How to use the VEGAHUB tools to assess fish acute toxicity

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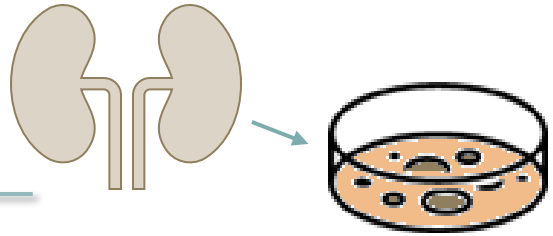
NAMs

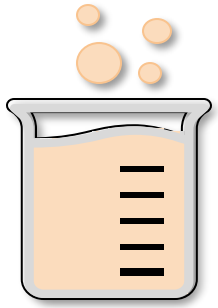
(New Approach Methodologies)

To reduce the dependency on animal testing for the chemical hazard and risk assessment

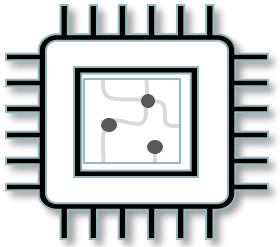
In vitro



Ex vivo

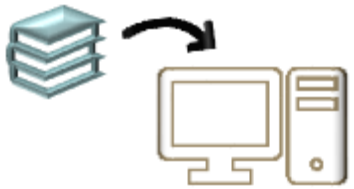


In chemico



In silico

The *in silico* methods



Take advantage of existing data

Can be used to fill the data-gaps

Cheap

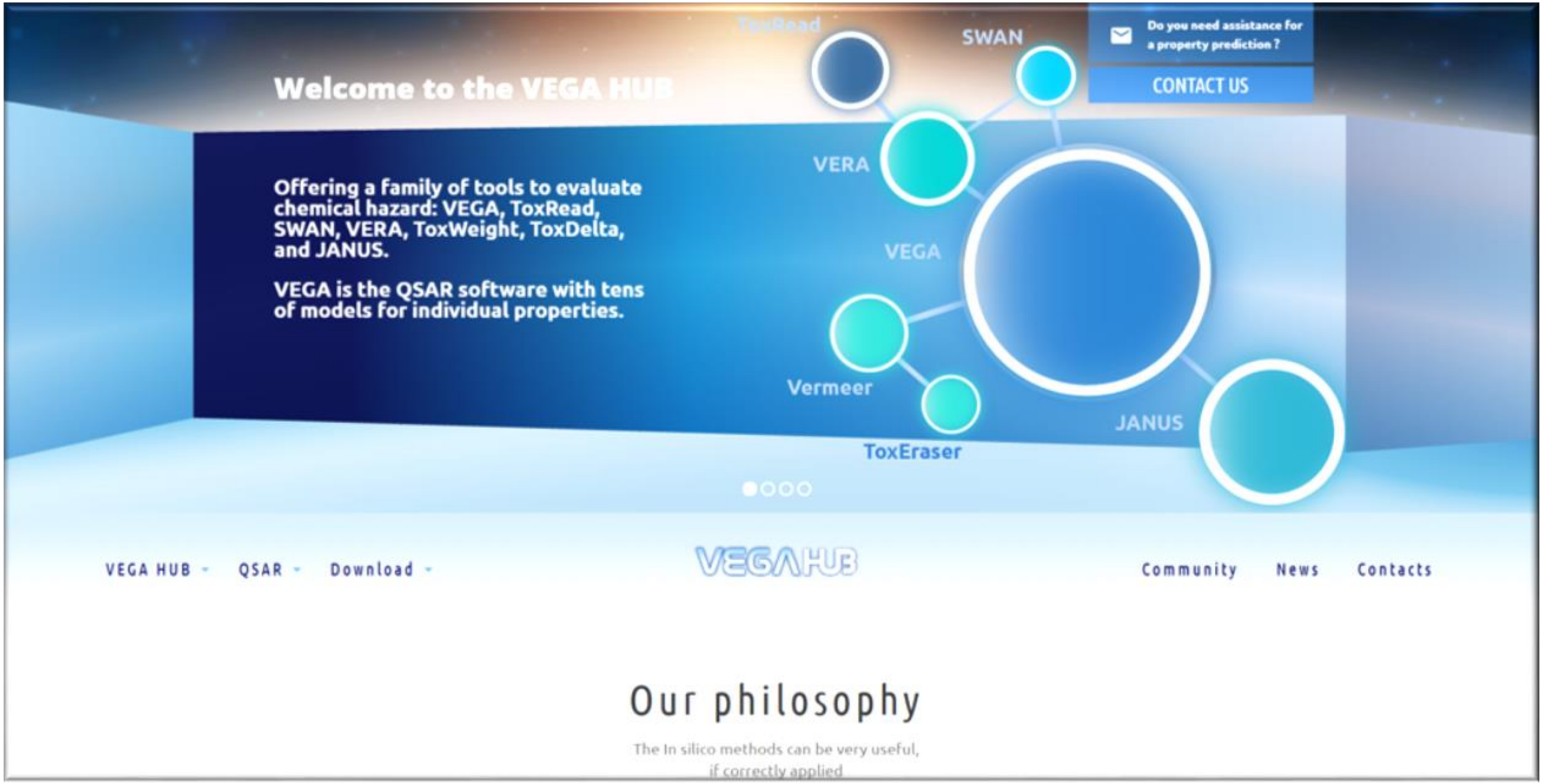
Fast

Batch processing

Green (no solvent, no waste)

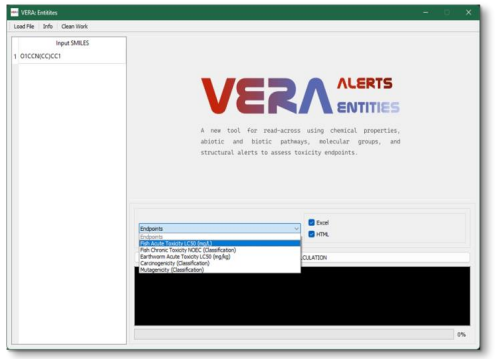
Ethical issues: (no lab. animals)

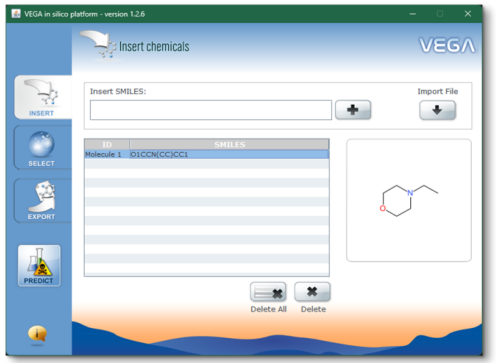
Pre-synthesis studies

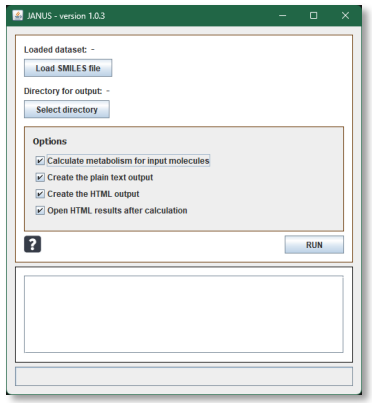


https://www.vegahub.eu/

The VEGAHUB tools: general information







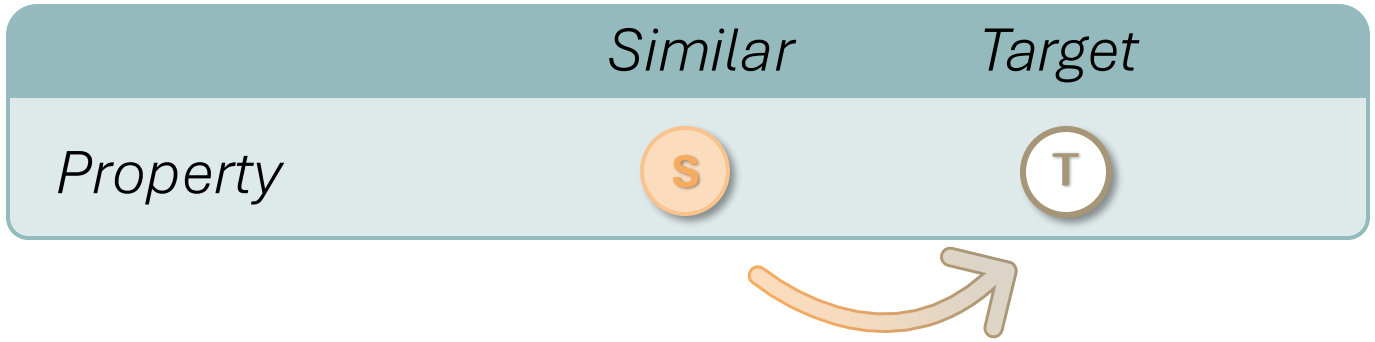
- **User-friendly** tools
- **Transparent** and **information-rich outputs**
- **Goal**: **help**, not substitute assessors

- **Input**: chemical structure (SMILES*)
- **Output**: **tabular** (for batch) + **PDF/HTML** (detailed)

**Alphanumeric strings to represent the molecule (atoms, bonds,…)*

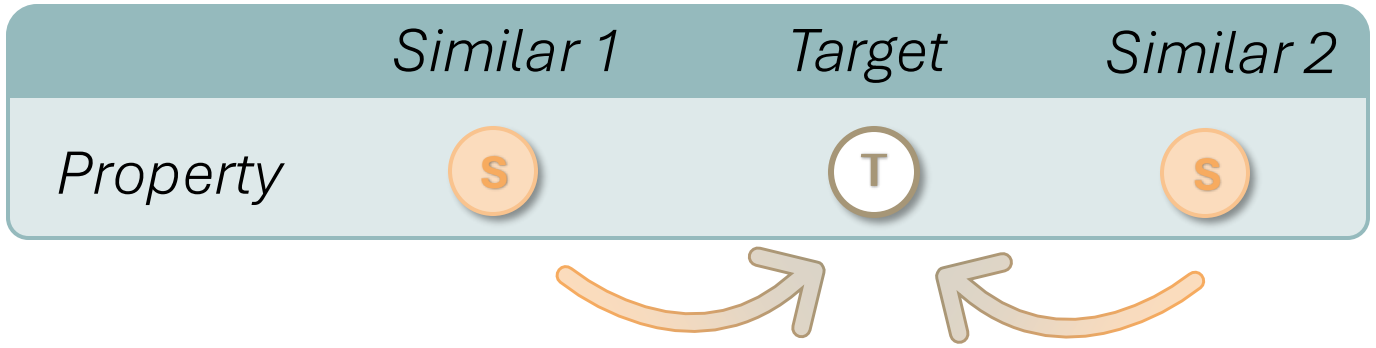
Read-across - Analogue approach

⇨ estimates a property/activity from **similar chemical(s)**

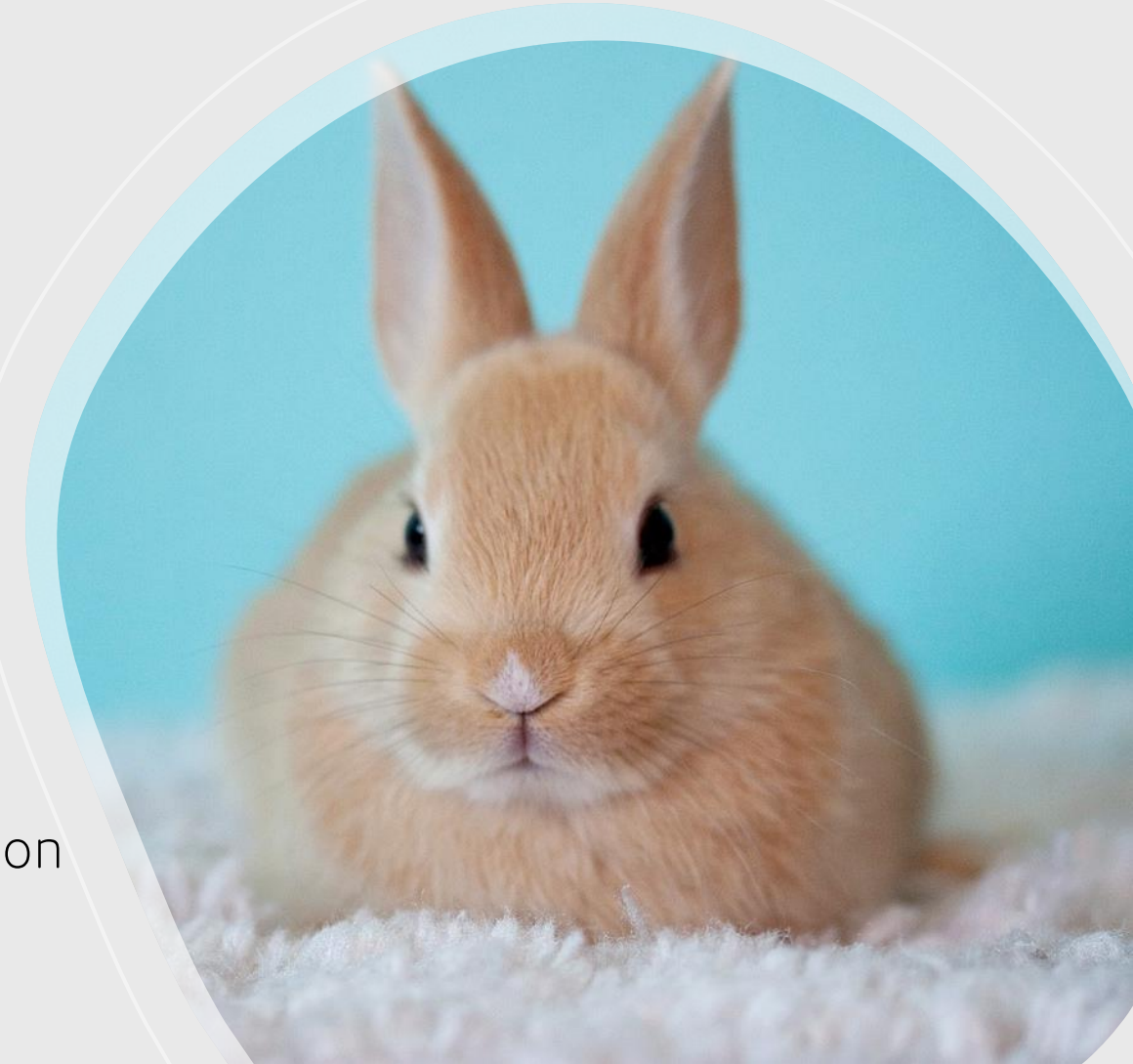


One-to-one

Interpolation (extrapolation)

