Re-Thinking Experimentation

QSAR Model Reporting Format (QMRF) for iSafeRat® HA-QSAR and QSAR for CHRONIC TOXICITY TO FISH (32-DAY EC10), DAPHNIDS (21-DAY EC10) and TOXICITY TO ALGAE (72-HOUR ErC10) – Version 2.8

1 QSAR identifier

1.1 QSAR identifier (title)

iSafeRat® fishEC10
iSafeRat® daphEC10
iSafeRat® algErC10

1.2 Other related models

None

1.3 Software coding the model

The ecotoxicological models are implemented to the commercial software iSafeRat® Desktop.
The version of the ecotoxicological models presented in this QMRF are present in the version 5.2.3 and later versions of iSafeRat® Desktop.
Additional documentation: KREATiS (2025) – iSafeRat® Desktop v5.2.3 User Guide

2 General information

2.1 Abstract

This QMRF is related to all the chronic aquatic ecotoxicological models implemented to the iSafeRat® desktop commercial software. The QMRF deals with three families of model: iSafeRat® algErC10, iSafeRat® daphEC10 and iSafeRat® fishEC10. Each family of model contains a specific number of individual models (all are simple linear regressions). The models were developed to predict the chronic ecotoxicity to fish, daphnids and algae that would be expected in a laboratory experiment following the OECD Guideline 210, 212 and 215 (OECD, 2013, 1998, 2000), 211 (OECD, 2012) and 201 (OECD, 2011) respectively. The data present in the models were obtained from public sources in most cases and were checked internally, manually one by one to be sure that the experimental data are valid according to the OECD guidelines and that the experimental settings and parameters were adapted to the properties of the tested compounds. Those that did not meet the strict criteria were not considered fit for purpose regardless of the external validation scores given to them. Only organic compounds were considered in the models. Each aquatic toxicity model developed by KREATiS experts are implemented in the software iSafeRat® as simple linear-relationships between the Subcooled Liquid Water-Solubility (SLWS) and the ecotoxicity. Moreover, all these models were based on a mechanistic interaction between the chemical and the organisms (MechoA, Bauer et al., 2018). Therefore, one regression can contain compounds having different chemical functions. But these different chemical functions must be related to the same MechoA and are recognised as acting similarly based on scientific knowledge. The iSafeRat® algErC10, iSafeRat® daphEC10 and iSafeRat® fishEC10 contains 4, 7 and 4 independent models, respectively (one per MechoA). The statistics assessing the goodness-of-fit, the robustness and the predictivity of the models were calculated according to the OECD (2007) document and are described below for transparency.

2.2 Date of QMRF

16 June 2025

2.3 Date of QMRF update(s)

Table 1: Dates of QMRF updates

Date	QMRF update identifier
16 June 2025	KTS/QMRF/CHR/13
20 January 2025	KTS/QMRF/CHR/12
6 November 2024	KTS/QMRF/CHR/11
13 August 2024	KTS/QMRF/CHR/10
28 May 2024/ 3 June 2024	KTS/QMRF/CHR/09
21 December 2023	KTS/QMRF/CHR/08
10 July 2023/11 September 2023	KTS/QMRF/CHR/07
9 March 2023	KTS/QMRF/CHR/06
30 March 2022	KTS/QMRF/CHR/05
15 April 2019	KTS/QMRF/CHR/04
28 February 2019	KTS/QMRF/CHR/03
13 July 2017	KTS/QMRF/CHR/02
30 April 2015	KTS/QMRF/CHR/01

2.4 QMRF update(s)

Table 2: Contents of QMRF updates

QMRF update identifier	Content
KTS/QMRF/CHR/13	• New model: MechoA 1.2&5.2 daphEC10 • Update of the following ecotoxicological models: MechoA 3.1 for daphnids: Removal of two compounds. MechoA 2.1 for daphnids: addition of one compound.
KTS/QMRF/CHR/12	• Update of the following ecotoxicological models: MechoA 1.1 for daphnids: Addition of 1 lactam compound in the training set. MechoA 1.1 for algae: Addition of 2 lactam and 2 amide compounds in training set. • Update of structural fragment space of each model based on the update list of SMARTS used in the iSafeRat® models.
KTS/QMRF/CHR/11	• Update of the following ecotoxicological models: MechoA 1.1 for fish: update of the water solubility value of one compound of the training set. MechoA 2.1 for fish, daphnids and algae: addition of one lactate compound. • Update of section 7.7.
KTS/QMRF/CHR/10	• New ecotoxicological model: DaphEC10 MechoA5.2 (carboxylic acids). DaphEC10 models are now version 2.1.
KTS/QMRF/CHR/09	28 May 2024: • Update of QMRF format according to the QAF template (version 2.4 of QMRF). • Update of the information provided in sections 6 and 7 (calculation of statistical metrics). • New ecotoxicological models: MechoA 1.2 (polar narcosis) for fish and daphnids • Update of the following ecotoxicological models: MechoA 1.1 for fish: addition of a lactam compound in the training set. MechoA 2.1 for fish: addition of 2 phosphate compounds in the training set. Also, training and validation set were merged to obtain a more robust model. MechoA 2.1 for daphnids: addition of 3 phosphate compounds in the training set. MechoA 2.1 for algae: addition of 2 phosphate compounds in the training set. MechoA 3.1 for fish and daphnids: training and validation set were merged to obtain a more robust model.

	MechoA 3.2 for daphnids: training and validation set were merged to obtain a more robust model. 3 June 2024: *minor changes in 5.1.d section (no change in models so no change of QMRF version).*
KTS/QMRF/CHR/08	• New ecotoxicological model: MechoA 2.1 model for algae. • Update of the following ecotoxicological models: Improvement of structural applicability domain of the MechoA 2.1 model for fish and daphnids (lactones).
KTS/QMRF/CHR/07	• Update of the structural domain section/Improvement of structural applicability domain of the MechoA 1.1 model for fish and daphnids (amides).
KTS/QMRF/CHR/06	• One updated model (for non-polar narcotic compounds) and two new models (for hard-electrophile reactive compounds and soft-electrophile reactive compounds) for algae. • Two new models (for hard-electrophile reactive compounds and soft-electrophile reactive compounds) for daphnids. • One new model (for hard-electrophile reactive compounds) for fish.
KTS/QMRF/CHR/05	Release of the first version of the QMRF presenting the long-term toxicity models for fish, daphnids and algae. Before this version, algal models were presented in another document.

2.5 Model developer(s) and contact details

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2.6 Date of model development and/or publication

The results presented in this QMRF refer to the current version of the following models:
iSafeRat® fishEC10 – Version 2.1
iSafeRat® daphEC10 – Version 2.4
iSafeRat® algErC10 – Version 2.4
All models were validated on 16th June 2025.

2.7 Reference(s) to main scientific papers and/or software package

The ecotoxicological models are part of iSafeRat® – in Silico Algorithms For Environmental Risk And Toxicity, available through the following software tools:

- iSafeRat® Desktop version 5.2.3 and later versions. iSafeRat® Desktop version 5.2.3 for High-Accuracy QSAR predictions by KREATiS SAS. https://isaferat.kreatis.eu/

2.8 Availability of information about the model

The models are proprietary but limited information has been made publicly available for the validation/test set. Any queries concerning the model or its validity should be addressed at contact@kreatis.eu. Furthermore, KREATiS undertakes to provide supplementary information to sponsors or regulatory authorities upon request to demonstrate compliance of our QSARs with good practice.

2.9 Availability of another QMRF for exactly the same model

None

3 Defining the endpoint - OECD Principle 1

3.1 Species

The species for which relevant studies were included in the model's training set for the three ecotoxicological endpoints were:

- Chronic toxicity to fish: *Danio rerio, Pimephales promelas, Oryzias latipes, Jordanella floridae, Oncorhynchus mykiss*
- Chronic toxicity to invertebrates: *Daphnia magna*
- Toxicity to algae: *Raphidocelis subcapitata (synonyme: Pseudokirchneriella subcapitata), Desmodesmus subspicatus (synonyme: Scenedesmus subspicatus), Scenedesmus quadricauda*

3.2 Endpoint

The models can accurately predict the chronic aquatic toxicity to:

- Algae, based on the inhibition of the exponential growth rate that would be expected in a laboratory experiment following the OECD Guideline 201
- Daphnid, based on the inhibition of reproduction of the individuals that would be expected in a laboratory experiment following the OECD Guideline 211
- Fish, based on the hatching success, growth rate and mortality of the individuals that would be expected in a laboratory experiment following the OECD Guideline 210, the growth rate of the individuals that would be expected in a laboratory experiment following the OECD Guideline 212 and the growth rate and mortality of the individuals that would be expected in a laboratory experiment following the OECD Guideline 215.

