IN SILICO MODELS FOR THE REACH AND THE FOOD REGULATION : PERSPECTIVES FOR THE NEAR FUTURE

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WHICH REGULATIONS?

- **REACH** (industrial chemicals); ECHA
- **Food** (and related substances: ingredients, additivies, contaminants, pesticides, veterinary products, feed); EFSA
- Pharmaceuticals; EMA
- Cosmetics products; SCCS
- Biocides; ECHA
- Pollutants; EEA
- …

NB: Ingredients are under REACH

CONTEXT FOR IN SILICO

Assessment of substances

This covers:

- Hazard
- Environmental properties
- Phys-chem
- Toxicokinetics
- Exposure (internal and external)
- Risk assessment

NB: Other features not covered by in silico (substance identification, registrant, etc.)

1S1A

NB: Europe is moving towards one substance - one assessment

Individual regulations will remain, but much better harmonisation, and one single database

GHS - CLP

REGULATIONS AND IN SILICO MODELS (i)

In silico models can be used for different purposes
Different models are preferable for different purposes (assessment: conservative; prioritization; balanced)
The documentation should clarify the context and intended use

Not covered today:

In silico models for «**research**» (strange endpoints, new approaches, etc.)
In silico models for **R&D** for industrial purposes (different properties, confidential data, etc.)

REGULATIONS AND IN SILICO MODELS (ii)

- Cosmetics products (SCCS): **NO** in vivo data
- Food (EFSA): moving towards NAMs (new alternative methodologies); in vivo data on parental compound; in silico can be used for degradation products, etc.
- REACH / Biocides (ECHA): in silico can be used (a few percent of registered substances; 25% read across; < 30% experimental data)
- Pharmaceuticals (EMA): in vivo requested; in silico for impurities

- In silico for prioritization

REACH

About 50 different properties:

- Phys-chem
- Environ
- Ecotox
- Tox

Different requests depending on the tonnage of substance on the market (from 1 tonn/year, up; if > 1000 all properties)

QSAR: key study or weight-of-evidence

Weight of evidence (WoE): EFSA Guidance

SCIENTIFIC OPINION

EFSA Journal

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Guidance on the use of the weight of evidence approach in scientific assessments

EFSA Scientific Committee,
Anthony Hardy, Diane Benford, Thorhallur Halldorsson, Michael John Jeger,
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Geoff Frampton, Matthias Greiner, Andrew Hart, Christer Hogstrand, Claude Lambre,
Robert Luttik, David Makowski, Alfonso Siani, Helene Wahlstroem, Jaime Aguilera,
Jean-Lou Dorne, Antonio Fernandez Dumont, Michaela Hempen, Silvia Valtueña Martínez,
Laura Martino, Camilla Smeraldi, Andrea Terron, Nikolaos Georgiadis and Maged Younes

https://www.efsa.europa.eu/en/efsajournal/pub/4971

EFSA Guidance on WoE

Approach for WoE

1. Gather all info
2. Evaluate individual lines of evidence
3. Integrate the results

EFSA Guidance: integration

Criteria for integration

1. Relevance
2. Reliability
3. Agreement

In silico and read-across: integration

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journal homepage: www.elsevier.com/locate/envint

Review article

Integrating *in silico* models and read-across methods for predicting toxicity of chemicals: A step-wise strategy

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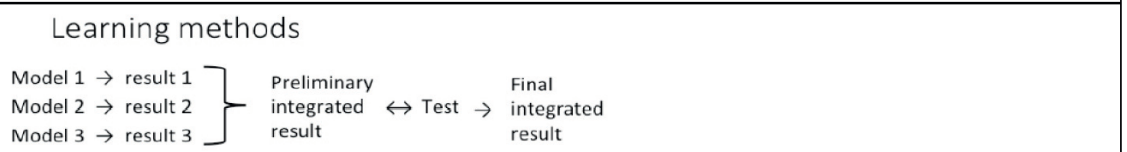
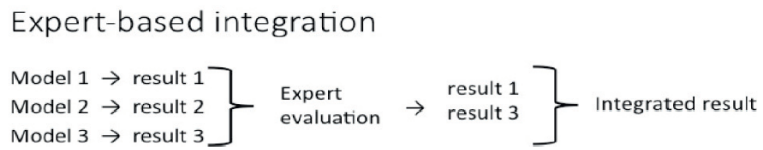
ABSTRACT

In silico methods and models are increasingly used for predicting properties of chemicals for hazard identification and hazard characterisation in the absence of experimental toxicity data. Many *in silico* models are available and can be used individually or in an integrated fashion. Whilst such models offer major benefits to toxicologists, risk assessors and the global scientific community, the lack of a consistent framework for the integration of *in silico* results can lead to uncertainty and even contradictions across models and users, even for the same chemicals. In

Integration of in silico

Algebraic and voting methods	
Weighing	
Hybrid	
Learning	
Expert-based	

Algebraic methods

Majority vote

Unanimity

Worst case

All models at the same level of reliability

Or you introduce thresholds (in / out: 2 levels or reliability)