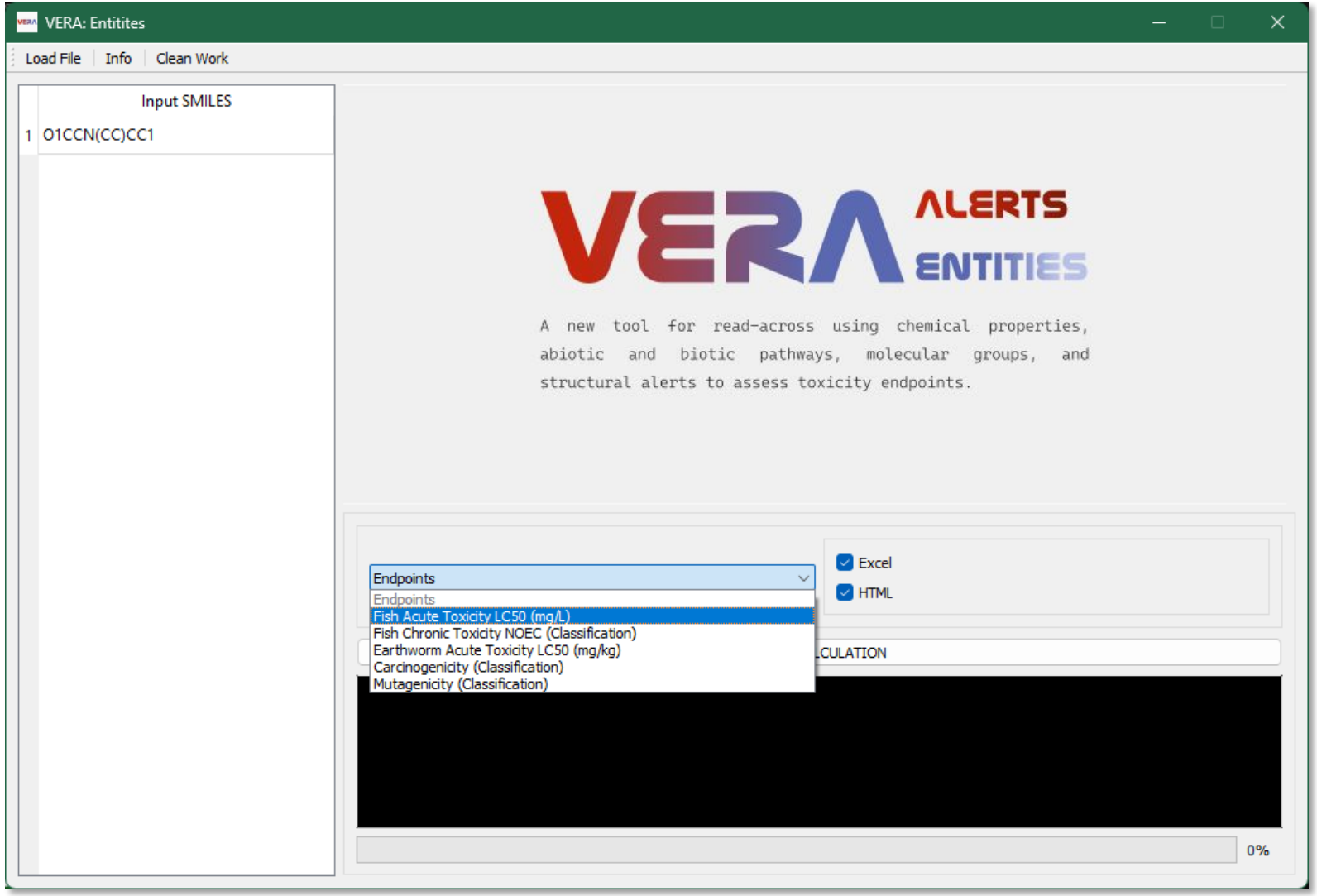


Read-across & similarity



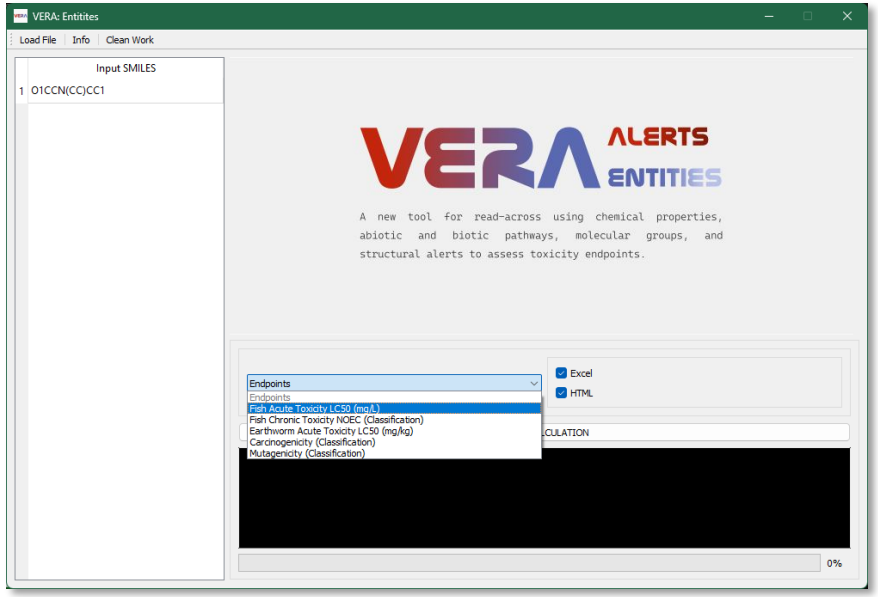
- No accepted definition
- Expert-dependent

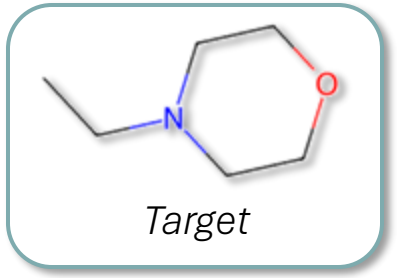
Virtual Extensive Read-Across: VERA

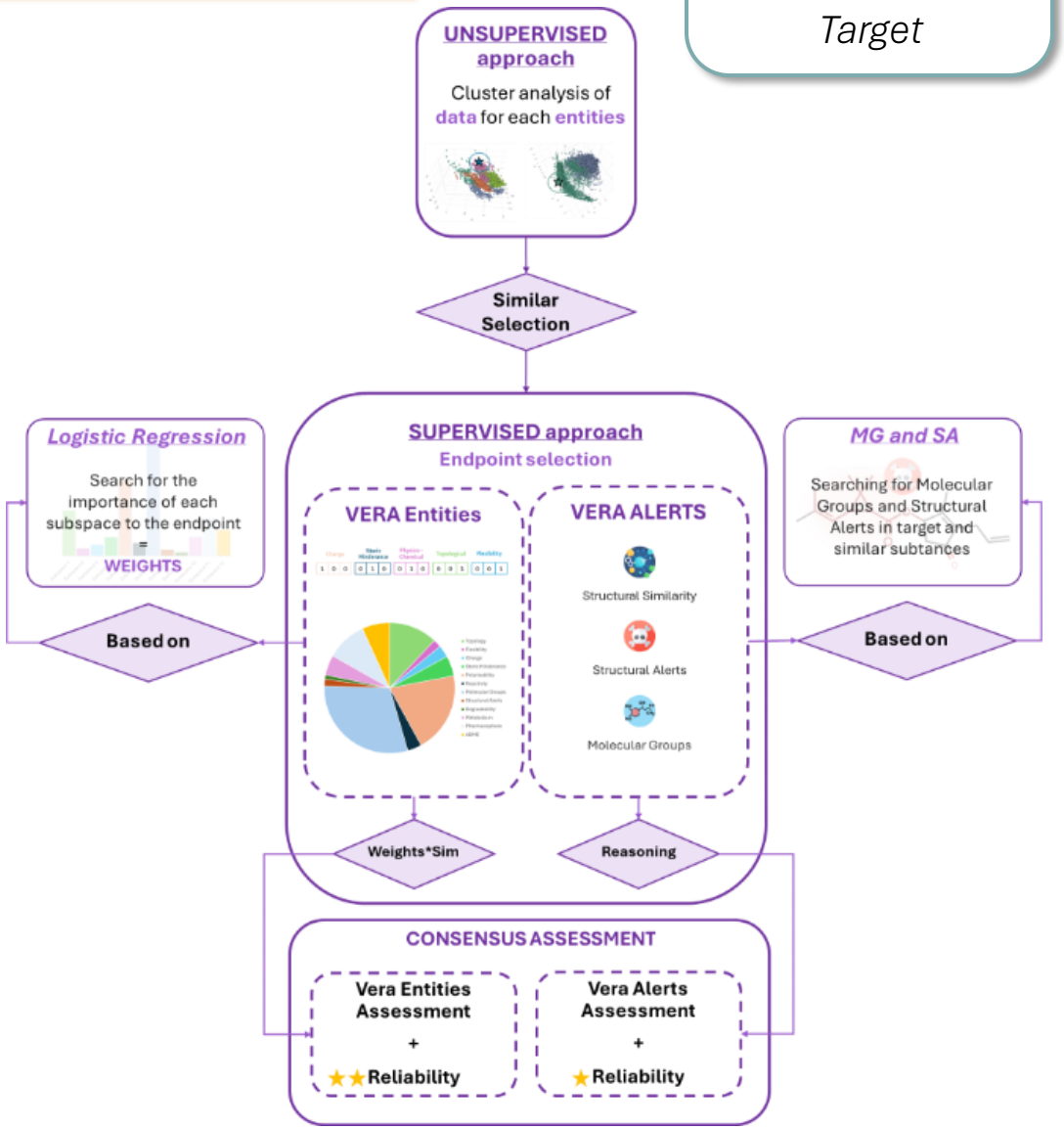


- Automated
- Transparent
- Reproducible
- Unique system with multiple similarity approaches
- Mathematical definition of reliability

Virtual Extensive Read-Across: VERA





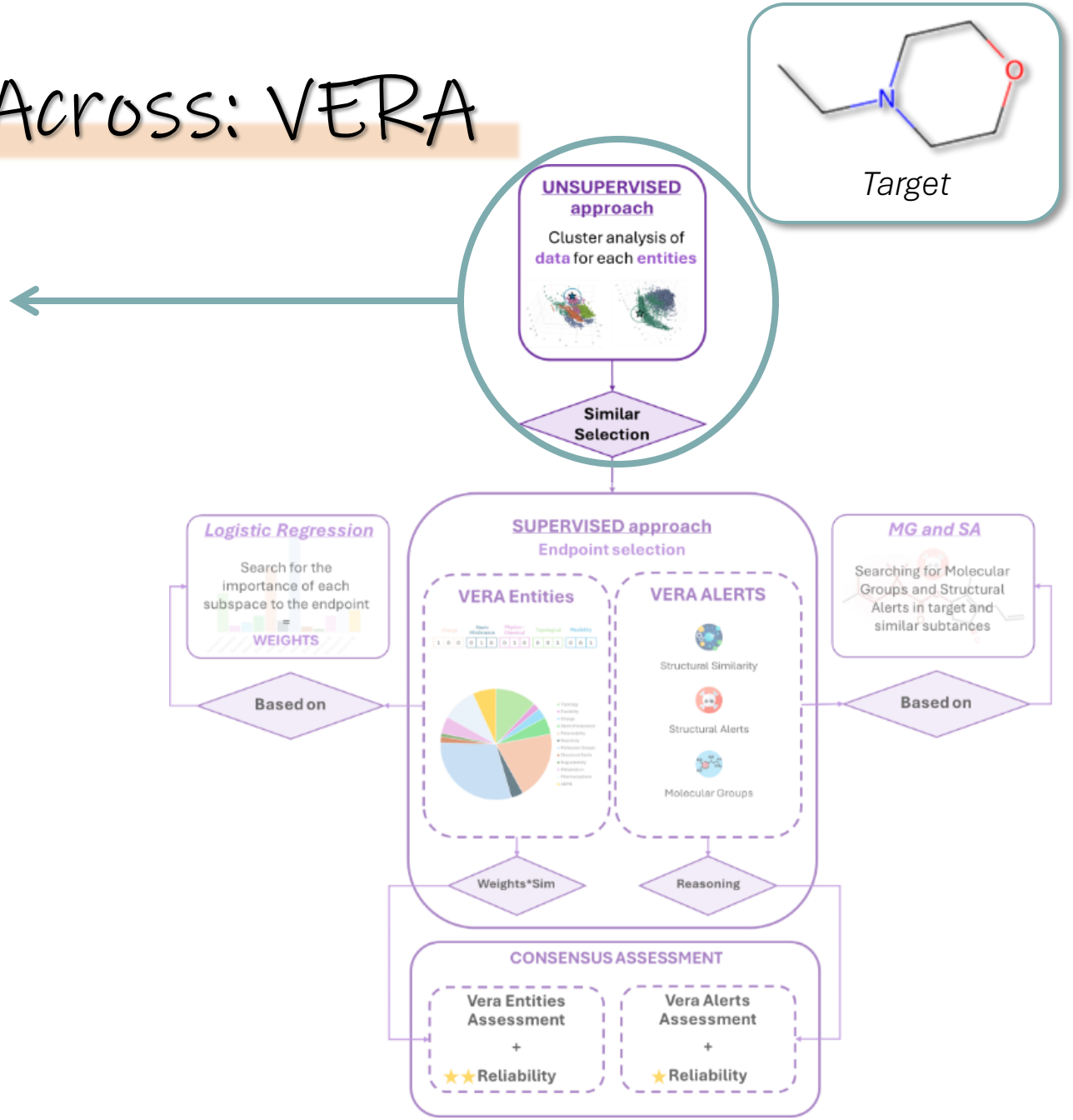


Virtual Extensive Read-Across: VERA

Goal: **similar selection**

Based on:

