External Validation of Common Aquatic Toxicity Prediction Tools:

Challenges and Opportunities.

Fjodor Melnikov

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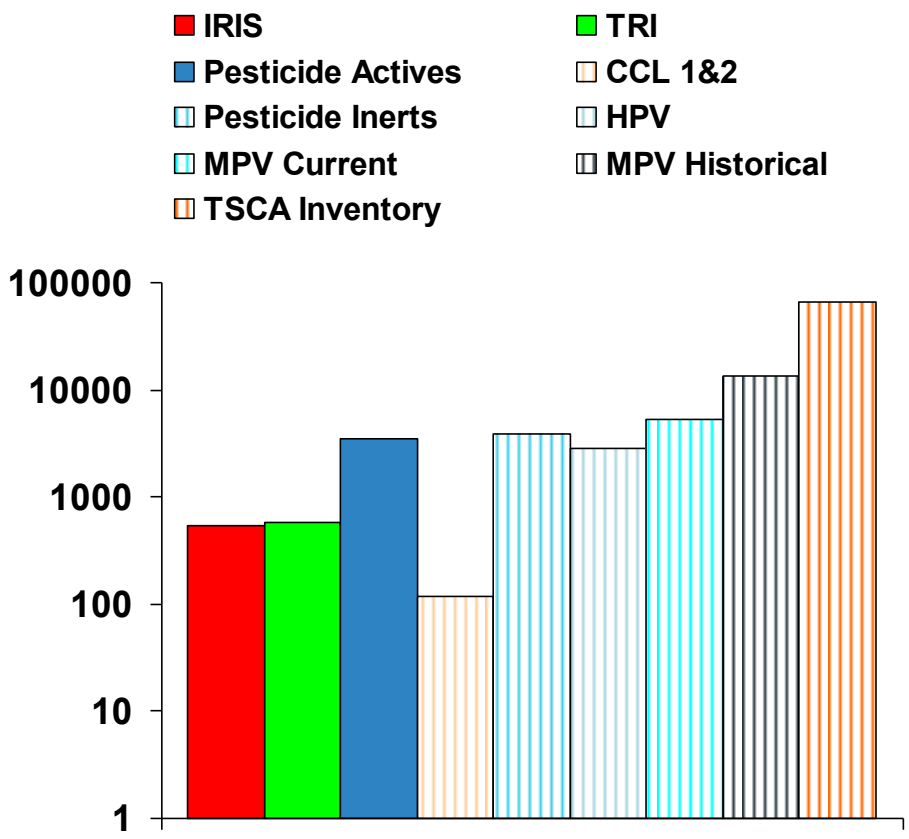
The Challenge of Toxicity Assessment

- Over 100,000 chemicals in global circulation
- 700+ chemicals are introduced each year to the US Market Alone
- 85% lack experimental data
- High testing costs
- <u>Increased interest in alternative toxicity assessment methods and chemical design</u>



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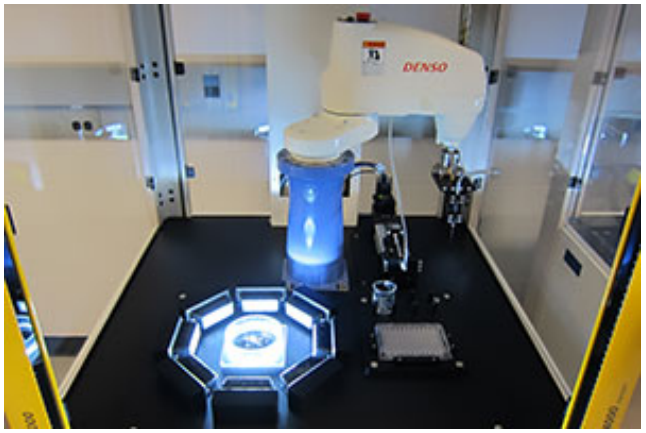
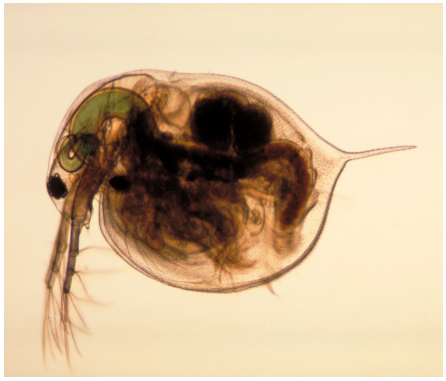
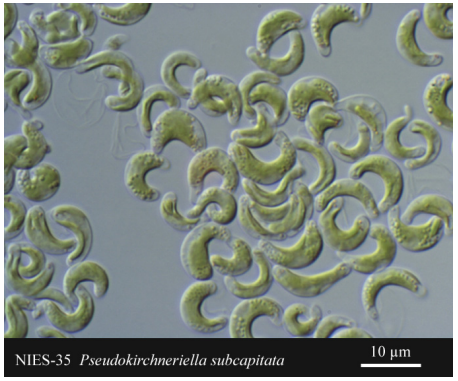
Why Acute Aquatic Toxicity?

- Data Quantity
- Data Quality
- Relevant to Mammals
  - Oxidative stress
  - High throughput toxicity assessment
  - Gene manipulation
- Spans multiple methods of action
  - Example: Endocrine disruption







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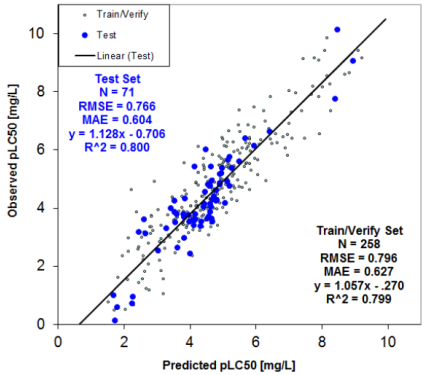
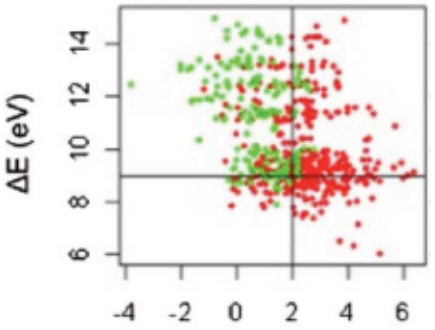
What are we looking for in a alternative assessment models?

- Have a clear association with the target endpoint
- Are based on a transparent and easily applicable algorithm
- Have a clear mechanistic basis
- Have a clearly defined applicability domain
- Include a description of the predicted estimates and goodness of fit.
- **Are externally validated**
- **Are assessed for accuracy by data sets distinct from its training set.**





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Available Software

Software	Public?	Method
ECOSAR (EPI Suite v. 4.1)	Yes	704 regression models (sometimes additive)
TEST (US EPA v.4.0.1)	Yes	Consensus method: average of neural network, FDA, clustering and regression models.
Cadre-AT	Development	Partitions chemical space base on bioavailability, reactivity, and metabolism.
KATE	Yes	6 regression models
ACD/Tox	No	Nested regression models w/ bootstrap
ADMET	No	Array of statistical approaches
DS TOPKAT	No	2D-QSAR
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TerraQSAR	No	Neural network