Weighing methods

VEGA and mutagenicity is an example

Use of all models, in a quantitative way

(not in or out, binary, qualitative approach)

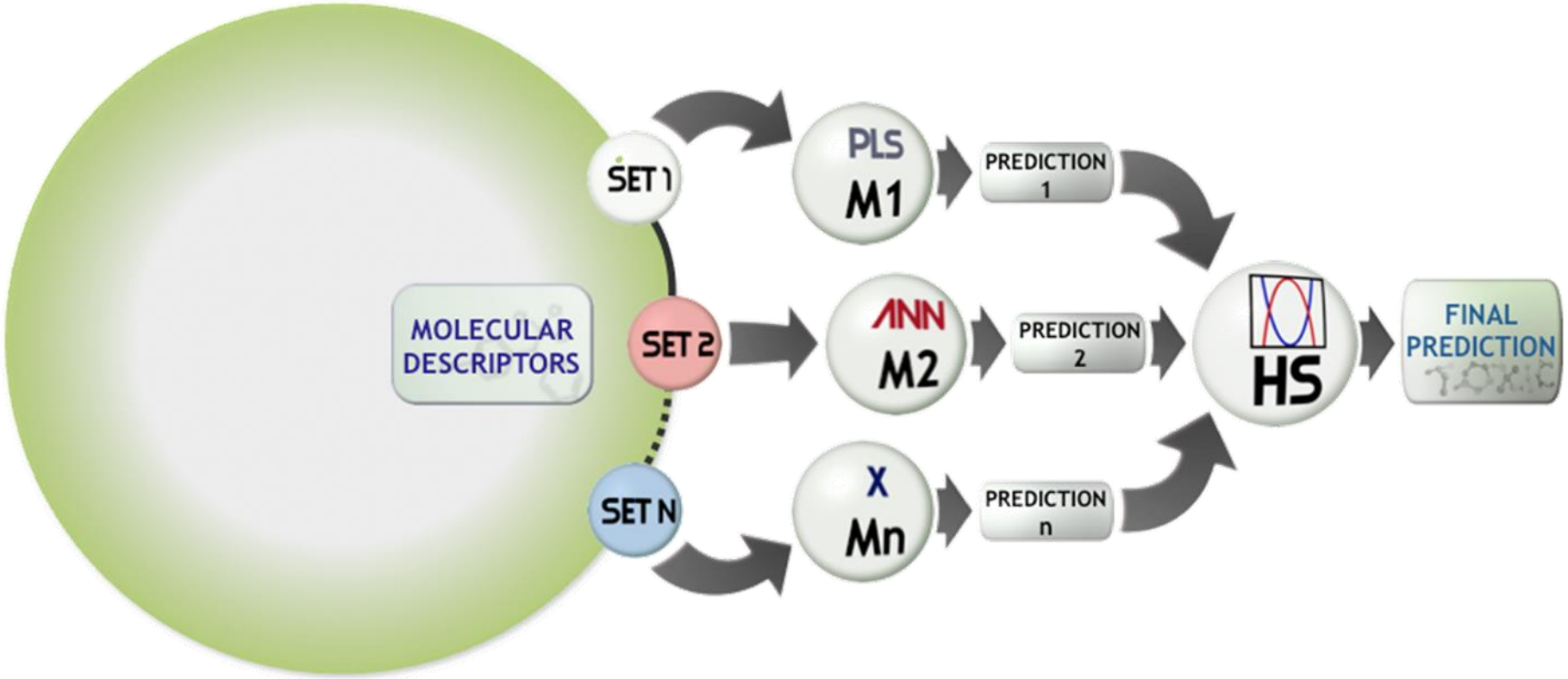
Consensus model (CNS-VEGA) : CAESAR + SARPY + TT-VEGA

$$\mathrm{CONSENSUS} = \frac{(\pm 1) * \mathrm{AD}_{\mathrm{CAESAR}} + (\pm 1) * \mathrm{AD}_{\mathrm{SARPY}} + (\pm 1) * \mathrm{AD}_{\mathrm{TTVEGA}}}{\mathrm{AD}_{\mathrm{CAESAR}} + \mathrm{AD}_{\mathrm{SARPY}} + \mathrm{AD}_{\mathrm{TTVEGA}}}$$

Algorithm extended now to 4 models

Hybrid models

The 5 CAESAR models in VEGA are hybrid models



Learning methods

Hybrid models are planned since their beginning to be within one single system

Learning methods takes pre-existing models, integrate them, and finds the best way to assemble them, ideally using a test set for this purpose.

The test set has to contain new substances, never used by any of the pre-existing models. This is often very difficult.

