

In silico approaches to replace acute fish
toxicity testing
ECONAMS Webinar

28 May 2026

KREATiS, 23 rue du Creuzat, 38080 L'Isle d'Abeau, France | Email: contact@kreatis.eu

Re-thinking safety experimentation with confidence



Why we exist

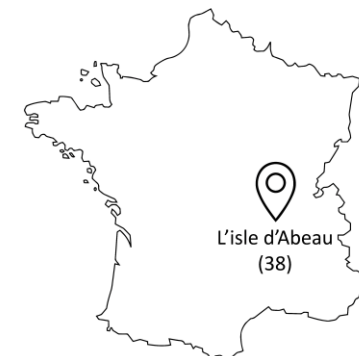
To understand the **mechanistic interactions** between chemical and biological matrices and **quantify** the impact on **human health** and the **environment**

Who we are

- Pure *in silico* R&D company specialising in hazard predictions for human health and environment

What we do

- State of the science modelling *i.e.*, including AI
- Ecodesign modelling for innovative substances
- Bespoke *in silico* solutions to industry for regulatory purposes

**13**

years of *in silico*
experience

15

multidisciplinary
expert members
(8 PhDs & post-doc)

>25

years of regulatory
(eco)toxicology
experience

What is a NAM?

New Approach? Is HYBIT really a new approach?

New Alternative to vertebrates? Is FET (OECD 236) an alternative to vertebrates

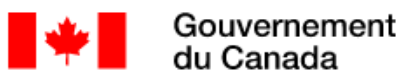
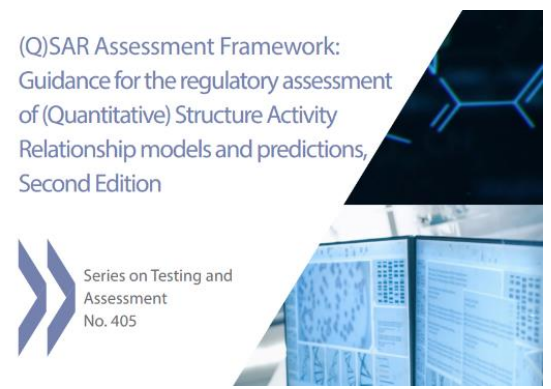
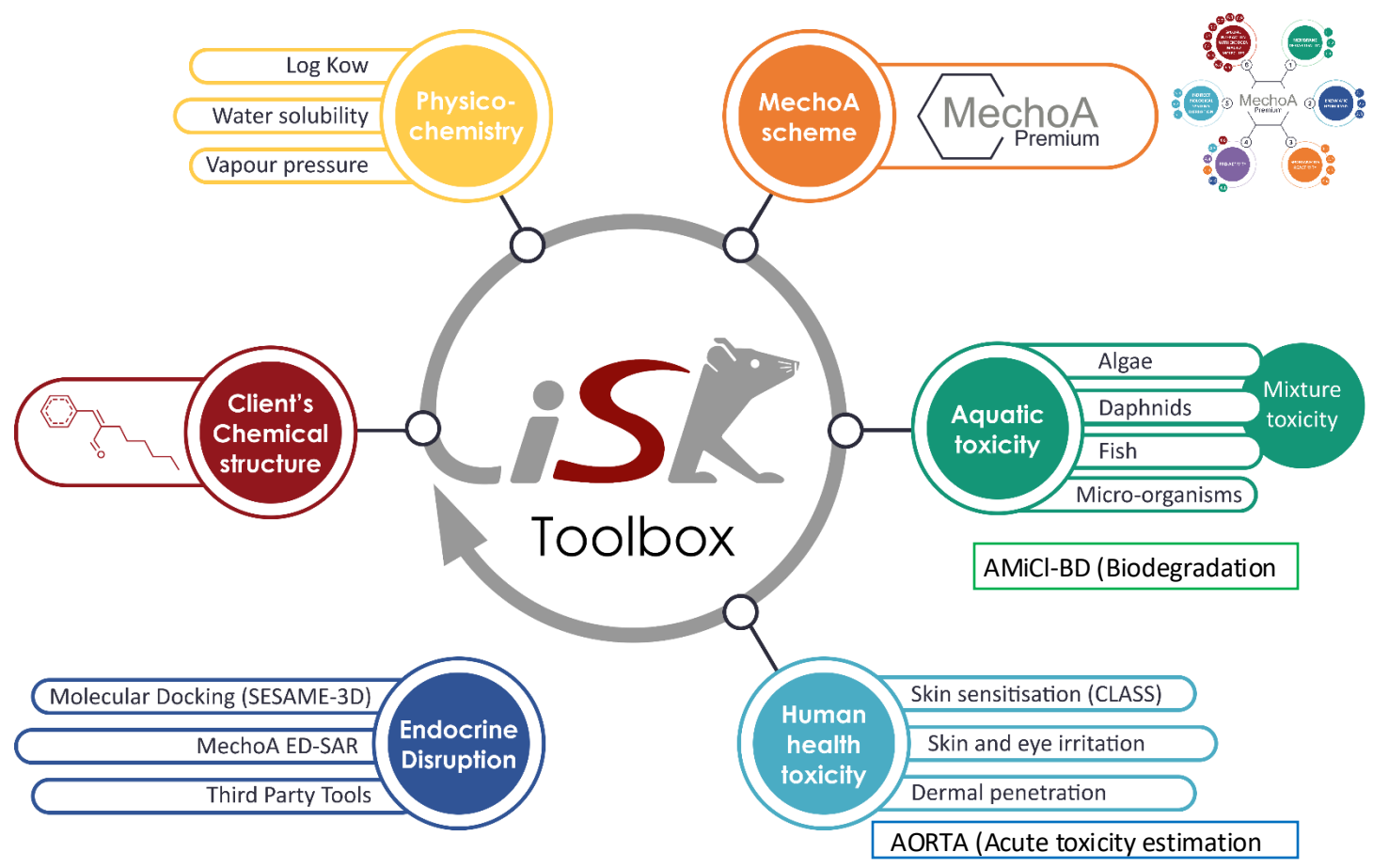
Non-Animal? Are invertebrates not animals?