- 12 **entities** (chemical, biotic, abiotic, physico-chemical)
- 10k chemicals
- **Clusters**



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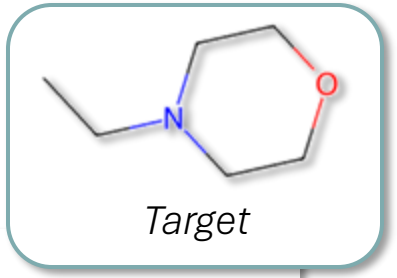


Target

UNSUPERVISED

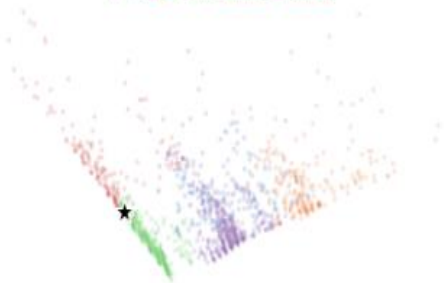
MolecularGroupsSpace

TopologicalSpace

ReactiveSpace

AdmeSpace

PhysChemSpace



PolarizabilitySpace

StericHinderenceSpace

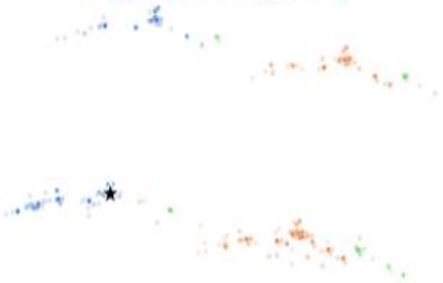
PharmacophoreSpace

ChargeDistributionSpace

MetaboliteSpace

FlexibilitySpace

DegradabilitySpace



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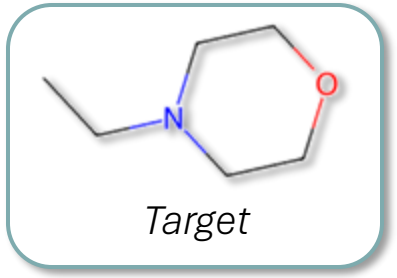
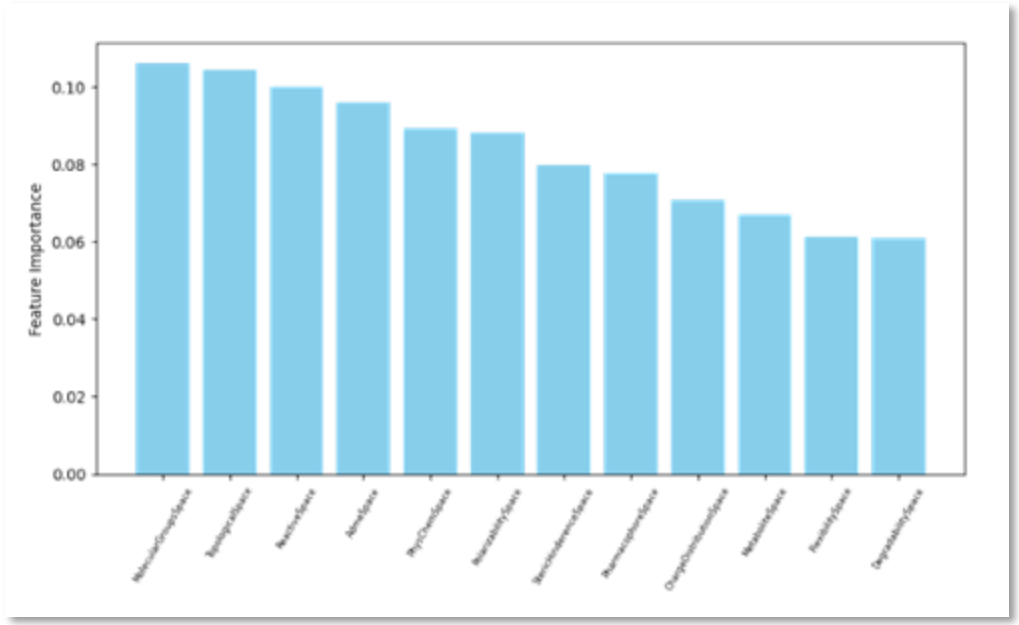
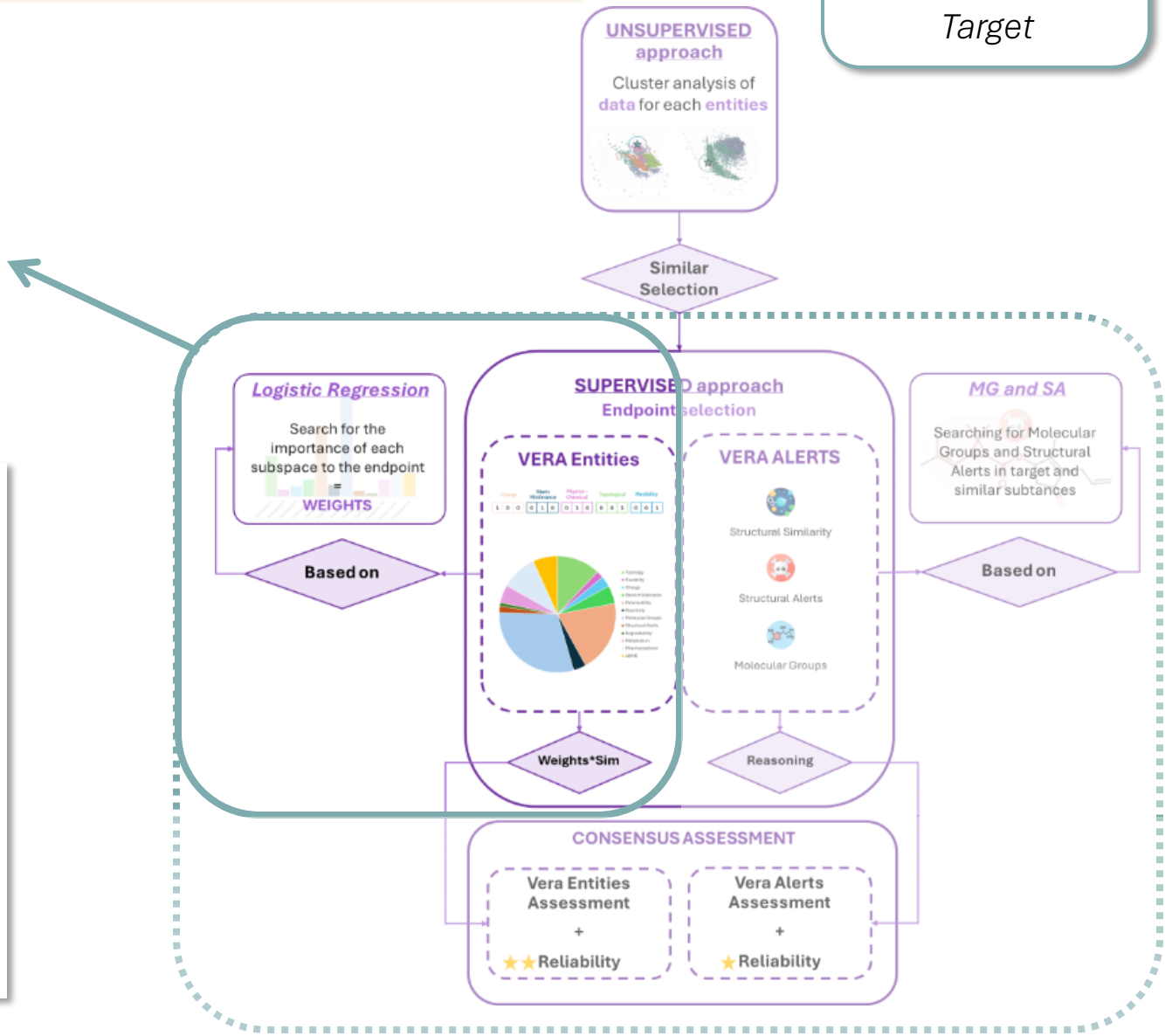


Goal: **endpoint assessment**

- VERA Entities
  - Entities importance
  - Similar (with value) selection
  - Prediction





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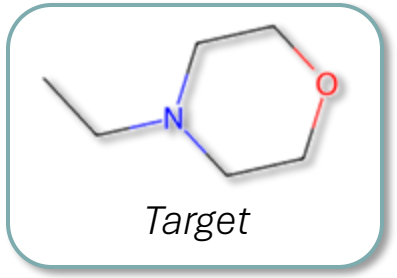
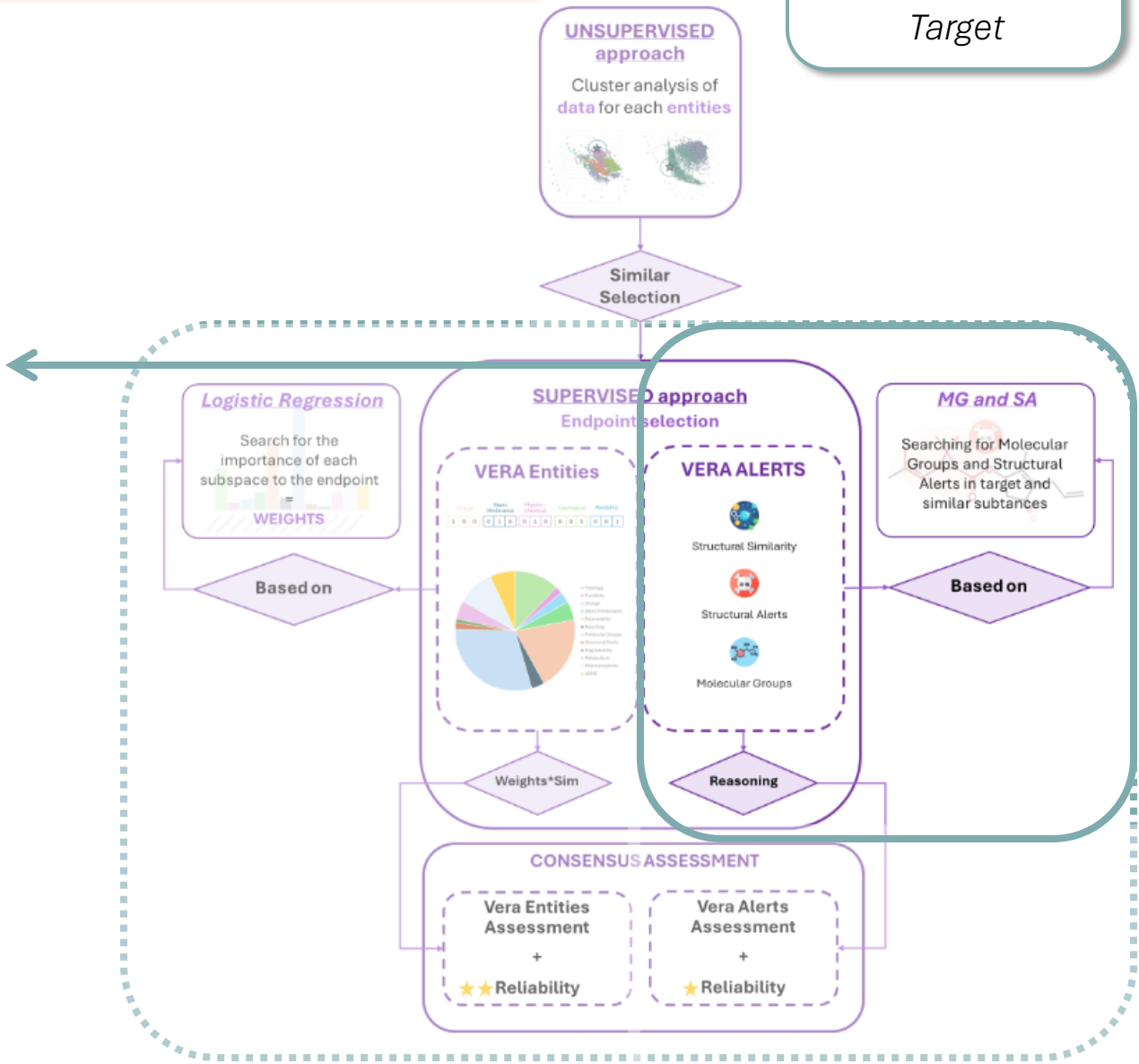
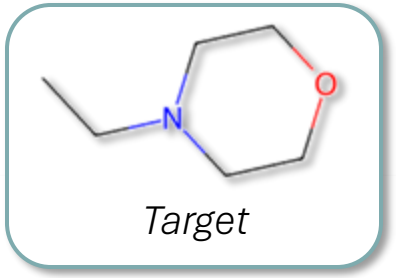