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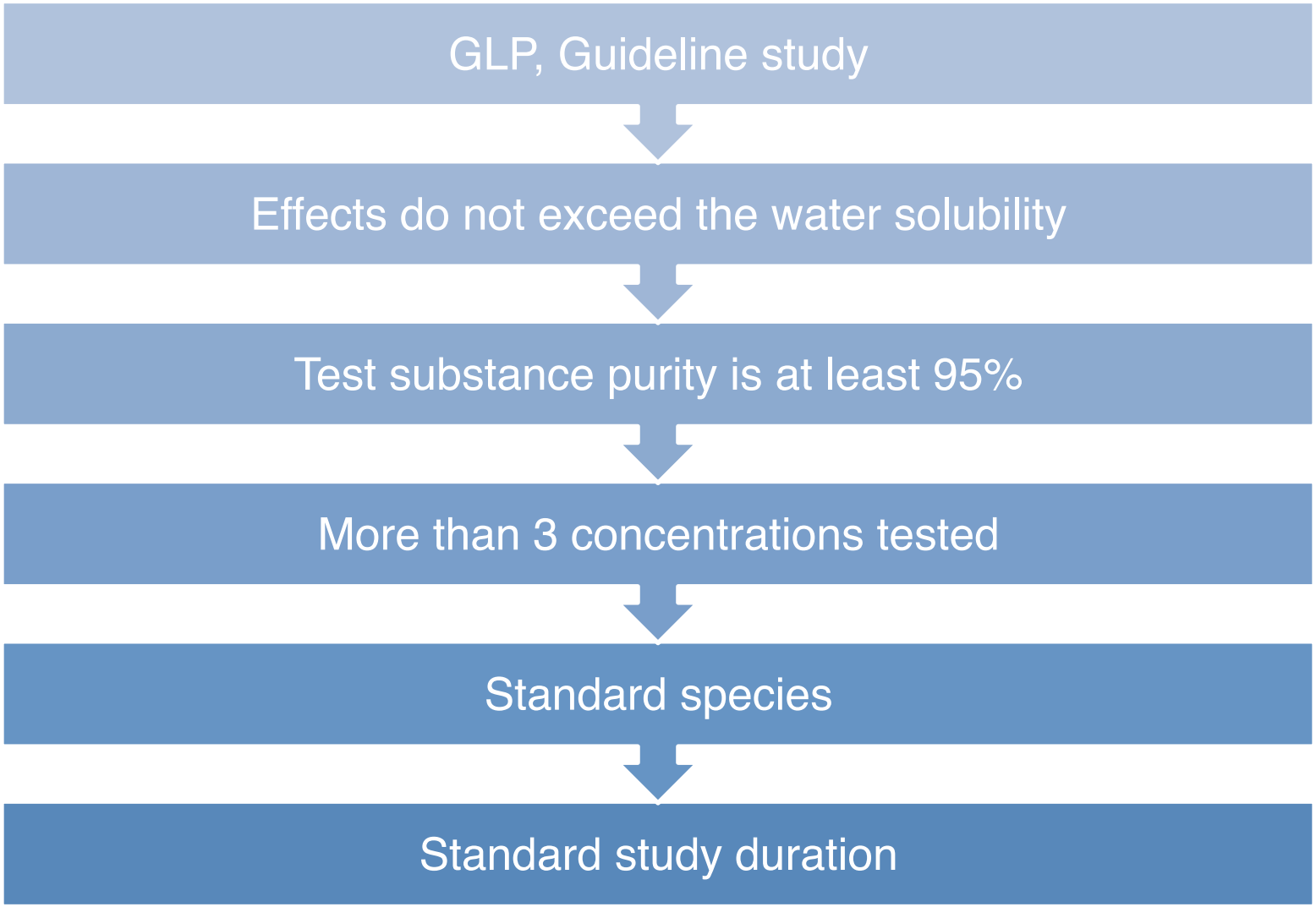
Data Selection

GOAL: Identify a diverse set of compounds with experimental data distinct from the training set of all evaluated models

ECHA Data	
Specie	Fish
# of Studies	274
# of Chemicals	190

ECOTOX Data	
Specie	Fish
# of Studies	1006
# of Chemicals	192
# of Chemicals (+ curation, - training sets)	84

Refined Data Selection Based on OECD Guidelines



Aquatic Toxicity Endpoints and OECD Guidelines

Criteria	Daphnia	Alga and Cyanobacteria	Fish
Endpoint	LC50	EC50	LC50
Duration	*48 h.*	*72 or 96 hours*	*48, 72, 96, 120 hours*
Specie	*Daphnia magna*	Green algae: *Pseudokirchneriella subcapitata, Selenastrum capricornutum, Scenedesmus subspicatus* Diatoms: *Navicula pelliculosa* Cyanobacteria: *Anabaena flos-aquae, Synechococcus leopoldensis*	Tier1: Oncorhynchus mykiss (Rainbow trout), Pimephales promelas(Fathead minnow), Brachydanio rerio (Zebra fish), Oryzias latipes (Ricefish), Cyprinodon variegatus (Sheepshead minnow); Cyprinodon variegatus (Sheepshead minnow) [SW].*

*Additional Acceptable Fish Species: Oncorhynchus kisutch (Coho salmon) Oncorhynchus tschawytscha (Chinook salmon) Salmo trutta (Brown trout) Salmo salar (Atlantic salmon) Salvelinus fontinalis (Brook trout) salvelinus namaycush (Lake trout) Esox Lucius (Northern pike), Catostomus commersoni (White sucker), Lepomis macrochirus (Bluegill), Ictalurus punctatus (Channel catfish) Jordanella floridae (Flagfish), Gasterosteus aculeatus (Three-spined stickleback) Cyprinus carpio.

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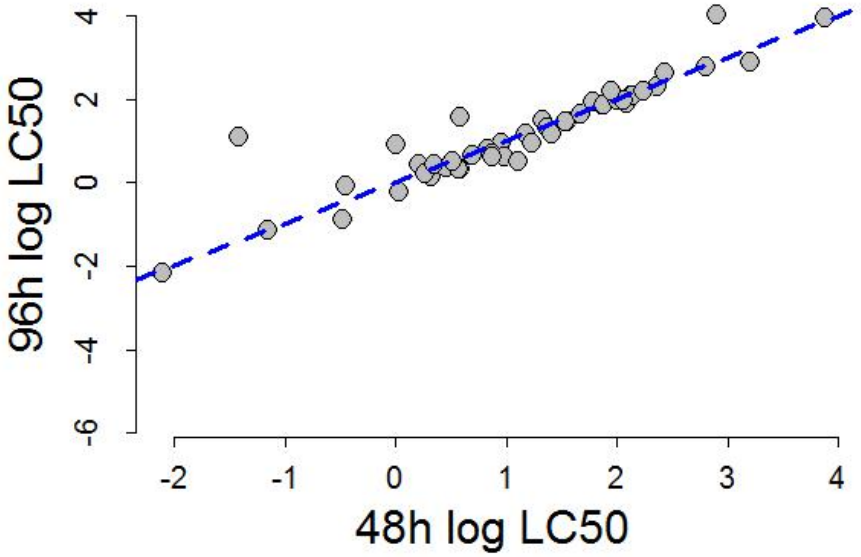
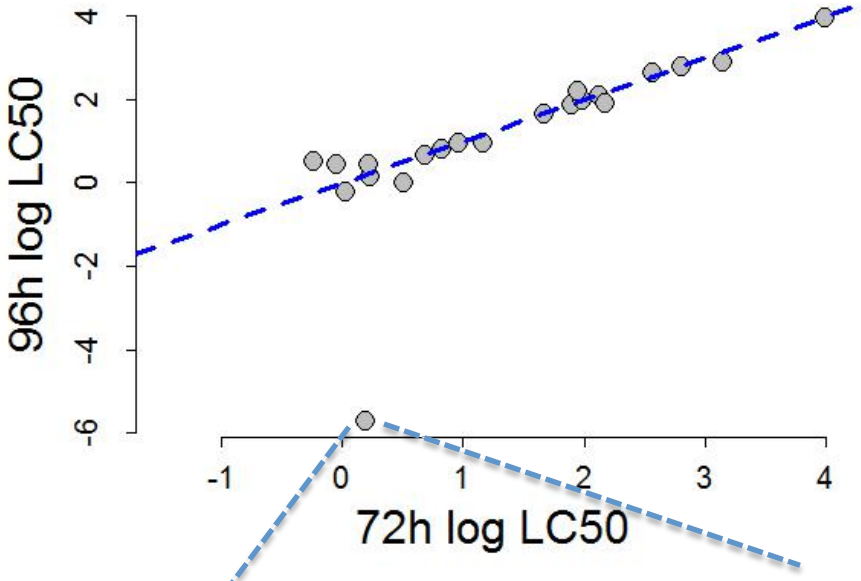
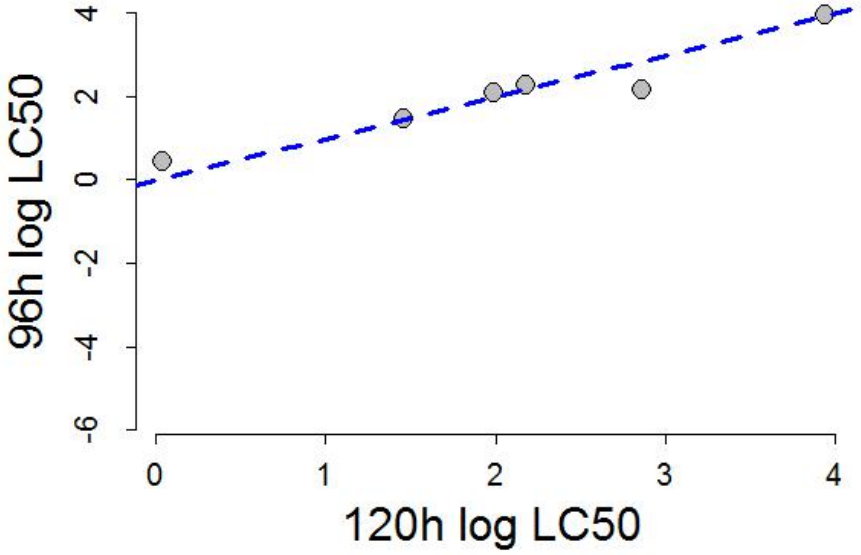
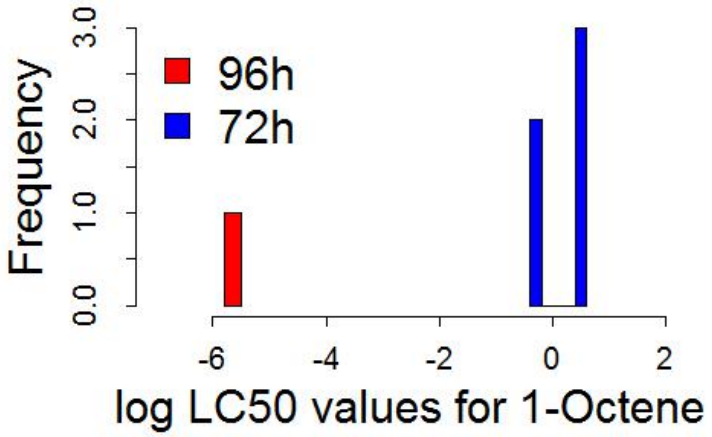
LC50 Comparison for Test Duration Across Species

