Modelling Chronic Toxicity to Fish Considering Data Uncertainty

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Introduction and Aim

Chronic aquatic toxicity is a critical endpoint in environmental hazards assessment. However, the conventional No-Observed-Effect Concentration (NOEC) testing is resource-intensive, time-consuming, and associated with ethical concerns. The development of predictive models is in accordance with the 3R principles (Replacement, Reduction, and Refinement) and provides a mechanistically informed methodology for screening substances, thereby reducing the need for animal testing.

The aim of this work is to develop models for predicting chronic toxicity in fish, accounting for data uncertainty. Two complementary methodologies have been develop. The first approach utilizes quantitative structure–activity relationship (QSAR) models to establish acute-to-chronic toxicity relationships [1] within mechanistically augmented Verhaar modes of action [2]. The second approach applies a read-across/trend analysis workflow, enabling the dynamic selection of structurally and mechanistically similar analogues. For both approaches, applicability domains incorporating mechanistic relevance, structural similarity, and parametric constraints were defined. Uncertainty in experimental NOEC data and model predictions was quantified using probabilistic distributions which results in a probabilistic Globally Harmonized System (GHS) chronic hazard categorization.

Both approaches have been implemented in the QSAR Toolbox, providing a transparent and practical framework for regulatory applications.

Materials and Methods

Data and Endpoint

The modeled endpoint is chronic fish NOEC (mg/L) derived from 21–60-day OECD TG 210 studies. A dataset consisting of 300 chemicals with 5,803 exp NOEC (Mortality or Growth rate) and LC(EC)50 (Mortality) data values was assembled from multiple QSAR Toolbox databases.

Modeling approaches

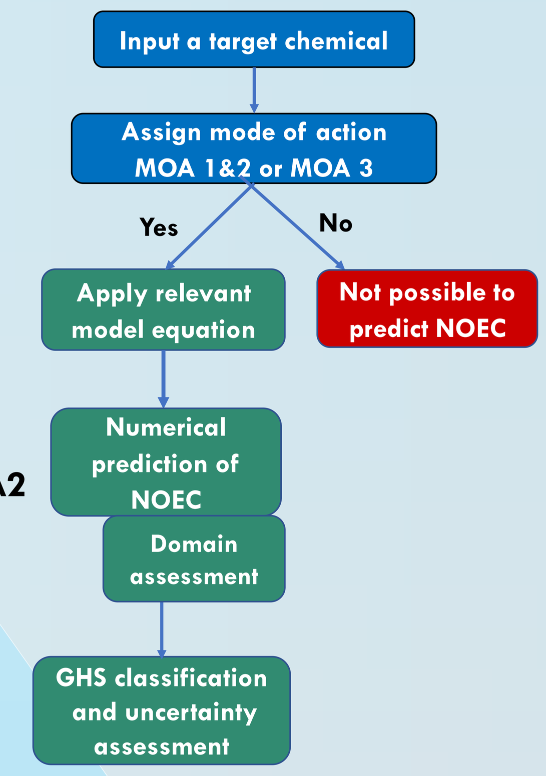
Modeling approach 1

Modelling Approach 1 establishes quantitative relationships between chronic (NOEC) and acute (LC50/EC50) fish toxicity using mechanistically consistent grouping of chemicals according to augmented Verhaar classification scheme, where an extra reactivity component is added consisting of protein and estrogen receptor (ER) binding alerts. In this way, the augmented Verhaar scheme **provides more precise classification** with respect to biological effects including (non)polar narcotics, non-specific protein and ER binding interactions, as follows:

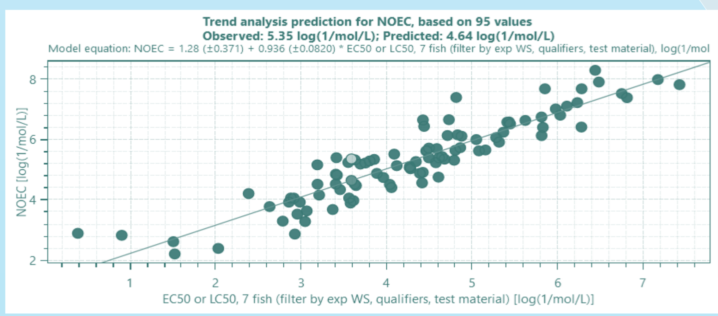
- ***MoA1 and MoA2** - baseline narcotics and less inert compounds **with NO protein and ER reactivity;***
- ***MoA3** - reactive chemicals extended with protein binding alerts;*
- ***MoA4** - pesticides under special regulatory considerations*
- ***MoA5** - substances that do not fit other categories (currently, excluded for modeling purposes)*

Individual linear regressions (models) were developed for each MoA using log-acute toxicity to predict log-NOEC, supported by applicability domain that consists of mechanistic, parametric, and structural layers. The resulting NOEC predictions are then translated into GHS chronic hazard categories, accounting for data and model uncertainty assessments.