Goal: **endpoint assessment**

- VERA Entities
- VERA Alerts
  - Selection 1: Entities
  - Selection 2: Structural Alerts and Molecular Groups
  - Reasoning



Virtual Extensive Read-Across: VERA



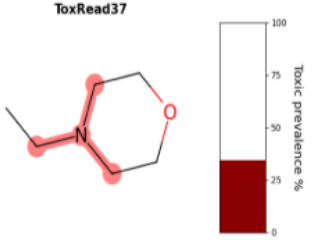
Target

VERA Alerts: similar substances used for the assessment

Endpoint: Fish Acute Toxicity LC50 (mg/L)

SMILES: CCN1CCOCC1

ToxRead37

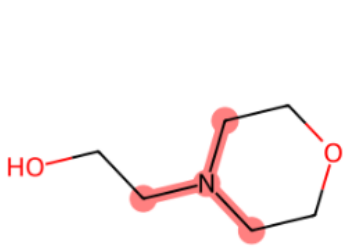


Warning:

Expert-based structural alerts (SAs) were found in the target molecule:

ToxRead37

Fish acute toxicity alert ToxRead37



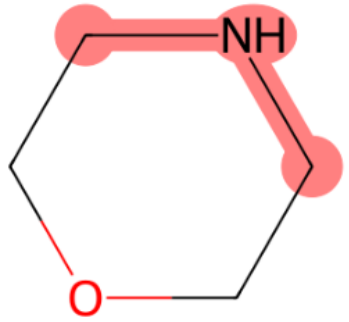
SMILES:

OCCN1CCOCC1

Experimental value mg/l:

2716.439

- VEGA Similarity: 0.906



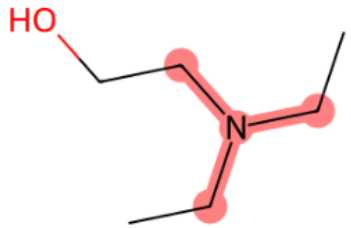
SMILES:

C1COCCN1

Experimental value mg/l:

181.97

- VEGA Similarity: 0.879



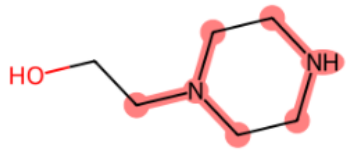
SMILES:

CCN(CC)CCO

Experimental value mg/l:

1774.189

- VEGA Similarity: 0.854



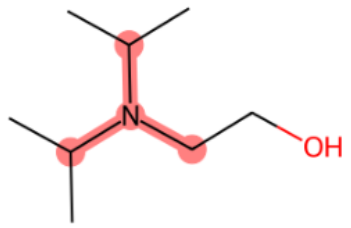
SMILES:

OCCN1CCNCC1

Experimental value mg/l:

6382.635

- VEGA Similarity: 0.806



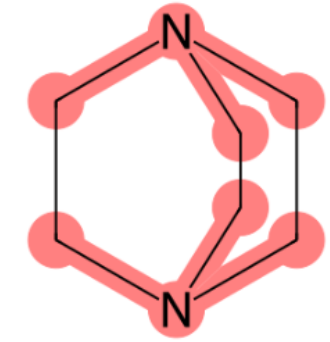
SMILES:

CC(C)N(CCO)C(C)C

Experimental value mg/l:

200.447

- VEGA Similarity: 0.799



SMILES:

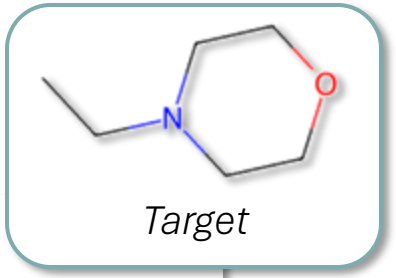
C1CN2CCN1CC2

Experimental value mg/l:

1729.816

- VEGA Similarity: 0.792

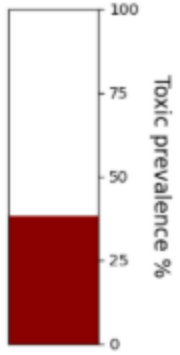
Virtual Extensive Read-Across: VERA



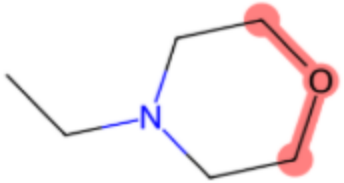
VERA Details

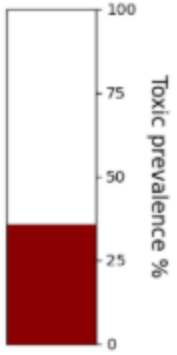
Molecular Groups

NH0

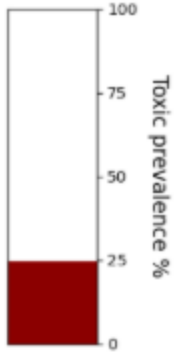


ether

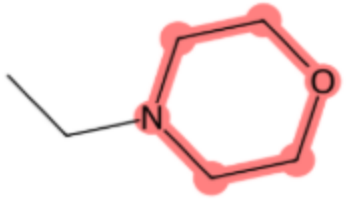


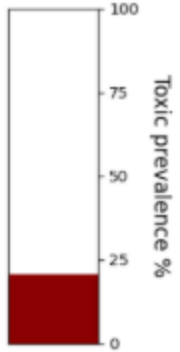


Ndealkylation1



morpholine





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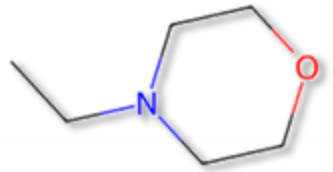
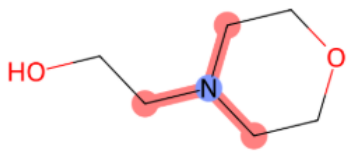


Reasoning

Coopresence analysis of:

1. Toxicity substructure (in red): could be expert base substructure or statistical relevant substructure
2. Specific molecular groups found in target [MGs]

MG: NH0



SMILES:

OCCN1CCOCC1

Experimental value (mg/l):

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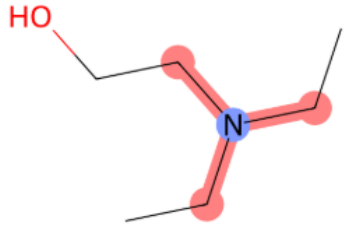
- VEGA Similarity: 0.906



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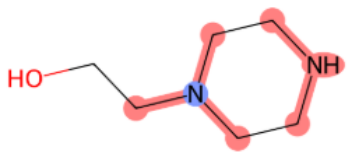

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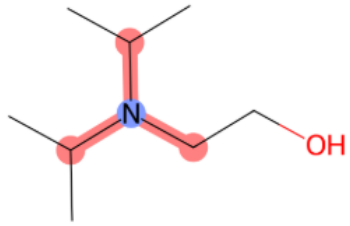

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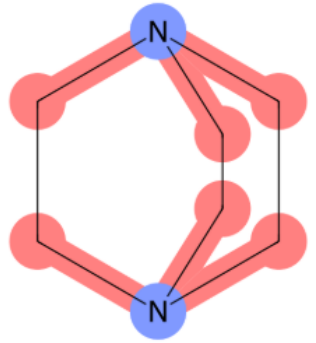

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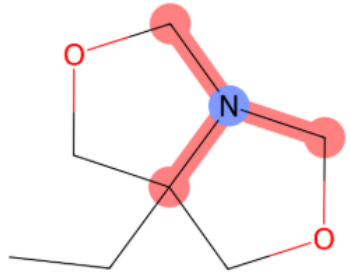


SMILES:

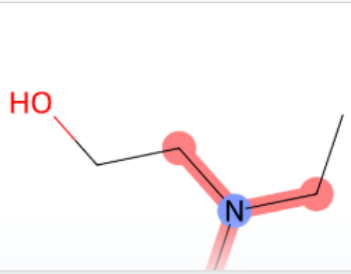
CCC12COCN1COC2

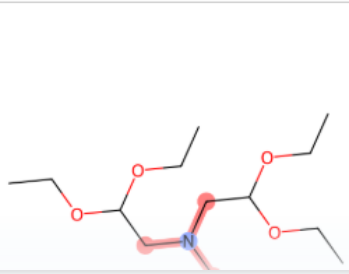
Experimental value (mg/l):