KREATiS position on NAMs:

- 1) Ideally **100% animal free** (e.g., *in silico*, *in vitro* from cell lines) **directly replacing or contributing to replacement of *in vivo* studies** i.e. not just non-vertebrate or reduction of animals
- 2) **When not possible, then a NAM must significantly reduce animal use** (e.g. S9 still needs fish liver)
- 3) **When essential to use living animals, can we limit to invertebrates as sentinel species?**

- The **chemical structure (SMILES)** is used to predict PC properties which are themselves used to predict ecotoxicity and human health endpoints.
- Meet REACH requirements fulfills the five OECD principles in **compliance with the QAF application, validated by the Joint Research Centre (JRC)** and increasingly recognized by regulatory authorities across the world.
- Potential for **endocrine disruptor modalities** is currently predicted in-house (not yet included in iSafeRat® platform).

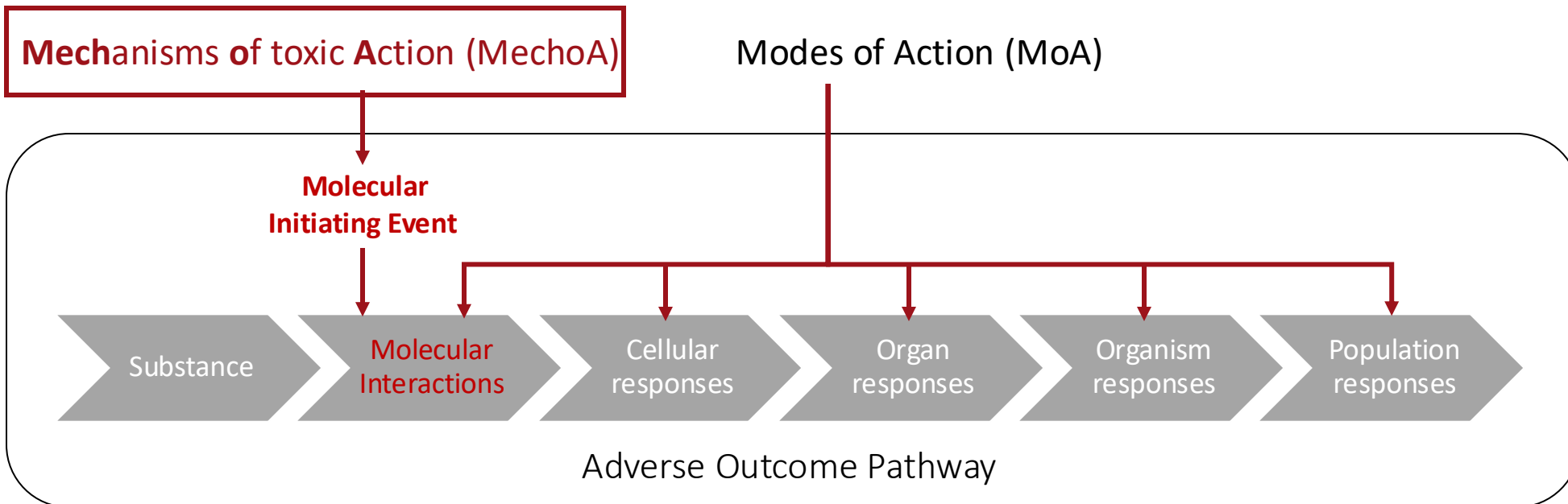
iSafeRat® fully regulatory compliant QSARs