Expert-based methods

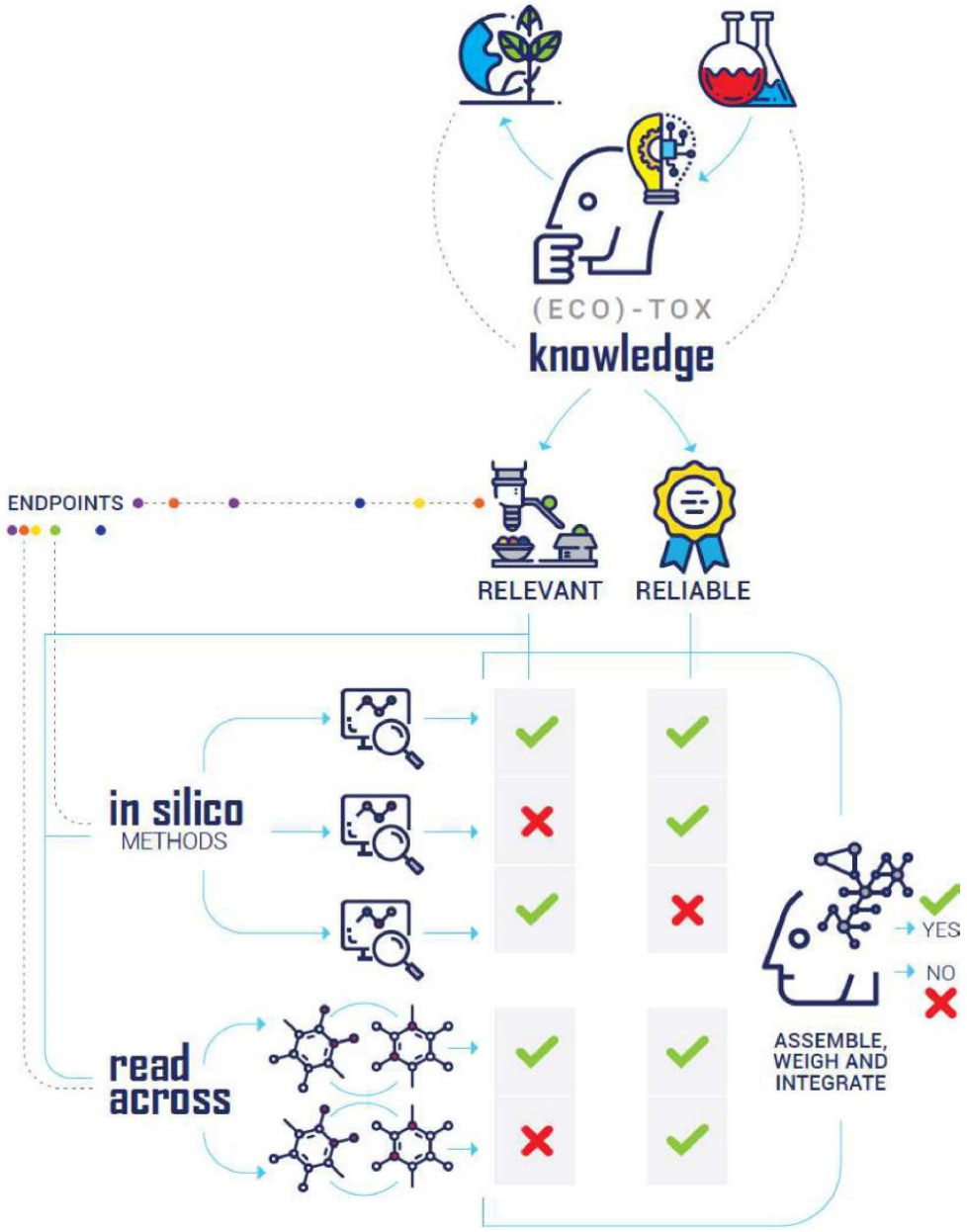
Experts may identify a preferred way to integrate results.

Pragmatic approach.

Often combining some criteria for reasoning, and introducing thresholds, and conservative assumptions.

Thus, the criteria are not only statistical. They should be declared.