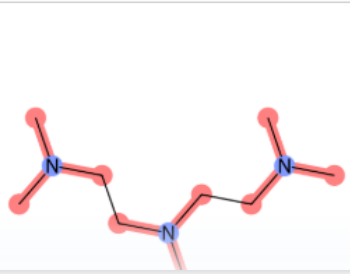
175.792

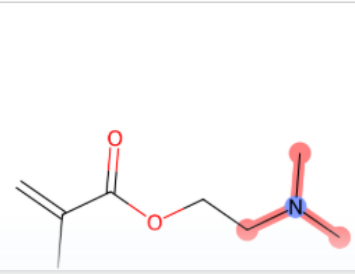
- VEGA Similarity: 0.781

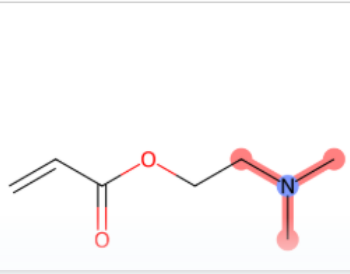
MG: Ndealkylation1



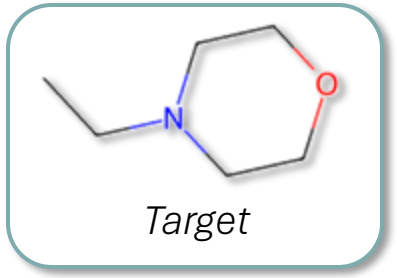






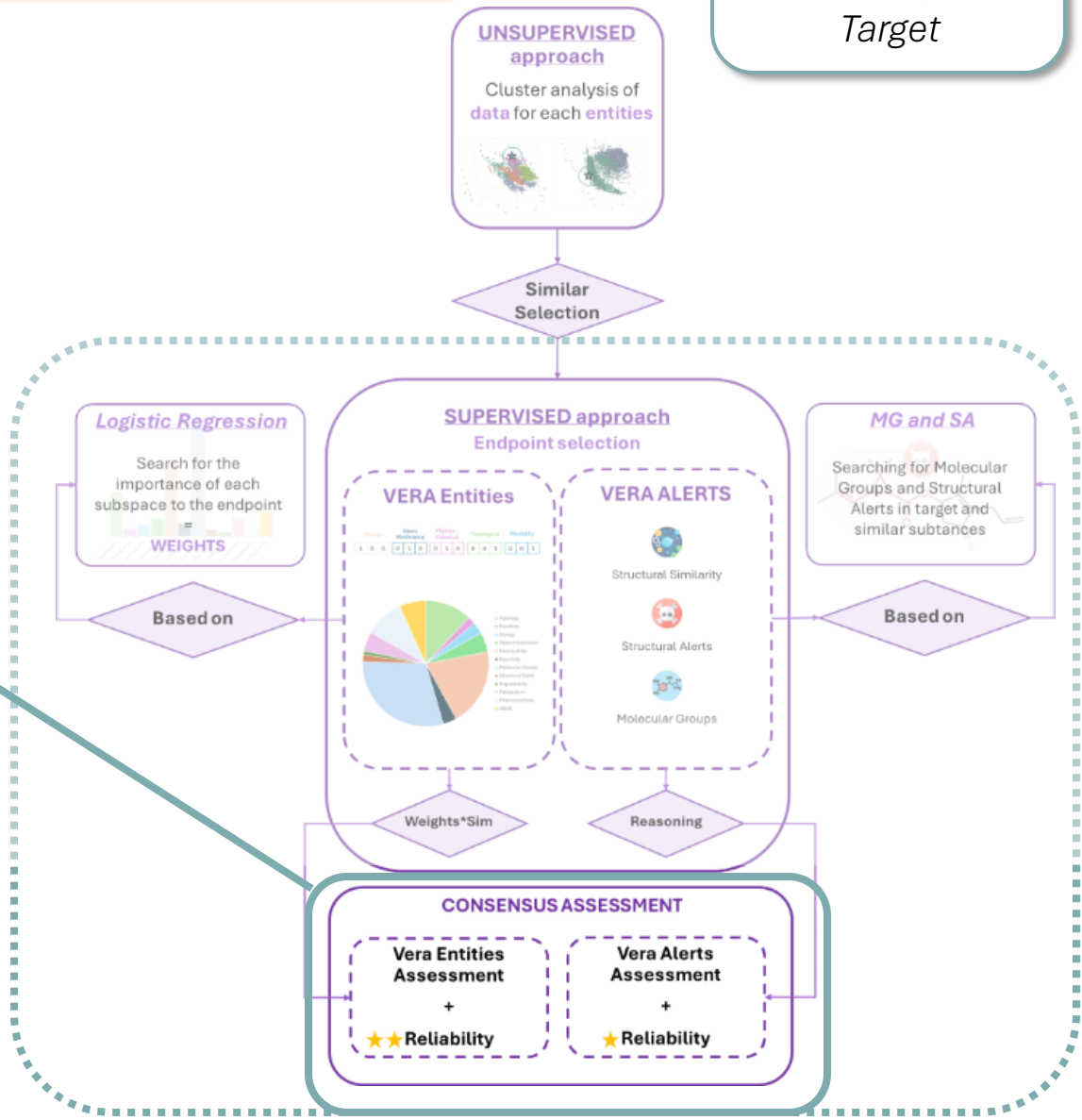


Virtual Extensive Read-Across: VERA



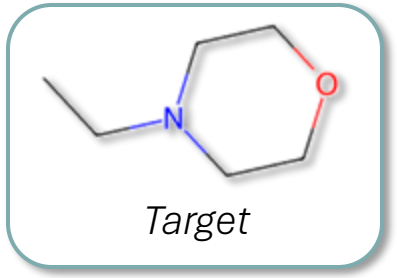
Goal: **endpoint assessment**

- VERA Entities
- VERA Alerts
- Consensus and **reliability**



Virtual Extensive Read-Across: VERA

UNSUPERVISED



Target

Assessment Summary

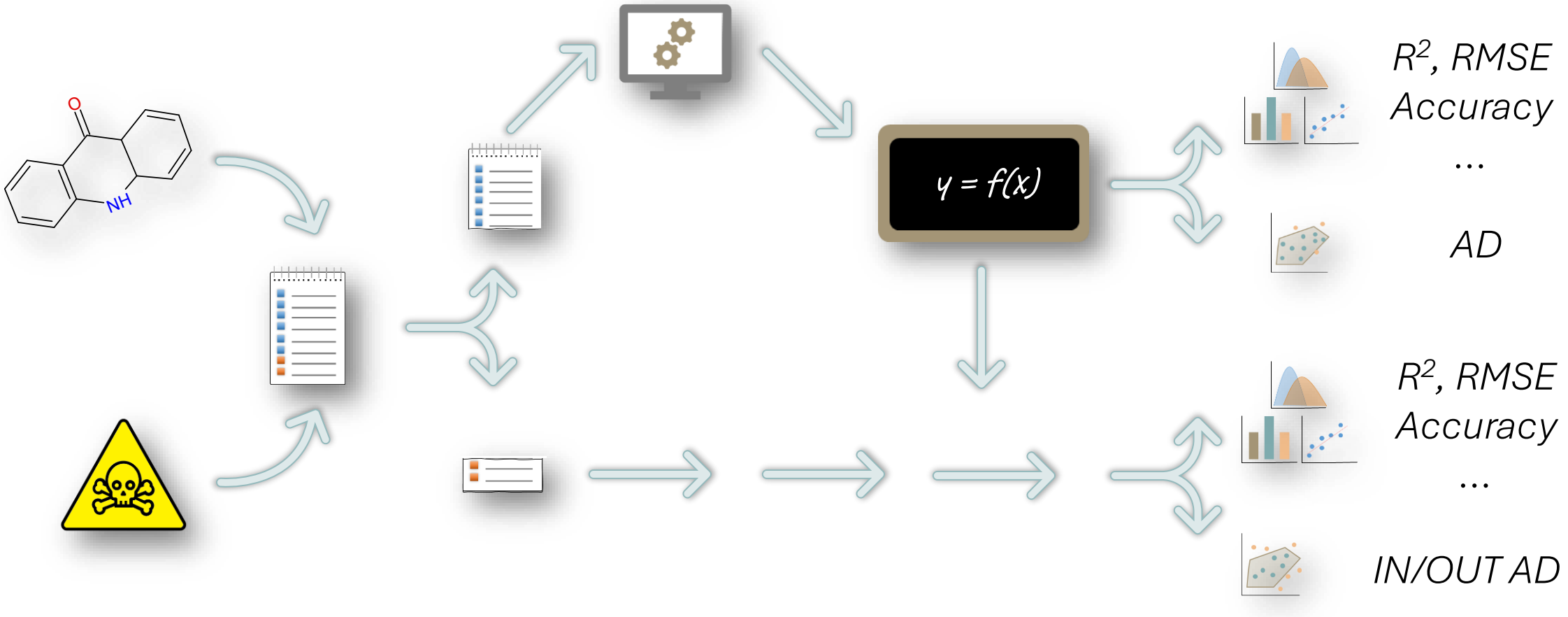
VERA Alerts (mg/l)	Reliability	VERA Entities (mg/l)	**Consensus**
606.644	High reliability	[223.872]	[415.258]

Chemicals used for Read Across

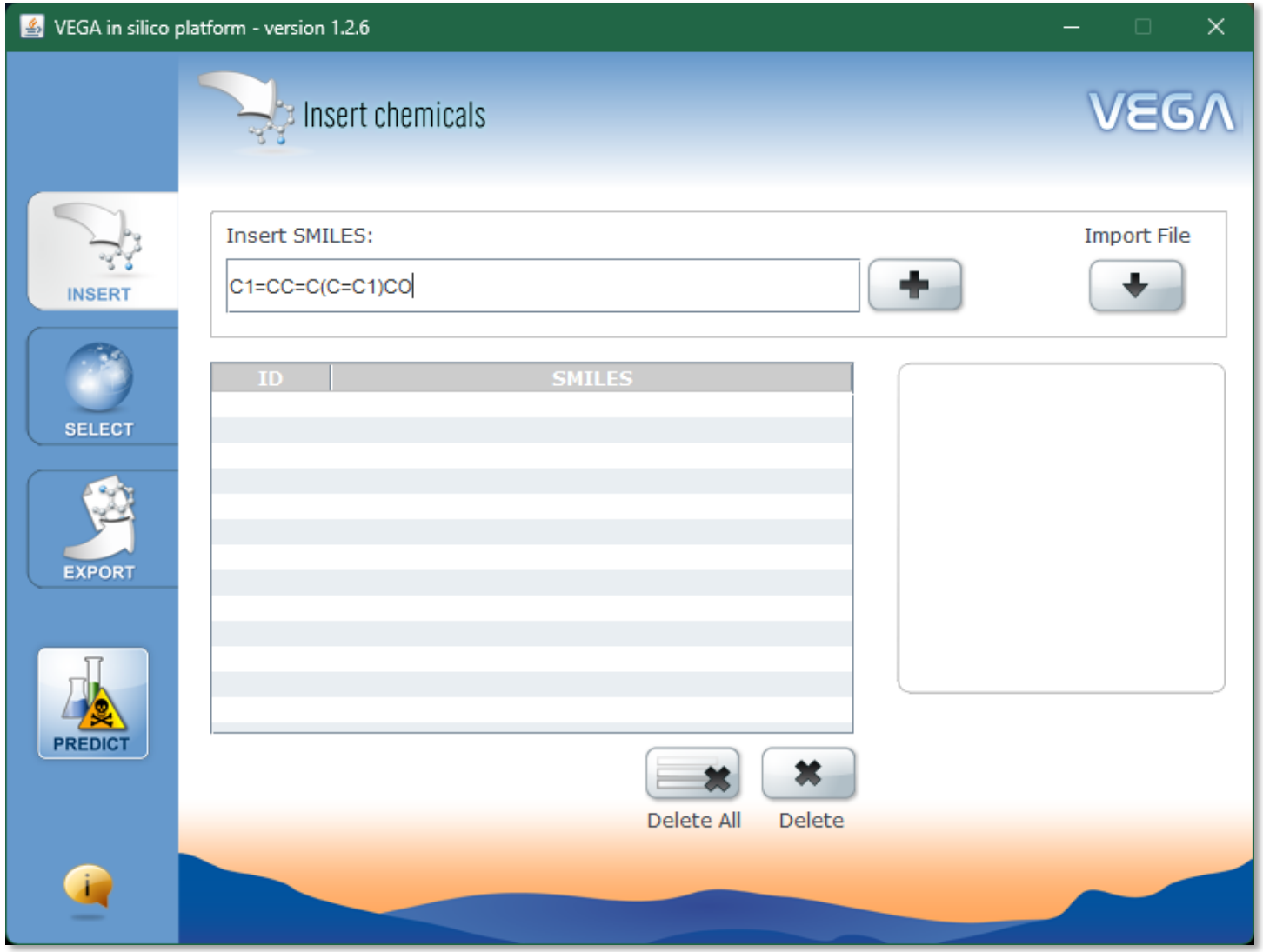
SMILES	VEGA_similarity	Experimental value mg/l	MGs	Alerts	Module
CCN1CCOCC1			NH0, Ndealkylation1, ether, morpholine	ToxRead37-Fish acute toxicity alert ToxRead37,	Target
OCCN1CCOCC1	0.906	2716.439	Al_OH, HOCCN, NH0, ether, morpholine	ToxRead37-Fish acute toxicity alert ToxRead37,	A, E
C1COCCN1	0.879	181.97	NH1, ether, morpholine	ToxRead37-Fish acute toxicity alert ToxRead37,	A, E
CCN(CC)CCO	0.854	1774.189	Al_OH, NH0, Ndealkylation1	ToxRead37-Fish acute toxicity alert ToxRead37,	A
OCCN1CCNCC1	0.806	6382.635	Al_OH, HOCCN, NH0, NH1, piperzine	ToxRead37-Fish acute toxicity alert ToxRead37,	A, E
CC(C)N(CCO)C(C)C	0.799	200.447	Al_OH, NH0	ToxRead37-Fish acute toxicity alert ToxRead37,	A
C1CN2CCN1CC2	0.792	1729.816	NH0, Al_bicycle, piperzine	ToxRead37-Fish acute toxicity alert ToxRead37,	A
CCN(CC)CC	0.774	23.878	NH0	ToxRead37-Fish acute toxicity alert ToxRead37,	E
CCOC(CN(C)CC(OCC)OCC)OCC	0.734	632.412	NH0, Ndealkylation1, ether	ToxRead37-Fish acute toxicity alert ToxRead37,	E
CN(C)CCN(C)CCN(C)C	0.698	109.648	NH0, Ndealkylation1	ToxRead37-Fish acute toxicity alert ToxRead37,	E
CCOc1cc(N2CCOCC2)c(OCC)cc1N	0.586	21.928	ArN, NH0, aniline, ether, morpholine, aniline_term	ToxRead37-Fish acute toxicity alert ToxRead37, ToxRead39-Fish acute toxicity alert ToxRead39,	E