3.3 Comment on endpoint

Long term aquatic toxicity to fish can be assessed according to different tests. In the Fish Early Life Stage and short-term toxicity on embryo and sac-fry studies, hatching success, growth rate and mortality are the classical endpoints, while in the juvenile growth test, only the growth rate is measured. In the dataset used for chronic toxicity to fish, results based on growth were preferred in 95% of studies for consistency purpose. For a few studies, results based on the survival of juvenile fish were used because no effect on growth were reported.
For chronic toxicity to Daphnia magna, only results based on the inhibition of reproduction from OECD 211 studies were used.
For toxicity to algae, only results based on the inhibition of growth from OECD 201 studies were used.

Since the 10% effective concentration (EC10) was not determined in most of the studies for several models, some QSAR have been designed using partially or only the Maximum Acceptable Toxicant Concentration (MATC) determined as the geometric mean of the No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC). The MATC represents the highest concentration that should cause minimum chronic effects and can be considered as equivalent as an EC10 value (KREATiS, 2024). The results from this study were as follows: The RMSE (<0.4) observed in the models containing both EC10 and MATC values is a relevant indicator of the adequacy of the use of both types of values in a same regression. Also, the Spearman coefficient correlation between the MATC and the EC10 of eight compounds present in the MechoA 2.1 regression for daphnids provided a value close to 1. Therefore, EC10 and MATC are considered as compatible for a common use in a same regression.

3.4 Endpoint units

The predicted endpoint value (EC10 or ErC10) is first derived from the model in log 10 (mol/L), then converted in mol/L and finally given in **mg/L**.

3.5 Dependent variable

Table 3 provides an overview of the dependent variables of all the endpoints involved (and additionally, test durations are provided for all ecotoxicological endpoints).

Table 3: Endpoints and model dependent variables

Endpoint	Dependent Variable
chronic toxicity to fish *(Lowest value based on growth (i.e. weight/length) or mortality effect on juvenile fish)*	32 day-EC10 value in log10 (mol/L)
chronic toxicity to daphnids *(Reproduction)*	21 day-EC10 value in log10 (mol/L)
toxicity to algae *(inhibition of the growth rate)*	72 hour-ErC10 value in log10 (mol/L)

3.6 Experimental protocol

- **Dependent variable (ecotoxicity to fish, daphnids and algae)**

For fish, the ecotoxicity values were obtained from studies which were carried out using the method recommended in the OECD guideline 210, 212 and 215. The study durations varied between 28 and 35 days, with one exception at 8 days. The median duration is 32 days and this endpoint can be considered as a 32-day MATC/EC10), thus falling under the recommendations of OECD guideline 210.

The majority of the data for *Daphnia magna* was based on 21-28 day studies. The median duration is 21 days and therefore this endpoint can be considered as a 21-day MATC/EC10), thus falling under the recommendations of OECD guideline 211.

For algal studies, the study duration was 72 hours, with one exception at 48 hours and two exceptions at 96 hours. The median duration is 72 hours and this endpoint can be considered as a 72-hours MATC/EC10, thus falling under the recommendations of OECD guideline 201.

- **Independent variable (descriptor: subcooled liquid water solubility or octanol-water partition coefficient)**

For all models, the descriptor of the model is the subcooled liquid water solubility (SLWS, calculation explained in section 4.3) of the compounds. The data used for water solubility values come from studies which have been carried out using the method recommended in the OECD guideline 105, *i.e.,* shake-flask (OECD, 1995). An adaptation (slow-stir method) is recommended for liquids with an expected solubility below 10 mg/L (European Chemicals Agency, 2016; Letinski et al., 2002), and data with this method have been used as often as possible. The aim of this method is to prevent the formation of emulsions that can occur with the shake flask method and lead to a solubility overestimation. In rare cases data has been taken from studies performed using a second method described in OECD guideline 105: the column elution method which is recommended for solids (not liquids) with water solubility below 10 mg/L.

To calculate the subcooled liquid water solubility value, the state liquid *vs.* solid of the compounds at 25°C is considered. The melting point values come from studies which have been carried out using one of the methods recommended in the OECD guideline 102, i.e., capillary, Kofler hot bar, melt microscope, differential thermal analysis, differential scanning calorimetry, freezing temperature or pour point method.

3.7 Endpoint data quality and variability

One of the essentials to derive a HA-QSAR model is to perform a strict validation on the study results included within the training set. This validation was carried out using expert judgement from the dedicated KREATiS model development team. If for any reason the quality of the study results was compromised (for instance, due to unacceptable experimental conditions or issues with laboratory protocol), their corresponding results were withdrawn from the training set and the reason for their removal labelled in the internal database.
The training set data comprised of quality results derived from multiple laboratories and as a result, inter-laboratory differences may be expected. In many cases, diverse experimental methodologies were followed for the same endpoint (for instance, different testing protocol was followed, test durations may have varied). For such cases, the results were not simply averaged but the validity of each result was then justified on a case by case basis. Cases with large differences in validated values for the same substance were treated with best possible modelling practice (for instance, potential outlier detection, data verification from available literature resources).

For consistency reason, HA-QSAR have been designed using comparable study results. For fish, toxicity values at 28 to 32 days determined following OECD 210 guideline were preferred. For Daphnia magna, toxicity values at 21 days determined following OECD 211 guideline were preferred. For algae, toxicity values at 72 hours determined following OECD 201 guideline were preferred.

4 Defining the algorithm - OECD Principle 2

4.1 Type of model

Each model is based on a Simple Linear Regression.

4.2 Explicit algorithm

In each model, this approach correlates the solubility of a compound to the chronic toxicity. For compounds with a non-polar narcotic mechanism of action (MechoA 1.1) according to the MechoA Scheme (Bauer et al., 2018), the predicted toxicity is based on a thorough understanding of the thermodynamic relationship between the activity and toxicity that is well discussed in the literature (Mackay et al., 2009; Thomas et al., 2015). For other MechoAs, a similar relationship is considered between subcooled liquid solubility values and ecotoxicological effects because subcooled liquid solubility is still a relevant descriptor. Indeed, the regressions will heavily rely on the MechoA and therefore, depending on different series of MechoA the obtained slopes and intercepts may vary significantly. Depending on the mechanism of action and the structural profile of the input chemical, it will be allocated to one of the local QSAR chronic aquatic toxicity models.
The model algorithm is an affine equation of the type:

$$\log MATC/EC10 = a \times \log SLWS + b$$

$MATC$: Maximum Acceptable Toxicant Concentration (mol.L^{-1}).
$EC10$: Effective Concentration with 10% effect (mol.L^{-1}).
$SLWS$: Subcooled Liquid Water Solubility (mol.L^{-1})

4.3 Descriptors in the model

The ecotoxicological models were based on the clear understanding of the thermodynamic relationship between ecotoxic effects and water solubility as the only descriptor. The selection of water solubility as a suitable descriptor for this model was based on the clear understanding of the thermodynamic relationship between the ecotoxicological effects and the chemical activity as proposed by (Mackay et al., 2009).

Therefore, the water solubility values are corrected to take into account the Subcooled Liquid Water Solubility (SLWS) which is an expression of the chemical activity of a pure compound within a given medium. The choice of the SLWS is justified in order to compare liquids and solids within the same dataset. Indeed, the solids have an entropy of fusion different to liquids as demonstrated by (Yalkowsky, 1979). Consequently, the water solubility of the solids is corrected using their melting point according to the following equation:

$$\mathrm{SLWS}_{solid} = \frac{WS_{solid}}{e^{(6.79\left(1-\frac{MP}{298}\right))}} \ (mol.L^{-1})$$

SLWS_{solid} : Subcooled Liquid Water Solubility (SLWS) of a given compound with solid state at 25°C
WS_{solid}: Water Solubility (SLWS) of a given compound with solid state at 25°C (as measured)
MP: Melting Point of the given compound (K)

For liquids, the SLWS is directly equal to the value of the water solubility as measured.

4.4 Descriptor selection

The mechanistic understanding of the thermodynamic principles was the driving factor for descriptor selection. No other variable selection approaches were implemented. For each iSafeRat® ecotoxicological model, the most important aspect is to achieve the highest accuracy possible without including inexplicable molecular descriptors and any other mechanistically unjustified complexities.

4.5 Algorithm and descriptor generation

No specific variable selection method was applied. No statistical tool or packages were used to generate a pool of molecular descriptors. Only validated experimental study results were used (for independent and dependent variables).

4.6 Software name and version for descriptor generation

The descriptors (independent variables) and endpoint values (dependent variables) were either experimentally derived and retrieved from various literature resources including some publicly disseminated databases as well as some confidential data available within KREATiS internal database, or, for the water solubility values, some were predicted using iSafeRat® WatSol model, ensured to be within its applicability domain.

