Overview of toxicity prediction methods

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The challenges

Contaminants, drugs…

Increased use of chemical substances

Transformation products

Decline of species

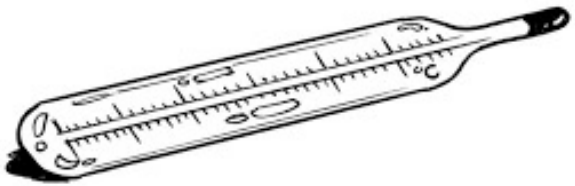
One health

Lack of data

….

Are we adequate?
Are we effective?

- Which are our objectives?
- Do we have priorities?
- What is our timeline?
- Do we measure impact of our activities?



Caravaggio, The conversion of S. Paul

Not apostasy
Not conversion

- Not to loose fundamental issues
- Need to focus our activities

Broad view

Priorities: hazard and exposure

One health

Single conceptual / in silico architecture

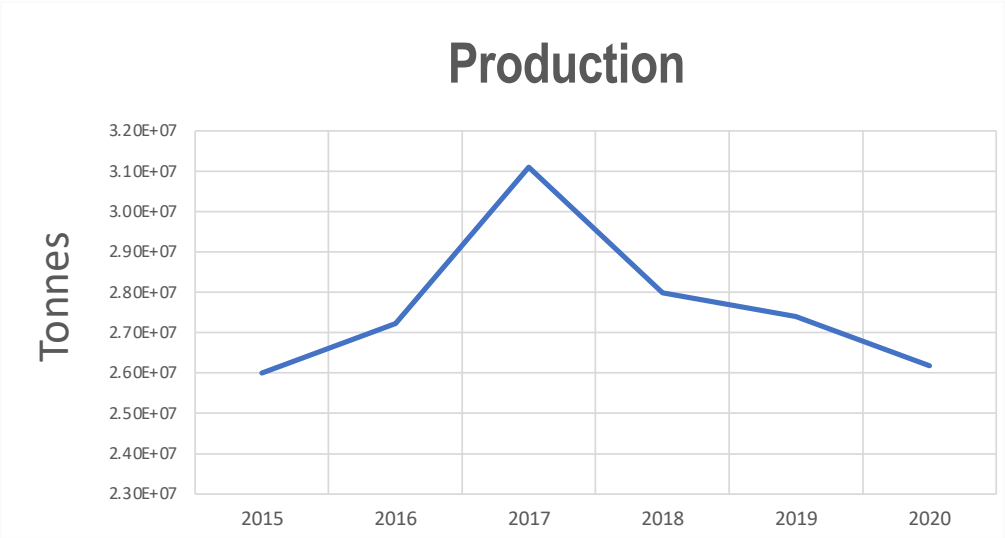
Safety (≠ lack of risk)

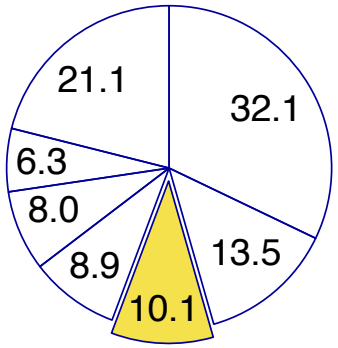
Beneficial aspects

Substitution

Green Deal

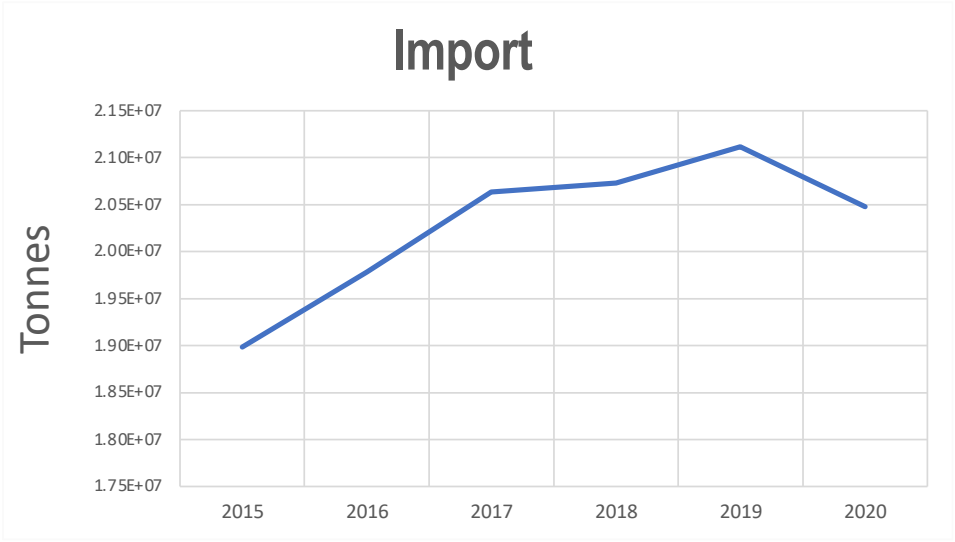
Risk. Global view. Are we exporting the risk?