Principle workflow



Correlation between NOEC and LC(EC)50 for MoA1&MoA2



Model equations, training sets (TS) and correlation statistics for developed QSAR models

MoA	Model equation	Local TS, n	Coefficient of correlation, R^2
MoA1&MoA2	NOEC=1.28 + 0.936*LC(EC)50	95	0.85
MoA3	NOEC=1.57 + 0.890*LC(EC)50	115	0.78
MoA4	NOEC=1.76 + 0.877*LC(EC)50	43	0.84

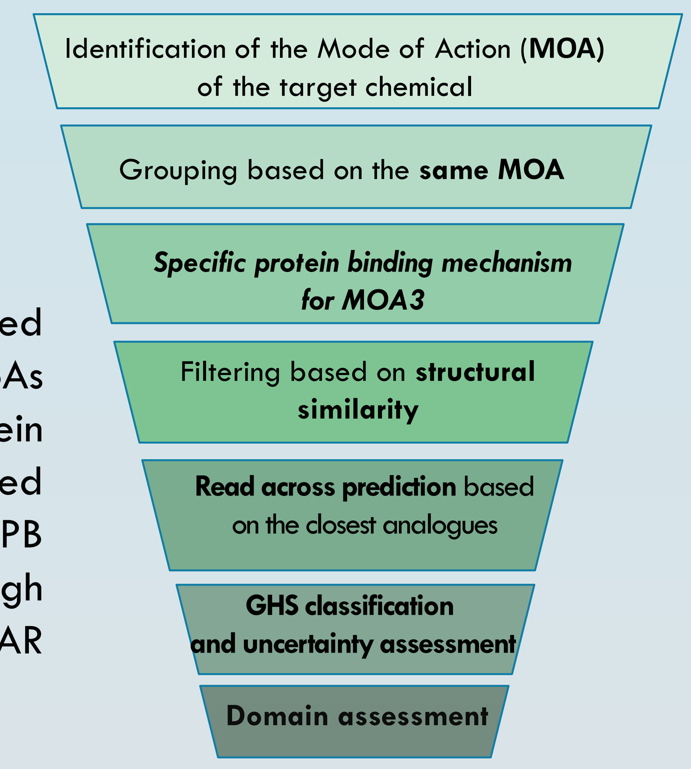
Modeling approach 2

Modeling approach 2 employs traditional category-based read-across strategy including a mechanistic and a structural similarity assessment components.

First, analogues are selected based on the augmented Verhaar MoAs and filtered according to protein binding (PB) mechanisms. The filtered analogues (having same MoA and PB mechanism) are refined through structural similarity using QSAR Toolbox profilers.

Depending on how many analogues remain, the model applies either trend analysis or read-across, producing both numerical NOEC predictions and GHS classifications.

Principle workflow



Training sets (TS) and model performance

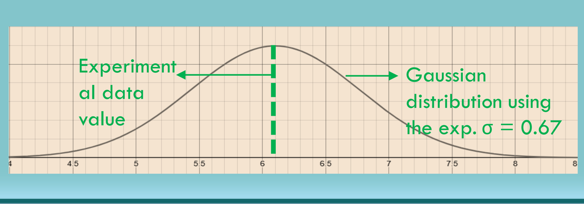
MoA	Local TS, n	Model performance
MoA1	64	0.83
MoA2	15	0.90
MoA3	78	0.59
MoA4	30	0.71