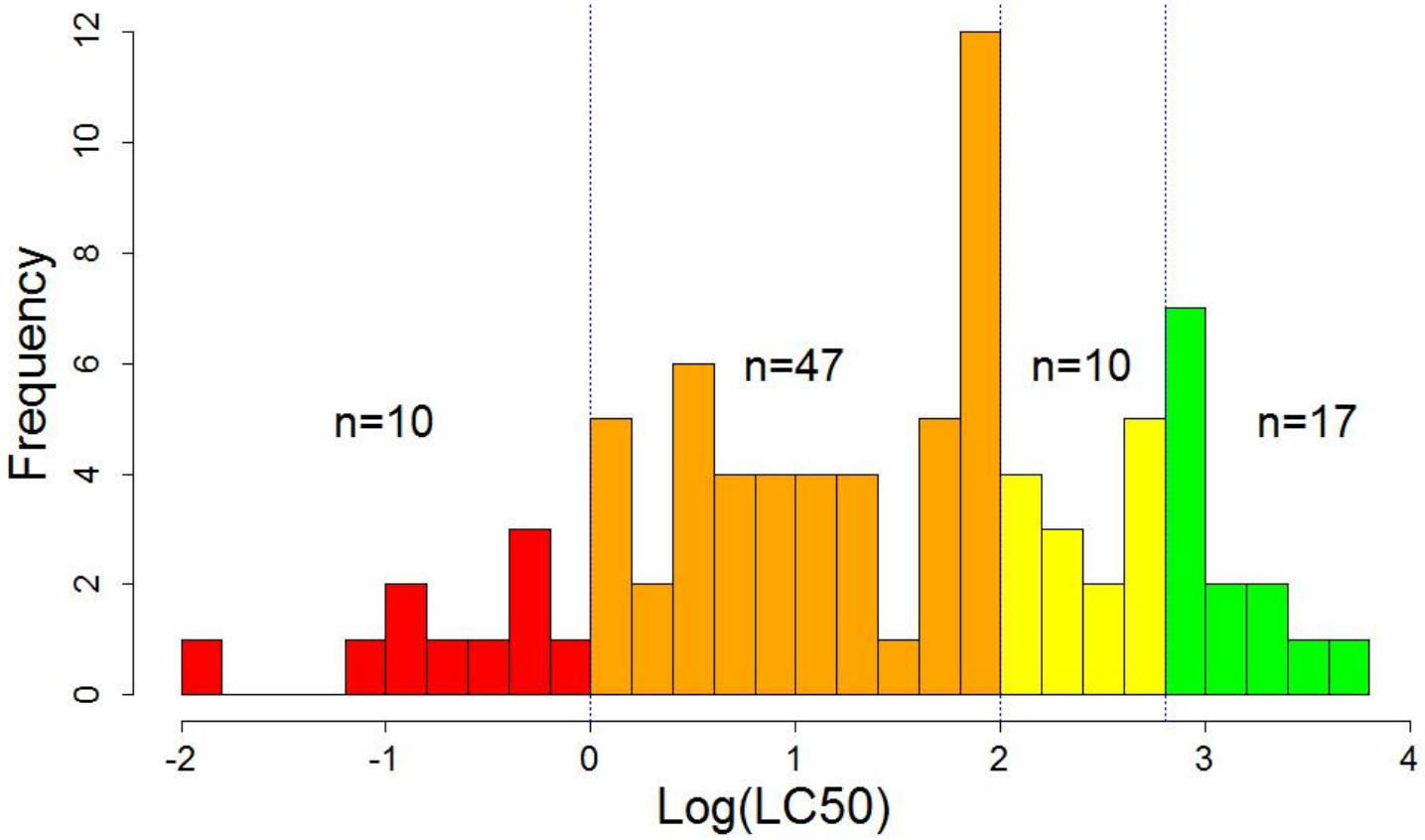








Toxicity Distribution in the Data Set



Acute LC_{50}/EC_{50} (mg/kg)	High	Medium	Low	No
	<1	1-100	100-500	>500

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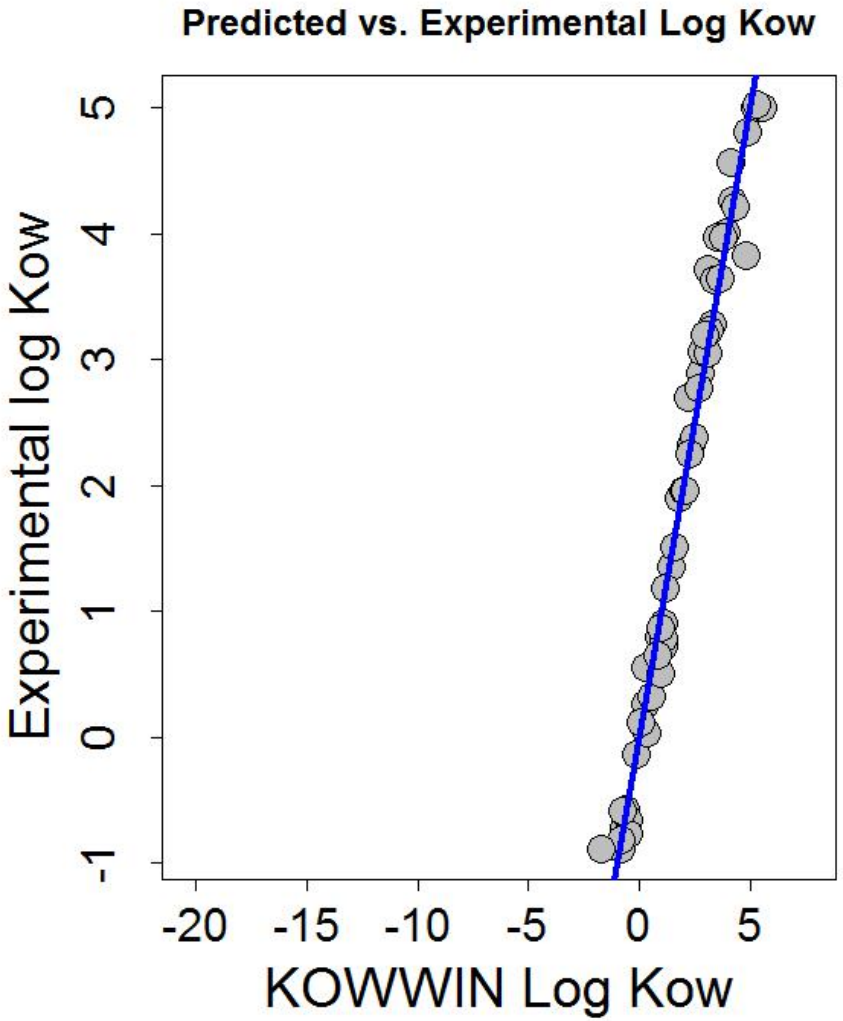
Categorical Accuracy