Italy: 10% of the chemical market in EU (after Germany and France)

Trend Production / Import in Italy, 2015-2020



CONCERTREACH
CONCERTING EXPERIMENTAL DATA AND IN SILICO MODELS FOR REACH

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Market of CMR and explosives

Previously we observed a similar shift in the period 2011-2015, in Italy, moving toward import, in particular for **CMR** (reduced diversity) **and explosive substances**

Marzo M, Leone C, Toma C, Roncaglioni A, Gianazzi S, Knauf R, Benfenati E. Impact of REACH legislation on the production and importation of CMR (carcinogen, mutagen and reproductive) and explosive chemicals in Italy from 2011 to 2015. Regul Toxicol Pharmacol 2019; 101 : 166-171 doi: 10.1016/j.yrtph.2018.11.013

Priorities and new methods

- Once we have the global view, we can cope with priorities
- Based on the global approach, global meter
- (1) In silico models for integrated view

Priorities: human (disease burden).

Ecotox (in The Netherlands 90% of the amphibians disappeared (for a disease), thus we should focus on amphibians, compared to fish)

(2) In silico models for priority endpoints

The new in silico tools for priorities

- Which in silico models are of higher relevance because addressing priority endpoints (hazard)?
- Which substances are of higher priorities (exposure)?

Mn Euro	EU28
Petrochemicals and Derivatives	**143.568**
Inorganic Industrial Chemicals	**77.629**
Fertilizers	*24.065*
Industrial Gases	*11.644*
Other inorganics	*41.920*
Specialty Chemicals	**153.394**
Paints & Inks	*42.860*
Dyes & Pigments	*16.918*
Auxiliaries for Industry	*82.337*
Crop Protection	*11.279*
Polymers	**120.330**
Plastics & Synthetic rubber	*109.031*
Man-Made Fibres	*11.299*
TOTAL	**494.922**
Pharmaceuticals	**313.236**
Personal Care Products	**69.957**

In silico platforms. Opportunities … and challenges

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Networks between different platforms

VEGA
OCHEM
Danish QSAR Database
AMBIT

Different models. Opportunities … and challenges

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increased confidence,
increased perspectives

different metrics, different info (overlap ?)

In silico models. Future?

- Integrating multiple tools for the same endpoint
- Covering AOP, same overall toxicological category (e.g. muta+geno+carcino)
- Integrating hazard and exposure
- SSbD
- Integrating risk/benefit

In silico models. Predictions and …

- **Reasoning** («*predicting*» mechamism, causality, …)
- **Heuristics and expert systems** (supervised/unsupervised)
- Link with

1. regulation,
2. confidence,
3. planning safer substances

Weight of evidence (WoE): EFSA Guidance

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 12 July 2017

doi: 10.2903/j.efsa.2017.4971

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,
Helle Katrine Knutsen, Simon More, Hanspeter Naegeli, Hubert Noteborn, Colin Ockleford,
Antonia Ricci, Guido Rychen, Josef R Schlatter, Vittorio Silano, Roland Solecki,
Dominique Turck, Emilio Benfenati, Qasim Mohammad Chaudhry, Peter Craig,
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

Environment International 131 (2019) 105060

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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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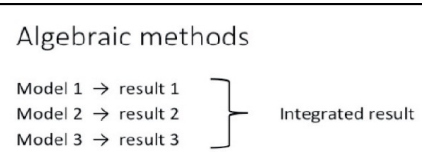
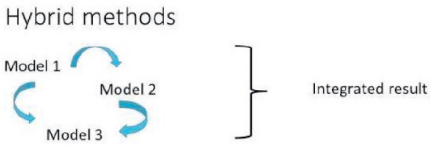
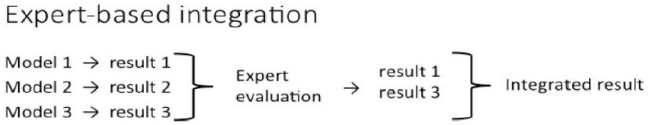
ARTICLE INFO

