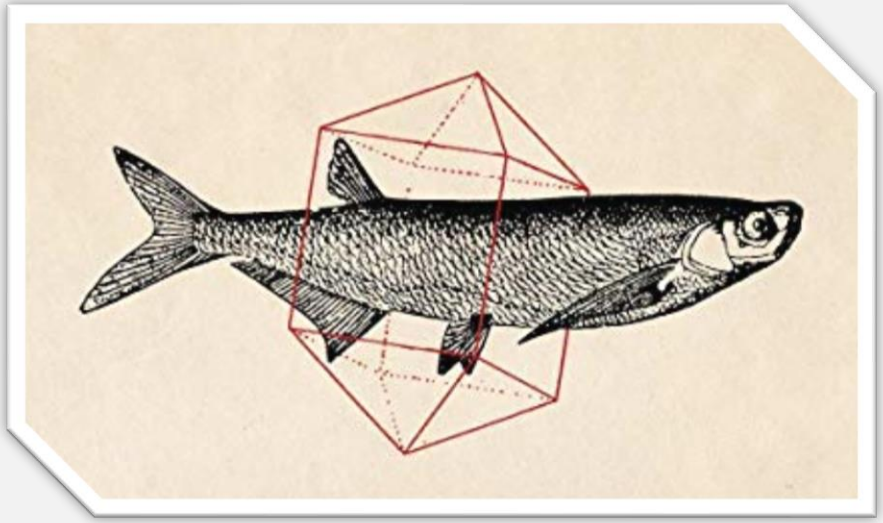
What (Q)SAR modelling can tell us about fish toxicity?



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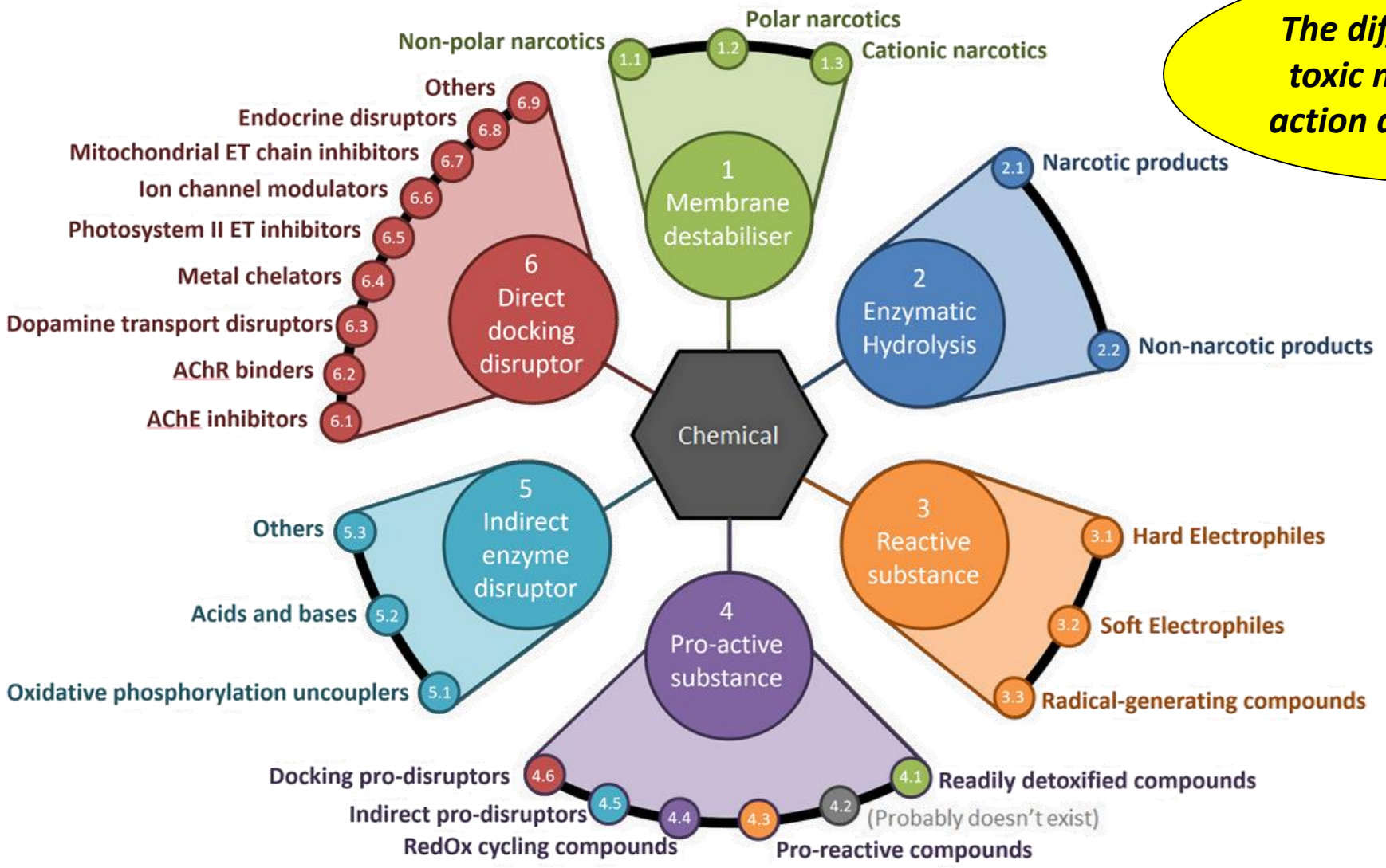
Introduction

- 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!