Integrating in silico and read-across

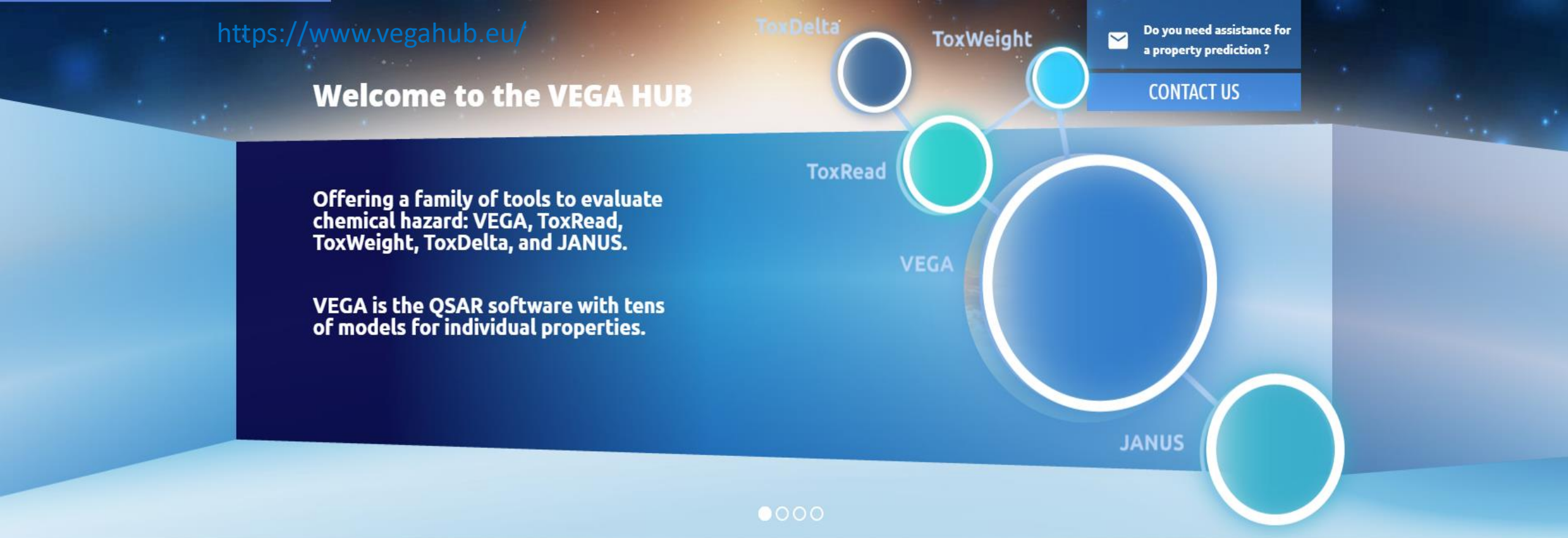


Use all lines of evidence

1. VEGA in silico models
2. Read-across
3. Reasoning

- Check agreement

https://www.vegahub.eu/



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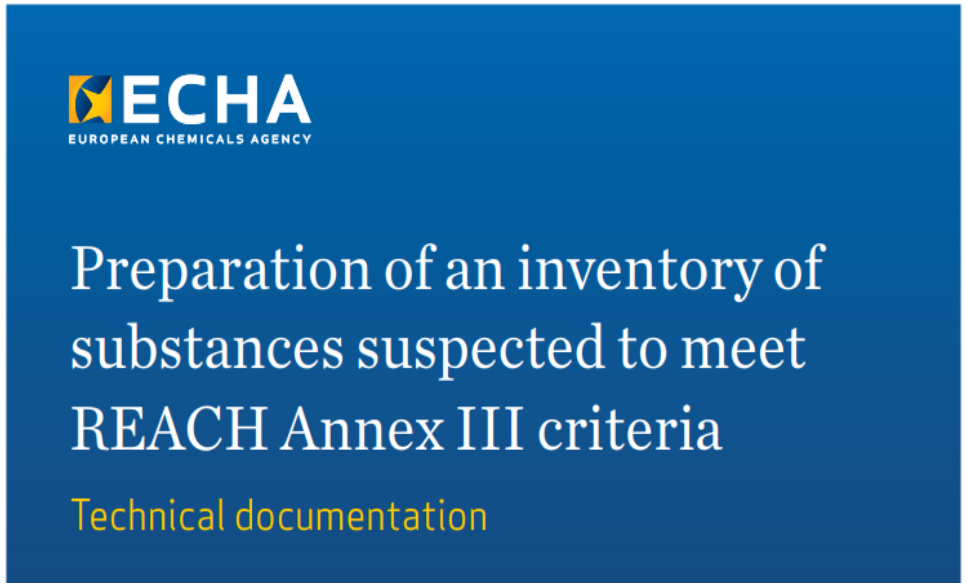
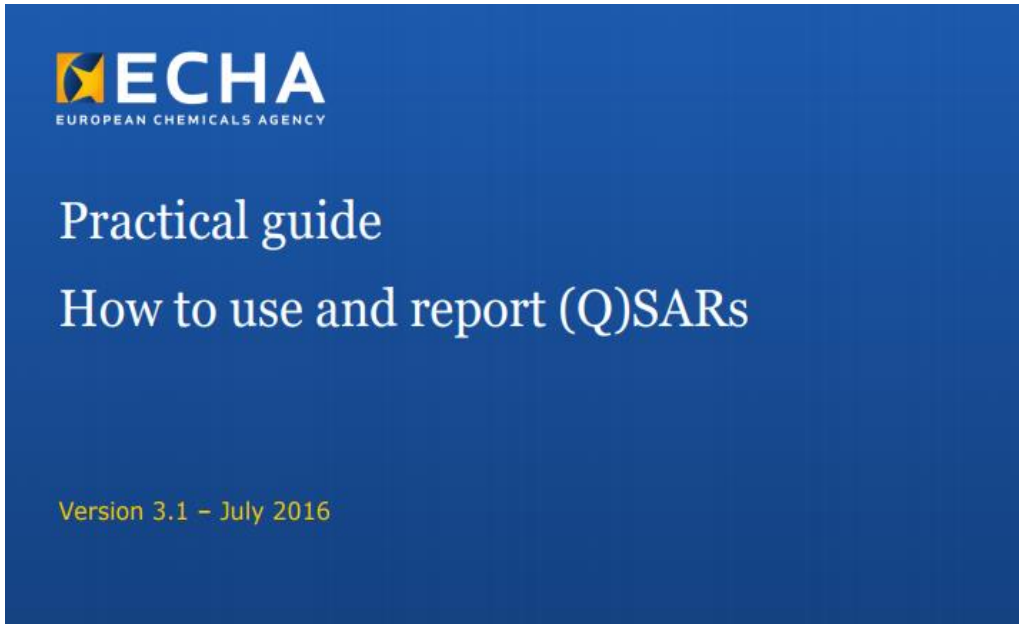
Our philosophy