(Q)SAR (Quantitative) structure-activity relationship

➡ estimates a property/activity using an **equation**

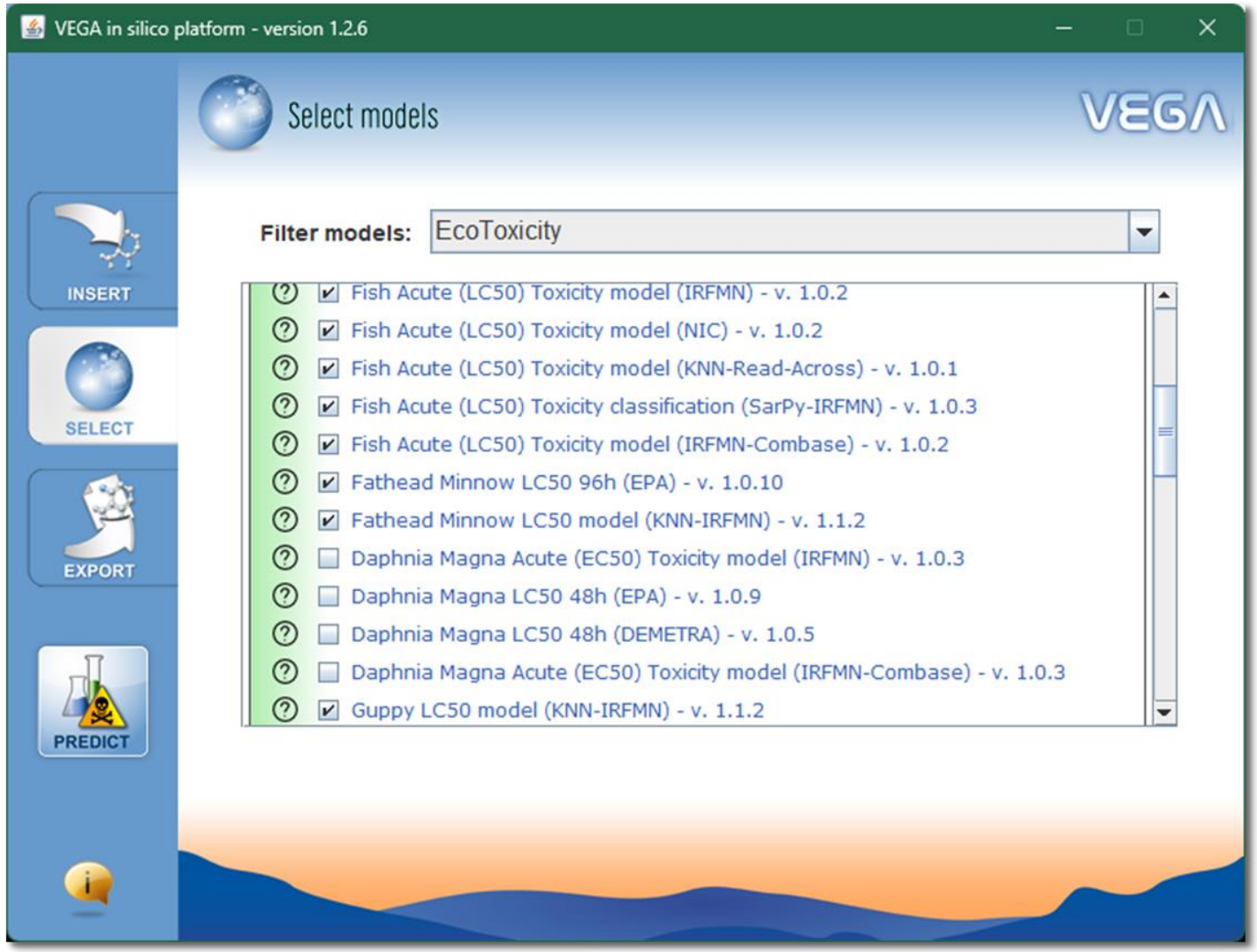


VEGA QSAR



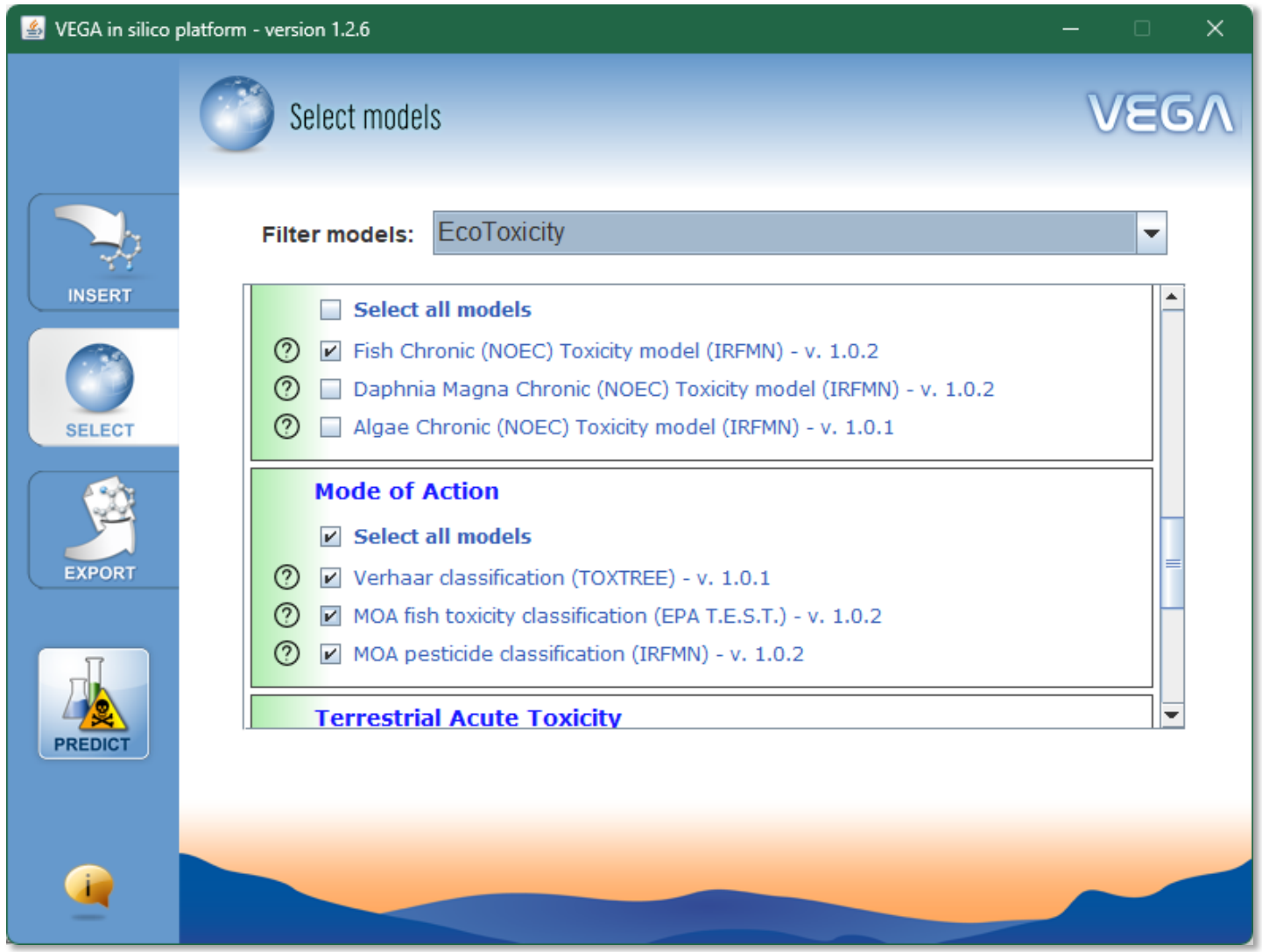
- 130+ models
- Toxicological, **ecotox**, environmental, phys-chem, toxicokinetic
- Applicability domain
- QMRF (https://www.vegahub.eu/portfolio-item/vega-qsar-models-qrmf/)

VEGA QSAR



- 5 **general models** for fish (1 in classification)
- 3 **species-specific** models

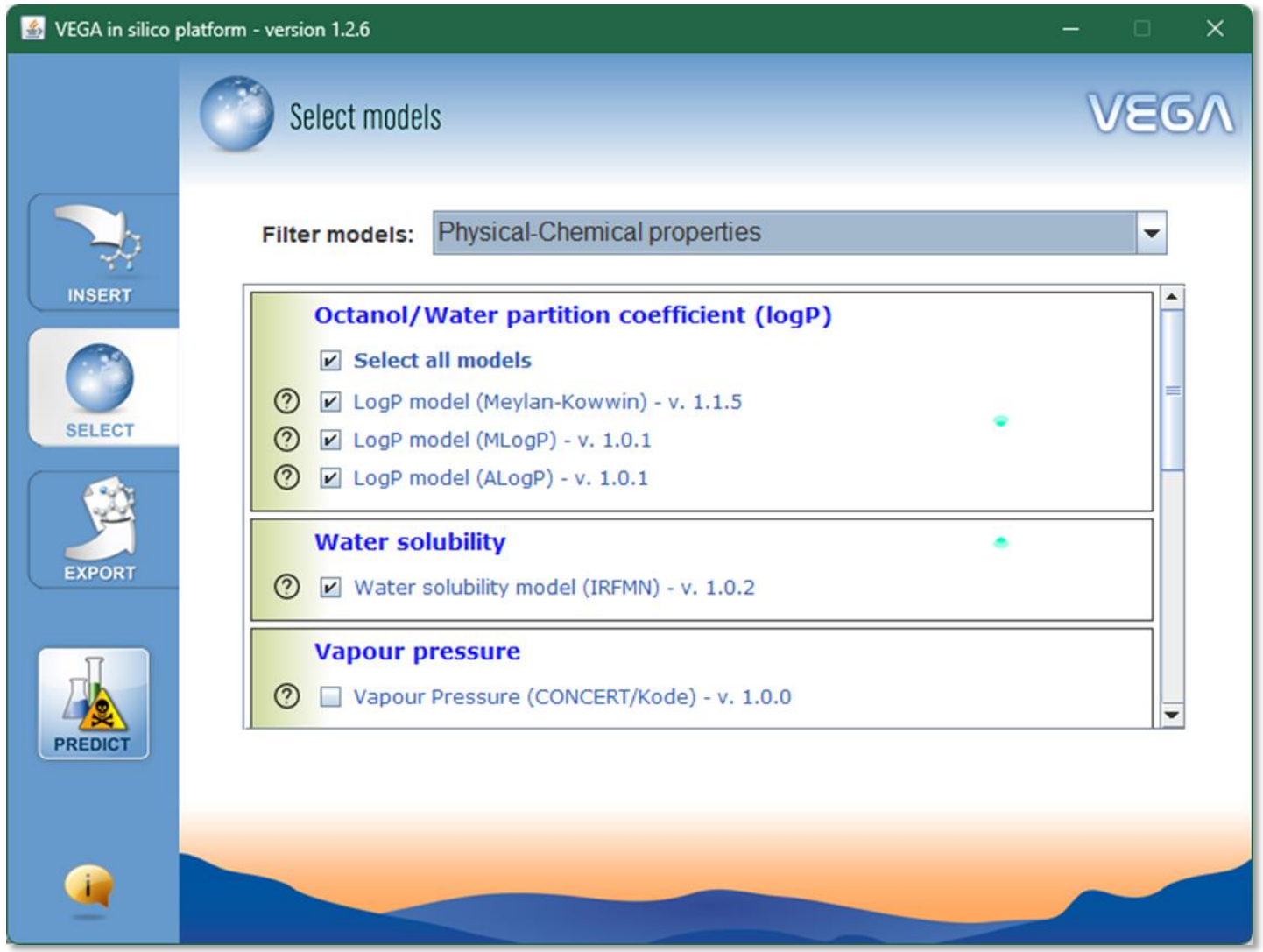
VEGA QSAR



Additional information

- Fish chronic toxicity
- MoA

VEGA QSAR



Additional information

- Fish chronic toxicity
- MoA
- Log Kow
- Water solubility

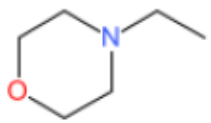
The PDF output

Summary page

- *Target*
- *Prediction*
- *Reliability*
- *Experimental value (if available)*
- *Eventual remarks*

1. Prediction Summary

Prediction for compound Molecule 1 -



Prediction: Reliability:

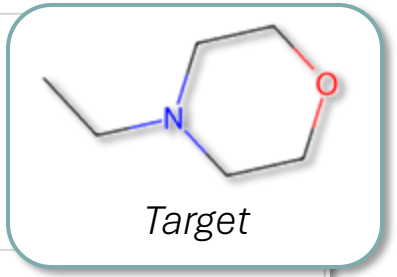
Prediction is 12.76 mg/L, but the result shows some critical aspects, which require to be checked:
- Only moderately similar compounds with known experimental value in the training set have been found

Compound: Molecule 1
Compound SMILES: O1CCN(CC)CC1
Experimental value: -
Predicted LC50 [a-dimensional]: -1.95
Predicted LC50 [mg/l]: 12.76
Molecular Weight: 115.2
Experimental value [mg/l]: -
Reliability: The predicted compound could be out of the Applicability Domain of the model
Remarks:
none

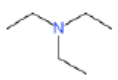
The PDF output

The six most similar chemicals

- *CAS and SMILES*
- *Training or test set*
- *VEGA similarity*
- *Experimental and predicted values*

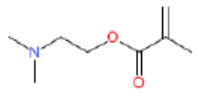


Target



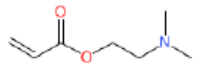
Compound #1

CAS: 121-44-8
Dataset id:335 (Training Set)
SMILES: N(CC)(CC)CC
Similarity: 0.774
Experimental value : -1.33
Predicted value : -1.331



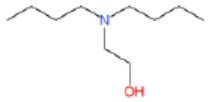
Compound #2

CAS: 2867-47-2
Dataset id:542 (Training Set)
SMILES: O=C(OCCN(C)C)C(=C)C
Similarity: 0.769
Experimental value : -1.885
Predicted value : -1.936



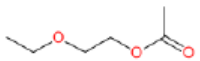
Compound #3

CAS: 2439-35-2
Dataset id:536 (Training Set)
SMILES: O=C(OCCN(C)C)C=C
Similarity: 0.766
Experimental value : -2.427
Predicted value : -2.204



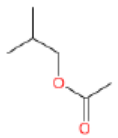
Compound #4

CAS: 102-81-8
Dataset id:215 (Training Set)
SMILES: OCCN(CCCC)CCCC
Similarity: 0.747
Experimental value : -1.622
Predicted value : -1.679



Compound #5

CAS: 111-15-9
Dataset id:288 (Training Set)
SMILES: O=C(OCCOCC)C
Similarity: 0.741
Experimental value : -1.076
Predicted value : -1.436



Compound #6

CAS: 110-19-0
Dataset id:271 (Training Set)
SMILES: O=C(OCC(C)C)C
Similarity: 0.737
Experimental value : -1.732
Predicted value : -1.935