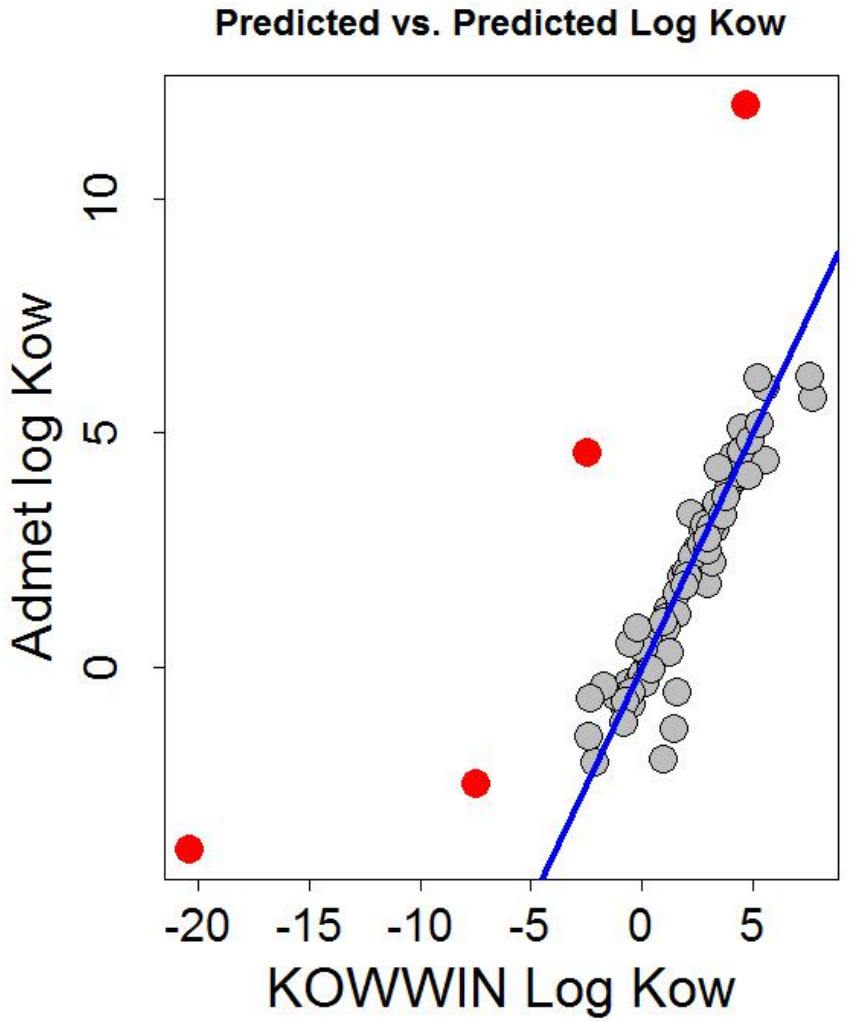
	Cadre AT	Admet	TEST	Ecosar	KATE
Accuracy	**80%**	**54%**	**50%**	**49%**	**56%**
Missing Predictions	**3**	**6**	**24**	**3**	**27**

WHY?

Bioavailability and Applicability Domains

Bioavailability and LogP Estimates





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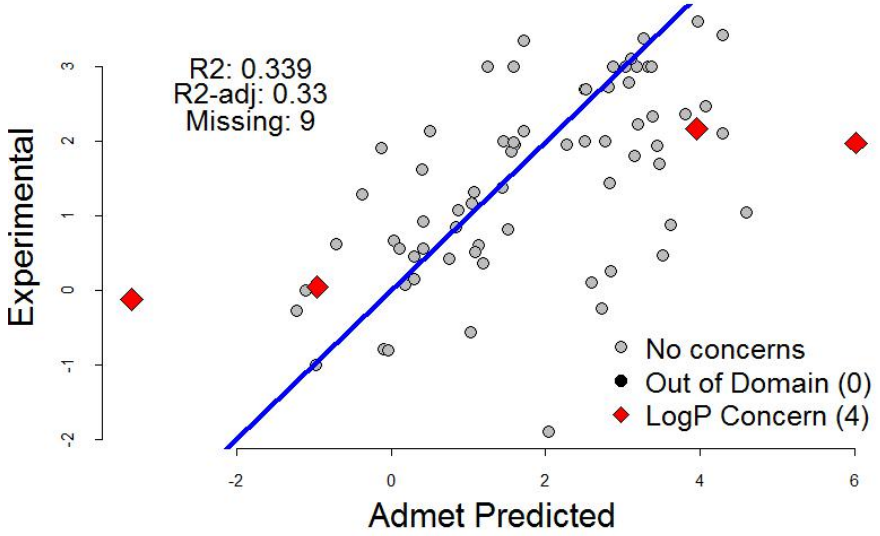
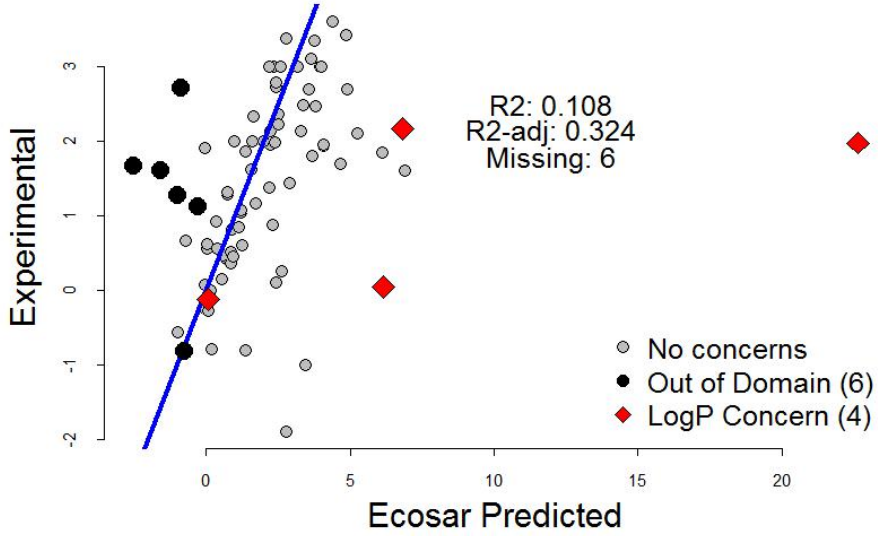
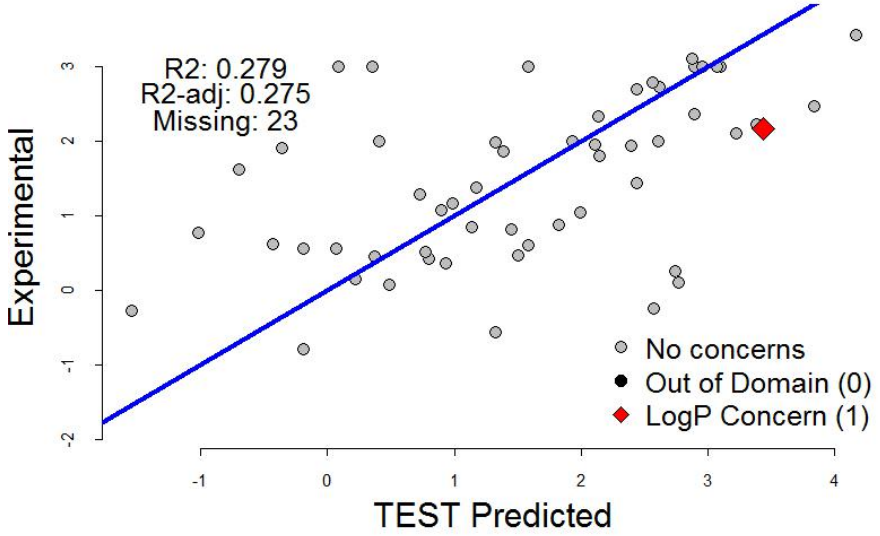
The Chemicals