The In silico methods can be very useful, if correctly applied

VEGA and ECHA





EFSA and OECD QSAR Toolbox

VEGA linked to the OECD QASR Toolbox
From OECD TB you can make the predictions using the VEGA models

However:

- No access to the graphical info for the ADI
- Less models (57 not 80)

EFSA and VEGA

SCIENTIFIC OPINION

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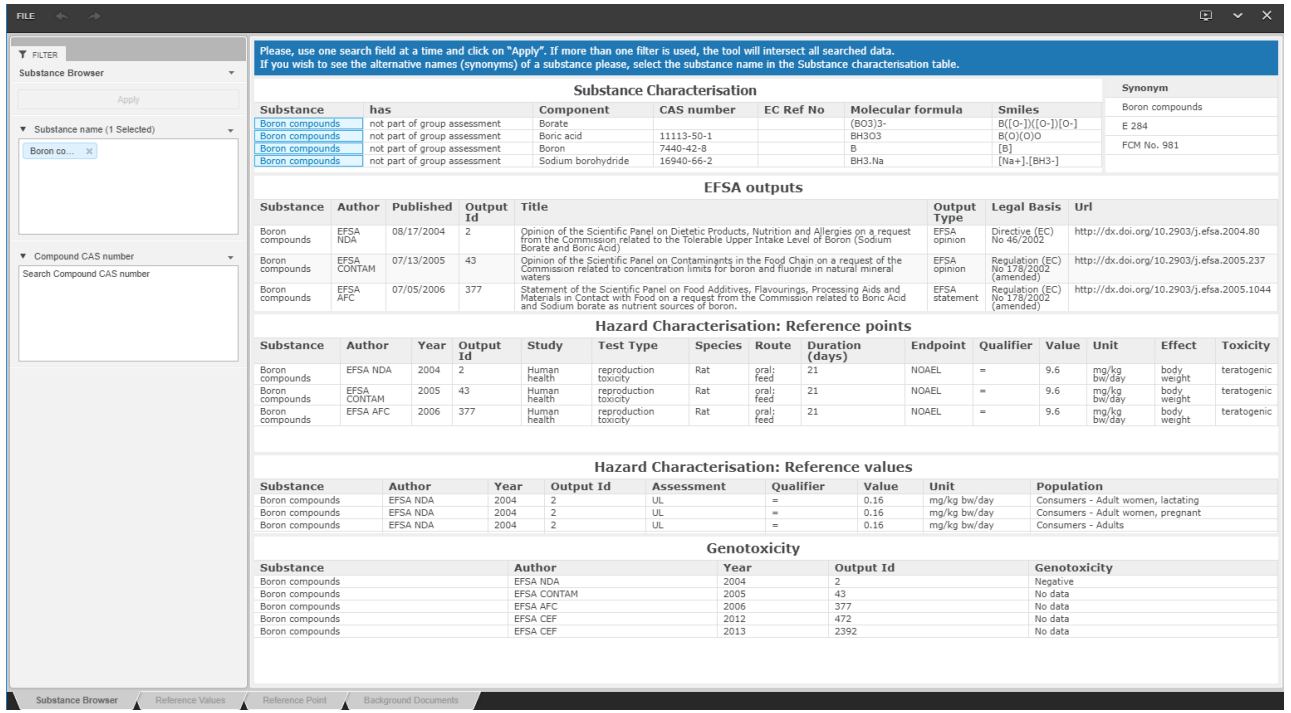


EFSA and CEFIC and Danish (Q)SAR DB

CEFIC: AMBIT

ADI parameters

- ✓ **Visualization of similar substances**
- ✓ **Similarity index** *(chemical; sub-indices)*
- ✓ **Chemometric check** *(descriptor space)*
- ✓ **Atom centered-fragment** *(chemical)*