4.7 Chemicals/Descriptors ratio

The **Tables 4.1, 4.2 and 4.3** provide with the Chemicals/Descriptors ratio for the QSAR models. Since the ratios were quite higher (equal to the number of training set compounds as the descriptor was equal to 1 in all the cases), it indicates that the models were not over fitted with a large number of descriptors.

Table 4.1: Chemicals/Descriptor ratios for different iSafeRat® fishEC10 models

MechoA	Fish chronic ecotoxicity models	Chem/Desc. ratio
Non-polar narcosis (MechoA 1.1)	iSafeRat® fishEC10	28
Polar narcosis (MechoA 1.2)	iSafeRat® fishEC10	9
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	iSafeRat® fishEC10	12
Hard-electrophile reactivity (MechoA 3.1)	iSafeRat® fishEC10	8

Table 4.2: Chemicals/Descriptor ratios for different iSafeRat® daphEC10 models

MechoA	Daphnids chronic ecotoxicity models	Chem/Desc. ratio
Non-polar narcosis (MechoA 1.1)	iSafeRat® daphEC10	31
Polar narcosis (MechoA 1.2)	iSafeRat® daphEC10	8
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	iSafeRat® daphEC10	20
Hard-electrophile reactivity (MechoA 3.1)	iSafeRat® daphEC10	10
Soft-electrophile reactivity (MechoA 3.2)	iSafeRat® daphEC10	10
Acidification or alkalinization of cells (MechoA 5.2)	iSafeRat® daphEC10	7
Polar narcosis & alkalinization of cells, corrosion if directly applied, for all species (MechoA 1.2&5.2)	iSafeRat® daphEC10	12

Table 4.3: Chemicals/Descriptor ratios for different iSafeRat® algErC10 models

MechoA	Algal ecotoxicity models	Chem/Desc. ratio
Non-polar narcosis (MechoA 1.1)	iSafeRat® algErC10	23
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and poly-ester)	iSafeRat® algErC10	26
Hard-electrophile reactivity	iSafeRat® algErC10	21

(MechoA 3.1)		
Soft-electrophile reactivity (MechoA 3.2)	iSafeRat® algErC10	14

5 Defining the applicability domain - OECD Principle 3

5.1 Description of the applicability domain of the model

a) Fixed or probabilistic boundaries

The following domains (response, descriptor, structural fragment, mechanistic and metabolic domains) are fixed boundaries based on training set data.

b) Response domain

The model is limited by its response with a minimum and a maximum value of ecotoxicity which define a zone of interpolation. Beyond these extreme values, the prediction is an extrapolation. The quantified limits are given in the T**ables 5.1, 5.2 and 5.3**:

Table 5.1: Response domain for the different iSafeRat® fishEC10 ecotoxicological local models

MechoA	Fish chronic ecotoxicity models	Response domain (EC10 in log10 (mol/L)) MIN	MAX
Non-polar narcosis (MechoA 1.1)	iSafeRat® fishEC10	-6.8559	-2.4084
Polar-narcosis (MechoA 1.2)	iSafeRat® fishEC10	-7.3539	-4.7639
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	iSafeRat® fishEC10	-6.8593	-3.8502
Hard-electrophile reactivity (MechoA 3.1)	iSafeRat® fishEC10	-6.7954	-4.6681

Table 5.2: Response domain for the different iSafeRat® DaphEC10 ecotoxicological local models

MechoA	Daphnids chronic ecotoxicity models	Response domain (EC10 in log10 (mol/L)) MIN	MAX
Non-polar narcosis (MechoA 1.1)	iSafeRat® daphEC10	-7.9510	-1.1324
Polar-narcosis (MechoA 1.2)	iSafeRat® daphEC10	-7.4954	-4.7252
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	iSafeRat® daphEC10	-6.7667	-3.3002
Hard-electrophile reactivity (MechoA 3.1)	iSafeRat® daphEC10	-6.6209	-4.1254
Soft-electrophile reactivity (MechoA 3.2)	iSafeRat® daphEC10	-6.9416	-4.6499
Acidification or alkalinization of cells (MechoA 5.2)	iSafeRat® daphEC10	-4.6972	-3.4998
Polar narcosis & alkalinization of cells, corrosion if directly applied, for all species (MechoA 1.2&5.2)	iSafeRat® daphEC10	-6.6679	-3.6878

Table 5.3: Response domain for the different iSafeRat® algErC10 ecotoxicological local models

MechoA	Algal ecotoxicity models	Response domain (ErC10 in log10 (mol/L)) MIN	MAX
Non-polar narcosis (MechoA 1.1)	iSafeRat® algErC10	-5.2247	-1.7477
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and poly-ester)	iSafeRat® algErC10	-5.9038	-2.6606
Hard-electrophile reactivity (MechoA 3.1)	iSafeRat® algErC10	-5.9338	-2.8346
Soft-electrophile reactivity (MechoA 3.2)	iSafeRat® algErC10	-6.5222	-3.8456

c) Descriptor domain

The model is limited by its descriptor with a minimum and a maximum value of SLWS which define a zone of interpolation. Beyond these extreme values, the prediction is an extrapolation. The quantified limits are given in the **Tables 6.1, 6.2 and 6.3**:

Table 6.1: Descriptor domain for the different iSafeRat® fishEC10 ecotoxicological local models

MechoA	Fish chronic ecotoxicity models	Descriptor domain (SLWS in log10 (mol/L)) MIN	MAX
Non-polar narcosis (MechoA 1.1)	iSafeRat® fishEC10	-5.0524	0.0484
Polar-narcosis (MechoA 1.2)	iSafeRat® fishEC10	-7.3539	-0.5962
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	iSafeRat® fishEC10	-5.0649	-0.8158
Hard-electrophile reactivity (MechoA 3.1)	iSafeRat® fishEC10	-4.1422	0.9576

Table 6.2: Descriptor domain for the different iSafeRat® DaphEC10 ecotoxicological local models

MechoA	Daphnids chronic ecotoxicity models	Descriptor domain (SLWS in log10 (mol/L)) MIN	MAX
Non-polar narcosis (MechoA 1.1)	iSafeRat® daphEC10	-5.9102	1.0305
Polar-narcosis (MechoA 1.2)	iSafeRat® daphEC10	-6.5293	-0.1737
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	iSafeRat® daphEC10	-6.7462	-0.0852
Hard-electrophile reactivity (MechoA 3.1)	iSafeRat® daphEC10	-5.1516	0.9576
Soft-electrophile reactivity (MechoA 3.2)	iSafeRat® daphEC10	-5.1256	0.9351
Acidification or alkalinization of cells (MechoA 5.2)	iSafeRat® daphEC10	-3.4785	0.5261
Polar narcosis & alkalinization of cells, corrosion if directly applied, for all species (MechoA 1.2&5.2)	iSafeRat® daphEC10	-2.9729	3.7646

Table 6.3: Descriptor domain for the different iSafeRat® algErC10 ecotoxicological local models

MechoA	Algal ecotoxicity models	Descriptor domain (SLWS in log10 (mol/L)) MIN	MAX
Non-polar narcosis (MechoA 1.1)	iSafeRat® algErC10	-4.2167	0.4903
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and poly-ester)	iSafeRat® algErC10	-5.8073	0.5832
Hard-electrophile reactivity (MechoA 3.1)	iSafeRat® algErC10	-5.1516	0.2572
Soft-electrophile reactivity (MechoA 3.2)	iSafeRat® algErC10	-5.1256	0.5694

The toxicity for a compound with SLWS outside of the descriptor range is predicted by the model but it has to be considered as an extrapolation. In that case, the prediction can still be considered as reliable if there is no contrary indication. Moreover, it recognised that there is a solubility cut-off beyond which no aquatic toxicity is observed, even for long-term exposure. This solubility cut-off value is here defined as the intersection between the solubility cut-off regression and the observed toxicity regression. The **Table 7** gives the values of the solubility cut-off:

Table 7: Solubility cut-off of the iSafeRat® chronic ecotoxicity models

iSafeRat® model	Solubility cut-off (log mol/L)
MechoA 1.1 – fishEC10	-14.988
MechoA 1.1 – daphEC10	-17.386
MechoA 1.1 – algErC10	-8.372
MechoA 1.2 – fishEC10	-8.041
MechoA 1.2 – daphEC10	-7.896
MechoA 2.1 – fishEC10 (mono and poly-ester)	-12.405
MechoA 2.1 – daphEC10 (mono and poly-ester)	-7.04
MechoA 2.1 – algErC10 (mono and poly-ester)	-6.512
MechoA 3.1 – fishEC10	-7.780
MechoA 3.1 – daphEC10	-6.955
MechoA 3.1 – algErC10	-6.933
MechoA 3.2 – daphEC10	-7.454
MechoA 3.2 – algErC10	-6.191
MechoA 5.2 – daphEC10	-4.540
MechoA 1.2&5.2 -daphEC10	-8.982

d) Structural fragment domain

The description of the structural fragments present in the molecules of the training set of each model is given in the **Tables 8.1, 8.2, 8.3**:

Table 8.1: Structural fragments present in the training set of the iSafeRat® fishEC10 ecotoxicity models

MechoA	Fish chronic ecotoxicity Models	Structural fragments
Non-polar narcosis (MechoA 1.1)	iSafeRat® fishEC10	amide; lactam; ketone; 1° alcohol; 2° alcohol; ether; chloride; fused aromatic cycles; non-aromatic heterocycle; phenyl; alkene; cycloalkyl; alkyl; C_2; C_3; C_4; C_5; C_6; C_7; C_8; C_{10}; C_{11}; C_{12}; C_{16}; C_{21}
Polar-narcosis (MechoA 1.2)	iSafeRat® fishEC10	C-aromatic ester; salicyl; phenol; phenyl; alkyl; C_7; C_{11}; C_{12}; C_{13}; C_{14}; C_{15}; C_{26}
Hydrolysis to non-polar narcotic products	iSafeRat® fishEC10	triaromatic phosphate; 3° phosphate; C-aromatic ester; ester; allylic ester; a,b-unsat.