Handling Editor: Da Chen

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)

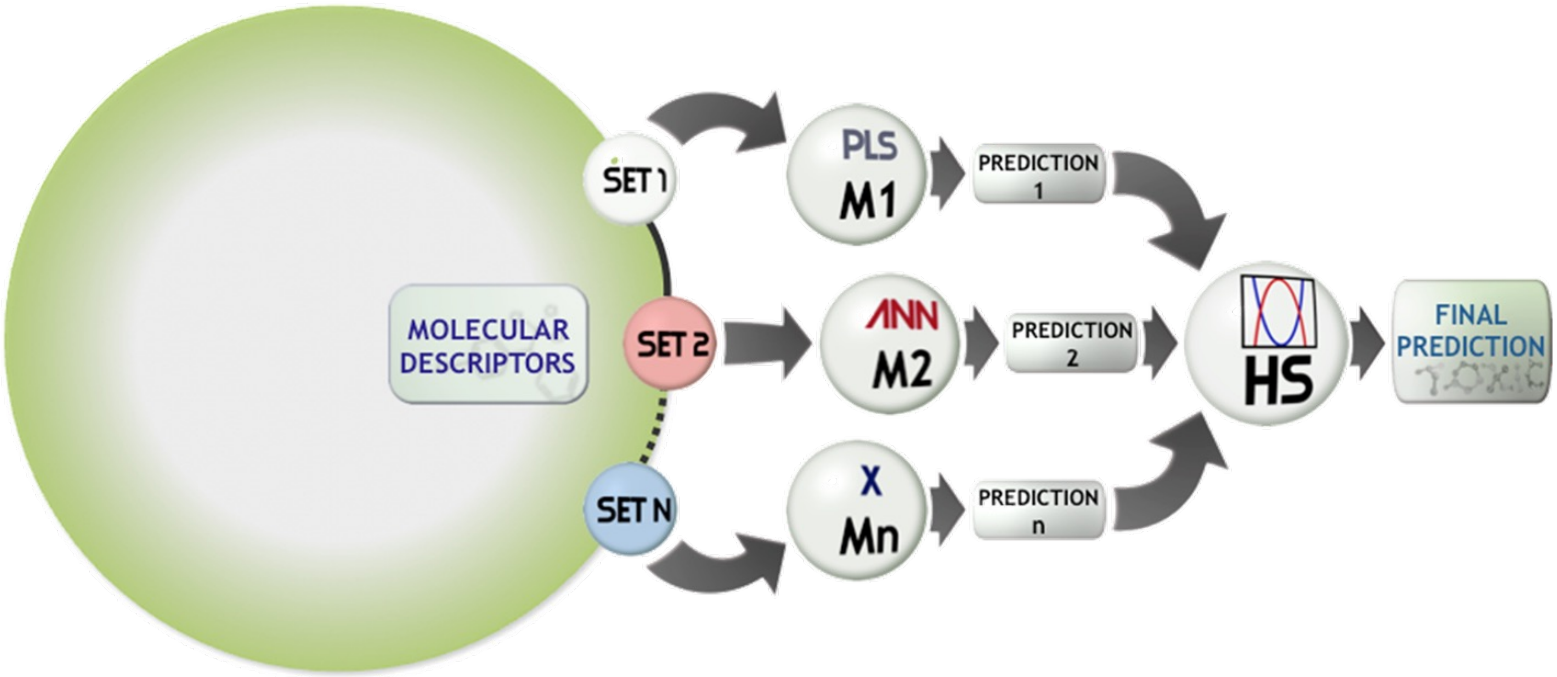
Consensus model (CNS-VEGA) : CAESAR + SARPY + TT-VEGA