MechoA Premium in iSafeRat®

**KREATiS QSARs are based on
Mechanistic structural alerts**

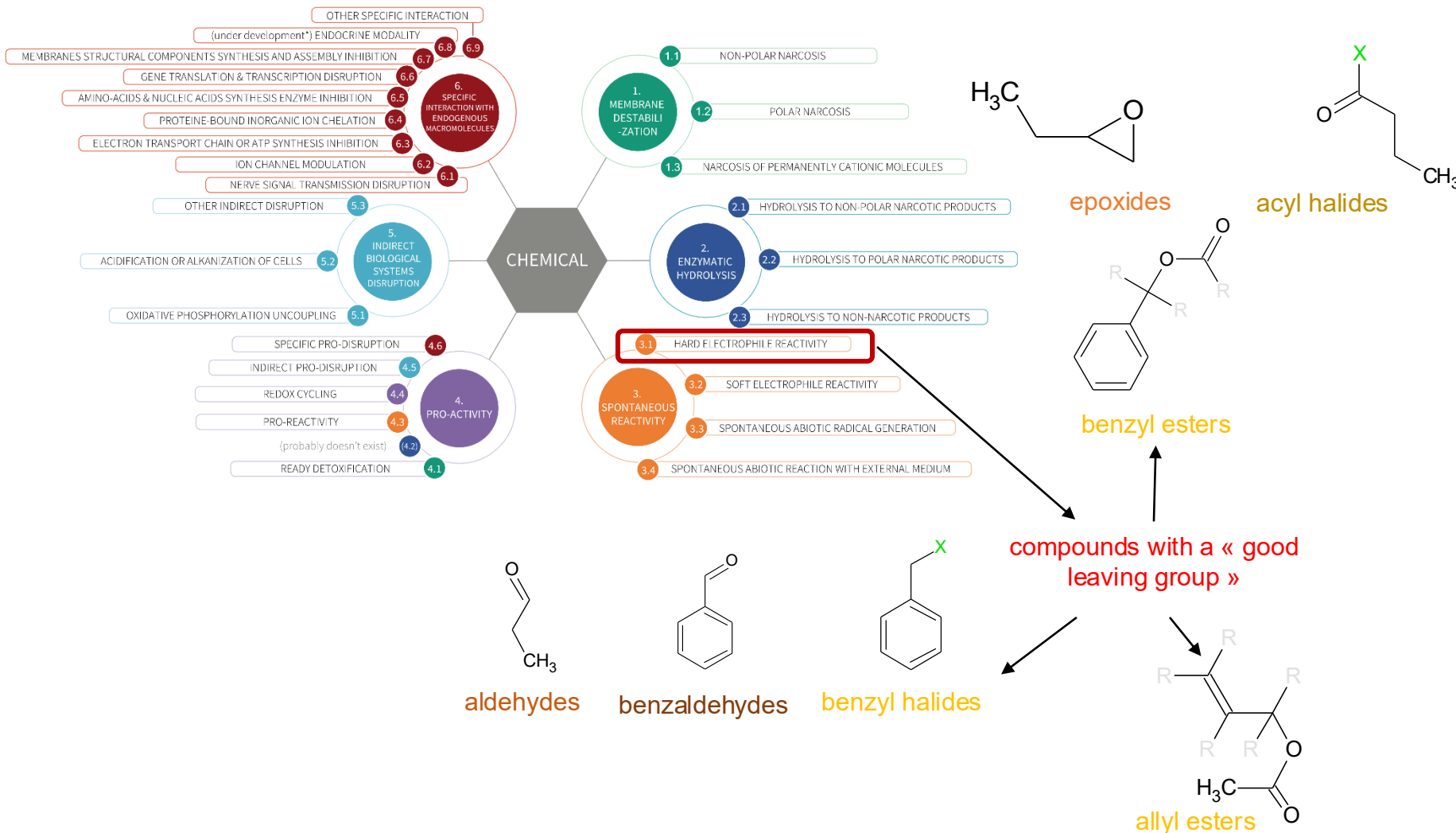




- Need of a general, accurate classification of chemicals according to their MechoA
 - Both in ecotoxicology and in toxicology, with a **unified language**

Adapted from Ankley, G. T. *et al. Environ. Toxicol. Chem.* **29**, 730–741 (2010).

Example of methodology used for reactive compounds (MechoA 3.1) :



A universal language for both Ecotoxicologists and Human health toxicologists alike



MECHANISMS OF TOXIC ACTION (MechoA) :