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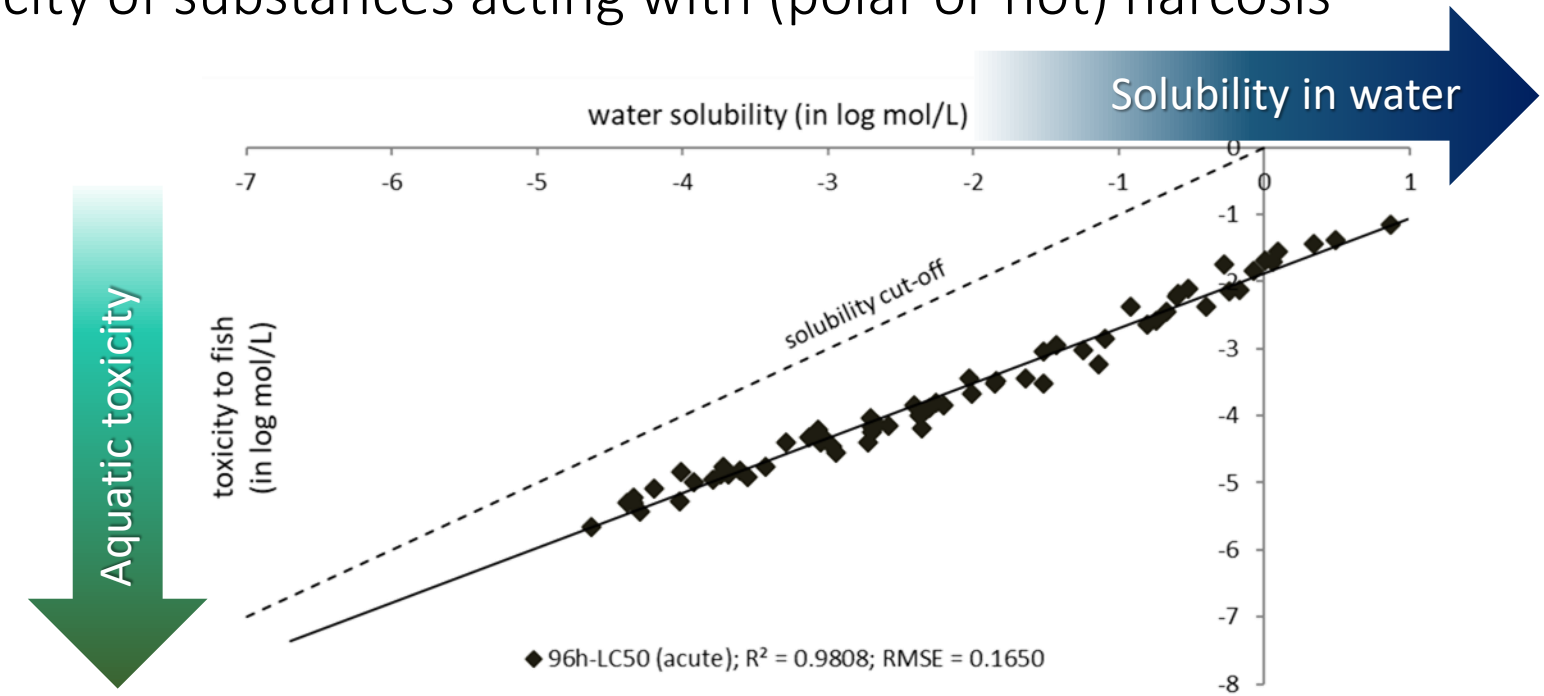
Regarding narcotic substances