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

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

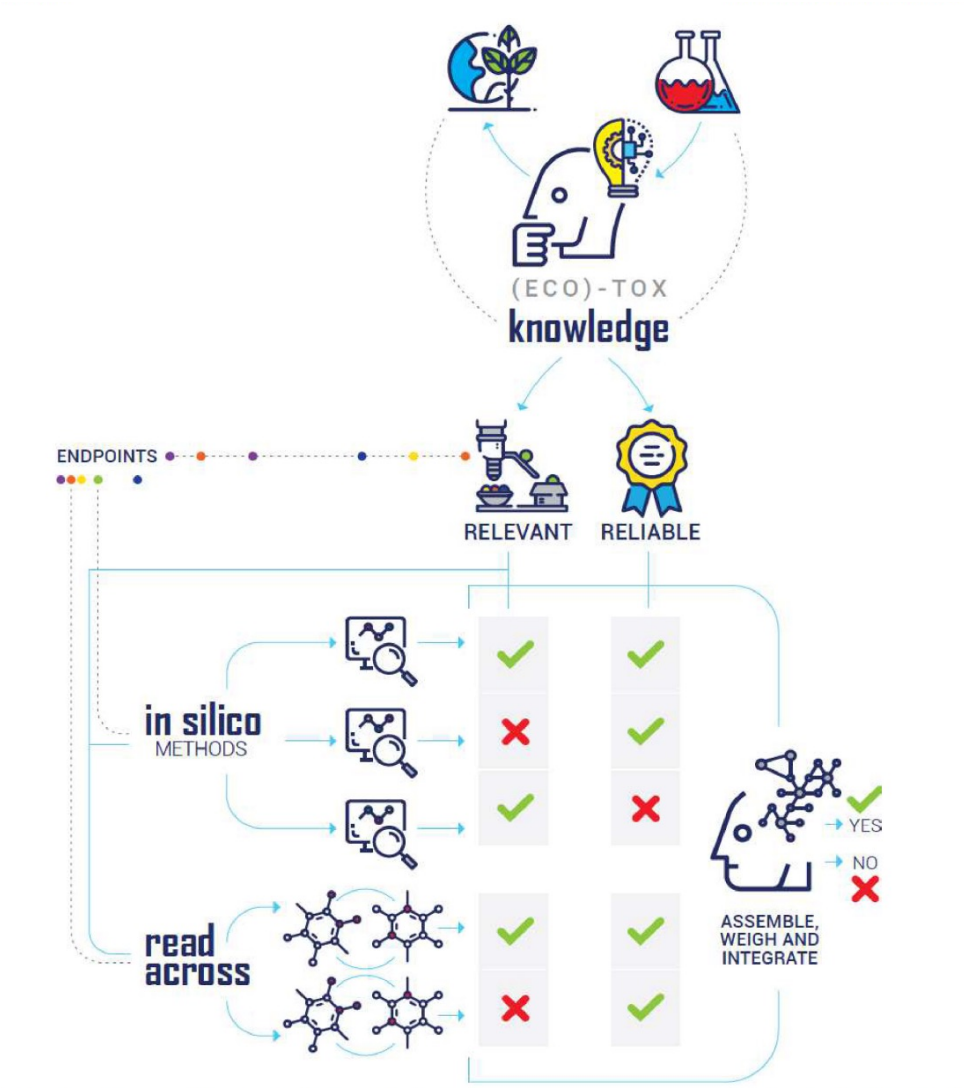
Experts may identify a preferred way to integrate results.

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

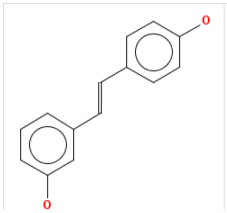


Use all lines of evidence

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

- Check agreement

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

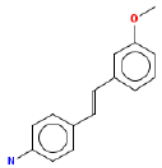
Global AD Index
AD index = 0.719
Explanation: the predicted compound could be out of the Applicability Domain of the model.

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.