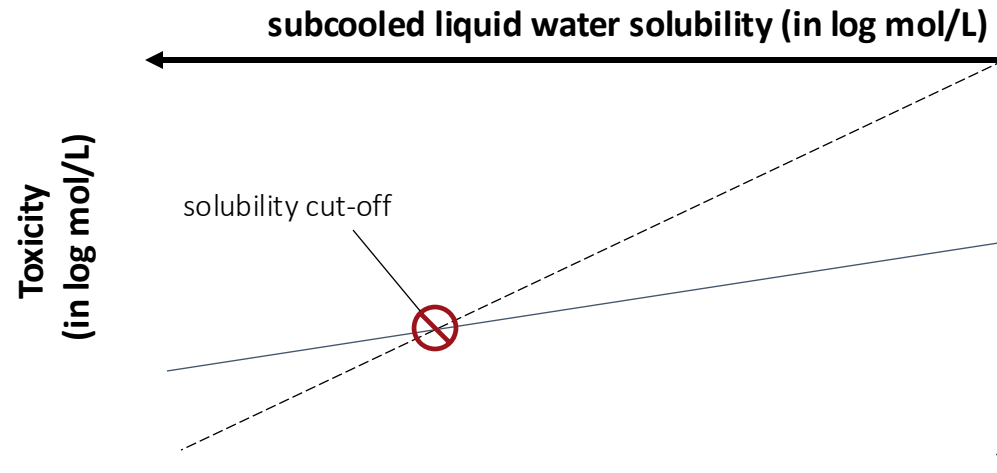
Work performed and published by KREATiS based on **structural alerts** that allow a chemical structure to be classified into specific categories of toxicity

Currently updated to MechoA+ / MechoA Premium, collaboration with Unilever & LJMU

Bauer *et al.* (2017 & 2018) publications
 Firman *et al.* (2025); Levet *et al.* (in Press);

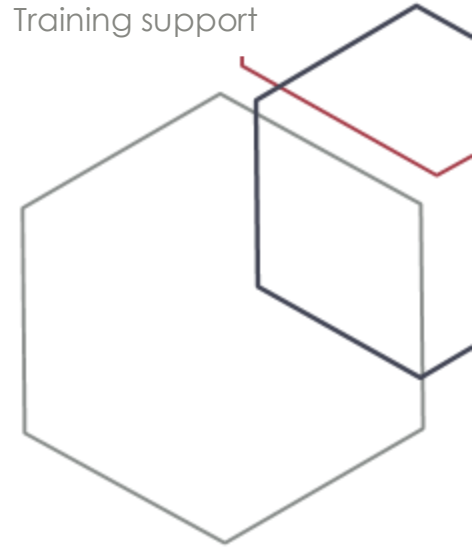
CONCEPTUAL MODEL

iSafeRat® QSAR models predict aquatic toxicity according to activity theory developed by Mackay *et al.* (2009) and Thomas *et al.* (2015).

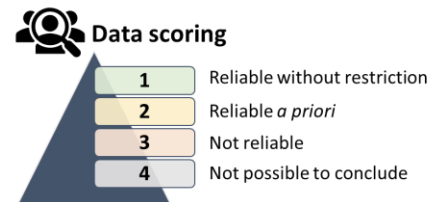
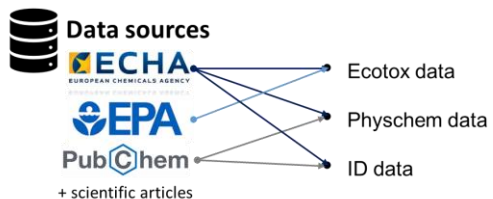
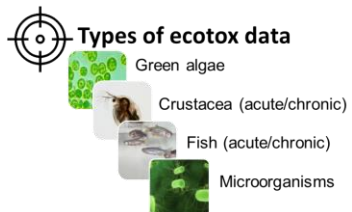


- linear regression as the simplest model
- water solubility as the descriptor
- **one regression per MechoA sub-class** expected for parent toxicity driven MechoAs = **QMARs rather than QSARs**

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DATA COLLECTION AND VALIDATION