(MechoA 2.1, mono and ployester)		carbonyl/iminyl/thionyl/nitrile; lactate; 2° alcohol; diaromatic ether; aromatic ether; ether; ethoxylation; chloride; phenyl; alkene; cycloalkyl; alkyl; C_5; C_{10}; C_{11}; C_{12}; C_{13}; C_{14}; C_{16}; C_{18}; C_{19}
Hard-electrophile reactivity (MechoA 3.1)	iSafeRat® fishEC10	carbonate; C-aromatic ester; ester; benzyl ester; allylic ester; aromatic aldehyde; aldehyde; ether; ethoxylation; fluoride; furan; phenyl; alkene; cycloalkyl; alkyl; C_5; C_8; C_9; C_{12}; C_{14}

Table 8.2: Structural fragments present in the training set of the iSafeRat® DaphEC10 ecotoxicity models

MechoA	Daphnids chronic ecotoxicity models	Structural fragments
Non-polar narcosis (MechoA 1.1)	iSafeRat® daphEC10	amide; lactam; ketone; 1° alcohol; 2° alcohol; benzyl alcohol; ether; chloride; pyridine; fused aromatic cycles; non-aromatic heterocycle; phenyl; alkene; cycloalkyl; alkyl; C_1; C_2; C_3; C_4; C_5; C_6; C_7; C_8; C_{10}; C_{12}; C_{13}; C_{14}; C_{16}; C_{21}
Polar-narcosis (MechoA 1.2)	iSafeRat® daphEC10	C-aromatic ester; salicyl; phenol; aromatic ether; fused aromatic cycles; phenyl; alkyl; C_7; C_9; C_{10}; C_{12}; C_{14}; C_{15}
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	iSafeRat® daphEC10	triaromatic phosphate; 3° phosphate; C-aromatic ester; ester; lactone; a,b-unsat. carbonyl/iminyl/thionyl/nitrile; lactate; 2° alcohol; aromatic ether; ether; ethoxylation; chloride; non-aromatic heterocycle; phenyl; alkene; cycloalkyl; alkyl; C_5; C_6; C_7; C_8; C_9; C_{10}; C_{11}; C_{12}; C_{13}; C_{14}; C_{15}; C_{16}; C_{18}; C_{19}; C_{20}
Hard-electrophile reactivity (MechoA 3.1)	iSafeRat® daphEC10	C-aromatic ester; ester; aromatic aldehyde; aldehyde; a,b-unsat. carbonyl/iminyl/thionyl/nitrile; phenol; aromatic ether; epoxide; glycidyl; 3° aromatic amine; chloride; fluoride; triazole; furan; non-aromatic heterocycle; phenyl; alkene; cycloalkyl; alkyl; C_5; C_7; C_8; C_{12}; C_{13}; C_{14}; C_{15}; C_{17}; C_{19}
Soft-electrophile reactivity (MechoA 3.2)	iSafeRat® daphEC10	ester; aldehyde; a,b-unsat. carbonyl/iminyl/thionyl/nitrile; 2° alcohol; benzyl alcohol; aromatic ether; ethoxylation; pyridine; pseudoheteroaromatic cycle with carbonyl; pseudoheteroaromatic O; non-aromatic heterocycle; phenyl; alkene; cycloalkyl; alkyl; C_5; C_7; C_8; C_{12}; C_{13}; C_{14}; C_{15}; C_{33}
Acidification or alkalinization of cells (MechoA 5.2)	iSafeRat® daphEC10	Aromatic carboxylic acid; carboxylic acid; a,b-unsat. carbonyl/iminyl/thionyl/nitrile; phenyl; alkene; alkyl; C_3; C_4; C_7; C_8; C_{11}
Polar narcosis & alkalinization of cells, corrosion if directly applied, for all species (MechoA 1.2&5.2)	iSafeRat® daphEC10	1° amine ; 2° amine; 3° amine; non-aromatic heterocycle ; phenyl; cycloalkyl ; alkyl; C_3 ; C_4 ; C_5 ; C_6 ; C_7 ; C_8 ; C_9; C_{10}; C_{16};

Table 8.3: Structural fragments present in the training set of the iSafeRat® algErC10 ecotoxicity models

MechoA	Algal ecotoxicological models	Structural fragments
Non-polar narcosis (MechoA 1.1)	iSafeRat® algErC10	amide; lactam; aromatic ketone; ketone; a,b-unsat. carbonyl/iminyl/thionyl/nitril; 1° alcohol; 2° alcohol; 3° alcohol; aromatic ether; ether; chloride; non-aromatic heterocycle; phenyl; alkene; cycloalkyl; alkyl; C_4; C_5; C_7; C_8; C_9; C_{10}; C_{11}; C_{12}; C_{13}; C_{15}
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and poly-ester)	iSafeRat® algErC10	3° phosphate; C-aromatic ester; ester; lactone; ketone; a,b-unsat. carbonyl/iminyl/thionyl/nitrile; lactate; 2° alcohol; aromatic ether; ether; ethoxylation; chloride; non-aromatic heterocycle; phenyl; alkene; cycloalkyl; alkyl; C_2; C_8; C_9; C_{10}; C_{11}; C_{12}; C_{13}; C_{15}; C_{16}; C_{17}; C_{18}; C_{20}; C_{22}
Hard-electrophile reactivity (MechoA 3.1)	iSafeRat® algErC10	C-aromatic ester; ester; benzyl ester; allylic ester; aromatic aldehyde; aldehyde; a,b-unsat. carbonyl/iminyl/thionyl/nitrile; phenol; 3° alcohol; aromatic ether; epoxide; glycidyl; mustard; 3° aromatic amine; chloride; non-aromatic heterocycle; phenyl; alkene; cycloalkyl; alkyl; C_3; C_5; C_6; C_7; C_9; C_{10}; C_{11}; C_{12}; C_{13}; C_{14}; C_{15}; C_{18}
Soft-electrophile reactivity (MechoA 3.2)	iSafeRat® algErC10	ester; nitrile; aldehyde; a,b-unsat. carbonyl/iminyl/thionyl/nitrile; 2° alcohol; benzyl alcohol; aromatic ether; ether; ethoxylation; pyridine; pseudoheteroaromatic cycle with carbonyl; pseudoheteroaromatic O; non-aromatic heterocycle; phenyl; alkyne; alkene; cycloalkyl; alkyl; C_3; C_7; C_8; C_{10}; C_{11}; C_{13}; C_{14}; C_{15}; C_{33}

e) Mechanistic domain

Currently, each chronic ecotoxicological model is related to one MechoA. Each MechoA is represented by one or several structural alerts (Bauer et al., 2018). The iSafeRat® fishEC10, daphEC10 and algErC10 models can reliably predict the aquatic toxicity for chemicals with the MechoA:

- Non-polar narcosis (MechoA 1.1)
- Polar narcosis (MechoA 1.2, except for algae)
- Esters whose hydrolysis products are narcotic compounds (MechoA 2.1)
- Hard-electrophile reactivity (MechoA 3.1)
- Soft-electrophile reactivity (MechoA 3.2, except for fish)
- Acidification or alkalinization of cells (MechoA 5.2, only for daphnids)
- Polar narcosis & alkalinization of cells, corrosion if directly applied, for all species (MechoA 1.2&5.2, only for daphnids)

f) Metabolic domain, if relevant

Information is provided specifically to each case in QPRF.

g) Possible defined (graphical) expression of how the descriptor values of the chemicals in the training set are distributed in relation to the endpoint values predicted by the model.

The graphics of the linear regressions composing the models and the associated data are proprietary and have not been made publicly available. They have been shared with some regulatory authorities and can be shared with other regulatory authorities on request.

