Development of a Non-Animal Integrated Approach to Testing and Assessment for Acute Aquatic Toxicity Hazard for Classification and Labeling

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What is AFSA?

The HSI-coordinated Animal-Free Safety Assessment (AFSA) Collaboration works with industrial partners to accelerate global adoption of a modern, species-relevant approach to safety assessment that will better protect people and our planet, and hasten the replacement of animal testing (Figure 1).

Figure 1. The Animal-Free Safety Assessment Collaboration (AFSA) workstreams.

Introduction

Aquatic toxicity

Aquatic toxicity is an ecotoxicological endpoint which provides important information about a chemical's potential to elicit adverse effect(s) on aquatic organisms. Historically, within regulatory toxicology, three trophic levels are typically considered as a proxy of the ecosystem: fish, crustaceans and algae. Acute aquatic toxicity effects were traditionally studied using one or more OECD Test Guideline assays such as the Fish Acute Toxicity Test (OECD 203), the Fish Embryo Acute Toxicity Test (OECD 236), and the recently validated Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay (OECD 249).

For animal welfare reasons as well as the quest for increased relevance, biological coverage and throughput, there have been significant efforts in recent years to reduce or eliminate the use of vertebrate fish for regulatory environmental hazard and risk assessment. Specifically, this also concerns hazard classification schemes such as the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and the EU Classification, Labelling and Packaging of Substances and Mixtures (EU CLP) Regulation (EC No 1272/2008) (Table 1).

Category	Pictogram	H-Phrase	Statement	Conc (mg/l)	Global C&L Schemes
Acute 1		H400	Very toxic	$L(E)C_{50} \leq 1$	EU CLP + GHS
Acute 2	None	H401	Toxic	$1 < L(E)C_{50} \leq 10$	GHS
Acute 3	None	H402	Harmful	$10 < L(E)C_{50} \leq 100$	GHS

Table 1. GHS and EU CLP classifications for acute aquatic toxicity.

Integrated Approaches to Testing and Assessment (IATA)

IATA combine several lines of evidence from multiple models or assays to provide a prediction of the toxicity of a chemical.

This project is focused on the development of an IATA, consisting of discrete modules, which can predict acute aquatic toxicity categories to be used within the GHS and EU CLP frameworks (Figure 2).

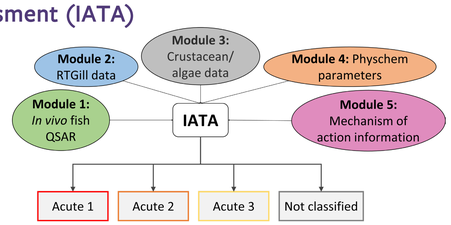


Figure 2. An illustration of the modular IATA being developed in this project.

Compilation of high-quality dataset

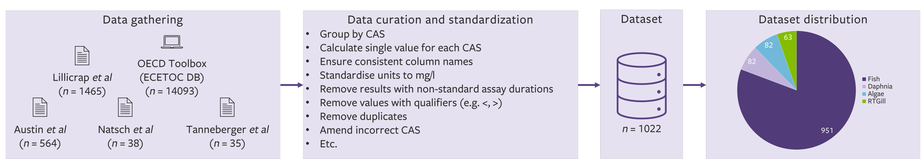


Figure 3. The steps undertaken to compile a high-quality dataset for use in IATA development.

Module development for IATA

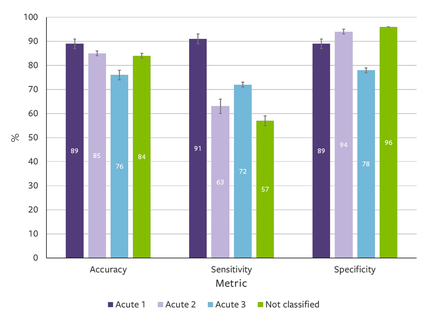
Module 1: QSAR model to predict *in vivo* fish LC_{50}

Methodology:

- To reduce uncertainty in the model, substances with multiple LC50 values where the LC50s spanned more than one GHS category (acute 1/2/3, not classified) were excluded from the dataset. This means that those chemicals may not be well-represented in the model – these substances will be assessed further. After elimination of substances with incorrect SMILES, inorganic/organometallic compounds and mixtures, 596 unique substances remained, and were used to develop the QSAR model.
- 4,676 molecular descriptors were calculated for each chemical, using in-house scripts, which include RDKit and Mordred descriptors, as well as others. Correlated, constant and null descriptors were filtered out. A feature importance algorithm was used to select the 12 most relevant descriptors for QSAR model development.
- The dataset was split into a training set (75%) and a test (25%) set, based on a K-means clustering algorithm and a random split of resulting clusters.
- The training set was used to develop a Random Forest model based on the 12 descriptors (Table 2) and the test set used to assess the performance of the model (Figure 5-6). A 5-fold cross-validation was also performed (Figure 4).