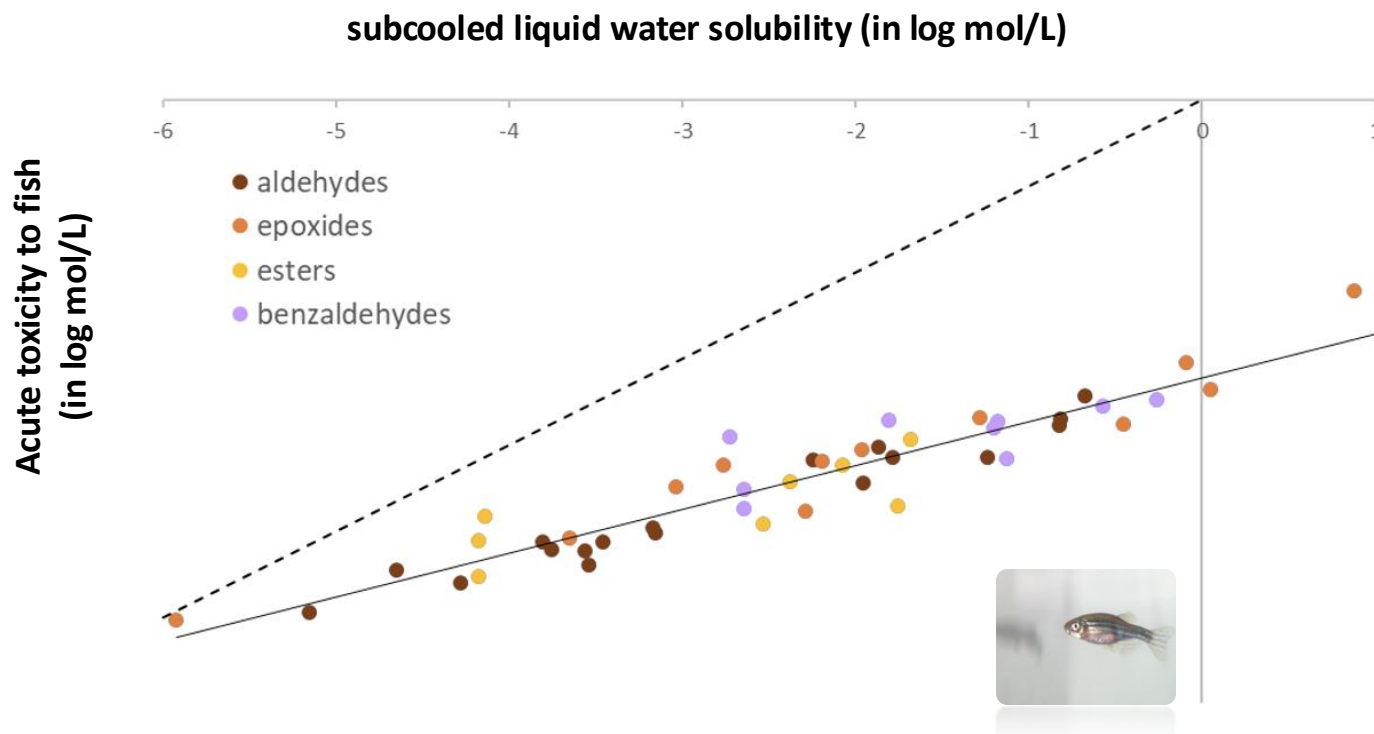
Example for one MechoA sub-class (MechoA 3.1)

	Algae	Crustacea (acute)	Crustacea (chronic)	Fish (acute)	Fish (chronic)	Microorganisms
Collected data (reliable)	431	343	113	343	53	18
1st OECD principle for validated QSAR: « a defined endpoint »						
Species	<i>Pseudokirchneriella s.</i> <i>Desmodesmus s.</i>	<i>Daphnia magna</i>	<i>Daphnia magna</i>	multiple	multiple	multiple (activated sludge)
Time	72h (+/- 24h)	48h	21d	96h	32d	30-180 min
Measure(s)	EC50 EC10	EC50	EC10 (MATC)	LC50	EC10 (MATC)	EC50
Effect(s)	growth rate	mobility	reproduction	mortality	growth	respiration
Selected data	72	79	20	47	9	39

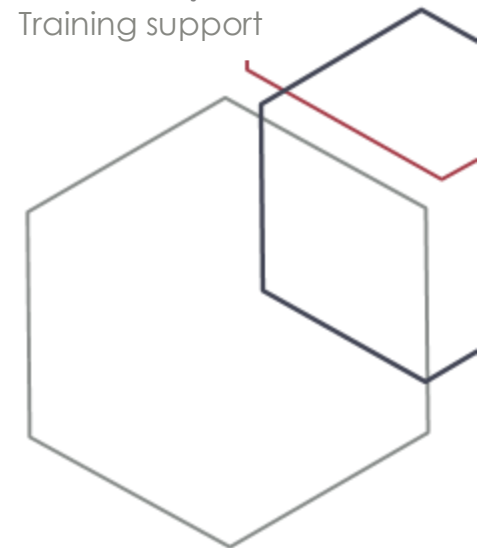
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MODEL FOR REACTIVE COMPOUNDS

The **MechoA 3.1 model** is based on hard electrophile reactive compounds, not just on structural similarity.



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Statistical results of Acute fish test from ECHA poster presented at SETAC Maastricht last week

Advancing aquatic hazard assessment: curated fish toxicity data and benchmarking of predictive models