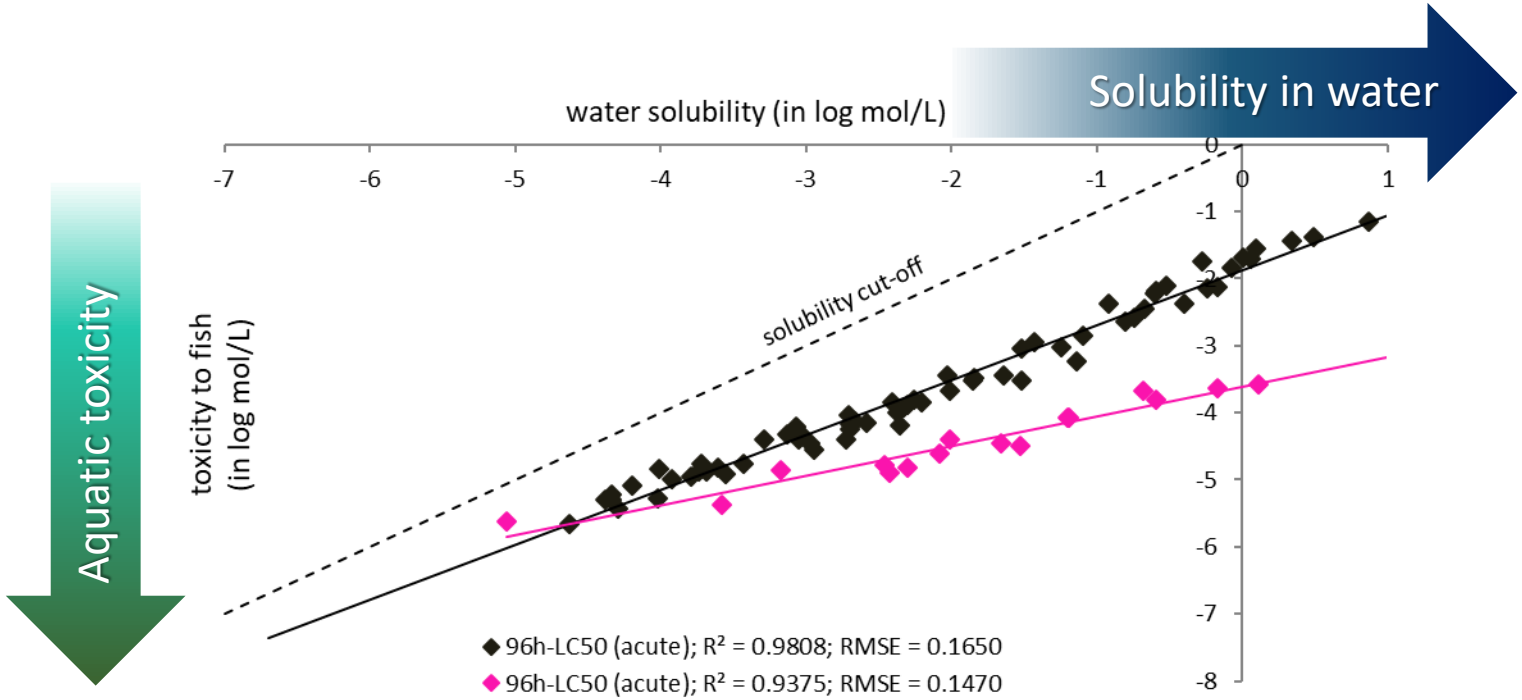
Water Solubility (or hydrophobicity as K_{OW}) is a very good descriptor to predict aquatic toxicity of substances acting with (polar or not) narcosis



- Non-polar narcotic chemicals (baseline toxicity)