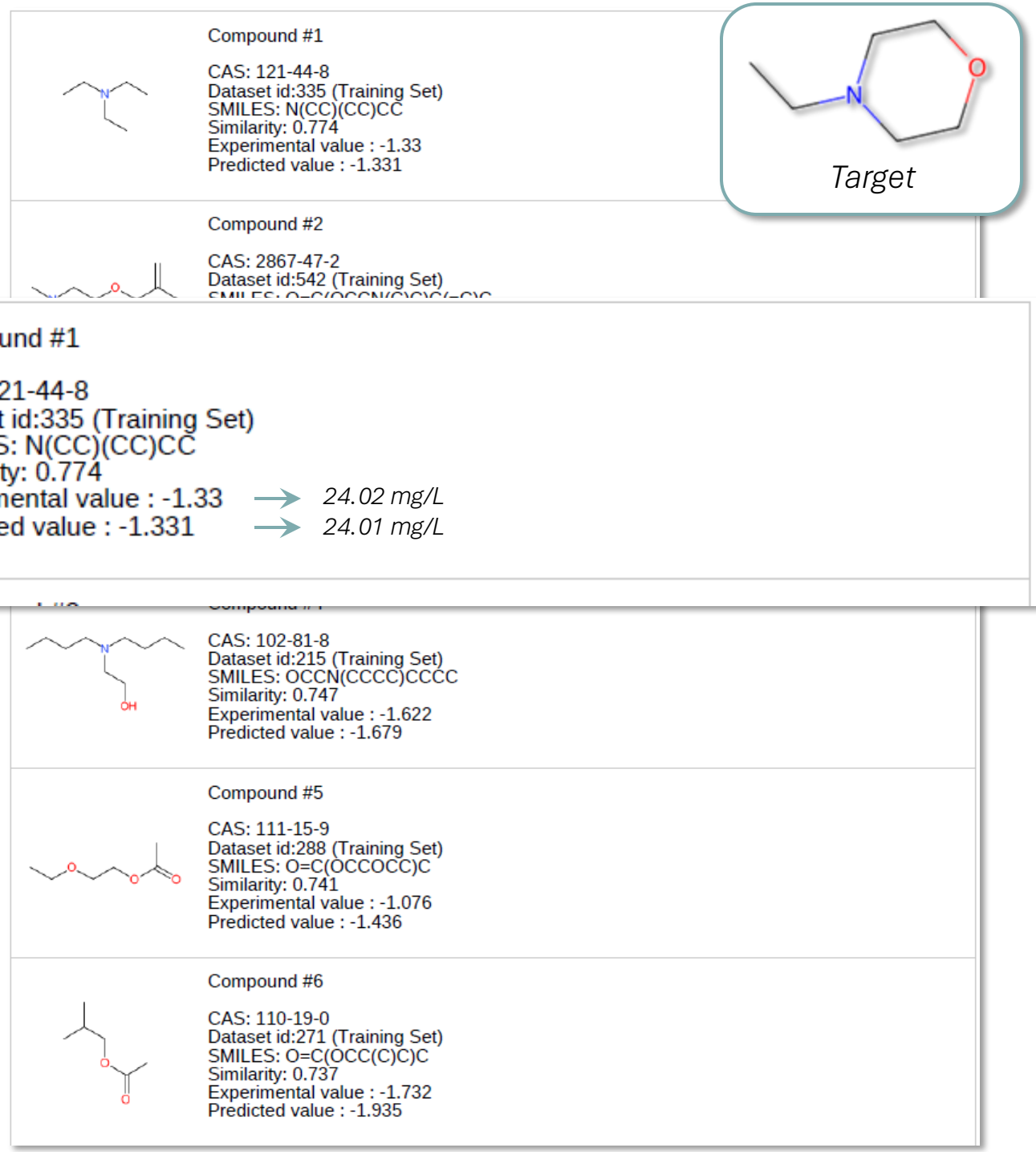
The PDF output

The six most similar chemicals

- *CAS and SMILES*
- *Training or test set*
- *VEGA similarity*
- *Experimental and predicted values*

Pay attention to the measure unit!!!

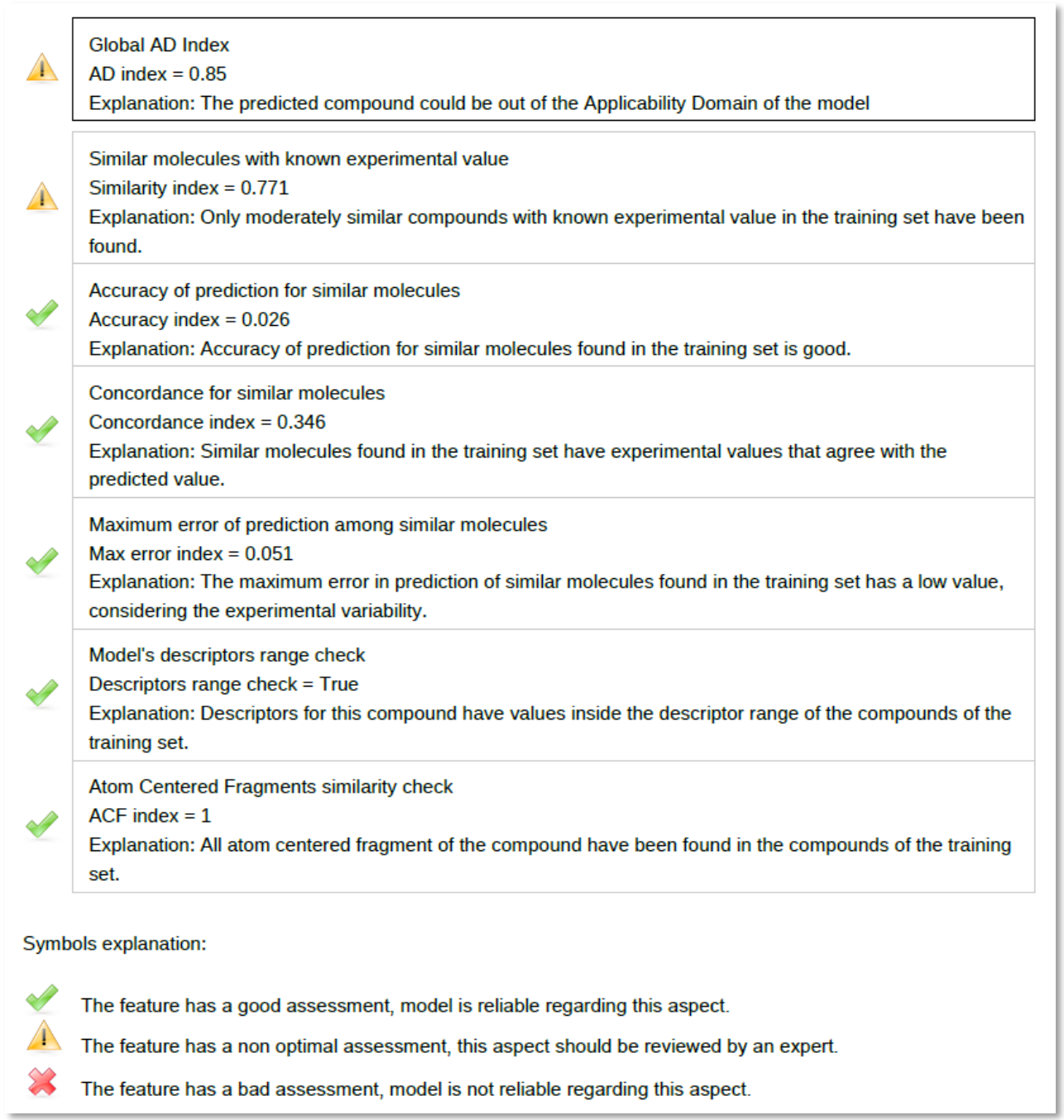
$$LD50_{mg/L} = \left((LD50_{a-dim} * 0.11 + 1)^{(1.0/0.11)}\right) * MW$$



The PDF output

The Applicability Domain Index (ADI)

- *Global AD index [0-1]*



Global AD Index
AD index = 0.85
Explanation: The predicted compound could be out of the Applicability Domain of the model

Similar molecules with known experimental value
Similarity index = 0.771
Explanation: Only moderately similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 0.026
Explanation: Accuracy of prediction for similar molecules found in the training set is good.

Concordance for similar molecules
Concordance index = 0.346
Explanation: Similar molecules found in the training set have experimental values that agree with the predicted value.

Maximum error of prediction among similar molecules
Max error index = 0.051
Explanation: The maximum error in prediction of similar molecules found in the training set has a low value, considering the experimental variability.

Model's descriptors range check
Descriptors range check = True
Explanation: Descriptors for this compound have values inside the descriptor range of the compounds of the training set.

Atom Centered Fragments similarity check
ACF index = 1
Explanation: All atom centered fragment of the compound have been found in the compounds of the training set.

Symbols explanation:

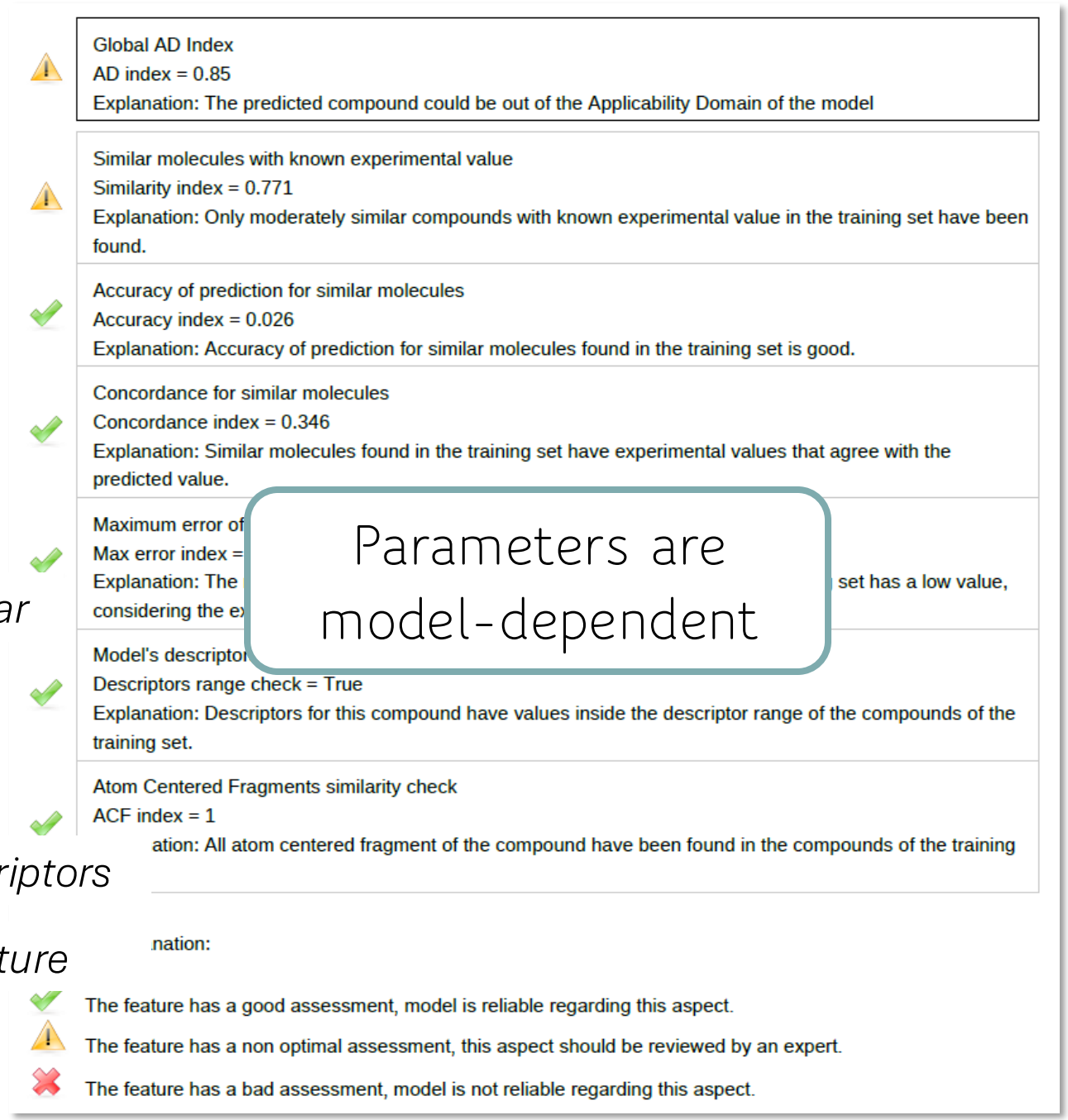
The feature has a good assessment, model is reliable regarding this aspect.
The feature has a non optimal assessment, this aspect should be reviewed by an expert.
The feature has a bad assessment, model is not reliable regarding this aspect.