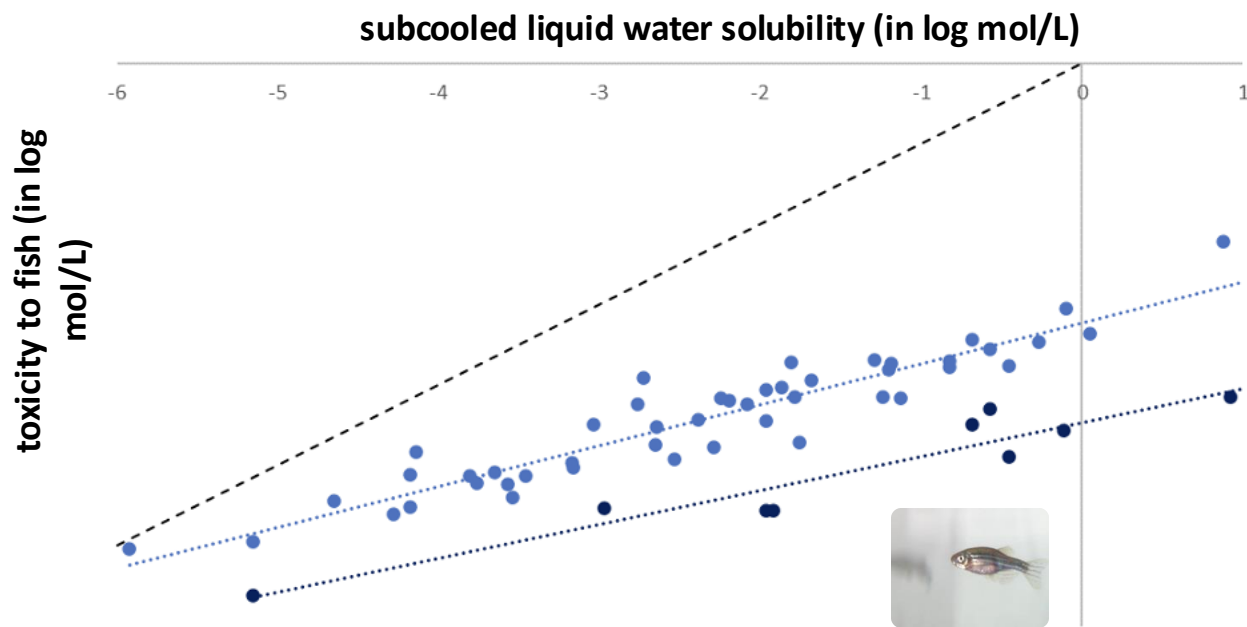
Panagiotis G. Karamertzanis¹, Heidi Ekholm¹, Aliisa Yli-Tuomola^{1,2}, Anna-Maija Nyman¹, Kostas Andreou¹, Romanas Cesnaitis¹, Vincent Bonnomet¹, and Konstantinos Prevedouros¹

iSafeRat[®] came out with extremely good results (best in class):

			under protective >2 log units	under protective 1-2 log units	within ±1 log units	over protective 1-2 log units	over protective >2 log units	
ECOSAR	Fish Acute Toxicity model	acute	4 %	6 %	85 %	5 %	0 %	435
	Fish Acute Toxicity model (single class)		2 %	4 %	88 %	6 %	0 %	226
KATE	Fish Acute Toxicity model		4 %	6 %	87 %	3 %	1 %	194
T.E.S.T.	Fathead minnow LC50 (96 hr) MoA		6 %	18 %	67 %	7 %	2 %	397
	Fathead minnow LC50 (96 hr) consensus		3 %	10 %	78 %	8 %	2 %	439
Trident	fish acute EC50 (96 hr) combo model		2 %	11 %	79 %	8 %	1 %	292
iSafeRat	96h-LC50 to fish (Opera MP), extr. AD excluded		2 %	3 %	94 %	1 %	0 %	158
	96h-LC50 to fish (Opera MP), extr. AD included		6 %	9 %	84 %	1 %	0 %	310
	96h-LC50 to fish (Opera MP), extr. AD included (str. AD in domain)		2 %	5 %	93 %	1 %	0 %	171
	96h-LC50 to fish (no MP), extr. AD excluded		2 %	3 %	94 %	1 %	0 %	163
	96h-LC50 to fish (no MP), extr. AD included		6 %	9 %	84 %	1 %	0 %	325
	96h-LC50 to fish (no MP), extr. AD included (str. AD in domain)		2 %	4 %	93 %	1 %	0 %	179
vega	Fathead Minnow LC50 96h (EPA)		2 %	11 %	84 %	3 %	0 %	237
	Fathead Minnow LC50 model (KNN-IRFMN)		4 %	16 %	76 %	4 %	0 %	180
	Fish Acute (LC50) Toxicity model (IRFMN)		2 %	4 %	85 %	6 %	3 %	224
	Fish Acute (LC50) Toxicity model (IRFMN-Combase)	0 %	5 %	83 %	11 %	2 %	103	
	Fish Acute (LC50) Toxicity model (KNN-Read-Across)	2 %	10 %	85 %	2 %	0 %	249	
	Fish Acute (LC50) Toxicity model (NIC)						0	
	Guppy LC50 model (KNN-IRFMN)	5 %	16 %	75 %	4 %	0 %	57	
	Zebrafish embryo AC50 (IRFMN-CORAL)	1 %	3 %	52 %	38 %	6 %	143	