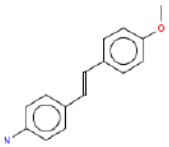


Compound #1

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

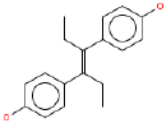


Compound #2

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

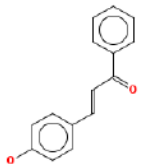


Compound #3

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



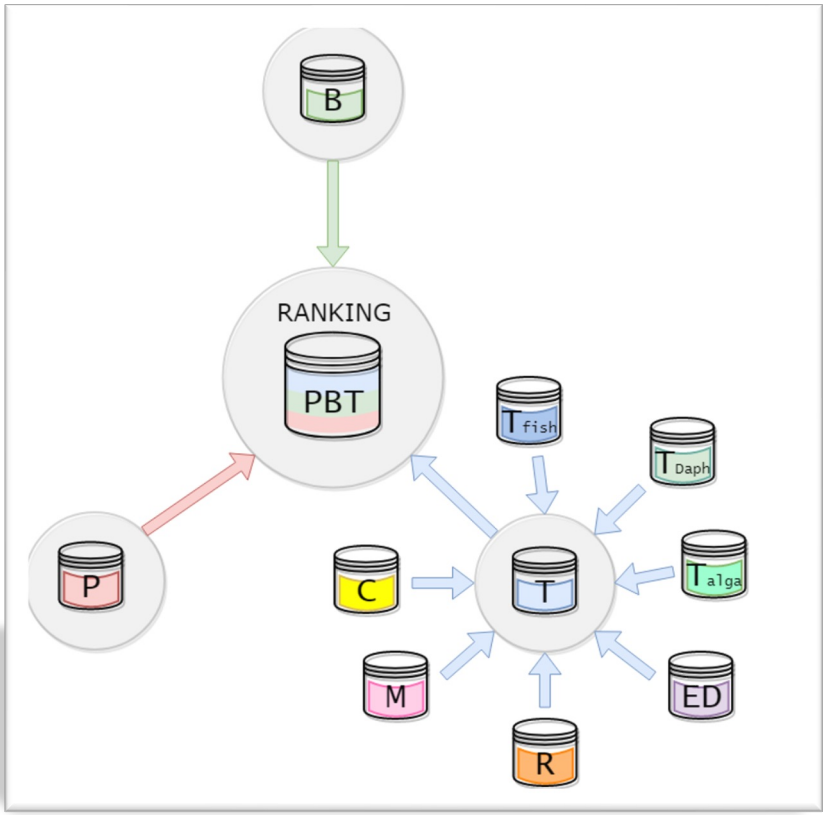
Compound #4

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

Alerts (not found in the target): SM158; SM172