Chemical input space

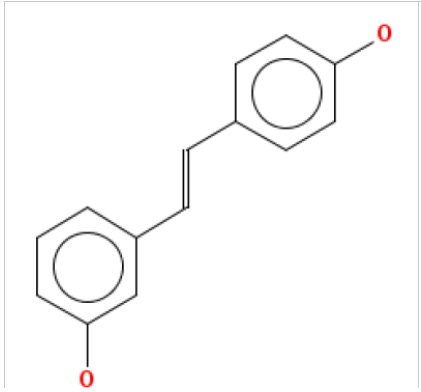
- ✓ **Check of the descriptor sensitivity** *(algorithm)*
- ✓ **Uncertainty** *(algorithm)*

Characteristics of the algorithm

- ✓ **Fragments for outliers** *(output space)*
- ✓ **Prediction Accuracy** *(output space)*
- ✓ **Prediction Concordance** *(tox exploration)*

Input and output toxicological space

ADI concordance



Prediction: Reliability:

Prediction is Possible NON-Mutagenic, but the result shows some critical aspects, which require to be checked:
- similar molecules found in the training set have experimental values that disagree with the predicted value

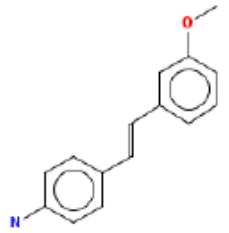
Global AD Index
AD index = 0.719
Explanation: the predicted compound could be out of the Applicability Domain of the model.

Similar molecules with known experimental value
Similarity index = 0.901
Explanation: strongly similar compounds with known experimental value in the training set have been found.

Accuracy of prediction for similar molecules
Accuracy index = 1
Explanation: accuracy of prediction for similar molecules found in the training set is good.

Concordance for similar molecules
Concordance index = 0.33
Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.

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.

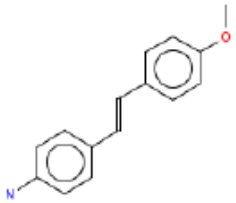


Compound #1

CAS: 154028-32-7
Dataset id: 2989 (Training set)
SMILES: O(c2cccc(C=Cc1ccc(N)cc1)c2)C
Similarity: 0.907

Experimental value: Mutagenic
Predicted value: Mutagenic

Alerts (not found in the target): SM44; SM104

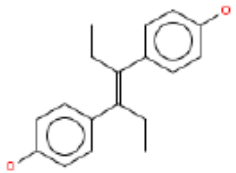


Compound #2

CAS: 7570-37-8
Dataset id: 1345 (Training set)
SMILES: O(c1ccc(cc1)C=Cc2ccc(N)cc2)C
Similarity: 0.905

Experimental value: Mutagenic
Predicted value: Mutagenic

Alerts (not found in the target): SM44; SM104

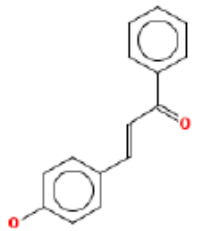


Compound #3

CAS: 56-53-1
Dataset id: 2731 (Test set)
SMILES: Oc1ccc(cc1)C(=C(c2ccc(O)cc2)CC)CC
Similarity: 0.893

Experimental value: NON-Mutagenic
Predicted value: NON-Mutagenic

Alerts (not found in the target): SM158



Compound #4

CAS: 20426-12-4
Dataset id: 561 (Test set)
SMILES: O=C(C=Cc1ccc(O)cc1)c2ccccc2
Similarity: 0.888

Experimental value: NON-Mutagenic
Predicted value: NON-Mutagenic

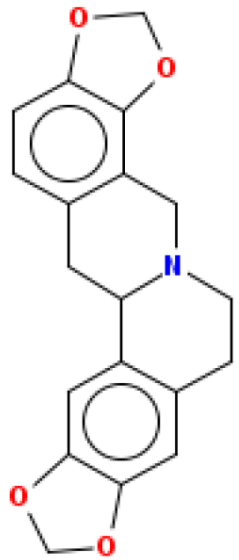
WoE mutagenicity

In silico model higher reliability than initial

Read-across: choise based on relevance

Reasoning about mechanism used

Elements of warning indicated by VEGA appropriate



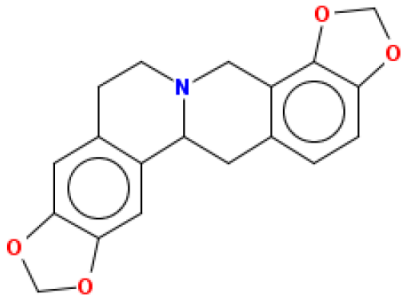
Prediction:

Prediction is NON-Mutagenic with a consensus score of 0.5, based on 4 models.