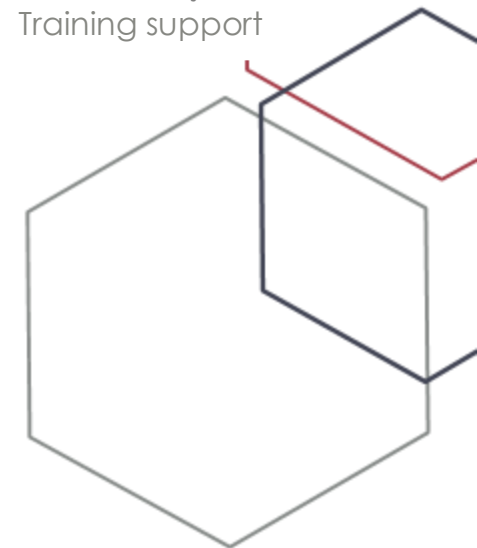
MODEL FOR REACTIVE COMPOUNDS

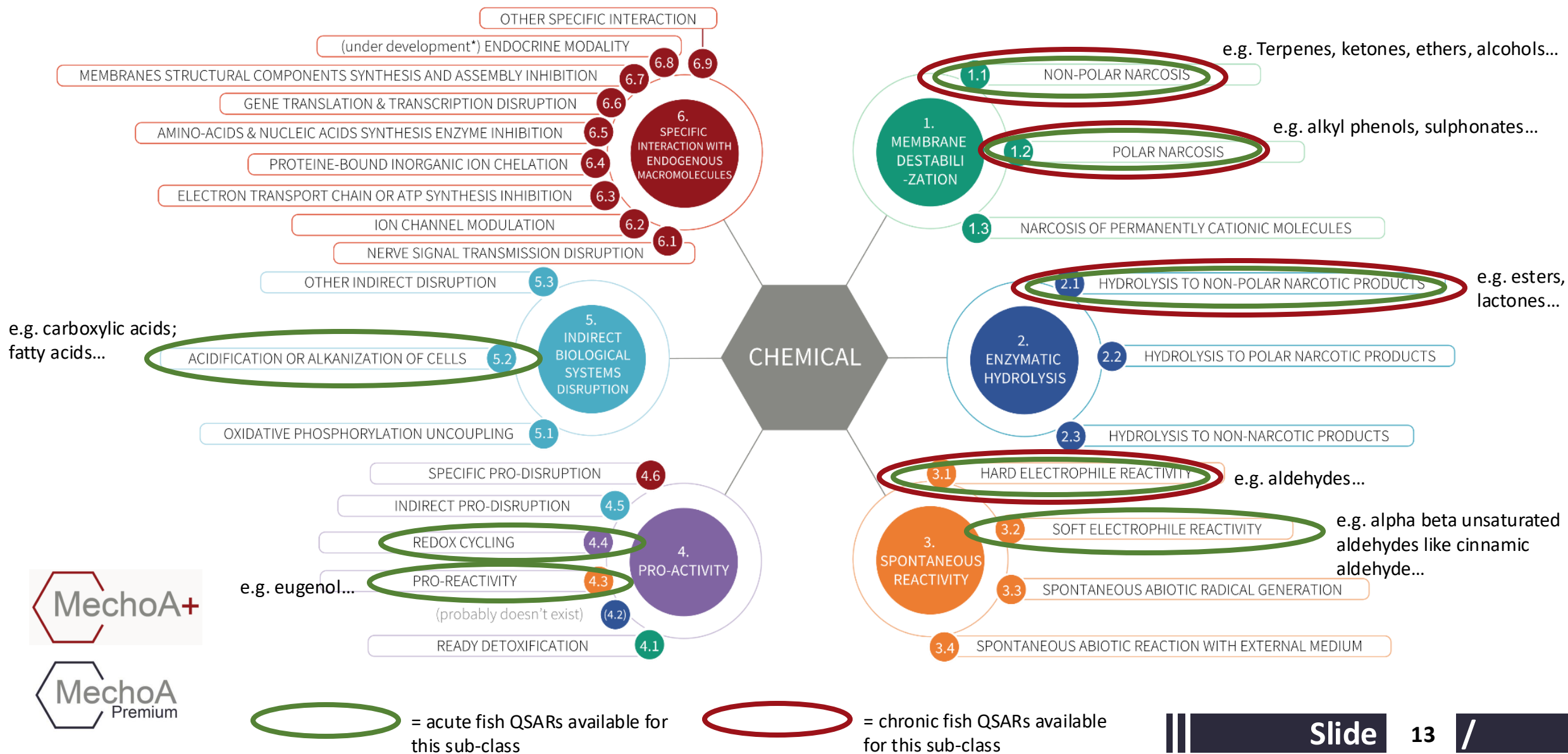
The MechoA 3.1 model is based on hard electrophile reactive compounds, not just on structural similarity.



- MechoA 3.1 compounds (96h-LC50) – training set; N = 47; $R^2 = 0.89$; RMSE = 0.27
- MechoA 3.1 compounds (32d-EC10) – training set; N = 9; $R^2 = 0.90$; RMSE = 0.27

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Screenshots of iSafeRat® Homepage and ecotox with Chronic fish predictions

Mono-Constituent Mode

Model Input

Enter SMILES code here:

Enter substance name (for report purpose only):

Melting point (°C, at 1 atm):

Boiling point (°C, at 1 atm):

Density (mg/mL, at 25°C and 1 atm):

Batch Mode | Reset | **Predict**

2D Structure

MechoA Premium v1.3

CCCCCCCC=O

Molecular weight: 142.24 g/mol

Zoom

Mixture Mode

iSafeRat® Predictions

Physicochemical Endpoints

n-octanol/water partition coefficient (log Kow, at 25°C)