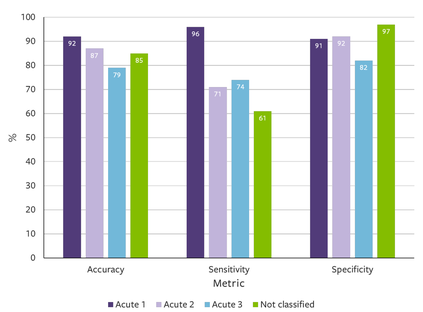
Descriptor	Description	Descriptor	Description
SLogP	logarithm of n-octanol and water partition coefficient	L2e	2nd component size directional WHIM index, Sanderson electronegativity-weighted
PEOE_VSA6	MOE Charge VSA descriptor 6	AATS0i	Averaged Broto-Moreau autocorrelation of lag 0 (log function), ionization potential-weighted
Mor22s	3D MoRSE signal 22, I-state-weighted	AATSC0i	Averaged centred Broto-Moreau autocorrelation of lag 0 (log function), ionization potential
HATSi	Leverage-weighted total index, ionization potential-weighted	AATSC0p	Averaged centred Broto-Moreau autocorrelation of lag 0 (log function), polarizability weighted
H2s	H autocorrelation of lag 2 / weighted by I-state	AATS0p	Averaged Broto-Moreau autocorrelation of lag 0 (log function) polarizability-weighted
R1p+	R maximal autocorrelation of lag 1 / weighted by polarizability	ATSC0p	centred Broto-Moreau autocorrelation of lag 0 (log function) polarizability-weighted

Table 2. Descriptors used to develop Random Forest QSAR model and a brief description of each.



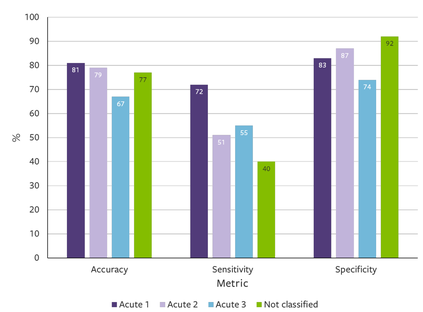
		Metric					
		Accuracy	Error	Sensitivity	Error	Specificity	Error
GHS category	Acute 1	89	±2	91	±2	89	±2
	Acute 2	85	±1	63	±3	94	±1
	Acute 3	76	±1	72	±3	78	±1
	Not classified	84	±1	57	±2	96	±0

Figure 4. 5-Fold cross-validation results.



		Predicted category			
		Acute 1	Acute 2	Acute 3	Not classified
Actual category	Acute 1	**55**	1	0	1
	Acute 2	12	**86**	21	2
	Acute 3	10	17	**89**	5
	Not classified	12	7	39	**89**

*Figure 5. Training set results – correct predictions in **bold**.*



		Predicted category			
		Acute 1	Acute 2	Acute 3	Not classified
Actual category	Acute 1	**13**	4	1	0
	Acute 2	11	**18**	5	1
	Acute 3	7	10	**30**	8
	Not classified	5	1	19	**17**

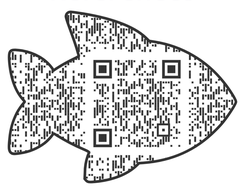
*Figure 6. Test set results – correct predictions in **bold**.*

Conclusions

To summarize:

- A large dataset of *in vivo* and *in vitro* data has been compiled, covering three trophic levels, fish, crustacean and algae.
- Fish toxicity data were used to develop the first module of an IATA, a multiclass QSAR model, which shows good predictivity for EU CLP and GHS categories for acute aquatic toxicity.
  - → Trends amongst those chemicals not predicted well are being investigated, to improve knowledge on the applicability domain of the model.
- Other modules covering crustacean and algae data (including species selectivity analysis), the *in vitro* RTgill assay, physicochemical parameters and mechanism of action are under development.
- Each module will be used as a discrete line of evidence combined within the IATA to predict acute aquatic toxicity classification, suitable for use within the CLP and/or GHS frameworks, without the need for vertebrate fish testing.

References



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