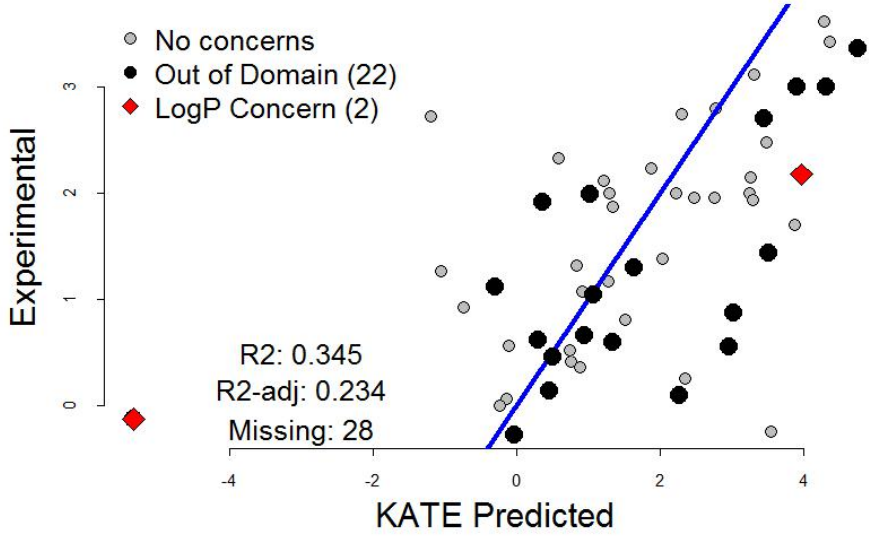
#	Name	CAS	LogP (ADMET)	LogP (KOWWIN)	LogP (Exp)
1	Didecyldimethylammonium chloride	7173-51-5	12	4.66	NA
2	Diallyldimethylammonium chloride	7398-69-8	4.59	-2.49	NA
3	Tetrakis(hydroxymethyl)phosphonium sulfate	55566-30-8	-3.86	-20.39	NA
4	Diazolidinyl urea	78491-02-8	-2.45	-7.49	NA

Predicted vs. Experimental Results









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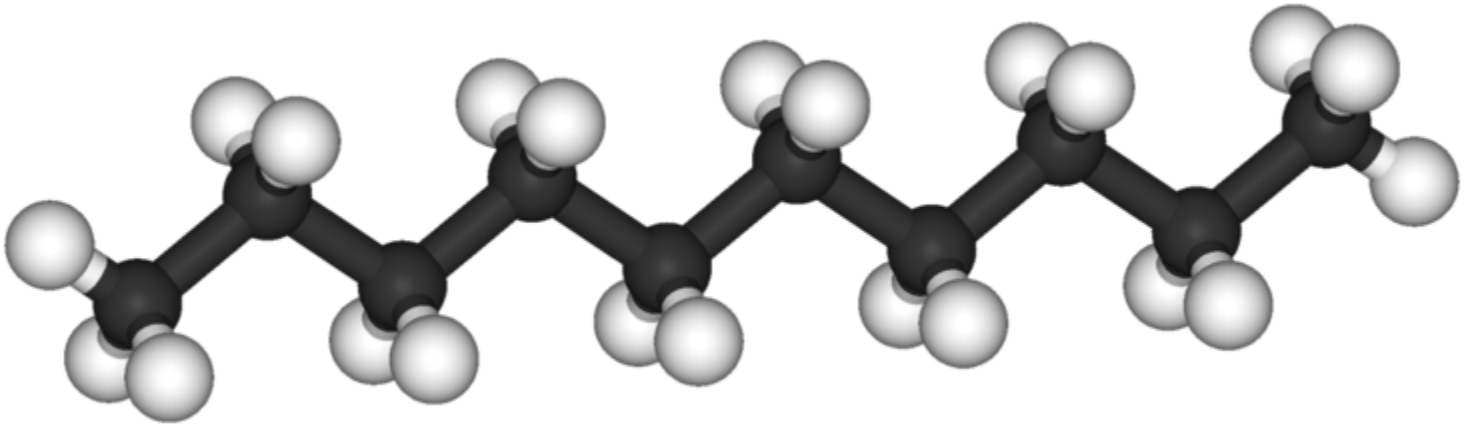
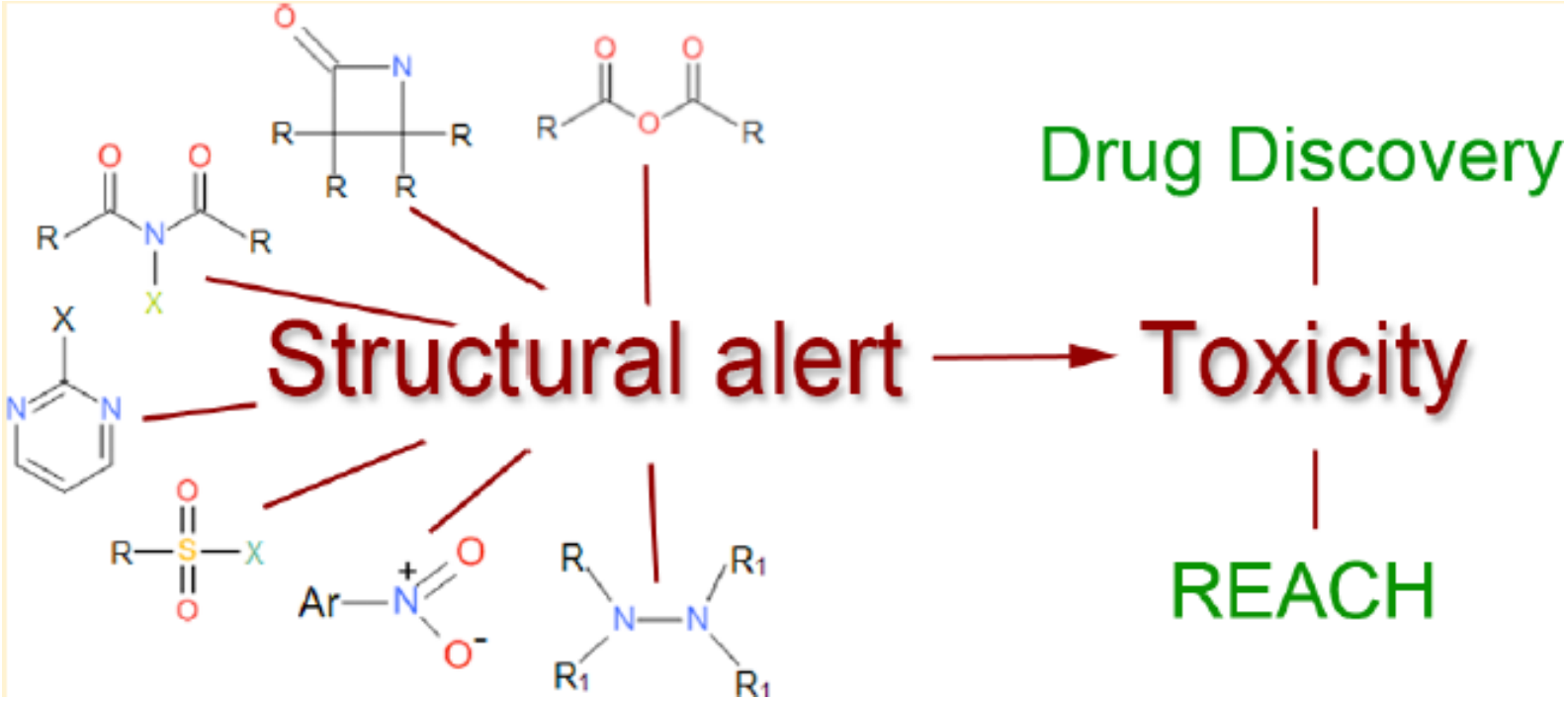
Accuracy (in applicability domain)	80%	54%	50%	52%	48%
Missing Predictions	3	6	24	9	56