Log KOW from C2SM v2.1: **3.6** (95% Pred Interval: Unknown) Applicability domain status: **Inside**

Log KOW from ALPS beta version: **3.3** (95% Pred Interval: Unknown) Applicability domain status: **Inside**

Selected Log KOW v1.0: **3.6** (95% Pred Interval: Unknown) Applicability domain status: **Inside**

Other physico-chemical endpoints

Water Solubility (mg/L, at 25°C) WATSOL v2.2: **6.36E+01** (95% Pred Interval: 1.70E+01 - 2.38E+02) Applicability domain status: **Inside**

Vapour Pressure (Pa, at 25°C) VP v1.5: **1.36E+02** (95% Pred Interval: 7.08E+01 - 2.62E+02) Applicability domain status: **Inside**

Henry's law constant estimation (Pa.m3.mol-1, at 25°C): **3.05E+02** (95% Pred Interval: Unknown) Applicability domain status: **Inside**

Additional user input? **Edit**

Prediction Summary Report

Mechanism of action | Physicochemistry | **Ecotoxicity** | Environmental fate | Human health

Acute aquatic toxicity

96h-LC50 to fish (fishLC50 v2.1): **2.2 mg/L** (95% Pred Interval: 5.3E-01 - 9.3) **Inside AD - using MechoA 3.1**

48h-EC50 to daphnids (daphEC50 v2.1): **1.7 mg/L** (95% Pred Interval: 4.5E-01 - 6.0) **Inside AD - using MechoA 3.1**

72h-ErC50 to algae (algErC50 v2.2): **4.9 mg/L** (95% Pred Interval: 1.6 - 1.5E+01) **Inside AD - using MechoA 3.1**

Chronic aquatic toxicity

32d-EC10 to fish (fishEC10 v2.1): **1.2E-01 mg/L** (95% Pred Interval: 1.2E-02 - 1.2) **Inside AD - using MechoA 3.1**

21d-EC10 to daphnids (daphEC10 v2.4): **3.0E-01 mg/L** (95% Pred Interval: 6.1E-02 - 1.5) **Inside AD - using MechoA 3.1**

72h-ErC10 to algae (algErC10 v2.4): **1.6 mg/L** (95% Pred Interval: 2.9E-01 - 9.3) **Inside AD - using MechoA 3.1**

Other ecotox endpoints

Prediction summary report

QMRFs are available on our website
 QPRFs are produced by our team of experts

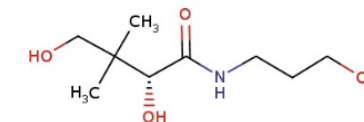
100s of HA-QSARs submitted by KREATiS in REACH dossiers since 2015 tacitly accepted (pers.com. Computational Unit)

NOTE THAT ECHA **NEVER** COMMUNICATES THAT STUDIES OF ANY KIND ARE ACCEPTED. ONLY THAT THEY ARE **NOT** ACCEPTED

- **Demonstrated acceptance for iSafeRat[®] Chronic Fish QSAR on Dexpanthenol**

Sent in comments on draft decision of REACH dossier EC No. 201-327-3

In your comments on the draft decision request for the ecotoxicological endpoint Long-term toxicity testing on fish (Annex IX, Section 9.1.6), you provided a new adaptation based on Quantitative Structure-activity relationship (QSAR), according to Annex XI, Section 1.3. Please include these QSAR predictions and any other relevant data in the same registration dossier update (by 17 June 2024).



If a successful dossier update is submitted by 17 June 2024, ECHA will exceptionally take this information into account in the ongoing dossier evaluation process.

- **IUCLID dossier:**

<p>6.1.2 Long-term toxicity to fish 4</p> <ul style="list-style-type: none"> <ul style="list-style-type: none"> S-01 Summary <ul style="list-style-type: none"> 001 No specified stud... 002 Key (Q)SAR 003 Supporting (Q)S... 	<p>Justification for type of information</p>	<ol style="list-style-type: none"> SOFTWARE iSafeRat[®] – in Silico Algorithms For Environmental Risk And Toxicity MODEL (incl. version number) iSafeRat[®] fishEC10 v1.7 SMILES OR OTHER IDENTIFIERS USED AS INPUT FOR THE MODEL CC(C)(CO)C(O)C(=O)NCCCCO SCIENTIFIC VALIDITY OF THE (Q)SAR MODEL See attached QMRF
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- 1) Mechanistically based *in silico* models (**QMARs**) can **replace** *in vivo* studies
- 2) Further, they can **mechanistically explain** empirical results
- 3) High accuracy quantitative predictions can be obtained from a **single descriptor**
- 4) The same methodology functions for **acute** and **chronic** studies for **algae, daphnids and fish**
- 5) The method is **increasingly recognised by regulators** as a functional alternative to *in vivo* studies

Thank you for your attention
Questions?

Contact me at paul.thomas@kreatis.eu

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