Integrating multiple endpoints/pathways. The JANUS example

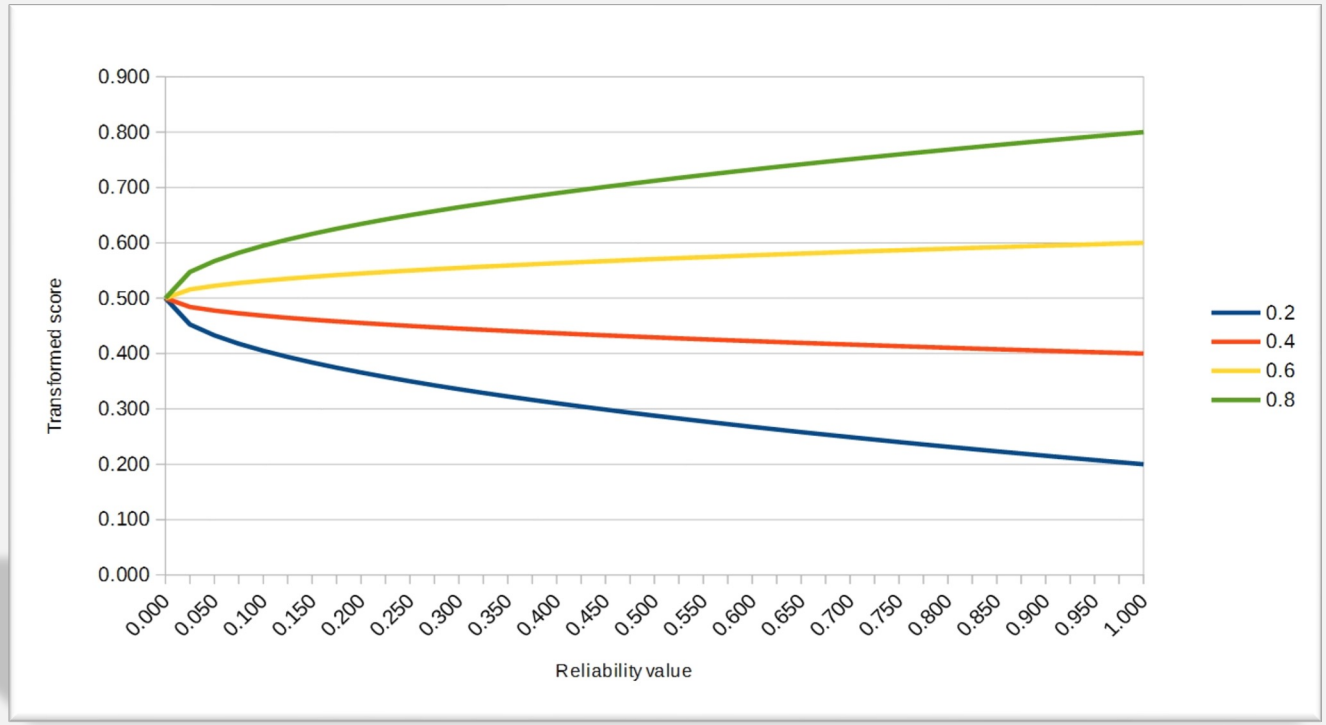


One single platform covering multiple endpoints,
using 48 separate in silico models for CMR, PBT and ED (parental and degradation products).
Done for German UBA

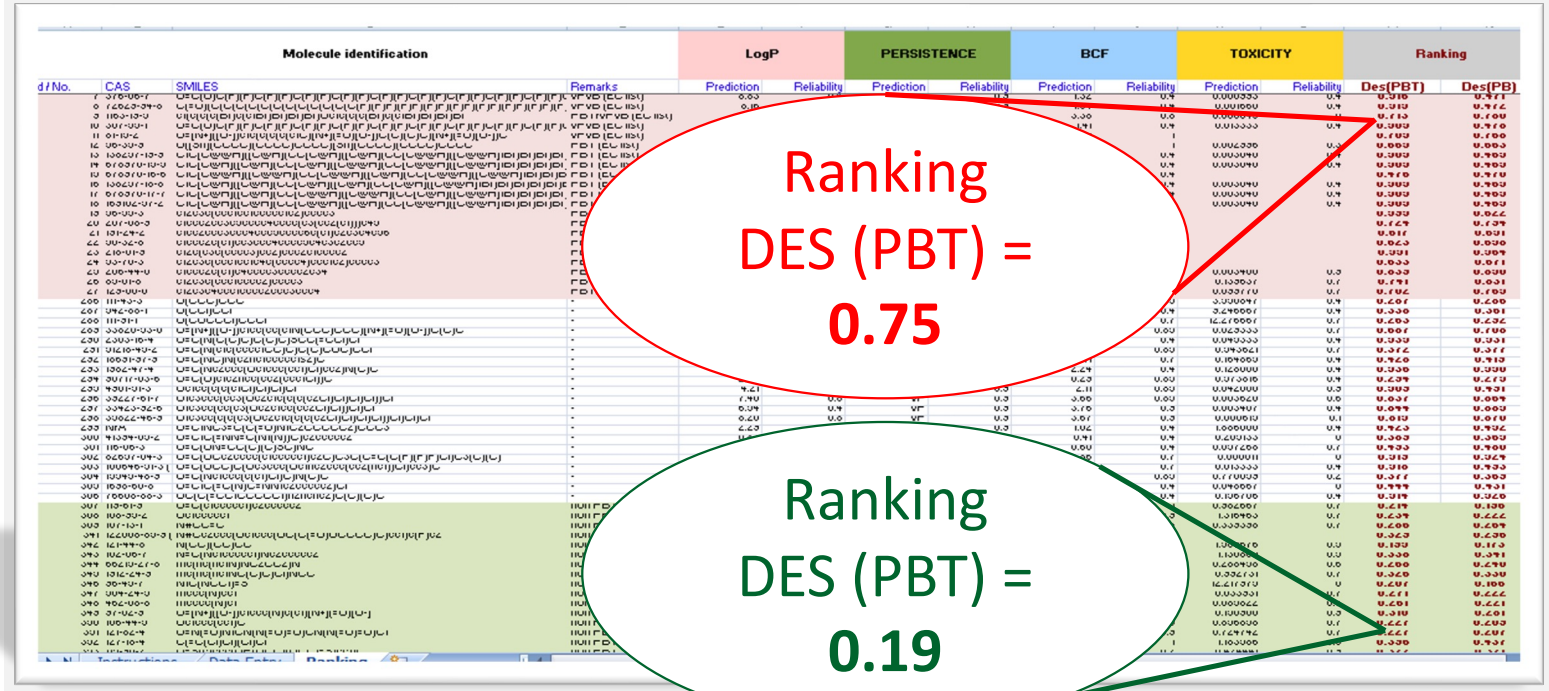
www.vegahub.eu

Uncentainty and effect

Example: how the score (on the Y axis) changes depending on the reliability value (X axis) for four example property's value (0.2, 0.4, 0.6 and 0.8)