Regarding narcotic substances

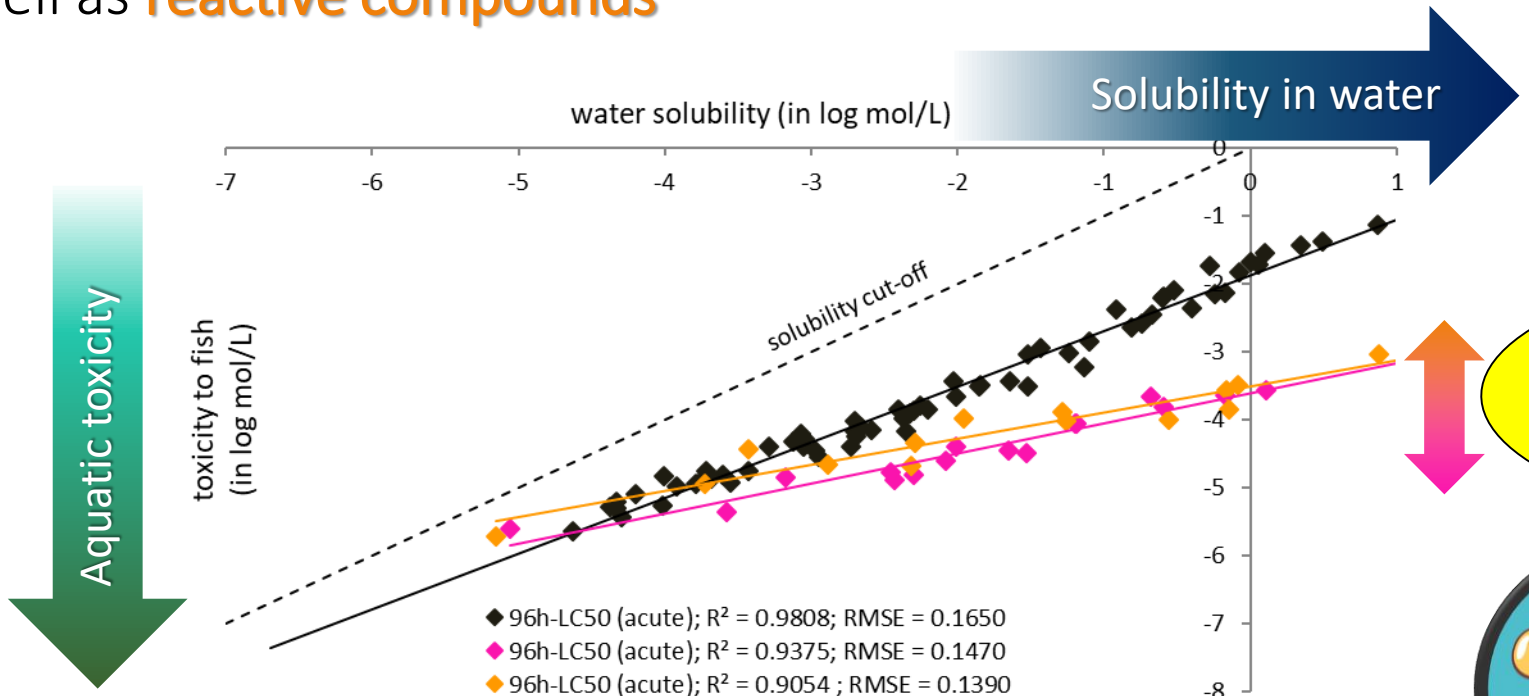
Polar narcotic compounds are subjected to “hydrophobic drift”