Solubility

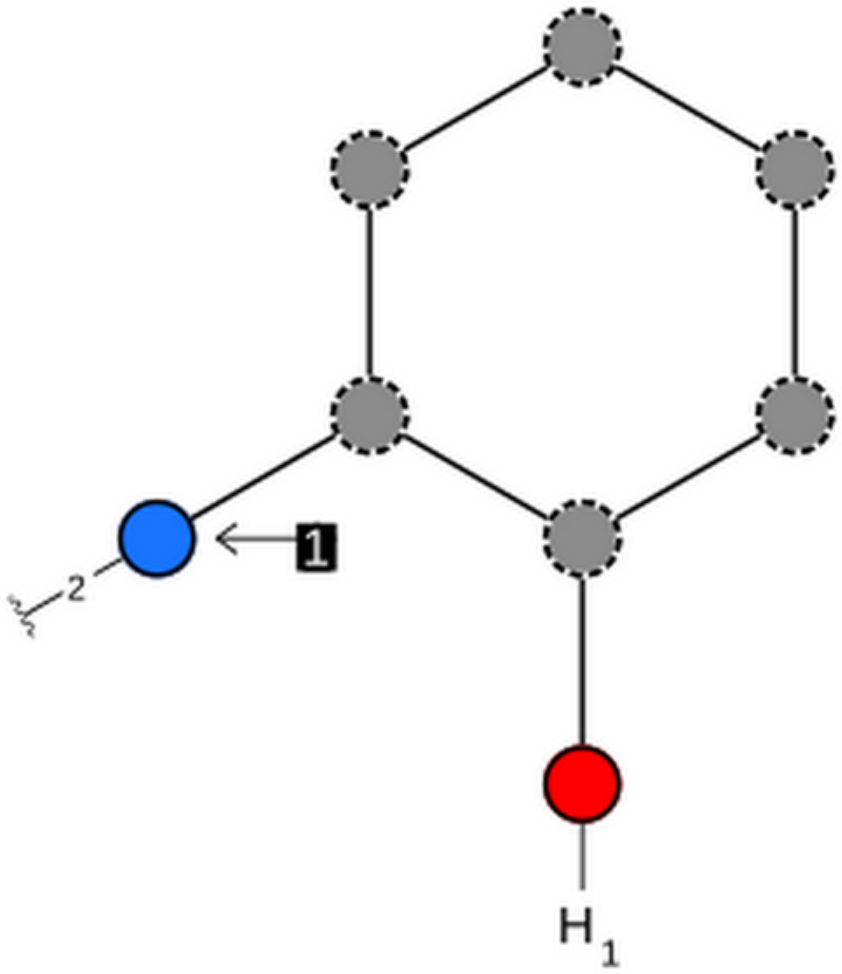


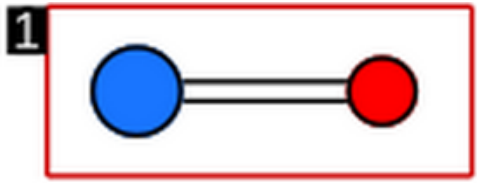
Solubility: 0.052 mg/L

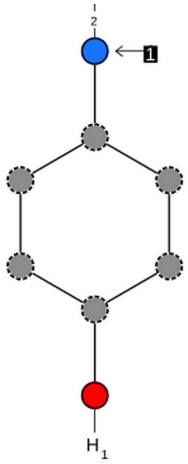
Structural Alerts, Expert Systems and MOA

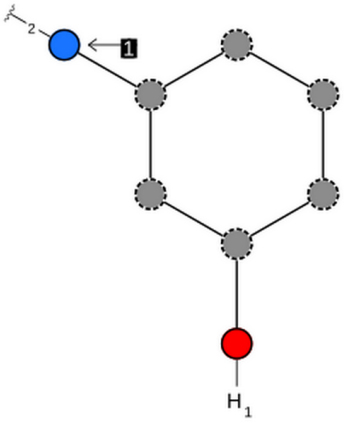


Example: Amino Phenols









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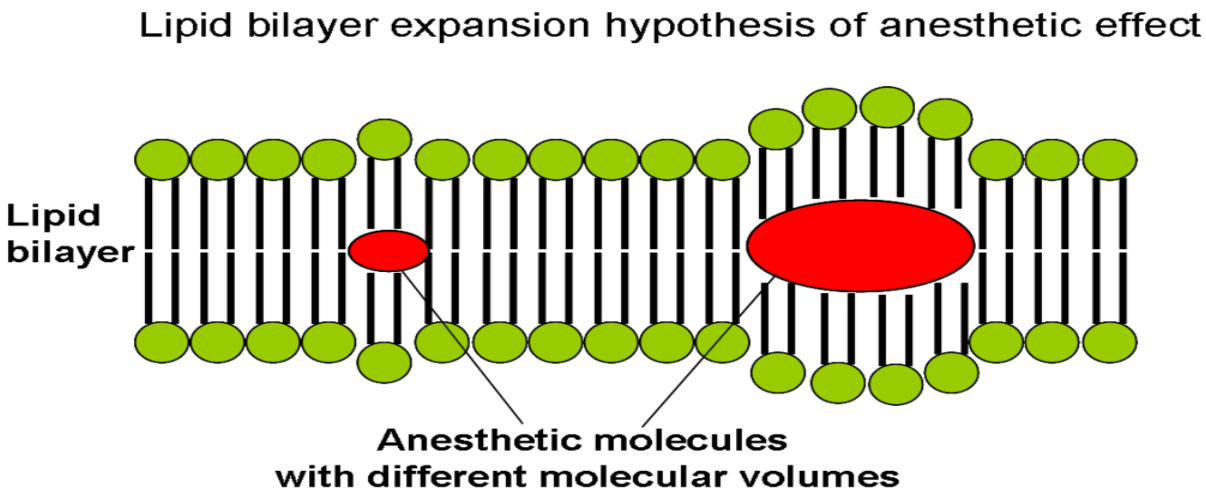
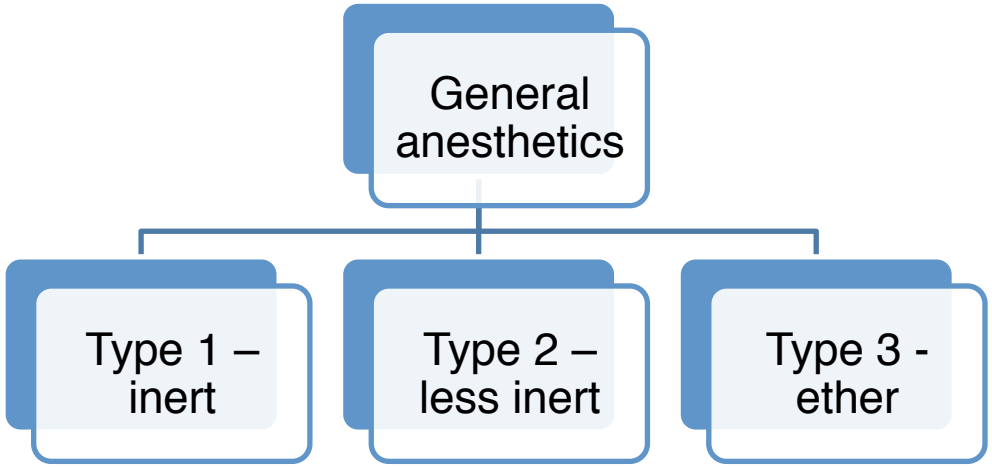
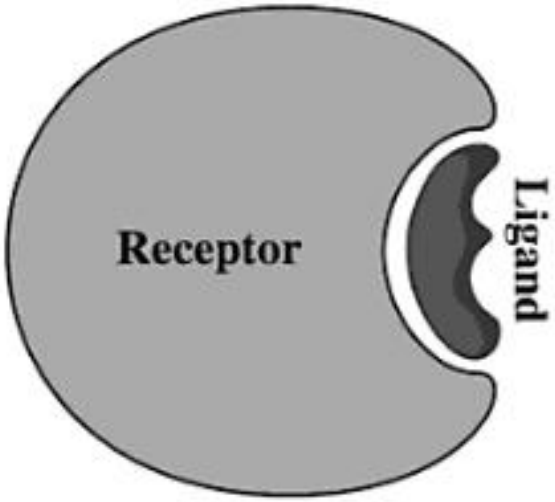
Mechanism of Toxicity - Narcosis