5.2 Method used to assess the applicability domain

To assess if the compound under investigation falls within the applicability domain of the model, different features are evaluated:

- **1/ the mechanistic domain (section 5.1.e):**

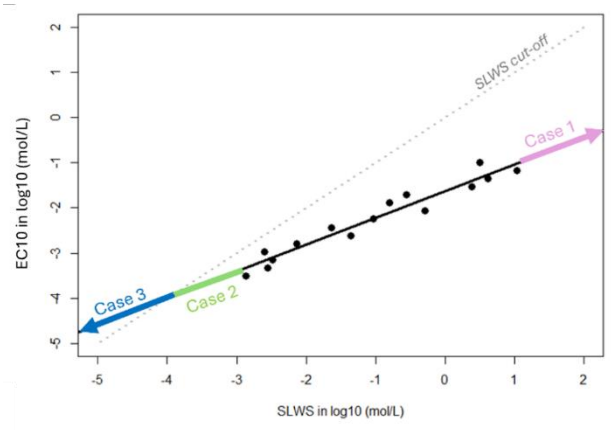
If the compound is known to correspond to a mechanism of toxic action of an existing iSafeRat® ecotoxicological model, the compound is considered as falling within the mechanistic domain of the model. Otherwise, it is considered as outside the mechanistic domain and no prediction is performed.

- **2/ the descriptor domain:**

Then, the log10 SLWS (mol/L) of the compound used as model input is compared to the range of log10 SLWS (mol/L) of the compounds present in the training set of the model.

- If the descriptor value falls between the range of minimum and maximum, the test item is considered as falling within the descriptor domain of the model and the prediction is considered as an interpolation.
- If the descriptor value falls beyond the defined range of minimum and maximum values of the training set, the prediction is considered as extrapolated. However, this extrapolated status does not have the same impact on the prediction reliability, depending on the space where the descriptor value falls (**Figure 1**). Three different cases are identified and are interpreted differently:
  - **Case 1:** the SLWS value of the compound is higher than all the values of the compounds of the training set (the compound is more soluble than the ones of the training set). This is especially the case for miscible compounds where solubility measurements have not reached a limit to dissolution within the limits of the test. According to our models, the toxicity decreases as water solubility increases. Therefore, one can expect that even if the SLWS value of the compound is higher than the ones present in the training set, it is quite unlikely to obtain toxicity values leading to a CLP classification. Nevertheless the water solubility values may be predicted. Therefore an alternative is to use the a predicted water solubility value as descriptor to predict the ecotoxicity although this will be higher than the highest measurable descriptor value. The prediction can be considered as extrapolated but reliable with restrictions.
  - **Case 2**: the descriptor value of the compound is lower than the lowest value of the descriptor and greater than the water-solubility cut-off value. The prediction can be considered as extrapolated but reliable with restrictions depending on the extent of deviation from the lowest SLWS value of the training set.
  - **Case 3:** The descriptor value falls below the water solubility cut-off value of the regression as defined in section 5.1.c. In such cases, a compound is not expected to exert intrinsic toxicity but may induce physical effects due to undissolved test compound coming into contact with the respiratory system/cell membranes of the test organism: the value is not strictly considered as an extrapolation and is considered as reliable with restrictions. The result of the (Q)SAR will be “no toxicity up to the solubility limit”.

Figure 1: Graphical representation of the three different cases where a test substance falls beyond the descriptor range (example on a randomly created model).



- **3/ Response domain:**

Then, the ecotoxicological value of the compound to investigate (in log10 (mol/L)) is compared to the range of log10 ecotoxicological values (mol/L) of the compounds present in the training set of the given model. If the predicted ecotoxicological value falls within the range, the test item is considered as falling within the response domain of the model. If the predicted value falls beyond the defined range, the prediction is considered as extrapolated. Also, if the predicted value is higher than the water solubility of the test item, that means that the test item crosses the water-solubility cut-off and that the test item is not expected to exert any intrinsic toxicity.

- **4/ Structural domain:**

The molecular structure of the test compound is decomposed into structural fragments defined by KREATiS and compared to those present in the compounds of the training set of the model. Different cases can be identified:

  - If all the fragments of the test compound are present in the compounds of the training set of the model, the compound is considered as falling within the structural applicability domain of the model.
  - If one or several fragments are missing, the test item is considered as partially falling within the applicability domain. This is more or less problematic depending on the nature of the missing fragment(s). If the missing fragments are unrelated to the MechoA of the test item, and are related to non-polar narcosis also known as baseline toxicity (e.g. alkyl, alkene, alcohol) or to another MechoA defined as less toxic in the MechoA decision tree, the prediction is still considered reliable as the missing fragments will be only interact as a contributor to hydrophobicity (which will be taken into account by the SLWS descriptor) and the prediction will still be considered as reliable.
  - Alternatively, a test item may fall within the mechanistic domain but not entirely within the structural domain because too few reliable studies were found to validate the assumption and they are not yet implemented in the models. In this case, analogues are provided to demonstrate the fit to the regression.

- **5/ Metabolic domain:**

This domain is not crucial for the iSafeRat® ecotoxicological models but if relevant, information will be provided in the related QPRF.

Synthesis of the applicability domain:

A compound falling in all domains is considered fully in the applicability domain and the prediction can be considered as reliable without restrictions.

A compound falling under some, but not all, domains is considered partially within the applicability domain and the prediction should be considered as an extrapolation. While treated on a case by case basis, this extrapolation should be considered as reliable with restrictions or unreliable, depending on the deviation(s) from the applicability domain.

A compound falling outside all domains is considered outside applicability domain and the prediction is considered as unreliable (or at least unverifiable).

5.3 Software name and version for applicability domain assessment

The software iSafeRat® Desktop provides some information regarding the applicability domain. However, a full assessment of the applicability domain must be performed manually by the user based on the information provided in the QMRF.

5.4 Limits of applicability

The limits of applicability are defined in sections 5.1.

6 Defining goodness-of-fit and robustness (internal validation) - OECD principle 4

6.1 Availability of the training set

The training set of the model is proprietary and has not been made publicly available. The training set of the model has been shared with some regulatory authorities and may be shared with other regulatory authorities upon request.

6.2 Available information for the training set

CAS RN: YES (confidential business information)
Chemical Name: YES (confidential business information)
SMILES: YES (confidential business information)
Formula: NO
INChI: NO
MOL file: NO

6.3 Data for each descriptor variable for the training set

The training set of the model is proprietary and has not been made publicly available.

6.4 Data for the dependent variable for the training set

The training set of the model is proprietary and has not been made publicly available.

6.5 Other information about the training set

The training set of the model is proprietary and has not been made publicly available.

6.6 Pre-processing of data before modelling

All the descriptor and endpoint values were converted into mol/L and used as decimal logarithm for modelling purposes. The solubility values for training compounds in solid state were converted to their corresponding Subcooled Liquid Water Solubility (SLWS) values taking into account the Melting Point as an additional parameter.

All the modelling steps and goodness-of-fit and robustness calculations were performed with R software (R Core Team, 2022; version 4.2.1, 2022-06-23). The presented metrics were obtained according to the formulae presented in the OECD document related to the validation of QSAR models (OECD, 2007). The R-script is available upon request for the competent authorities.

6.7 Statistics for goodness-of-fit

For each model, the respect of the hypothesis for a simple linear regression was investigated (residual homoscedasticity and normality).

The **Tables 9.1, 9.2 and 9.3** present the following metrics related to the goodness-of-fit of each simple linear regression: **The coefficient of multiple determination (Multiple-R^2), the adjusted coefficient of determination (Adjusted-R^2), the Root Mean Squared Error (RMSE)**.

Additionally, a significance test for linear regression was performed based on the F statistic. The H_0 assumes that there is no significant relationship between the dependent and the independent variables. If the p-value > 0.05, the hypothesis is accepted and no significant relationship occurs. If the p-value < 0.05, the hypothesis is rejected and the relationship is significant. For the models where a significant relationship was observed (p-value < 0.05), the coefficients of determination (R^2) and the RMSE are consider as relevant indicators of a model goodness-of-fit. The closer the R^2 is to 1 and lower the RMSE value is, the better the goodness-of-fit of the model. For the chronic endpoints, all the regression are characterized by a p-value < 0.05. However, the models related to MechoA 1.2, MechoA 3.2 and MechoA 3.1 for fish provided the highest p-values and among the lowest Q^2 (when it was possible to calculate one). However, the residuals (deviation between the observed and predicted values) remained acceptable.

For several models, it was not possible to retrieve enough data to build both training and validation sets. In such cases, all the data were used to constitute the training set of the model and enhance its goodness-ot-fit and robustness metrics and no validation set was used, according to the Q&A document of ECHA Webinar (2024)[1].