Data uncertainty assessment

Uncertainty of experimental data

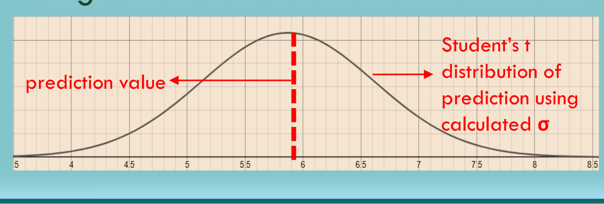
Experimental uncertainty was assessed using chemicals with multiple NOEC values, and the variability of these measurements was modeled with a Gaussian distribution, resulting in a uniform experimental error of σ = 0.67 for all substances.

$$\sigma \approx \sqrt{S^2_{\text{avg}}}$$



Uncertainty of predicted data

Uncertainty of predicted data reflects variation around QSAR-derived NOEC values. Each chemical is assigned an augmented Verhaar MoA, an acute-to-chronic equation is applied, and uncertainty is modeled using a Student's t-distribution based on training set size and deviation from the mean.



$$\sigma = s_e \cdot \sqrt{1 + \frac{1}{n} + \frac{(x_0 - \bar{x})^2}{\sum(x_i - \bar{x})^2}}$$

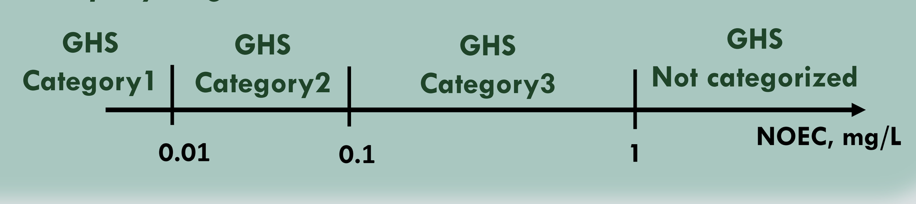
Where Se is dispersion error for the model; n - number of TS chemicals and the last part of the equation describes deviation of the target from the TS mean

GHS classification

The ***Globally Harmonized System of Classification and Labelling of Chemicals*** [3] classifies chronic aquatic toxicity based on long-term chemical effects on aquatic organisms, using the NOEC and biodegradability data. Low-NOEC chemicals are more poisonous, and non-biodegradable chemicals are more harmful since they last longer. Rapidly biodegradable chemicals are less hazardous at the same level.

- ***Non-rapidly degradable chemicals***

- ***Rapidly degradable chemicals***



Results and Discussions

Modeling approach 1

Internal validation

Internal validation is essential in QSAR modeling to ensure predictive reliability and avoid overfitting. The training sets were randomized via bootstrapping, leaving around one-third of the original data to serve as the validation set. The models were then rebuilt using new training sets, and both the training and validation sets were predicted. The procedure was repeated 1000 times. The distributions of performance measures (RMSE, R2) in the training and validation sets clearly showed no over-fitting.

Statistics	MOA 1,2	MOA 3
Number of training data	95	115
RMSE training data	0.558 (0.415 ÷ 0.700)	0.521 (0.401 ÷ 0.640)
Coeff. determination (R^2) ,training data	0.739 (0.590 ÷ 0.888)	0.836 (0.745 ÷ 0.927)
Number of validated data	18.2 (13.8 ÷ 22.5)	17.5 (13.3 ÷ 21.6)
RMSE validated data	0.606 (0.393 ÷ 0.820)	0.563 (0.389 ÷ 0.736)
Coeff. determination (R^2), validated data	0.738 (0.519 ÷ 0.957)	0.836 (0.703 ÷ 0.968)

External validation

A set of 80 chemicals with simultaneously experimental NOEC and LC(EC)50 data were used for the external validation. The chemicals and their data were not used for the model development. Fifty three chemicals were predicted by Modeling approach 1 and categorized into one of the three model equations – MoA1&2, MoA3 and MoA4.

Model performance was estimated by the standard deviation of differences between predicted and observed values. With large datasets, uncertainty mainly reflects experimental error and model limitations, while agreement is assessed through the distribution of these differences

MOA	Predicted chemicals	Model Performance
MOA 1&2	12	0.75 (9/12)
MOA 3	25	0.88 (22/25)
MOA 4	16	1.00 (16/16)

Modeling approach 2

External validation

For the validation of Model 2, a set of 63 new chemicals with experimental NOEC data according to TG210 were used. The model successfully predicted 17 chemicals, of which 11 were correctly predicted. This corresponds to an overall performance of 66% of the validation set.