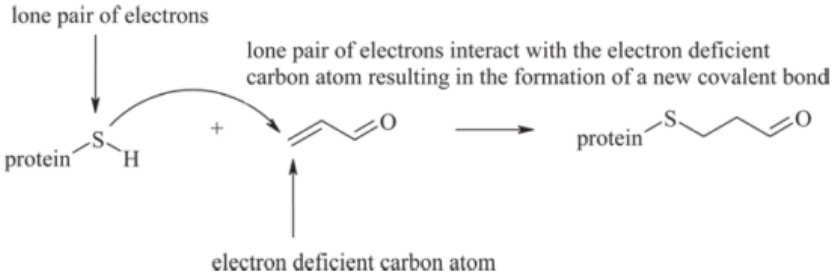


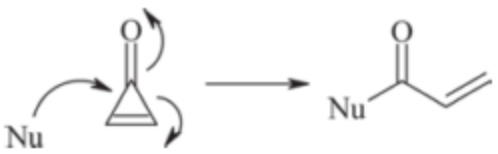
Reactivity

Specific



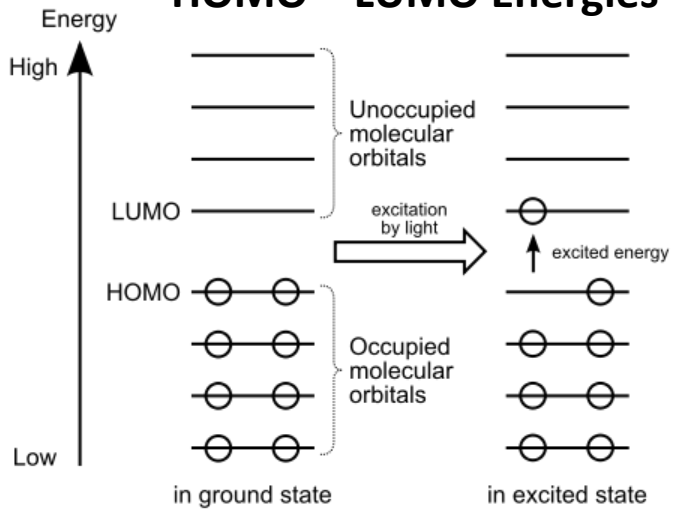
Non-Specific



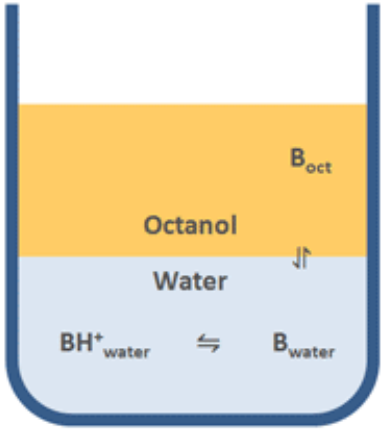


Molecular properties

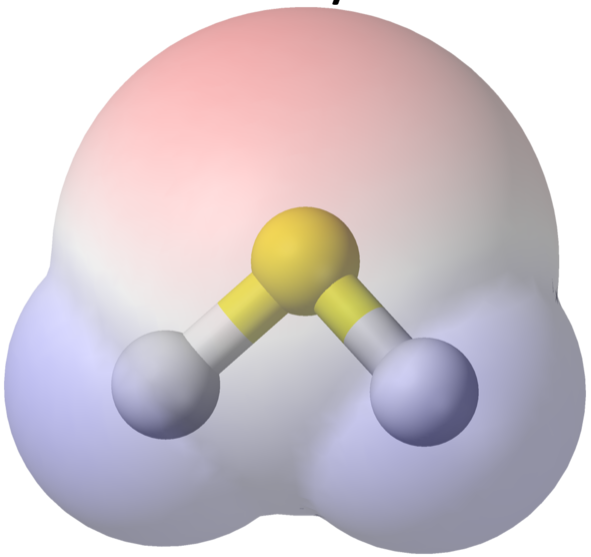
HOMO – LUMO Energies



Octanol – Water partition coefficient



Molecular volume/Surface area



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MOA

Russom et al. *Env. Tox. Chem.* (1997)

Accuracy	N	Cadre	Admet	Test	Ecosar	Kate
Narcosis I	41	78%	51%	48%	50%	50%
Gen. React.	46	82%	45%	52%	51%	52%

Verhaar et al., *Chemosphere*. (2000)

Accuracy	N	Cadre	Admet	Test	Ecosar	Kate
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Narcosis II	5	80%	80%	100%	100%	75%
Gen. React.	6	67%	33%	50%	50%	40%

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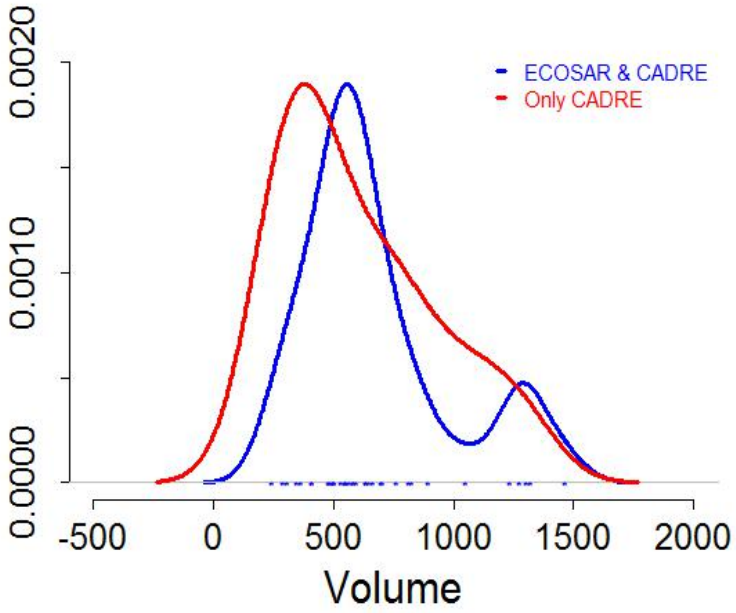
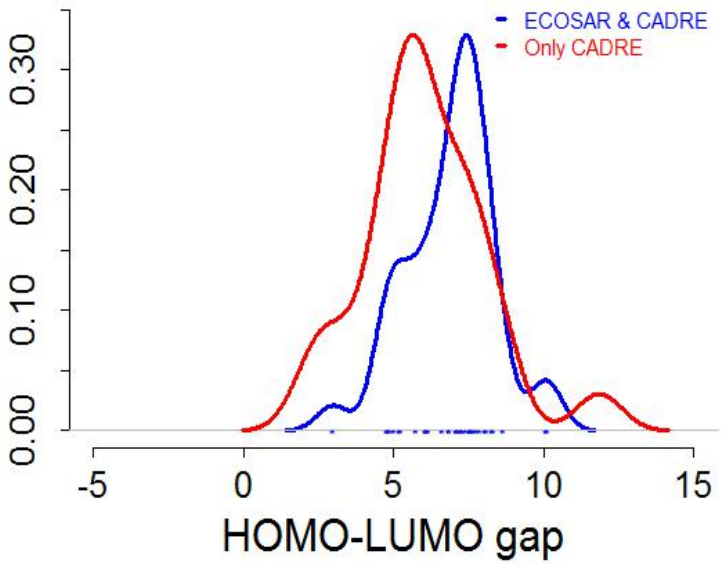
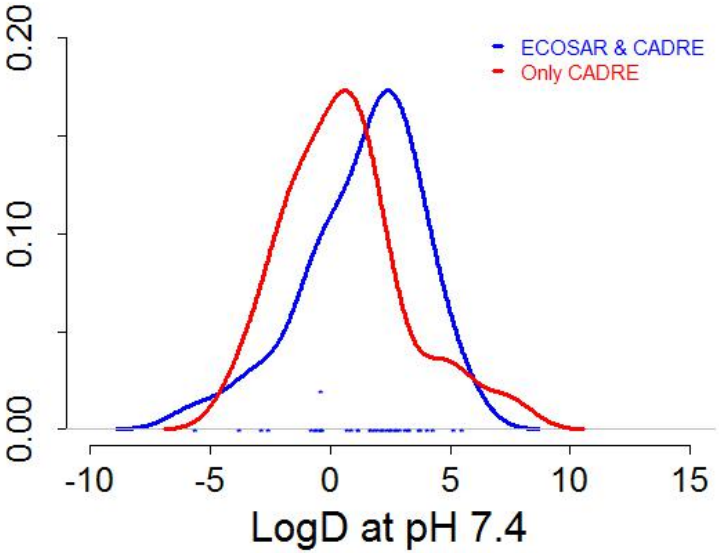
Chemical Properties