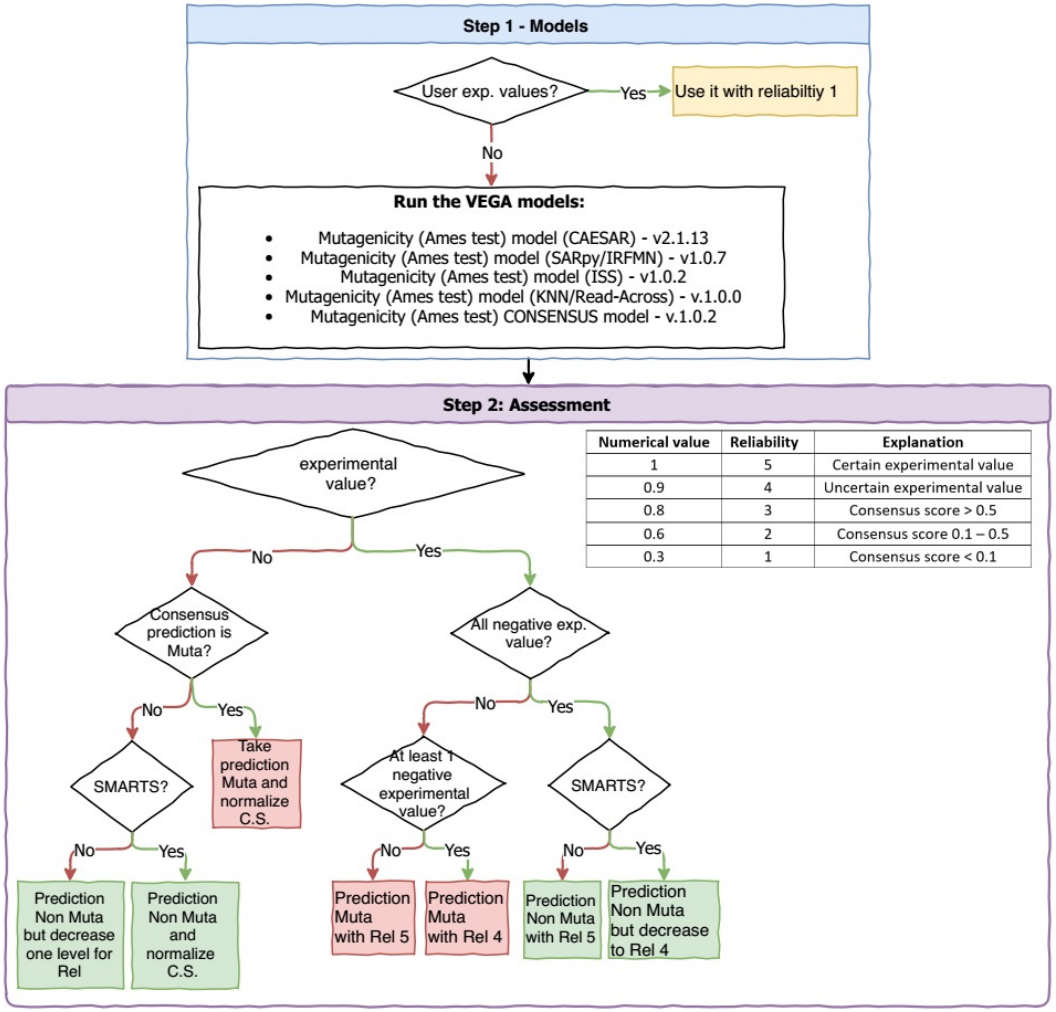
Ranking


Molecule identification
LogP
PERSISTENCE
BCF
TOXICITY
Ranking
Prediction
Reliability
Des(PBT)
Des(PB)
Ranking
DES (PBT) =
0.75
Ranking
DES (PBT) =
0.19

JANUS. Details for mutagenicity

- ✓ **Only in classification**
  - Based on **4 qualitative models** + a **consensus** model
- ✓ **Metabolism SMARTS** (5 SMARTS associated to mutagenicity)
  - Used **only with Non-Muta** values (if matched → reliability reduce)
  - **Reliability based on** the **output** and the **consensus score**