Compound: Molecule 0
Compound SMILES: O1c2ccc5c(c2(OC1))CN4CCc3cc6OCOc6(cc3C4C5)
Used models: 4
Predicted Consensus Mutagen activity: NON-Mutagenic
Mutagenic Score: 0
Non-Mutagenic Score: 0.5
Model Caesar assessment: NON-Mutagenic (low reliability)
Model ISS assessment: NON-Mutagenic (moderate reliability)
Model SarPy assessment: NON-Mutagenic (moderate reliability)
Model KNN assessment: NON-Mutagenic (moderate reliability)

1. Prediction Summary

Prediction for compound Molecule 0



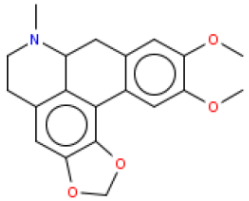
Prediction: Reliability:

Prediction is NON-Mutagenic, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues:
- accuracy of prediction for similar molecules found in the training set is not adequate
- similar molecules found in the training set have experimental values that disagree with the predicted value

Compound: Molecule 0
Compound SMILES: O1c2ccc5c(c2(OC1))CN4CCc3cc6OCOc6(cc3C4C5)
Experimental value: -
Predicted Mutagen activity: NON-Mutagenic
Structural alerts: -
Reliability: the predicted compound is outside the Applicability Domain of the model
Remarks:
none

3.1 Applicability Domain:

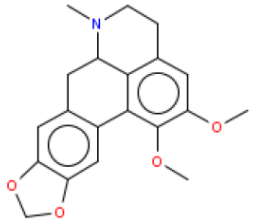
Similar Compounds, with Predicted and Experimental Values



Compound #1

CAS: 517-66-8
Dataset id: 1275 (Training set)
SMILES: O(c3cc4c1c5OCOc5(cc2c1C(N(C)CC2)Cc4(cc3(OC))))C
Similarity: 0.909

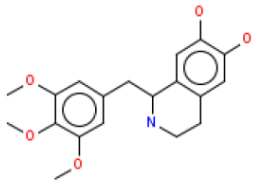
Experimental value: Mutagenic
Predicted value: NON-Mutagenic



Compound #2

CAS: 2565-01-7
Dataset id: 2067 (Training set)
SMILES: O(c1cc4c3c(c1(OC))c2cc5OCOc5(cc2CC3N(C)CC4))C
Similarity: 0.908

Experimental value: Mutagenic
Predicted value: NON-Mutagenic



Compound #3

CAS: 30418-38-3
Dataset id: 4202 (Training set)
SMILES: Oc1cc2c(cc1(O))C(NCC2)Cc3cc(OC)c(OC)c(OC)c3
Similarity: 0.898

Experimental value: NON-Mutagenic
Predicted value: NON-Mutagenic

3.2 Applicability Domain:
Measured Applicability Domain Scores

✗	**Global AD Index** AD index = 0.547 Explanation: the predicted compound is outside the Applicability Domain of the model.
✓	**Similar molecules with known experimental value** Similarity index = 0.905 Explanation: strongly similar compounds with known experimental value in the training set have been found.
✗	**Accuracy of prediction for similar molecules** Accuracy index = 0.33 Explanation: accuracy of prediction for similar molecules found in the training set is not adequate.
✗	**Concordance for similar molecules** Concordance index = 0.33 Explanation: similar molecules found in the training set have experimental values that disagree with the predicted value.
✓	**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

STYLOPINE mutagenicity

Read-across prevails

Not possible to exclude mutagenicity of stylopine

In silico models contradicted by the similar compounds

Elements of warning indicated by VEGA appropriate

FOOD: THE VERMEER-FCM MODEL

EXPOSURE X HAZARD = RISK

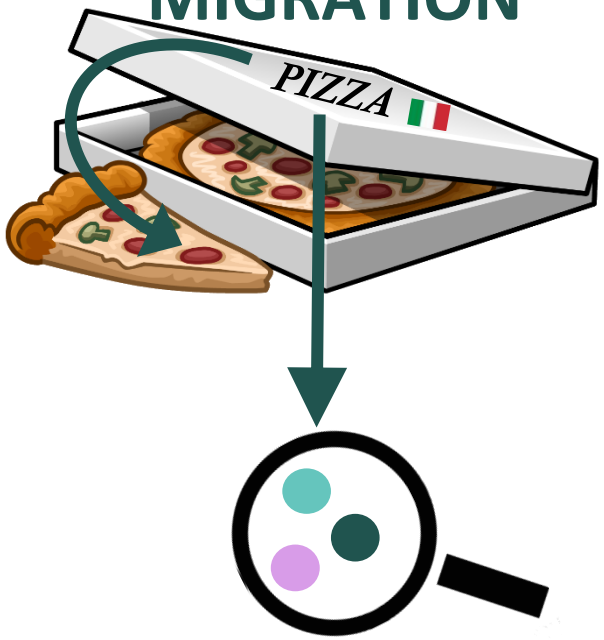
Several case studies including food contact materials - FCM

Food software

VERMEER FCM

Background: FCM

MIGRATION



FCM

HAZARD

related to FCM substances

Food contamination

Consumption

EXPOSURE

AIM: Combine information on exposure collected with MERLIN EXPO with information on hazards collected with VEGA to support risk assessment of FCM compounds

