<td>•</td>
</tr>
<tr>
<td>sisculi/otoliths</td>
<td>•</td>
</tr>
<tr>
<td>tail</td>
<td>•</td>
</tr>
<tr>
<td>heart</td>
<td>•</td>
</tr>
<tr>
<td>modified structure of the corda</td>
<td>•</td>
</tr>
<tr>
<td>scoliosis</td>
<td>•</td>
</tr>
<tr>
<td>rachischisis</td>
<td>•</td>
</tr>
<tr>
<td>deformity of yolk</td>
<td>•</td>
</tr>
<tr>
<td>growth-retardation</td>
<td>•</td>
</tr>
<tr>
<td>Length of tail**</td>
<td>•</td>
</tr>
</tbody>
</table>

*Focus is not on Modes of Action or Adverse Outcome Pathways*

From Nagel *et al*
Expand the ZFET to better understand mechanisms of toxicity

SMART ZFET

- Angiogenesis
- Neurodevelopment
- Energy metabolism
### Phenotypic effects of anti-cancer drugs

- **ZFET (4hpf-6dpf)**

**Compound X**

<table>
<thead>
<tr>
<th>mM</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
<th>144</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
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<td></td>
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<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>malformed</td>
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<td>10</td>
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<td>15</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>dead</td>
</tr>
</tbody>
</table>
Heartbeat

Cardiac edema 4dpf, y mM Compound X
Heartbeat

Heart beat

Heart beat per minute

concentration mM

Age in hpf

concentration mM
Blood vessel growth

- Cardiovascular toxicity (Casper::FLI)

![Image of blood vessel growth with Compound X at 4dpf]
Blood vessel growth

- Cardiovascular toxicity (Casper::FLI)
Expand the ZFET to better understand mechanisms of toxicity

SMART ZFET

- Angiogenesis
- Neurodevelopment
- Energy metabolism
# Phenotypic effects of carbamates

<table>
<thead>
<tr>
<th>MOA</th>
<th>24 hpf</th>
<th>48 hpf</th>
<th>72 hpf</th>
<th>144 hpf</th>
<th>behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>aldicarb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hyperactive</td>
</tr>
<tr>
<td>pirimicarb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hyperactive</td>
</tr>
<tr>
<td>methomyl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hyperactive</td>
</tr>
<tr>
<td>carbaryl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hyperactive</td>
</tr>
</tbody>
</table>

**Phenotypic Effects**
- **Tail Malformation**: curved, straight
- **Dead**: normal
- **Shorter Body Length**: normal
- **Edema**: normal
Viewpoint Zebrabox Behavior screen

a startle reflex assay (light response), in 96 well plates

Measure:
- Speed
- Distance
- Duration
Neurite outgrowth

Mauthner neuron (ventral side)

Rohon Beard neurons

Acetylated alpha-Tubulin Antibody
Expand the ZFET to better understand mechanisms of toxicity

SMART ZFET

- Angiogenesis
- Neurodevelopment
- Energy metabolism
Phenotypic effects of 19 OH-PBDEs

Acute (1h exposure)
LOEC 0.8 - 11 µM

Chronic (72h exposure)
LOEC 0.075 - 1.6 µM

Compound X (48 hpf)
 Developmental delay
 Unspecific
 Cardiotoxic
 Pigmentation
 Tail development

OXPHOSTOX
### Aerobic energy metabolism - oxidative phosphorylation (OXPHOS)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy demand</td>
<td>up</td>
</tr>
<tr>
<td>Oxygen consumption</td>
<td>up</td>
</tr>
<tr>
<td>CO2 production</td>
<td>up</td>
</tr>
</tbody>
</table>
New in vivo assays for OXPHOS disruption

Metabolic rate measurement in humans

Mineral oil

50 µl

Larva 3 days old
In vivo measurement of CO\textsubscript{2} production

Measure the CO\textsubscript{2} production as acid (H\textsubscript{2}CO\textsubscript{3}) in the medium via a pH-sensitive dye (phenol red)

Metabolic rate assay in a 96 well plate

Macky K. (2008), Journal of Biomolecular Screening
In vivo measurement of oxygen consumption

- OxoPlate® (PreSens)
  - oxygen sensitive dye at the well bottom
  - developed for cell and bacteria assays
  - measured with a standard multiplate reader

![Graph showing oxygen consumption over time](image)

- Negative Control
- DMSO (5)

**O_{2} (\mu M)**

**time (minutes)**

**OXPHOST X**

**IVM Institute for Environmental Studies**
Expand the ZFET to better understand mechanisms of toxicity

- Angiogenesis

Use SMART ZFET to expand the possibility of chemical analysis—Effect Directed Analysis (EDA)
Effect directed analysis

**Extraction (ASE DCM/Ac)**
**Clean up (GPC)**

**Fractionation (RP-HPLC)**

**Chemical screen (GC-MSD + LC Orbitrap MSD)**

**embryotoxicity**

**Series 1**
- Fraction 1
- Fraction 2
- Fraction 3
- Fraction 4
- Fraction 5

**Series 2**
- % malformations
- RP HPLC Fraction

**Series 3**
- % malformations

Legler 2011
Conclusion

We could link chemicals to mechanisms

- Pharmaceuticals -> Cardiotoxicity
- Pesticides -> Neurotoxicity
- Metabolites of Flameretardents -> Disruption of Energy metabolism

We could also link mechanisms to chemicals

- EDA using mechanistic bioassays in vivo
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www.pharmas-eu.org
www.denamic-project.eu
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