- 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**

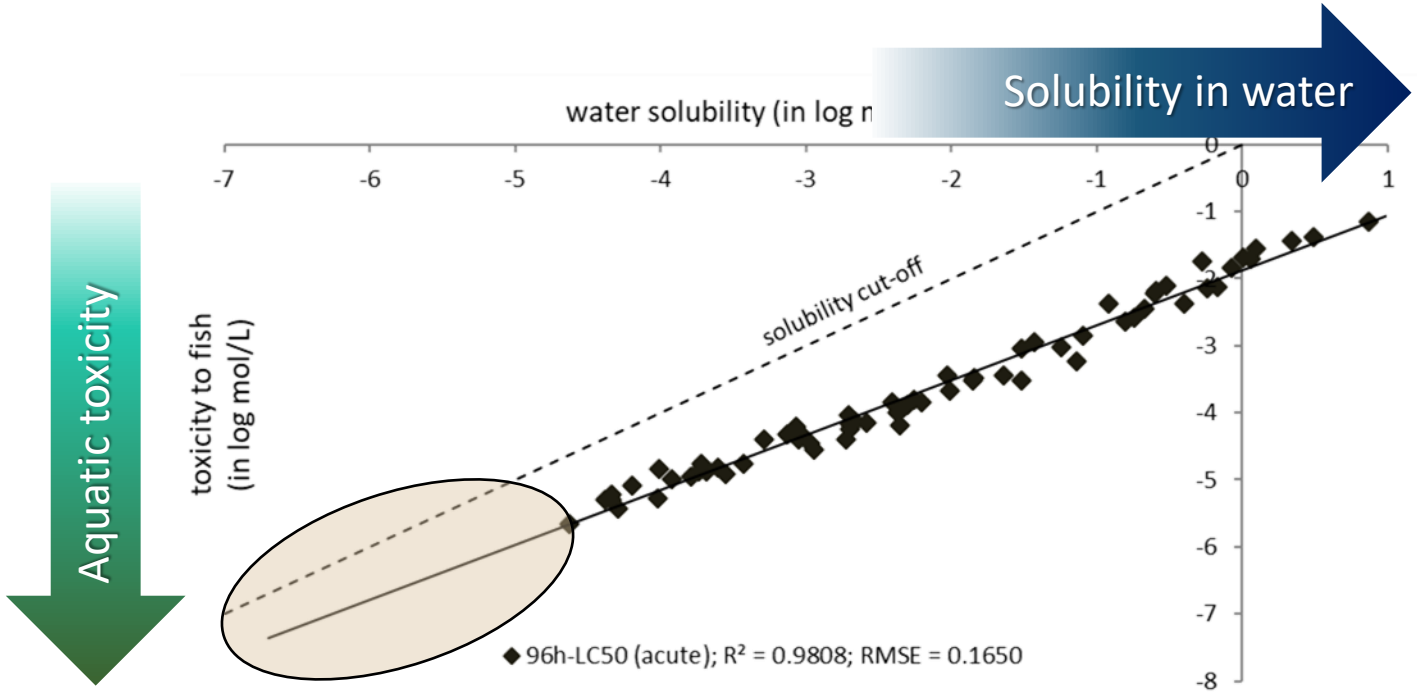


Fish are equally sensitive to polar narcotic and reactive compounds

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

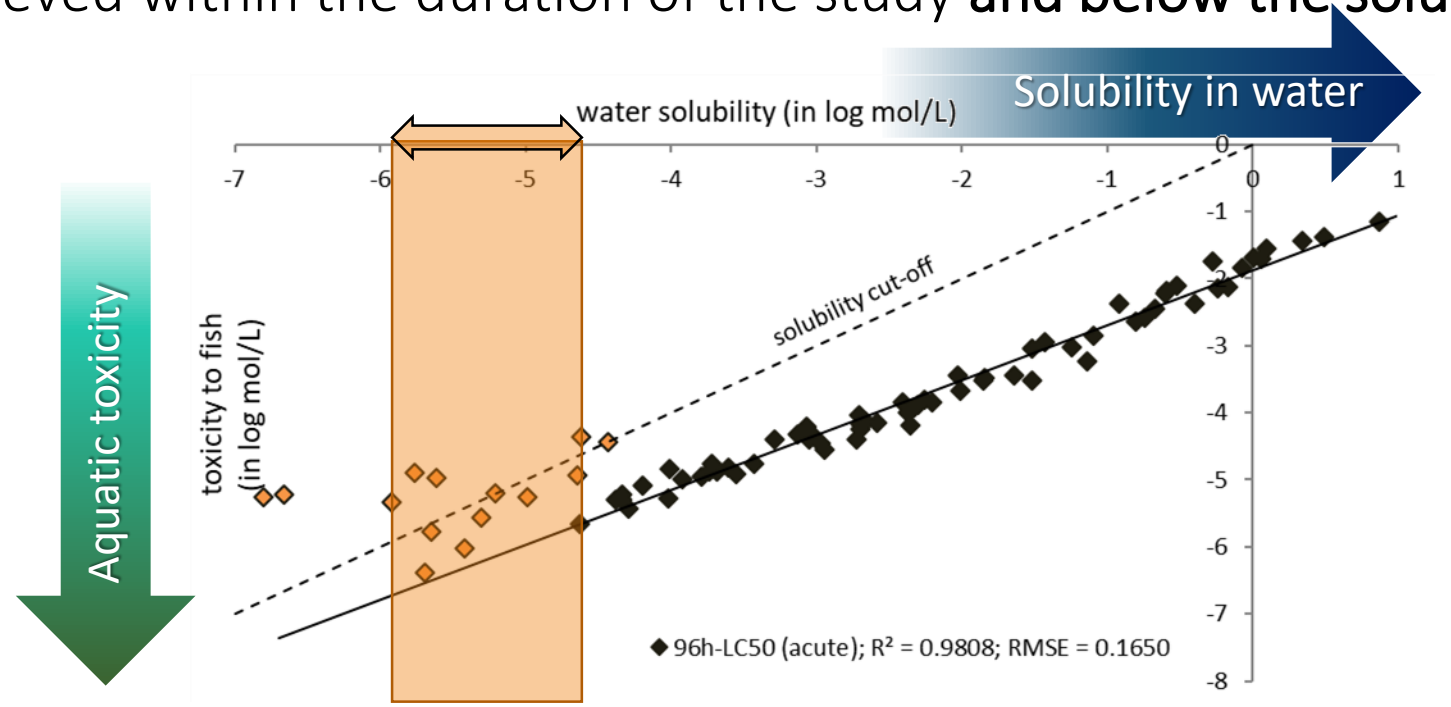
Regarding very hydrophobic substances

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.