MOA	Chemicals belonging to MOA	Predicted chemicals	Performance, % (correctly predicted)
MOA1&2	18	9	**67 (6/9)**
MOA3	36	7	**57 (4/7)**
MoA4	9	1	**100 (1/1)**

Model 1 vs. Model 2 comparison

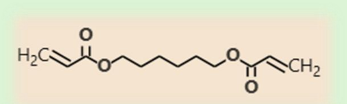
The predicted results for the 17 chemicals by Model 2 were compared to the predictions of Model 1 for the same chemicals. Model 1 and Model 2 show comparable performance for MoA1&2.

However, Model 1 performs significantly better for MoA3, (7/7 correct predictions), whereas Model 2 predicts correctly only 4 out of 7 chemicals. Model 2 predicts correctly the one chemical falling in MoA4, while Model 1 fails to correctly predict the chemical in this group.

MOA	Correct/total predictions Model 1	Correct/total predictions Model 2
MOA1&2	7/9	6/9
MOA3	7/7	4/7
MOA4	0/1	1/1

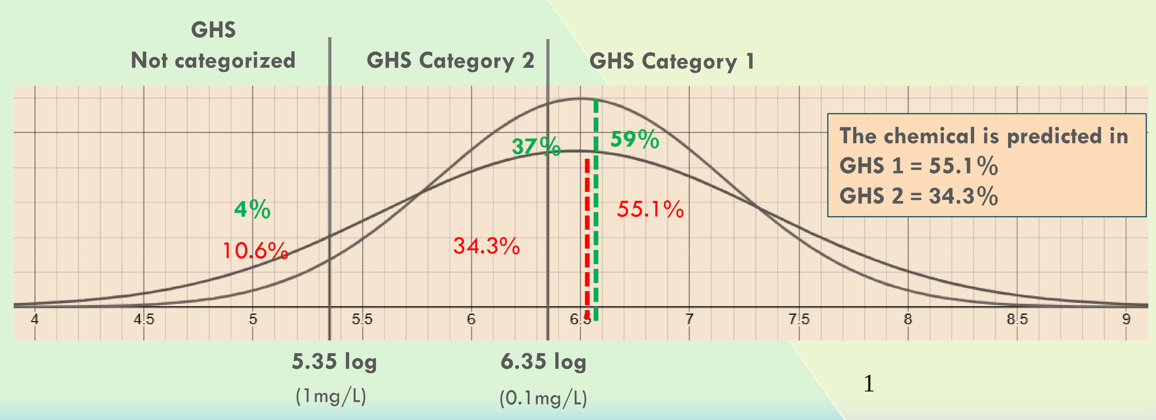
Probabilistic GHS categorization

A non-biodegradable substance is predicted to illustrate how the combined uncertainty framework results in a probabilistic GHS categorization. As seen, both the experimental (in green) and predicted (in red) distributions yield similar probabilistic classifications, with most likelihood in Category 1 (59% and 55.1% for experimental and predicted data, respectively), substantial probability in Category 2 (37% and 34.3%, for experimental and predicted data, respectively), and some in "Not classified" (4% and 10.6%, for experimental and predicted data, respectively). This demonstrates that the substance **cannot be assigned a single definitive GHS category.**



NOEC exp.: 0.0723 mg/L; 6.5 log(1/mol/L)
Exp. σ: 0.67

NOEC predicted: 0.0768 mg/L; 6.47 log(1/mol/L)
Pred. σ: 0.8893



Conclusions

This work demonstrates that chronic fish toxicity can be predicted using two complementary, uncertainty-aware computational methods in QSAR Toolbox. The QSAR acute-to-chronic models and the category-based read-across workflow both incorporate mechanistic information, applicability domains, and probabilistic evaluation of experimental and prediction data. Consistent results from both models ensure high confidence in the predictions. The NOEC predictions can then be translated into GHS chronic hazard categories with quantified certainty. The models provides a reliable and mechanistically transparent approach to screening substances and minimizing animal testing.

References:

[1] Kienzler A. et.al., *Toxicological & Environmental Chemistry*, 2016, 99(7–8), 1129–1151

[2] Verhaar H.J.M., et.al., *Chemosphere*, 1992, 25 (4), 471-491

[3] *United Nations. (2023). Globally Harmonized System of Classification and Labelling of Chemicals (GHS, Rev. 10). United Nations, New York; Geneva."*