Does the addition of perfluoro-moieties impact Mechanisms of toxic Action of organic chemicals? An *in-Silico* approach

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Introduction

The perfluorinated substances (PFAS), often described as "forever chemicals", are increasingly recognized as a threat to both aquatic and terrestrial environments. While their extreme environmental persistence has long been acknowledged, their "pan-phobe" characteristics is a huge benefit for certain applications and that has driven their widespread industrial use to over 4000 PFAS, most famously in applications such as non-stick coatings. The EU Commission has recently recognized that **potential for "forever chemicals" pose a threat to aquatic and terrestrial organisms alike**. Despite extensive research into their fate and bioaccumulation, **the underlying Mechanisms of toxic Action (MechoA) of PFAS remain underexplored.**

The aim of this work was to investigate the **potential for perfluoration to alter acute and chronic toxicity in fish, daphnids and algae compared to non-fluorinated substances** comparing a set of Internally validated experimental aquatic toxicity studies on PFAS with valid and robust High Accuracy Quantitative Structure-Activity Relationship (QSAR) from the iSafeRat® platform. Placing the PFAS data on the **existing robust non-PFAS regressions** would allow a determination of whether observed effects indicated **excess, reduced, or indifferent ecotoxicity for the same MechoA**. By comparing PFAS against conventional model boundaries, we aim to evaluate both the relevance of current QSARs to accurately predict toxicity for these atypical substances and the potential need to expand mechanistic models to better capture PFAS-specific effect on organisms. In doing so, this work highlights the value of **New Approach Methodologies (NAMs)** and especially **QSAR models i**n strengthening hazard profiling of substances that challenge traditional experimental approaches in ecotoxicology.

Methods

Database

Database: A comprehensive search of fluorine-containing substances was performed using the **OECD QSAR Toolbox[1]**. Substances were screened for completeness and relevance. Compounds lacking an iSafeRat® predicted Mechanism of Action (MechoA), a CAS number, or ecotoxicology endpoints (including NOEC, LOEC, LC50, EC50, ErC50, ErC10, or EC10) were removed from the dataset. This exclusion ensured that only substances with reliable experimental data and associated existing or future iSafeRat® QSAR models were included in the analysis.

Data selection

Internal data selection: Due to the extensive documentation on non-polar narcotics, and the completeness of our MechoA 1.1 QSAR models, we chose to begin the integration with the fluorinated substances predicted as **MechoA 1.1: Non-polar Narcosis[2]** focusing on **acute toxicity** values. The experimental data for ecotoxicity and sub-cooled liquid water solubility (SLWS) of each substance were **internally reviewed** using information in the ECHA registration dossiers. Substances without acute ecotoxicity studies or internally reviewed as irrelevant (internal K3) or with a lack of information (internal K4), were rejected. The SLWS was predicted using the iSafeRat® WATSOL v2.1 model, when no reliable experimental water solubility was available, and the substance fell inside the applicability domain of the model.

Modelisation

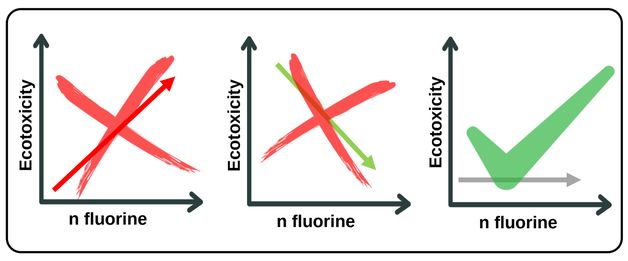
Modelling: Validated data were plotted on the appropriate iSafeRat® aquatic ecotoxicity QSAR models. **The distance of the FPAS points to the PFAS-free regression lines was assessed**. This evaluation helped identify whether fluorinated compounds exhibited excess, reduced or indifferent toxicity. **Statistical analyses** of the goodness of fit were performed to determine whether PFAS compounds could be reliably incorporated into the existing QSAR models.