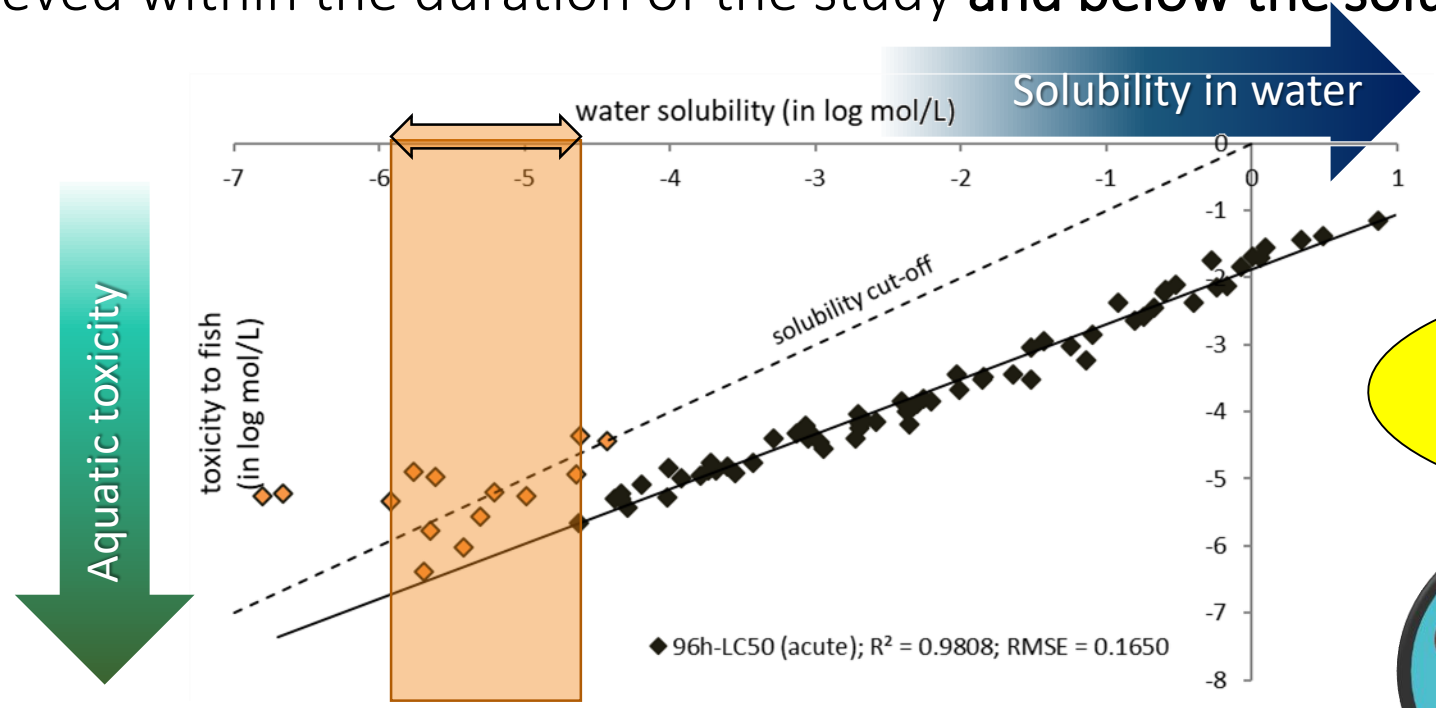
2. There is a zone where the toxicity is likely to be intermediate between the extrapolated prediction and the solubility

