What (Q)SAR modelling can tell us about fish toxicity?

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• There are many popular sayings that are far from reality e.g. pirañas
  → even in scientific domains
• Toxicity of chemicals to fish has been studied for decades
• Quantitative Structure-Activity Relationship models (QSARs) are one of the identified alternatives to experimental tests accepted for use in many regulatory contexts.
• In recent years, several QSARs have been developed to predict toxicity to fish. Despite their clear advantages of rapidity and cost, QSARs were not heavily employed as alternatives for REACH registration dossiers compared to other approaches.
• Yet, they are still useful tools to better understand the mechanisms of toxicity to fish for many chemicals
MechoA as centrepiece for toxicity prediction

The different kinds of toxic mechanisms of action are predictable!
Regarding narcotic substances

Water Solubility (or hydrophobicity as $K_{OW}$) is a very good descriptor to predict aquatic toxicity of substances acting with (polar or not) narcosis.

- Non-polar narcotic chemicals (baseline toxicity)
Regarding narcotic substances

**Polar narcotic** compounds are subjected to “hydrophobic drift”

- Non-polar narcotic chemicals (baseline toxicity)
- Polar narcotic chemicals
Regarding narcotic substances

**Polar narcotic** compounds are subjected to “hydrophobic drift” as well as **reactive compounds**

- Non-polar narcotic chemicals (baseline toxicity)
- Polar narcotic chemicals
- Reactive chemicals (hard electrophiles)

*Fish are equally sensitive to polar narcotic and reactive compounds*
Regarding very hydrophobic substances

What happens when hydrophobicity is high (i.e. log KOW > 4.5 or WatSol < 10 mg/L)?
Regarding very hydrophobic substances

Hydrophobicity plays a role in toxicity studies where time to equilibrium may not be achieved within the duration of the study and below the solubility limit!

1. The toxicity solubility cut-off occurs before the intersect of the toxicity regression and the solubility limit.

2. There is a zone where the toxicity is likely to be intermediate between the extrapolated prediction and the solubility.

→ Toxicity results with high experimental variability
→ Toxicity can still be estimated by the Geom. Mean of Water Solubility and theoretical toxicity expected by the regression
Regarding the very hydrophobic substances

Hydrophobicity plays a role in toxicity studies where time to equilibrium may not be achieved within the duration of the study **and below the solubility limit!**

- Long-term exposure is expected to overcome this limitation

Substances with log $K_{ow} > 6$ have systematically acute toxicity above their solubility
Regarding long-term toxicity

Model using Simple Linear Regression is still appropriate for hydrophobic substances, at least up to log $K_{OW}$ 6.

Good quality data for chronic toxicity is scarce due to the difficulties of maintaining test substances during the assays.

The Acute to Chronic ratio (ACR) for non-polar narcotics compounds.
Model using Simple Linear Regression is still appropriate for hydrophobic substances, at least up to log $K_{OW}$ 6.

PAHs and aromatic compounds (red circles) do not exhibit significative excess of toxicity compared to narcosis.

Metabolism by P-450 cytochromes might not to be a decisive step in AOP to explain chronic toxicity to fish for PAHs.
Regarding specific toxic chemicals

- Fish Embryo Toxicity (FET) test (OECD TG 236) initially developed to replace Acute Fish Toxicity (AFT) test (OECD TG 203)

- Some specific mechanisms of action are not detected by FET test, notably neurotoxicity. Test adaptations may increase predictivity of FET test (Klüver et al., 2015; Braunbeck et al., 2015).

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Compound Class</th>
<th>FET Test Predictivity</th>
<th>Predicted MechoA for Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>PAH</td>
<td>yes</td>
<td>MechoA 1.1: non-polar narcosis</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>organochlorine</td>
<td>yes</td>
<td>MechoA 1.1: non-polar narcosis</td>
</tr>
<tr>
<td>Esfenvalerate</td>
<td>insecticide (pyrethroid)</td>
<td>no</td>
<td>MechoA 3.1 &amp; 4.1: hard electrophile reactivity and metabolism to non-toxic compounds</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>reducing agent</td>
<td>no</td>
<td>MechoA 4.3 &amp; 4.4: oxidation into quinone leading to protein/DNA adducts &amp; RedOx cycling.</td>
</tr>
<tr>
<td>Endrin</td>
<td>insecticide (organochlorine)</td>
<td>no</td>
<td>MechoA 6.6: inhibition of GABAergic Cl- channel</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>insecticide (organochlorine)</td>
<td>no</td>
<td>MechoA 6.6: inhibition of GABAergic Cl- channel</td>
</tr>
<tr>
<td>Methomyl</td>
<td>insecticide (carbamate)</td>
<td>no</td>
<td>MechoA 6.1: AChE inhibition</td>
</tr>
<tr>
<td>Dicofol</td>
<td>miticide (organochlorine)</td>
<td>no</td>
<td>MechoA 6.8 &amp; 6.9: endocrine disruption and others MechoA</td>
</tr>
<tr>
<td>Rotenone</td>
<td>insecticide (ichtyotoxine)</td>
<td>no</td>
<td>MechoA 6.7: inhibition of mitochondrial electronic chain</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>antidepressant</td>
<td>no</td>
<td>MechoA 1.2 &amp; 5.2 / an6.2: probable binding to ACh receptors (muscarinic or nicotinic)</td>
</tr>
</tbody>
</table>

MechoA can be used to complement FET test result.
Role of (Q)SAR models for IATA

Development of an Integrated Approach to Testing and Assessment for Acute Fish Toxicity
Role of (Q)SAR models for IATA

Development of an Integrated Approach to Testing and Assessment for Acute Fish Toxicity

**Weight-of-Evidence analysis**

- WoE inconclusive
- WoE conclusive → no further test

**in vitro Fish Cell / Fish Embryo Toxicity test**

- Test inconclusive
- Test conclusive → no further test

**Acute Fish Toxicity**
Conclusion

Appropriate (Q)SAR models may be used to generate useful information:

• to replace the preliminary range-finding test, thus avoiding additional use of fish
• to predict toxicity of very hydrophobic compounds where experimentation (and analytical monitoring) is difficult to perform
• to predict chronic toxicity of compounds which are difficult to maintain stable during long-term exposure
• to anticipate toxic MechoA in an Adverse Outcome Pathway analysis, → like neurotoxicity (e.g. in a complement of FET test) → like endocrine activity in near future
Thank you for your attention