The statistical metrics of the chronic ecotoxicity models are presented in **Tables 9.1, 9.2 and 9.3** :

Table 9.1: Goodness-of-fit statistics for iSafeRat® fishEC10 ecotoxicological models

MechoA of FishEC10 models	Number of compounds (training set)	p-value	Multiple-R^2	Adjusted-R^2	RMSE
Non-polar narcosis (MechoA 1.1)	28	< 2.2e-16	0.9522	0.9504	0.2572
Polar-narcosis (MechoA 1.2)	9	0.002089	0.763	0.7292	0.4459
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	12	7.76e-08	0.9501	0.9451	0.2356
Hard-electrophile reactivity (MechoA 3.1)	8	0.003371	0.7855	0.7497	0.3215

Table 9.2: Goodness-of-fit statistics for iSafeRat® daphEC10 ecotoxicological models

MechoA of DaphEC10 models	Number of compounds (training set)	p-value	Multiple-R^2	Adjusted-R^2	RMSE
Non-polar narcosis (MechoA 1.1)	31	< 2.2e-16	0.9573	0.9558	0.3184
Polar-narcosis (MechoA 1.2)	8	9.62e-04	0.8573	0.8335	0.3439
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	20	2.47e-13	0.9522	0.9495	0.2218
Hard-electrophile reactivity (MechoA 3.1)	10	5.72e-05	0.8812	0.8664	0.2515
Soft-electrophile reactivity (MechoA 3.2)	10	3.20 e-03	0.6830	0.6434	0.3985
Acidification or alkalinization of cells (MechoA 5.2)	7	2.38e-01	0.2645	0.1174	0.3196
Polar narcosis & alkalinization of cells, corrosion if directly applied, for all species (MechoA 1.2&5.2)	12	5.08e-07	0.9275	0.9203	0.1976

Table 9.3: Goodness-of-fit statistics for iSafeRat® algErC10 ecotoxicological models

MechoA of AlgErC10 models	Number of compounds (training set)	p-value	Multiple-R^2	Adjusted-R^2	RMSE
Non-polar narcosis (MechoA 1.1)	23	7.64e-16	0.9571	0.9551	0.1737

[1] OECD QSAR Assessment Framework in REACH dossier evaluation: what you need to know. Events - ECHA (europa.eu). f19f111f-0896-709c-77f2-a45aa501e39f (europa.eu)

Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and poly-esters)	26	2.48e-11	0.8488	0.8425	0.2860
Hard-electrophile reactivity (MechoA 3.1)	21	9.52e-09	0.8301	0.8212	0.3311
Soft-electrophile reactivity (MechoA 3.2)	14	0.0002032	0.6970	0.6717	0.3656

The data presented in the above tables were obtained using the R software. The p-value, multiple-R^2 and adjusted-R^2 metrics were obtained using the summary() function applied to the linear model created using the lm() function. The RMSE was calculated using an in-house command line following the formula presented in the document OECD (2007).

6.8 Robustness - Statistics obtained by leave-one-out cross-validation

Leave-one-out (LOO) cross validation is carried out such that each compound present in the training is excluded once. A new model is built using the remaining compounds only (N-1). The ecotoxicity value of the excluded compound is then predicted using the new model. This step is reiterated as many times as there are data in the initial training set of the local model. Then, the **Q^2_{LOO}** (explained variance in prediction) and the **SDEP** (Standard Error in Prediction) was calculated. Ideally, the **Q^2_{LOO}** value approach 1 and the SDEP approach 0. As a general trend, the lower the number of compounds and the higher the p-value, the lower the Q^2 metrics. The models related to such data are presented in italics. We are continuing our search for reliable data to improve this QSAR to ultimately reach HA-QSAR status, but it should be recognised that the variability in the data of these models is directly related to the highly reactive properties (i.e., poor stability in test systems) of the test substances themselves. This leads to inherent variability in the final results between studies, even between valid experimental data.
Statistical values are presented in the **Tables 10.1, 10.2 and 10.3**.

Table 10.1: Leave-One-Out (LOO) results for the iSafeRat® fishEC10 ecotoxicological models

MechoA of FishEC10 models	Number of compounds (training set)	Q^2 LOO	SDEP
Non-polar narcosis (MechoA 1.1)	28	0.9443	0.2779
Polar-narcosis (MechoA 1.2)	*9*	*0.6205*	*0.5643*
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	12	0.9232	0.2923
Hard-electrophile reactivity (MechoA 3.1)	*8*	*0.6206*	*0.4275*

Table 10.2: Leave-One-Out (LOO) results for the iSafeRat® daphEC10 ecotoxicological models

MechoA of DaphEC10 models	Number of compounds (training set)	Q^2 LOO	SDEP
Non-polar narcosis (MechoA 1.1)	31	0.9484	0.3500
Polar narcosis (MechoA 1.2)	*8*	*0.7472*	*0.4577*
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	20	0.9427	0.2427
Hard-electrophile reactivity (MechoA 3.1)	10	0.8147	0.3141

Soft-electrophile reactivity (MechoA 3.2)	*10*	*0.5688*	*0.4647*
Acidification or alkalinization of cells (MechoA 5.2)	*7*	-	-
Polar narcosis & alkalinization of cells, corrosion if directly applied, for all species (MechoA 1.2&5.2)	12	0.8520	0.2823

Table 10.3: Leave-One-Out (LOO) results for the iSafeRat® algErC10 ecotoxicological models

MechoA of AlgErC10 models	Number of compounds (training set)	Q^2 LOO	SDEP
Non-polar narcosis (MechoA 1.1)	23	0.9441	0.1982
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and poly-esters)	26	0.8279	0.3051
Hard-electrophile reactivity (MechoA 3.1)	21	0.7911	0.3672
Soft-electrophile reactivity (MechoA 3.2)	14	0.5862	0.4272

The data presented in the above tables were obtained using the R software. The metrics Q^2 LOO were obtained from using the LOO() function of the DEMOVA package (version 1.0, Prana (2016)) applied to the training set of the local model. The SDEP was calculated using an in-house command line based on the LOO() function result, following the formula presented in the document OECD (2007). "-" means that no LOO analysis was run as all the conditions were not met to perform a reliable analysis (low amount of data and p-value>0.05).

6.9 Robustness - Statistics obtained by leave-many-out cross-validation

Leave-Many-Out (LMO) cross validation is similar to the Leave-one-out concept, however multiple training compounds are excluded from the training set and the remaining training set is used to predict the excluded compounds. The number of blocks of substance to exclude (iterations) was defined in order that the training set of the model created at each iteration contained ca. 75% of the original training set. Ideally, the **Q^2_{LMO}** and **SDEP** values approach 1 and 0, respectively. As a general trend, the lower the number of compounds and the higher the p-value, the lower the Q2 metrics and the higher the SDEP. The models related to such data are presented in italics. We consider these models to be of acceptable quality to provide reliable predictions, but we are continuing our search for reliable data to improve this QSAR to ultimately reach HA-QSAR status. Statistical values are presented in the **Tables 11.1, 11.2 and 11.3**:

Table 11.1: Leave-Many-Out (LMO) results for the iSafeRat® fishEC10 ecotoxicological models

MechoA of FishEC10 models	Number of compounds (training set)	Q^2 LMO	SDEP
Non-polar narcosis (MechoA 1.1)	28	0.9450	0.2760
Polar narcosis (MechoA 1.2)	*9*	*0.3581*	*0.7339*
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	12	0.9275	0.2841
Hard-electrophile reactivity (MechoA 3.1)	*8*	*0.6200*	*0.4278*

Table 11.2: Leave-Many-Out (LMO) results for the iSafeRat® daphEC10 ecotoxicological models

MechoA of DaphEC10 models	Number of compounds (training set)	Q^2 LMO	SDEP
Non-polar narcosis (MechoA 1.1)	31	0.9495	0.3462
Polar narcosis (MechoA 1.2)	*8*	*0.7472*	*0.4577*
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	20	0.9466	0.2343
Hard-electrophile reactivity (MechoA 3.1)	10	0.8048	0.3224
Soft-electrophile reactivity (MechoA 3.2)	*10*	*0.5678*	*0.4652*
Acidification or alkalinization of cells (MechoA 5.2)	*7*	-	-
Polar narcosis & alkalinization of cells, corrosion if directly applied, for all species (MechoA 1.2&5.2)	12	0.8335	0.2994

Table 11.3: Leave-Many-Out (LMO) results for the iSafeRat® algErC10 ecotoxicological models

MechoA of AlgErC10 models	Number of compounds (training set)	Q^2 LMO	SDEP
Non-polar narcosis (MechoA 1.1)	23	0.9437	0.1990
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and poly-esters)	26	0.8171	0.3145
Hard-electrophile reactivity (MechoA 3.1)	21	0.7451	0.4056
Soft-electrophile reactivity (MechoA 3.2)	14	0.5803	0.4302

The data presented in the above tables were obtained using the R software. The metrics Q^2 LMO were obtained from using the LMO() function of the DEMOVA package (version 1.0, Prana (2016)) applied to the training set of the local model. The SDEP was calculated using an in-house command line. "-" means that no LMO analysis was run as all the conditions were not met to perform a reliable analysis (low amount of data and p-value>0.05).