→ 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!**

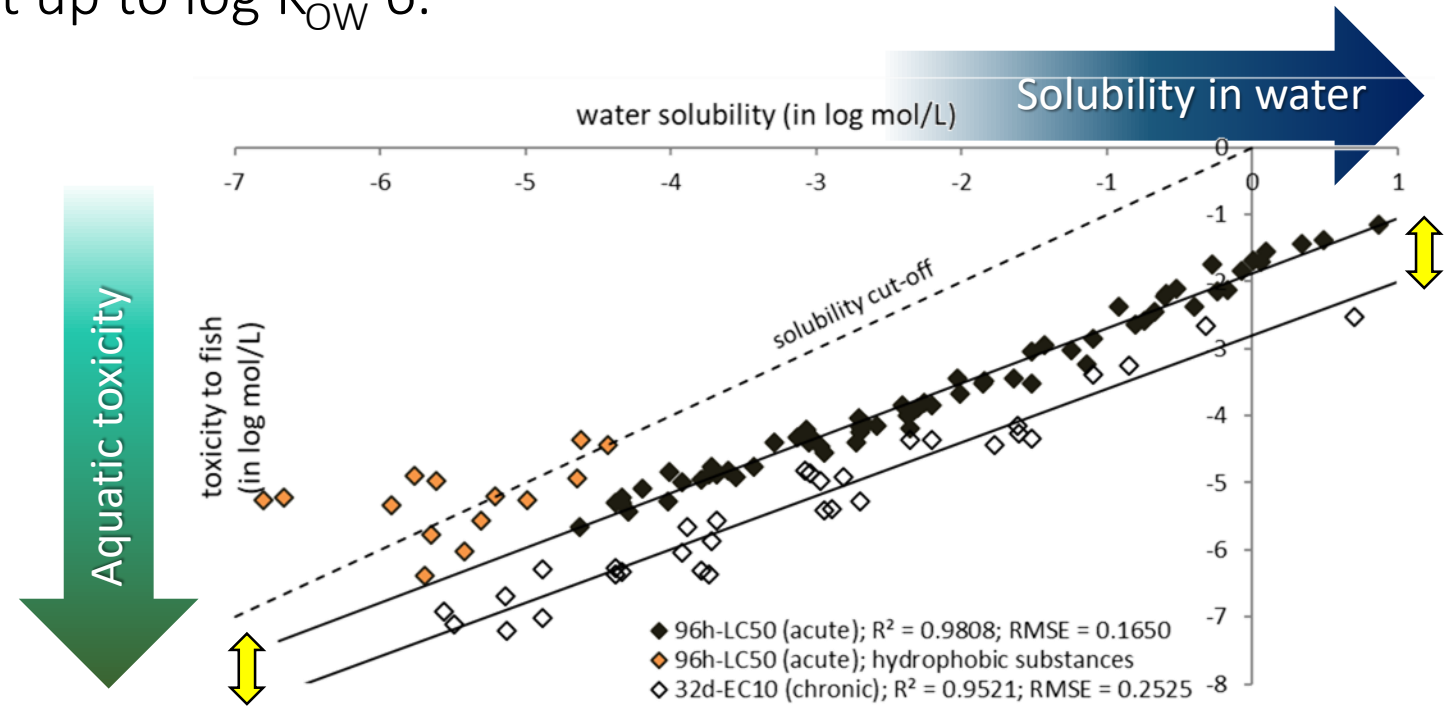


Substances with log K_{OW} > 6 have systematically acute toxicity above their solubility

→ **Long-term exposure is expected to overcome this limitation**

Regarding long-term toxicity

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

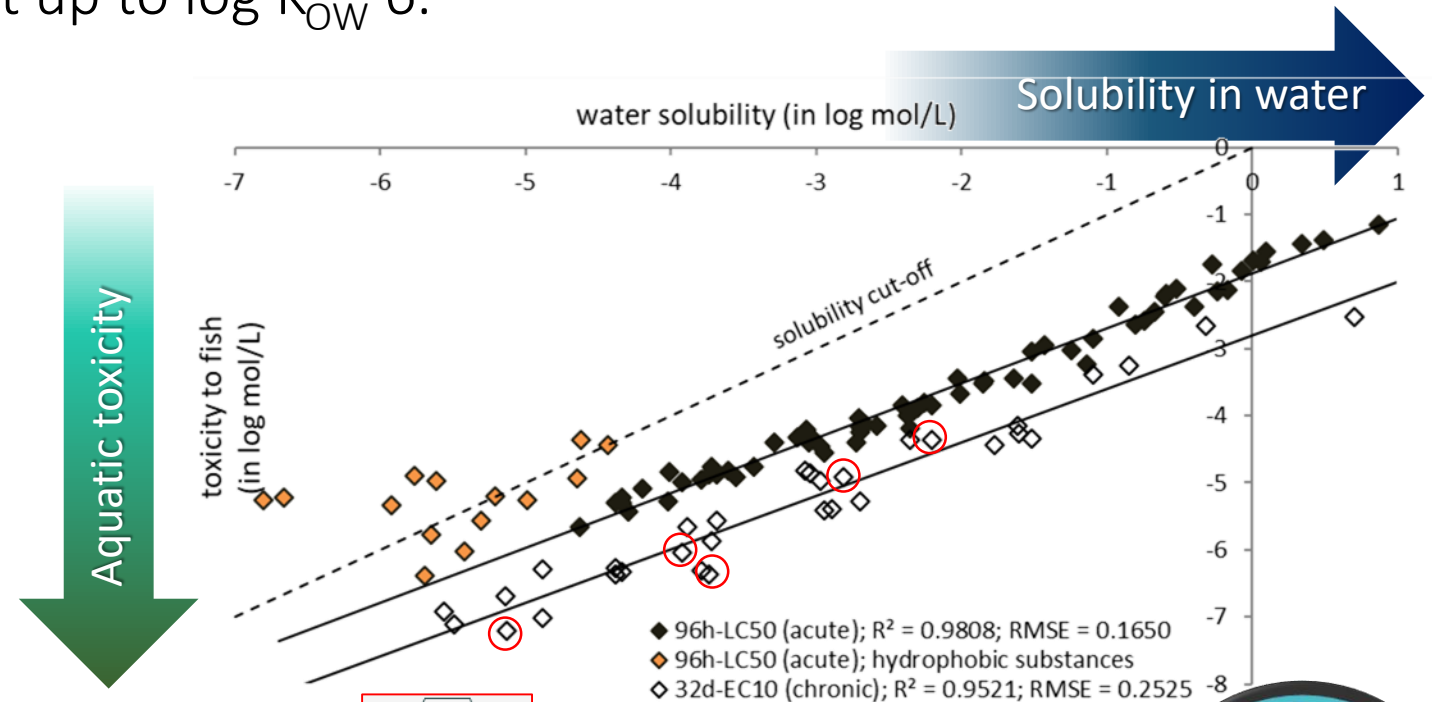


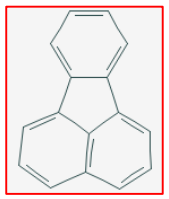
The Acute to Chronic ratio (ACR) for non-polar narcotics compounds.