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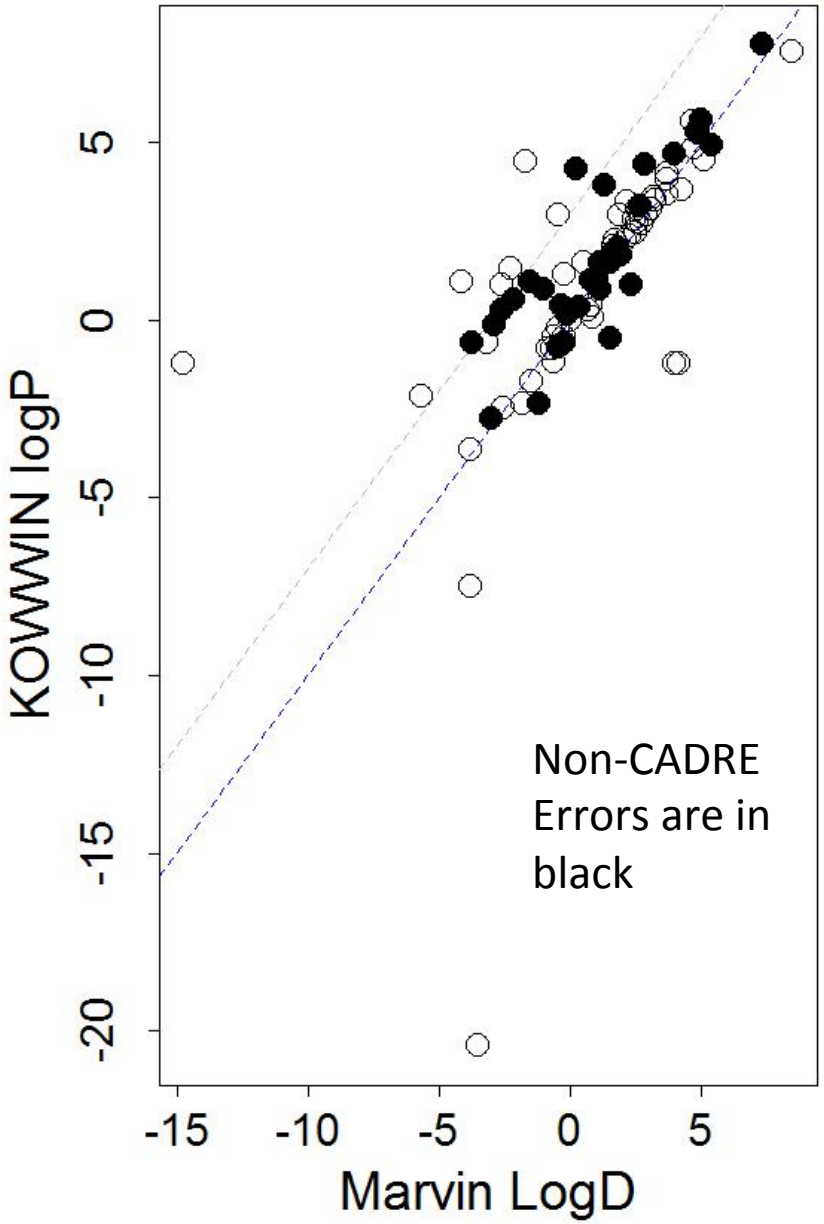
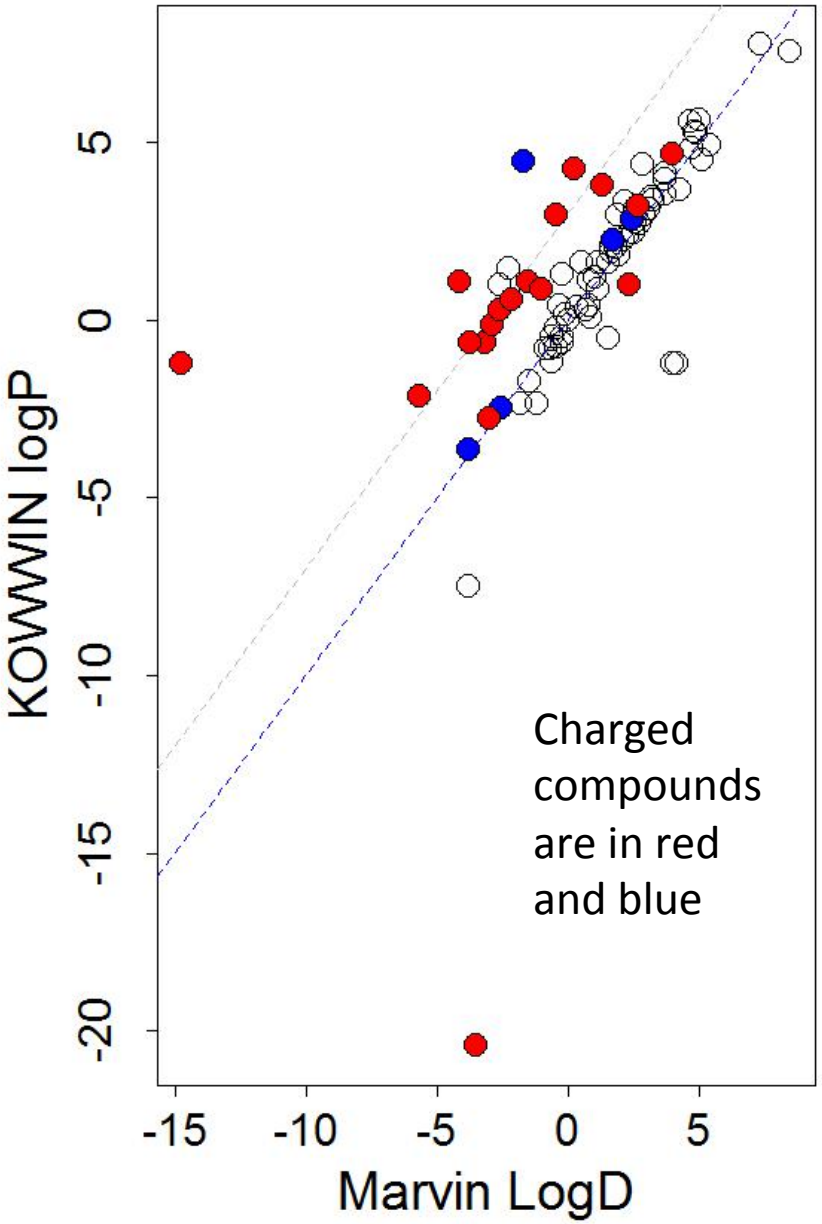
Charged Species

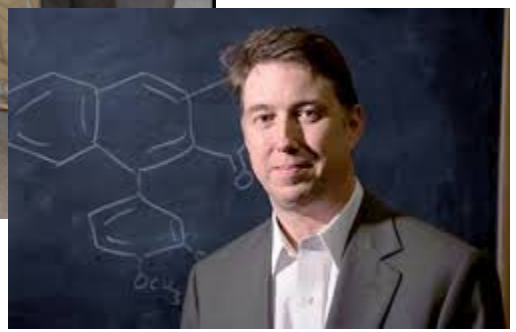




Conclusions

- Incorporation of multiple properties to specific and non-specific reactivity, as well as bioavailability improves model performance.
- Bioavailability predictions can still be improved; especially for charged chemical species.
- A relevant applicability domain should be defined with care
- It could be advantageous to the user to see predictions outside the applicability domain when possible.

Thank You!



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Thank You!

Most Relevant References:

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