Regulations: FCM

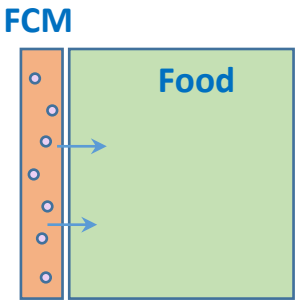
Plastic FCM: **positive list (Annex I of Regulation 10/2011)** (only starting substances such as monomers and additives)

Annex I provides overview of authorized substances to be used in plastic FCM

(with corresponding SML, if available)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
FCM substance No	Ref. No	CAS No	Substance name	Use as additive or polymer production aid (yes/no)	Use as monomer or other starting substance or macromolecule obtained from microbial fermentation (yes/no)	FRF applicable (yes/no)	SML [mg/kg]	SML(T) [mg/kg] (Group restriction No)	Restrictions and specifications	Notes on verification of compliance
1	12310	0266309-43-7	albumin	no	yes	no				
2	12340	—	albumin, coagulated by formaldehyde	no	yes	no				

VERMEER FCM: Migration model



- **One FCM layer**

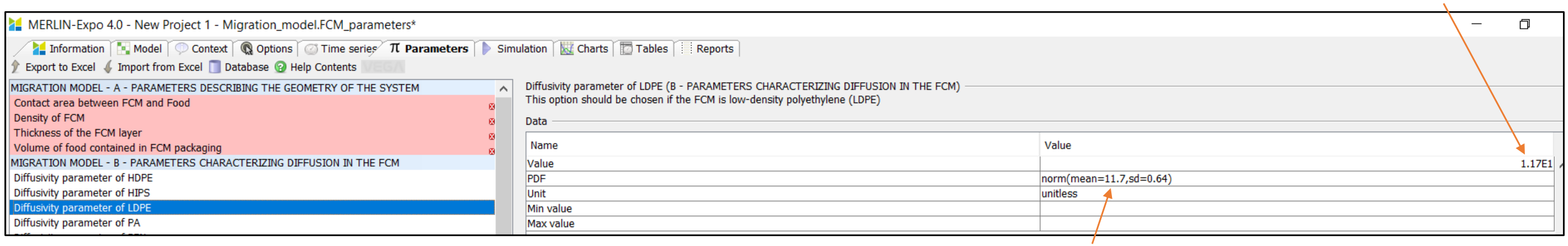
- **One dimensional (1D) diffusion** model between the FCM layer and Food

Fick's law: $\frac{\partial C_i}{\partial t} = D_i . \frac{\partial^2 C_i}{\partial x^2}$

- When only one FCM layer is considered, mass-balance equation based on Fick's law → **analytical solution** (Crank, 1975 ; Piringer et al, 2008)

VERMEER FCM: Model available

It works within the MERLIN-Expo platform. Best estimate of the parameter



First step: migration, then toxicity

https://www.life-vermeer.eu/

doi: 10.1016/j.fct.2022.113118

VERMEER FCM: Toxicity models (via VEGA)

Migration threshold	Toxicological data required	Models available in Vega
X < 0.05 mg/kg food	• Genotoxicity data	
	➢ Gene mutations	• Mutagenicity (Ames test) CONSENSUS model (version 1.0.3)
	➢ Structural and numerical chromosome aberration	• *In vitro* Micronucleus activity (IRFMN/Vermeer) (version 1.0.0)
0.05 ≤ X < 5 mg/kg food	• Genotoxicity data	• *See above*
	• Subchronic oral toxicity data (90-day study)	• NOAEL (IRFMN/CORAL) (version 1.0.0)
	• Data to demonstrate absence of accumulation potential in man	• LogP model (MLogP) (version 1.0.0)

VERMEER FCM: Hazard models

Migration threshold	Toxicological data required	Models available in Vega
5 mg/kg ≤ X < 60 mg/kg	• Genotoxicity data • Subchronic oral toxicity data (90-day study) • Toxicokinetic data	• *See above*
	• Data on reproductive and developmental toxicity	• Developmental Toxicity model (CAESAR) (version 2.1.7) • Developmental/Reproductive Toxicity library (PG) (version 1.1.0)
	• Data from long term toxicity/carcinogenicity studies	• Carcinogenicity model (Antares) (version 1.0.0) • Carcinogenicity model (ISSCAN-CGX) (version 1.0.0) • Carcinogenicity model (CAESAR) (version 2.1.9) • Carcinogenicity model (ISS) (version 1.0.2) • Carcinogenicity consensus model • Carcinogenicity oral classification model (IRFMN) (version 1.0.0) + Carcinogenicity oral Slope Factor model (IRFMN) (version 1.0.0)

CONCLUSIONS

In silico models assisting experts

Integratation of models for the same endpoint

Integration hazard + exposure

Network between multiple systems

Plus prioritization

Transparency, documentation, reasoning, weight-of-evidence