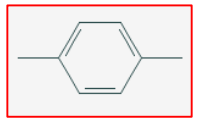
Good quality data for chronic toxicity is scarce due to the difficulties of maintaining test substances during the assays.

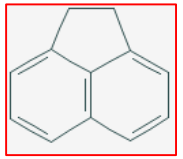
Regarding long-term toxicity

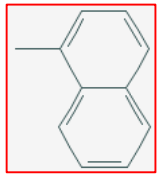
Model using Simple Linear Regression is still appropriate for hydrophobic substances, at least up to log K_{OW} 6.

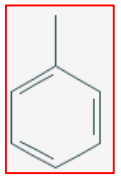



fluorenthene


p-xylene


acenaphtene


1-methylnaphthalene


toluene

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)

Figure: Fish embryos. Different malformations on the right.

- 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).

compound name	compound class	FET test predictivity	predicted MechoA for fish
Naphthalene	PAH	yes	MechoA 1.1: non-polar narcosis
Dichloromethane	organochlorine	yes	MechoA 1.1: non-polar narcosis
Esfenvalerate	insecticide (pyrethroid)	no	MechoA 3.1 & 4.1: hard electrophile reactivity and metabolisation to non-toxic compounds
Hydroquinone	reducing agent	no	MechoA 4.3 & 4.4: oxidation into quinone leading to protein/DNA adducts & RedOx cycling.
Endrin	insecticide (organochlorine)	no	MechoA 6.6: inhibition of GABAergic Cl- channel
Dieldrin	insecticide (organochlorine)	no	MechoA 6.6: inhibition of GABAergic Cl- channel
Methomyl	insecticide (carbamate)	no	MechoA 6.1: AChE inhibition
Rotenone	insecticide (ichtyotoxine)	no	MechoA 6.7: inhibition of mitochondrial electronic chain
Dicofol	miticide (organochlorine)	no	MechoA 6.8 & 6.9: endocrine disruption and others MechoA
Fluoxetine	antidepressant	no	MechoA 1.2 & 5.2 / an6.2: probable binding to ACh receptors (muscarinic or nicotinic)

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

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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

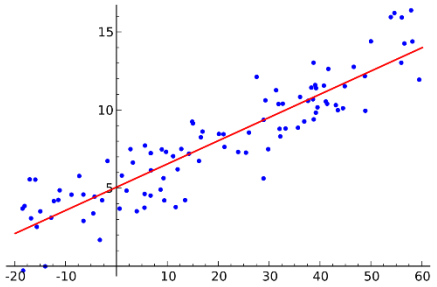
WoE conclusive → no further test

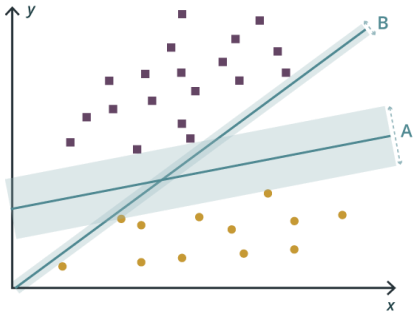
in vitro Fish Cell / Fish Embryo Toxicity test

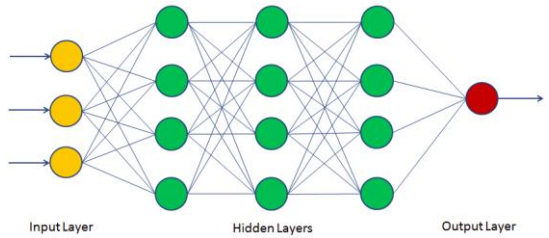
Test inconclusive

Test conclusive → no further test

Acute Fish Toxicity







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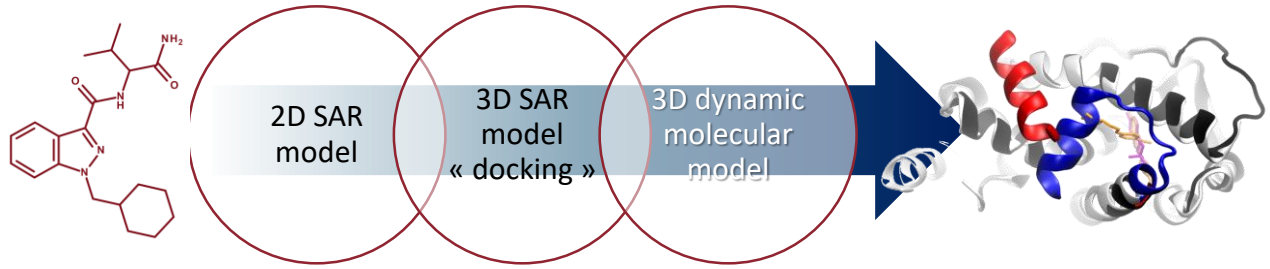
Conclusion

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

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