6.10 Robustness - Statistics obtained by Y-scrambling

This validation approach consists in identifying if the correlation was based on chance. The ecotoxicological data are randomly permuted and attributed to another compound of the training set. A new regression is built and its respective multiple-R^2 value is calculated. This operation is performed a certain number of times. Finally, the mean of all the R^2 obtained among the different iterations and its standard deviation (**SD**) is computed. It appears not relevant to use a number of iterations higher than the maximal number of possible permutations in the training set (factorial number) as in this case, a same regression will occur several times. Therefore, the number of iterations was set at 300 (factorial number for a training set of 6 substances in the training set). Statistical values are presented in the **Tables 12.1, 12.2 and 12.3**:

Table 12.1: Y-scrambling results for the iSafeRat® fishEC10 ecotoxicological models

MechoA of FishEC10 models	Number of compounds (training set)	Y-scrambling multiple-R^2	Y-scrambling SD
Non-polar narcosis (MechoA 1.1)	28	0.0368	0.0527
Polar-narcosis (MechoA 1.2)	*9*	*0.1292*	*0.1522*
Hydrolysis to non-polar narcotic products	12	0.0767	0.1072

(MechoA 2.1, mono and polyester)			
Hard-electrophile reactivity (MechoA 3.1)	*8*	*0.1280*	*0.1585*

Table 12.2: Y-scrambling results for the iSafeRat® daphEC10 ecotoxicological models

MechoA of DaphEC10 models	Number of compounds (training set)	Y-scrambling multiple-R^2	Y-scrambling SD
Non-polar narcosis (MechoA 1.1)	31	0.0342	0.0448
Polar-narcosis (MechoA 1.2)	*8*	*0.1271*	*0.1602*
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	20	0.0537	0.0683
Hard-electrophile reactivity (MechoA 3.1)	10	0.1076	0.1189
Soft-electrophile reactivity (MechoA 3.2)	*10*	*0.1016*	*0.1225*
Acidification or alkalinization of cells (MechoA 5.2)	*7*	*0.1607*	*0.1737*
Polar narcosis & alkalinization of cells, corrosion if directly applied, for all species (MechoA 1.2&5.2)	12	0.1089	0.1637

Table 12.3: Y-scrambling results for the iSafeRat® algErC10 ecotoxicological models

MechoA of AlgErC10 models	Number of compounds (training set)	Y-scrambling multiple-R^2	Y-scrambling SD
Non-polar narcosis (MechoA 1.1)	23	0.0423	0.0568
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and poly-ester)	26	0.0399	0.0546
Hard-electrophile reactivity (MechoA 3.1)	21	0.0478	0.0671
Soft-electrophile reactivity (MechoA 3.2)	14	0.0853	0.1042

The data presented in the above tables were obtained using the R software. The metrics Y-scrambling multiple-R^2 and Y-scrambling SD were obtained using the scramb() function of the DEMOVA package (version 1.0, Prana (2016)) applied to the training set of the local model.

6.11 Robustness - Statistics obtained by bootstrap

Bootstrapping is another form of internal validation in which a selected subset of the original training set forms the model. Each of the samples of this subset are reiterated in the model such that the newly created model is of the same size as the original training set and the excluded training samples form the validation set and predictions for the validation set are recorded each time. To obtain a Bootstrap-Q^2 value, the training set was randomly sampled at each iteration with a size representing 75% of the training set. Predictions were performed on the 25% excluded from the training set. Based on the derived predictions, a Q^2 was calculated at each iteration. For models with a limited number of data, bootstrap analysis was not performed (represented as "-" in the table). The results provided in the **Tables 13.1, 13.2 and 13.3** were derived from a bootstrap with 1000 iterations.

Table 13.1: Bootstrap results for the iSafeRat® fishEC10 ecotoxicological models

MechoA of FishEC10 models	Number of compounds (training set)	Bootstrap-Q^2	Bootstrap-SDEP
Non-polar narcosis (MechoA 1.1)	28	0.9367	0.2949
Polar-narcosis (MechoA 1.2)	9	-	-
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	12	0.9014	0.3271
Hard-electrophile reactivity (MechoA 3.1)	8	-	-

Table 13.2: Bootstrap results for the iSafeRat® daphEC10 ecotoxicological models

MechoA of DaphEC10 models	Number of compounds (training set)	Bootstrap-Q^2	Bootstrap-SDEP
Non-polar narcosis (MechoA 1.1)	31	0.9412	0.3663
Polar-narcosis (MechoA 1.2)	8	-	-
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	20	0.9297	0.2627
Hard-electrophile reactivity (MechoA 3.1)	10	0.6640	0.3471
Soft-electrophile reactivity (MechoA 3.2)	10	-	-
Acidification or alkalinization of cells (MechoA 5.2)	*7*	-	-
Polar narcosis & alkalinization of cells, corrosion if directly applied, for all species (MechoA 1.2&5.2)	12	0.6744	0.3001

Table 13.3: Bootstrap results for the iSafeRat® algErC10 ecotoxicological models

MechoA of AlgErC10 models	Number of compounds (training set)	Bootstrap-Q^2	Bootstrap-SDEP
Non-polar narcosis (MechoA 1.1)	23	0.9349	0.2056
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and poly-ester)	26	0.7815	0.3235
Hard-electrophile reactivity (MechoA 3.1)	21	0.7481	0.3872
Soft-electrophile reactivity (MechoA 3.2)	14	0.2506	0.5108

The data presented in the above tables were obtained using the R software. The bootstrap Q^2 and SDEP were obtained using an in-house script following the guideline provided in the OECD (2007) document.

6.12 Robustness - Statistics obtained by other methods

No additional statistical methods were implemented.

7 Defining predictivity (External validation) - OECD Principle 4

7.1 Availability of the external validation set

The validation set of the model is proprietary and has not been made publicly available. The validation set of the model has been shared with some regulatory authorities and can be shared with other regulatory authorities on request.

7.2 Available information for the external validation set

CAS RN: YES (confidential business information)
Chemical Name: YES (confidential business information)
SMILES: YES (confidential business information)
Formula: NO
INChI: NO
MOL file: NO

7.3 Data for each descriptor variable for the external validation set

The validation set of the model is proprietary and has not been made publicly available.

7.4 Data for the dependent variable for the external validation set

The validation set of the model is proprietary and has not been made publicly available.

7.5 Other information about the external validation set

The validation set of the model is proprietary and has not been made publicly available.

7.6 Experimental design of test set

The test set of compounds were retrieved from one of the following publicly available data resources:

a) EPISuite package (available experimental results)
b) ECHA dissemination database
c) Data from KREATiS inventory

Test set selection had been a crucial exercise to demonstrate the validity of the presented model. It was made sure that the measured values were derived under appropriate experimental conditions and using suitable methods. To qualify as a test set compound, the following verification checks were made:

a) should not be a part of the training set for the model
b) should be within the applicability domain of the model
c) must have experimentally derived values available

7.7 Predictivity - Statistics obtained by external validation

The external validation was performed on a set of compounds which are not present in the training set of the models. The ecotoxicity of each compound present in the external validation set of the models was predicted. The ecotoxicological predicted values were then used to calculate the **externally explained variance (Q^2 ext)** and **Standard Deviation Error of Prediction (SDEP)**. In theory, Q^2 values closer to 1 and SDEP closer to 0 indicates that the model is associated with a reliable predictivity. **Tables 14.1, 14.2** and **14.3** provide the results derived applying the iSafeRat® models on the external validation set for each model.

For some models, it was complicated to find enough reliable data to comfortably constitute both a training and a validation set. In such cases, it was decided that the model would be more accurate if all the data were used in the training set to obtain the most robust model. Therefore, it was not possible to compute an external Q^2 for now (although efforts are continuously made to increase the number of quality data in our models). Validation set is especially of importance to detect model overfitting and for models based on advanced statistical approaches and/or based on many descriptors. KREATiS QSARs are based on mechanistic understanding and

one descriptor. Therefore, these models are less susceptible to overfitting and recent information clarified that the EU Chemicals Agency, ECHA accepts exclusion of the external validation set for such cases[2].