Results

Model Fit with PFAS: The predicted MechoA 1.1 PFAS data aligned with the regression curves of the non-PFAS MechoA 1.1 acute toxicity models for **fish (Figure 1)**, **daphnids (Figure 2)**, and **algae (Figure 3)**. No evidence of either excess or reduced toxicity was observed, indicating that predicted MechoA1.1 PFAS appear to conform to the expected toxicity profiles of non-polar narcotics.

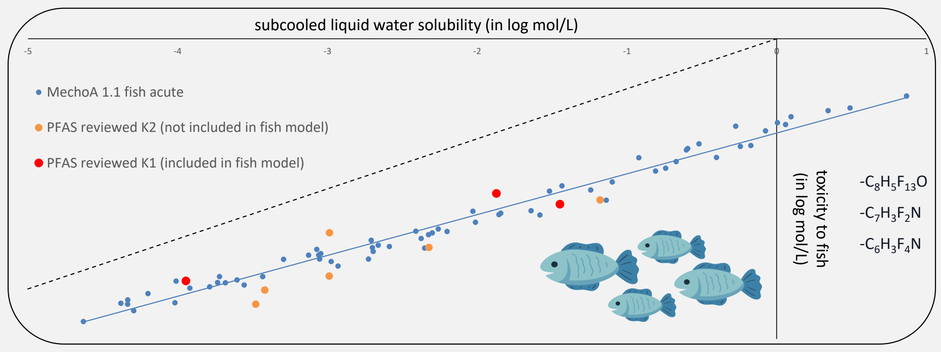
Data Quality and Filtering: Due to the experimental challenges associated with testing fluorinated substances high variability was observed in the reported ecotoxicity values. To ensure consistency and reliability, only studies internally reviewed and assigned as **only Klimisch score of 1 were considered accurate enough to be fit for purpose**. All Klimisch 2 studies were excluded from the statistical analysis.

Impact on Model Performance: The inclusion of K1 PFAS compounds in the MechoA 1.1 models led to a very slight decrease in R^2 and a modest increase in RMSE across all three organism models. These changes do not indicate a significant impact on the goodness-of-fit of the models.



Further analyses are currently being conducted to assess the integration of PFAS within additional mechanisms of toxic action models, including:
– **Polar narcosis (1.2),**
– **Hydrolysis to non-polar narcotics (2.1),**
– **Hard electrophile reactivity (3.1),**
– **Acidification or alkalinisation of cells (5.2)**.

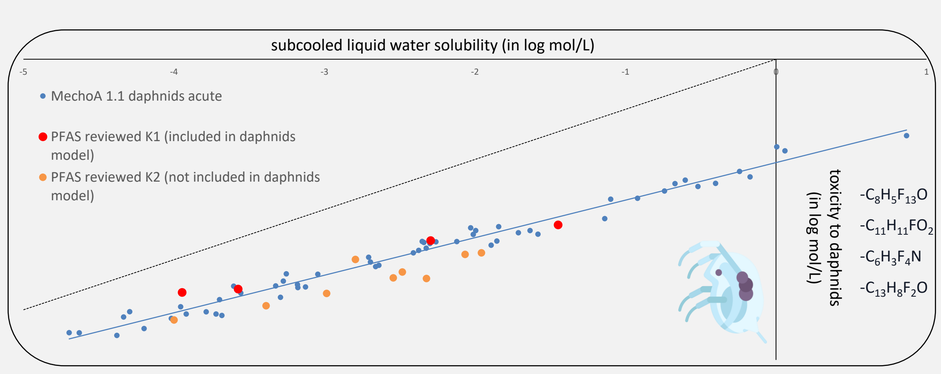
Ecotoxicity HA-QSAR



MechoA 1.1 compounds (96h-LC50) – training set; N = 66; R^2 = 0.9808; RMSE = 0.1664

MechoA 1.1 compounds + PFAS (96h-LC50) – training set; N = 68; R^2 = 0.9798; RMSE = 0.1697