The PDF output

The Applicability Domain Index (ADI)

- *Global AD index [0-1]*
  - *Similarity*
  - *Accuracy for similar*
  - *Concordance (target prediction – similar experimental)*
  - *Maximum error in prediction*

  Similar
  - *Descriptors range* — *Descriptors*
  - *Atom Centered Fragments* — *Structure*



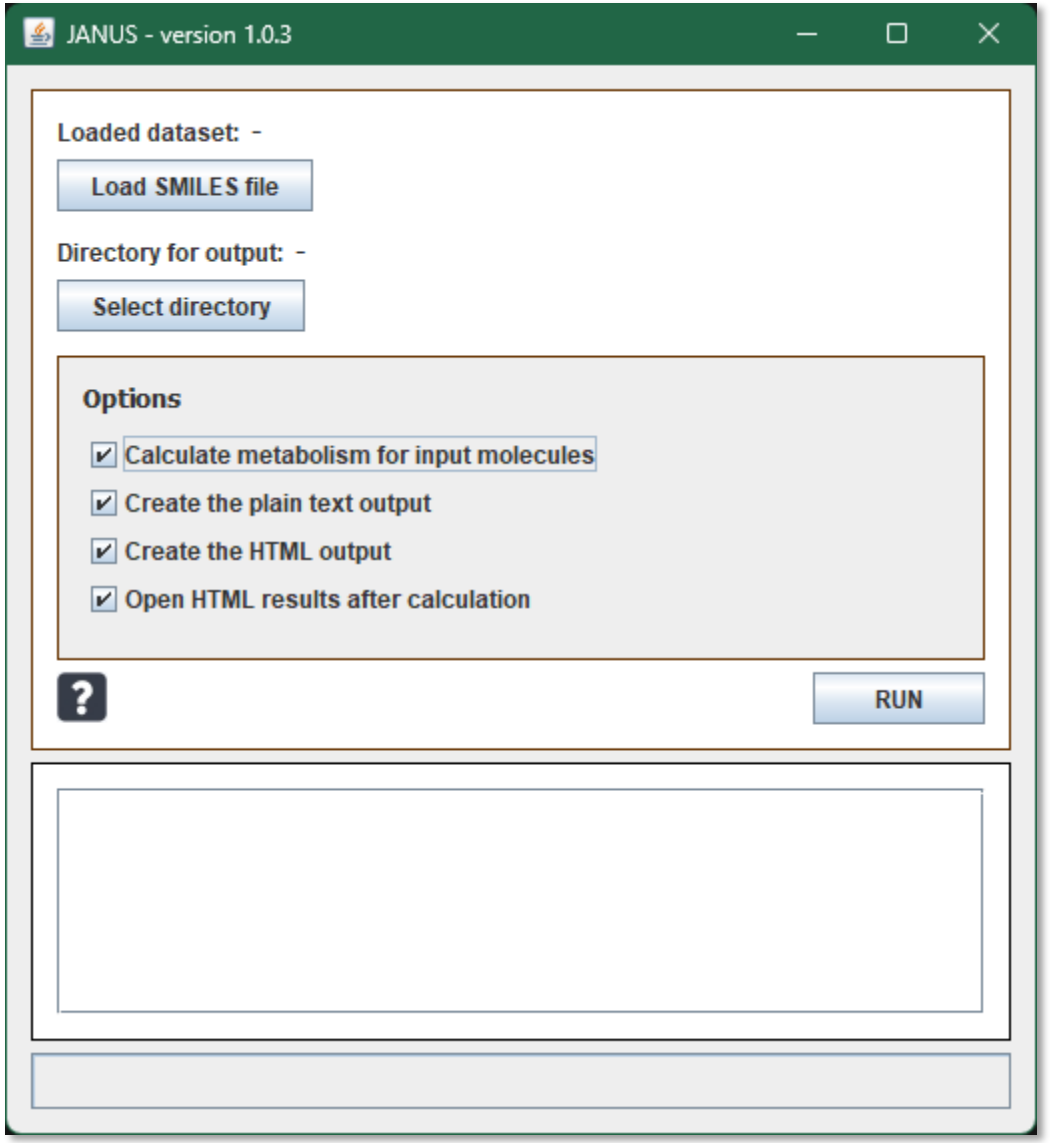
Parameters are model-dependent

Bundesministerium für Umwelt, Naturschutz und nukleare Sicherheit

Umwelt Bundesamt

The JANUS prioritization tool

Prioritization of chemical substances for **PBT**, **CMR** and **endocrine disrupting** activity



ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI · IRCCS

kode from data to knowledge

KnowledgeMiner Software

JANUS vs VEGA QSAR

- Prioritization
- Consensus
- Additional parameters (e.g. water solubility)
- Microbial metabolites*
- VEGA QSAR output more detailed

**Microbial biocatalytic reactions and biodegradation pathways from EAWAG Biocatalysis/Biodegradation Database (https://envipath.org).*

The HTML output

Janus Result

NUMBER OF COMPOUNDS **1**

PBT CMR ED PARTIAL SCORES FINAL SCORES

	No.	Metabolite	Id	SMILES	Label	P	rel.	score	B [log(L/kg)]	rel.	score	T [mg/l]	rel.	score	C	rel.	score	M	rel.	score	R	rel.	score	ED	rel.	score	Score(vPvB)	Score(SVHC)	Score(PBT)
	1		Molecule 1	O1CCN(CC)CC1	P B T - C M R E	**nP**	0.59	0.289	**0.05**	0.99	0.051	**5.77**	0.51	0.249	**NC**	0.8	0.142	**M**	1	1	**T**	0.6	0.887	**NA**	0.8	0.142	0.121	0.909	0.237

Export as plain text

Molecule ID: Molecule 1

Back to index

Molecule

Info

Molecule number: 1
Molecule id: Molecule 1
Molecule SMILES: O1CCN(CC)CC1

Note

SUMMARY

Persistence **nP** (reliability: 0.59)	Bioaccumulation **0.05** (reliability: 0.99)	Toxicity **5.77** (reliability: 0.51)
Carcinogenicity **NON Carcinogenic** (reliability: 0.8)	Mutagenicity **MUTAGENIC** (reliability: 1)	Reproductive toxicity **TOXICANT** (reliability: 0.6)
Endocrine Disruptor **Inactive** (reliability: 0.8)		

Toxicity assessment

Overall prediction [mg/l]	Overall Reliability
5.77	**0.51**

Values retrieved and/or calculated in the workflow:

Property	Value
Fish Acute (LC50) Toxicity model (KNN)	1039.26 mg/L (good reliability)
Fish Acute (LC50) Toxicity model (NIC)	155.59 mg/L (moderate reliability)
Fish Acute (LC50) Toxicity model (IRFMN)	12.7 mg/L (moderate reliability)
Fathead Minnow Acute (LC50) Toxicity model (EPA)	1831.3 mg/L (good reliability)
Fathead Minnow Acute (LC50) Toxicity model (KNN)	1083.88 mg/L (moderate reliability)
Guppy Acute (LC50) Toxicity model (KNN)	N/A
Fish Chronic (NOEC) Toxicity model (IRFMN)	5.77 mg/L (low reliability)
Daphnia Magna Acute (LC50) Toxicity model (DEMETRA)	12.31 mg/L (moderate reliability)
Daphnia Magna Acute (LC50) Toxicity model (EPA)	44.53 mg/L (low reliability)
Daphnia Magna Acute (LC50) Toxicity model (IRFMN)	195.47 mg/L (moderate reliability)
Daphnia Magna Chronic (NOEC) Toxicity model (IRFMN)	5.93 mg/L (moderate reliability)
Algae Acute (EC50) Toxicity model (IRFMN)	25.39 mg/L (moderate reliability)
Algae Chronic (NOEC) Toxicity model (IRFMN)	16.55 mg/L (low reliability)
Water Solubility model (IRFMN)	995697.81 mg/L (EXPERIMENTAL value)
Overall Fish Acute (LC50) Toxicity assessment (used in the workflow)	1379.6 mg/L (reliability: 0.83)
Overall Fish Chronic (NOEC) Toxicity assessment (used in the workflow)	5.77 mg/L (reliability: 0.45)
Overall Fish assessment (used in the workflow)	NON Toxic, 5.77 mg/L (reliability: 0.45)
Overall Daphnia Magna Acute (LC50) Toxicity assessment (used in the workflow)	103.9 mg/L (reliability: 0.68)
Overall Daphnia Magna Chronic (NOEC) Toxicity assessment (used in the workflow)	5.93 mg/L (reliability: 0.65)
Overall Daphnia Magna assessment (used in the workflow)	NON Toxic, 5.93 mg/L (reliability: 0.65)
Overall Algae Acute (EC50) Toxicity assessment (used in the workflow)	25.4 mg/L (reliability: 0.65)
Overall Algae Chronic (NOEC) Toxicity assessment (used in the workflow)	16.6 mg/L (reliability: 0.45)
Overall Algae assessment (used in the workflow)	NON Toxic, 16.6 mg/L (reliability: 0.45)

Janus Result

NUMBER OF COMPOUN

FINAL SCORES

	No.	Metaboli	VHC)	Score(PBT)
	1			0.237

Export as plain text

Molecule ID: Molecule 1

Back to index

Molecule

Info

Molecule number: 1
Molecule id: Molecule 1
Molecule SMILES: O1CCN(CC)CC1

Note

SUMMARY

Persistence
nP (reliability: 0.59)

Bioaccumulation
0.05 (reliability: 0.99)

Toxicity
5.77 (reliability: 0.51)

Carcinogenicity
NON Carcinogenic (reliability: 0.8)

Mutagenicity
MUTAGENIC (reliability: 1)

Reproductive toxicity
TOXICANT (reliability: 0.6)

Janus Result

NUMBER OF COMPOUN

FINAL SCORES

	No.	Metaboli	VHC)	Score(PBT)
	1			0.237

Toxicity assessment

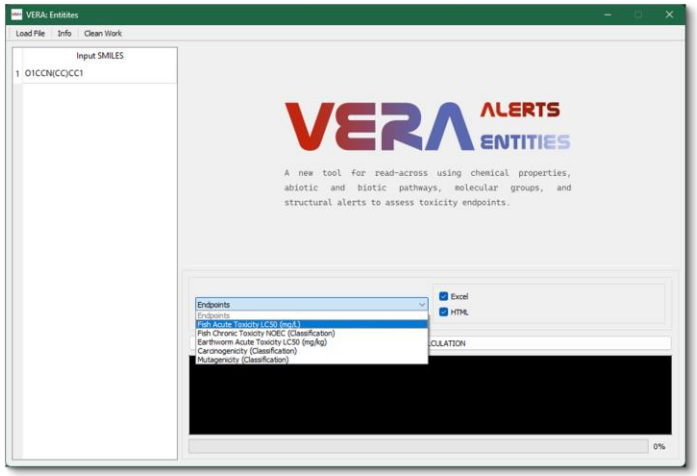
Values retrieved and/or calculated in the workflow:

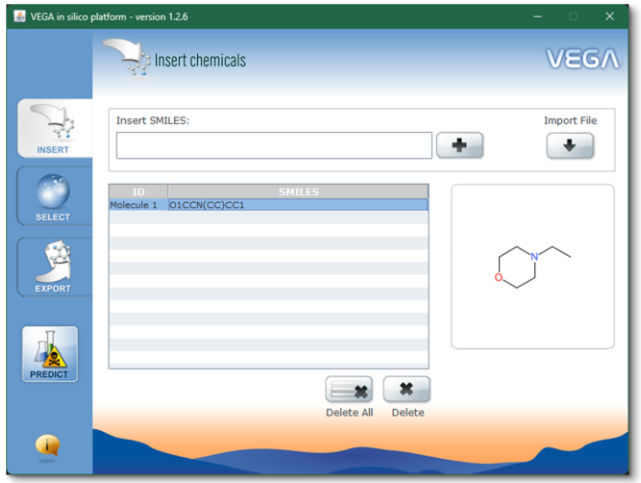
Property	Value
Fish Acute (LC50) Toxicity model (KNN)	1039.26 mg/L (good reliability)
Fish Acute (LC50) Toxicity model (NIC)	155.59 mg/L (moderate reliability)
Fish Acute (LC50) Toxicity model (IRFMN)	12.7 mg/L (moderate reliability)
Fathead Minnow Acute (LC50) Toxicity model (EPA)	1831.3 mg/L (good reliability)
Fathead Minnow Acute (LC50) Toxicity model (KNN)	1083.88 mg/L (moderate reliability)
Guppy Acute (LC50) Toxicity model (KNN)	N/A
Water Solubility model (IRFMN)	995697.81 mg/L (EXPERIMENTAL value)
Overall Fish Acute (LC50) Toxicity assessment (used in the workflow)	1379.6 mg/L (reliability: 0.83)
Algae Acute (EC50) Toxicity model (IRFMN)	25.39 mg/L (moderate reliability)
Algae Chronic (NOEC) Toxicity model (IRFMN)	16.55 mg/L (low reliability)
Overall Daphnia Magna Chronic (NOEC) Toxicity assessment (used in the workflow)	5.93 mg/L (reliability: 0.65)
Overall Daphnia Magna assessment (used in the workflow)	NON Toxic, 5.93 mg/L (reliability: 0.65)
Overall Algae Acute (EC50) Toxicity assessment (used in the workflow)	25.4 mg/L (reliability: 0.65)
Overall Algae Chronic (NOEC) Toxicity assessment (used in the workflow)	16.6 mg/L (reliability: 0.45)
Overall Algae assessment (used in the workflow)	NON Toxic, 16.6 mg/L (reliability: 0.45)

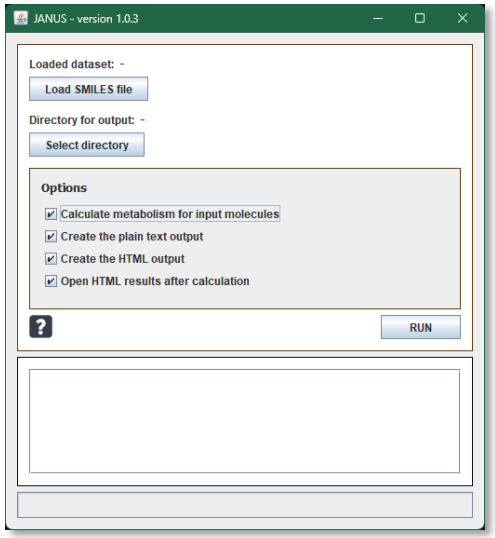
Export as plain text

Take-home message

Several ***in silico*** methods can be used to assess **fish acute toxicity**







Automatic read-across

Multiple approaches for similar selection

Reliability

QSAR models

ADI

Similar chemicals

Prioritization

Consensus (VEGA models)

Microbial metabolites

Next future: **SESAPI** (for pharmaceuticals)

Thank you for your attention!

anna.lombardo@marionegri.it

https://www.vegahub.eu/

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FARMACOLOGICHE
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