Table 14.1: External validation results for the iSafeRat® fishEC10 ecotoxicological models

MechoA of FishEC10 models	Number of compounds (training set)	Number of compounds (validation set)	External Q^2	SDEP
Non-polar narcosis (MechoA 1.1)	28	10	0.9354	0.2887
Polar-narcosis (MechoA 1.2)	9	No validation set	-	-
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	12	No validation set	-	-
Hard-electrophile reactivity (MechoA 3.1)	8	No validation set	-	-

Table 14.2: External validation results for the iSafeRat® daphEC10 ecotoxicological models

MechoA of DaphEC10 models	Number of compounds (training set)	Number of compounds (validation set)	External Q^2	SDEP
Non-polar narcosis (MechoA 1.1)	31	12	0.8790	0.3589
Polar-narcosis (MechoA 1.2)	8	No validation set	-	-
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and polyester)	20	7	0.9243	0.2601
Hard-electrophile reactivity (MechoA 3.1)	10	No validation set	-	-
Soft-electrophile reactivity (MechoA 3.2)	10	No validation set	-	-
Acidification or alkalinization of cells (MechoA 5.2)	*7*	No validation set	-	-
Polar narcosis & alkalinization of cells, corrosion if directly applied, for all species (MechoA 1.2&5.2)	12	No validation set	-	-

Table 14.3: External validation results for the iSafeRat® algErC10 ecotoxicological models

MechoA of AlgErC10 models	Number of compounds (training set)	Number of compounds (validation set)	External Q^2	SDEP
Non-polar narcosis (MechoA 1.1)	23	7	0.9465	0.2076
Hydrolysis to non-polar narcotic products (MechoA 2.1, mono and poly-ester)	26	10	0.7588	0.2533
Hard-electrophile reactivity (MechoA 3.1)	21	11	0.8212	0.3642
Soft-electrophile reactivity (MechoA 3.2)	14	7	0.4848	0.4152

The data presented in the above tables were obtained using the R software. The metrics external Q^2 and SDEP were obtained using an in-house script following the guideline provided in the OECD (2007) document.

[2] f19f111f-0896-709c-77f2-a45aa501e39f (europa.eu)

7.8 Predictivity - Assessment of the external validation set

It was made sure that the test set covered the chemical domain of the training data sufficiently to avoid any possible bias in the resulting statistical validation.

7.9 Comments on the external validation of the model

The validation set may be extended from time to time and the revised validation results can be presented as the updated version of this QMRF.

8 Providing a mechanistic interpretation - OECD Principle 5

8.1 Mechanistic basis of the model

The thermodynamic relationship between surrogates for chemical activity, such as SLWS, log K_{OW} and narcosis has been widely reported in the literature (Mackay *et al.*, 2009) but only recently was it postulated that this could be used to develop a method which can be applied to certain mechanisms of action to reliably predict endpoint values for standard regulatory guideline studies (ECETOC, 2013; Thomas *et al.,* 2015, 2019).

8.2 A priori or a posteriori mechanistic interpretation

A priori

8.3 Other information about the mechanistic interpretation

To allow a better understanding of the methodology, no inexplicable molecular descriptors or modelling algorithms were included. As hypothesised by Mackay *et al.* (2009) and validated by Thomas *et al.* (2015), aquatic toxicity of a non-polar narcotic (MechoA 1.1) compound is directly correlated to its chemical activity and thereby its SLWS. For other MechoAs, specific relationships with SLWS have also been determined by KREATiS although the slopes and intercepts vary from one MechoA to another. The toxicity of MechoA 1.1 compounds can be considered as a membrane saturation phenomenon. Reasons for excess toxicity for other mechanisms of action have been elucidated in Bauer et al. (2018) and more recently is Firman et al (2022) and when they are known they are indicated in the HA-QSAR study report accompanying the QMRF.

9 Miscellaneous information

9.1 Comments

This QMRF can be used as a reference document for QPRF providing:

- iSafeRat® fishEC10 v2.1 predictions for CHRONIC TOXICITY TO FISH (32-DAY EC10);
- iSafeRat® daphEC10 v2.4 predictions for CHRONIC TOXICITY TO DAPHNIDS (21-DAY EC10);
- iSafeRat® algErC10 v2.4 predictions for TOXICITY TO ALGAE (72-HOUR ErC10).

Finally, it is important to explain that KREATiS designates a QSAR as a High Accuracy QSAR or HA-QSAR when the model meets the following internally decided obligations:

1) the model is fully compliant with the 5 OECD Principles of QSAR development;
2) that the input data have been carefully checked internally to verify fitness for purpose and compliance with the OECD guidelines. This goes beyond meeting the Klimisch score applied in an ECHA dossier, for example. All data are revalidated internally according to KREATiS criteria often leading to a more restrictive classification than the ECHA Klimisch score;
3) All the HA-QSAR models are associated with statistics highlighting high model robustness like for example high R^2 and/or low RMSE. Such results are observed in the cases where the toxicity is highly dependent upon the descriptor and those models can be considered “HA-QSAR” models without reservation;
4) Conversely, for certain MechoA subclasses, there is a less clear relationship, or even no apparent link between the hydrophobicity descriptor (i.e. water solubility) and the predicted variable, resulting in a horizontal (or near horizontal) regression (cf. p-values in goodness-of-fit tables). However, the fact that the data are close to the regression line and are not dispersed in a heterogeneous manner indicate that whatever the water solubility, the toxicity remains in a specific range. This may be due to a direct

reaction between the compound and the biological matrix causing significant damage to the organism in question regardless of the water solubility of the compound until reaching the water solubility-toxicity cut-off line. This can result in low R^2 because of the poor relationship between solubility and toxicity. Nevertheless, the RMSE may still be low (potentially even lower than in HA-QSARs) as the toxicity is still dependent on the primary variable which is the MechoA. In these cases the models may still be considered as HA-QSARs as long as the RMSE is low enough (i.e. <0.4).

However, if the R^2 and RMSE limits are not met and the model does not have necessarily enough data to compute all the robustness statistics, the models do not receive the KREATiS "HA-QSAR" designation.

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9.3 Supporting information

Training set(s):
Proprietary. All the queries must be directly addressed to KREATiS SAS.

Test set(s):
Proprietary. All the queries must be directly addressed to KREATiS SAS.

Supporting information:
None

10 Summary (KREATiS QMRF Database)

10.1 QMRF number (For KREATiS internal records only)

KTS/QMRF/CHR/13

10.2 Publication date

16 June 2025

10.3 Keywords

iSafeRat®; QSAR; ecotoxicity; chronic; long-term toxicity; SLWS; chemical activity; non-polar narcosis; enzymatic hydrolysis; reactive substances; EC10; MATC; fish; daphnid; algae

10.4 Comments

None

Appendix 1: Applicability domains and statistical metrics of models before the training set and the validation set were merged.

These tables are related to the models which have a limited number of reliable data and for which the entire set of data was, in the previous version (version 1.7 of iSafeRat® fishEC10, version 1.8 daphEC10 and version 2.1 algErC10), splatted into a training and a validation set. In the updated model version (v2.0, v2.0 and v2.2 for fish, daphnids and algae respectively), the training set and the validation set were merged in a single training set to obtain more robust models. More information about the previous version of the models is available in QMRF v2.3.

Appendix-Table 1: Descriptor, response and mechanistic domains for iSafeRat® Ecotoxicity models

Models	Mechanistic domain	Reliable Descriptor range for subcooled liquids *(log10 SLWS mol/L)*	Reliable Prediction range *(log10 EC10 mol/L)*
Long-term toxicity to fish	MechoA 3.1	-4.142 to 0.95	-6.795 to -4.668
Long-term toxicity to daphnids		-5.152 to 0.958	-6.621 to -4.125
Long-term toxicity to daphnids	MechoA 3.2	-5.126 to 0.935	-6.450 to -4.650

Appendix-Table 2: Goodness-of-fit statistical values for iSafeRat® Ecotoxicity models

iSafeRat® models	n	R^2	RMSE
Long-term toxicity to fish – MechoA 3.1	5	0.8732	0.3387
Long-term toxicity to daphnids – MechoA 3.1	8	0.8652	0.3227
Long-term toxicity to daphnids– MechoA 3.2	6	0.6417	0.5216

Appendix-Table 3: Leave-One-Out cross validation for iSafeRat® Ecotoxicity models

iSafeRat® models	n	Q^2_{LOO}
Long-term toxicity to fish– MechoA 3.1	5	0.5216
Long-term toxicity to daphnids– MechoA 3.1	8	0.7644
Long-term toxicity to daphnids– MechoA 3.2	6	0.4388

Appendix-Table 4: Predictivity statistics for iSafeRat® Ecotoxicity models

iSafeRat® models	n	n_{Test}	Q^2	RMSEP
Long-term toxicity to fish– MechoA 3.1	5	3	0.6879	0.4375
Long-term toxicity to daphnids– MechoA 3.1	8	4	0.8095	0.3163
Long-term toxicity to daphnids– MechoA 3.2	6	4	0.9882	0.4198