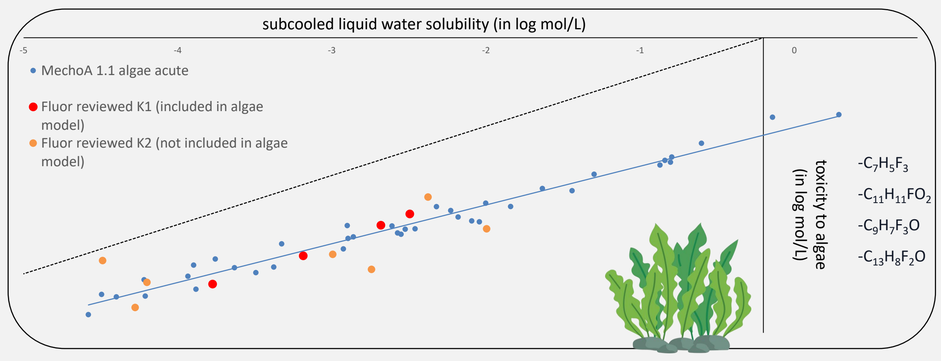
***Figure 1**. regression of MechoA 1.1 (Non-polar narcosis) QSAR for the fish 96h-LC50 v2.0. Dotted line is the water solubility limit. Goodness-of-fit statistics are provided for both acute fish toxicity QSARs and acute fish + PFAS toxicity QSAR.*



MechoA 1.1 compounds (48h-EC50) – training set; N = 57; R^2 = 0.9798; RMSE = 0.1459

MechoA 1.1 compounds + PFAS (48h-EC50) – training set; N = 59; R^2 = 0.9793; RMSE = 0.1462

***Figure 2**. regression of MechoA 1.1 (Non-polar narcosis) QSAR for the daphnid 48h-EC50 v2.0. Dotted line is the water solubility limit. Goodness-of-fit statistics are provided for both acute daphnid toxicity QSARs and acute daphnid + PFAS toxicity QSAR.*



MechoA 1.1 compounds (72h-ErC50) – training set; N = 57; R^2 = 0.9798; RMSE = 0.1459

MechoA 1.1 compounds + PFAS (27h-ErC50) – training set; N = 59; R^2 = 0.9793; RMSE = 0.1462

***Figure 3**. regression of MechoA 1.1 (Non-polar narcosis) QSAR for the algae 72h-ErC50 v2.0. Dotted line is the water solubility limit. Goodness-of-fit statistics are provided for both acute algae toxicity QSARs and acute algae + PFAS toxicity QSAR.*

Conclusion

For substances acting through the **non-polar narcosis** mode of action, the presence of perfluorination does not appear to significantly impact acute ecotoxicity. Based on this observation, fluorinated substances assessed in this study have been **integrated into the MechoA 1.1 acute models for fish, daphnids, and algae**. These updates are now included in **iSafeRat® software version 4.3.48**, which can reliably predict the acute aquatic toxicity of fluorinated substances within this mechanistic domain.

The considerable experimental challenges associated with testing PFAS highlight the critical need for robust **NAMs** to support risk assessment. Mechanistic models like iSafeRat® offer a promising pathway for improving our understanding of these substances where traditional methods fall short.

Further work is ongoing to incorporate fluorinated substances into **chronic toxicity models** under MechoA 1.1, as well as other relevant mechanisms such as **polar narcosis, hydrolysis to narcotic products**, and **hard electrophile reactivity**. Preliminary work on these additional models suggests **similar outcomes** to those observed for non-polar narcotics, with no consistent indication of increased or reduced toxicity due to the presence of fluorine.

Ultimately, this research contributes to **the development of reliable predictive tools[3]** for substances that are **difficult or impractical to test experimentally** and supports a broader application of NAMs for the evaluation of emerging chemical classes such as PFAS.

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References

1. OECD (2024). OECD QSAR Toolbox v.4.7.
2. Bauer, F.J et al. (2018). High-accuracy prediction of Mechanisms of Action using structural alerts. Comput. Toxicol., 7, 36–45.
3. Thomas, P.C et al. (2018) How in silico and QSAR approaches can increase confidence in environmental hazard and risk assessment. Integrated Environmental Assessment and Management 15, 1, 40–50.