Numeric value	Reliability	Explanation
1	5	Certain exp. values
0.9	4	Uncertain exp. values
0.8	3	Consensus score > 0.5
0.6	2	Consensus score 0.1 – 0.5
0.3	1	Consensus score < 0.1



JANUS. One score

Janus Result

NUMBER OF COMPOUNDS **3**

☑ PBT ☐ CMR ☐ ED ☑ PARTIAL SCORES ☑ FINAL SCORES

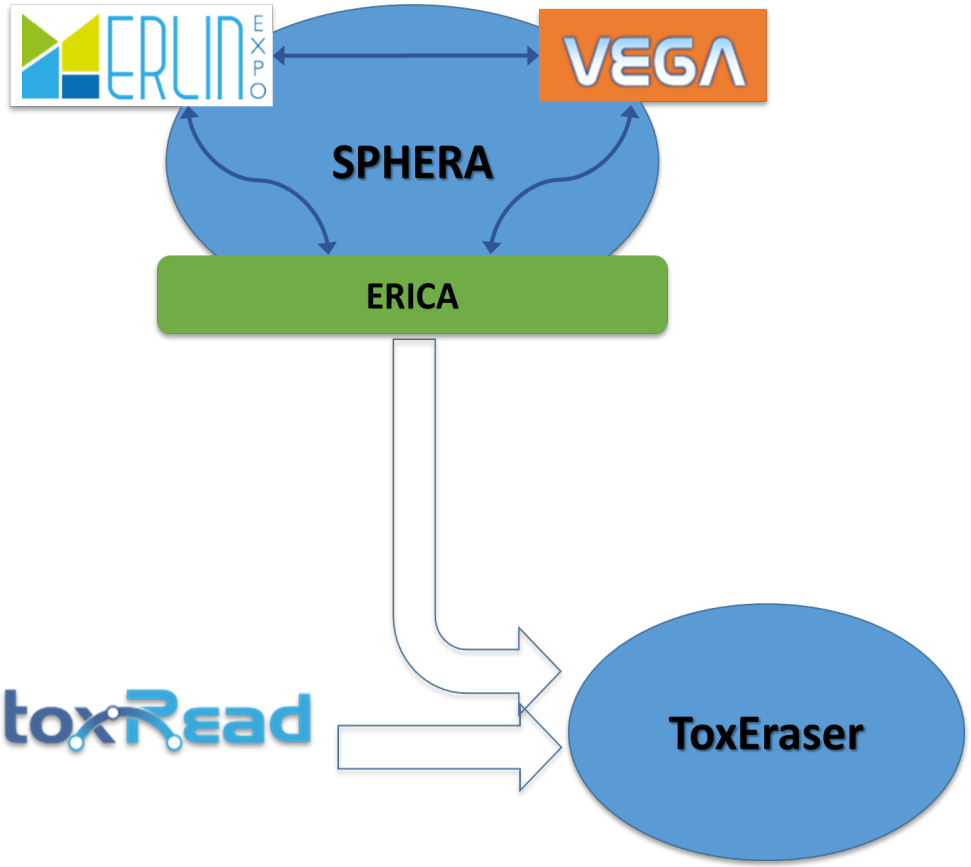
	No.	Metabolite	Id	SMILES	Label	P	rel.	score	B [log(L/kg)]	rel.	score	T [mg/l]	rel.	score	Score(vPvB)	Score(SVHC)	Score(PBT)
🔍	1		Molecule 1	OC(OC)C	P B T - C M R E	**nP**	0.45	0.211	**0.16**	0.9	0.078	**2.18**	0.65	0.264	0.129	0.853	0.242
🔍	2	Metabolite	Molecule 1 [M-01]	O=CC	P B T - C M R E	**nP**	0.5	0.243	**0.31**	0.5	0.191	**1.08**	0.7	0.301	0.215	0.523	0.291
🔍	3	Metabolite	Molecule 1 [M-02]	OC	P B T - C M R E	**nP**	0.8	0.174	**0.15**	0.75	0.114	**0.78**	0.7	0.325	0.141	0.875	0.26

LIFE16
ENV/IT/000167

life vermeer

Integrating VEGA, toxRead, MERLIN-Expo and ERICA in a platform for risk assessment and substitution of risky substance

Integrating hazard + exposure



Integrating **VEGA**, **ToxRead**, **MERLIN-Expo**, and **ERICA** in a **platform for risk assessment and substitution** of risky substances

1. Identification of the risky substances

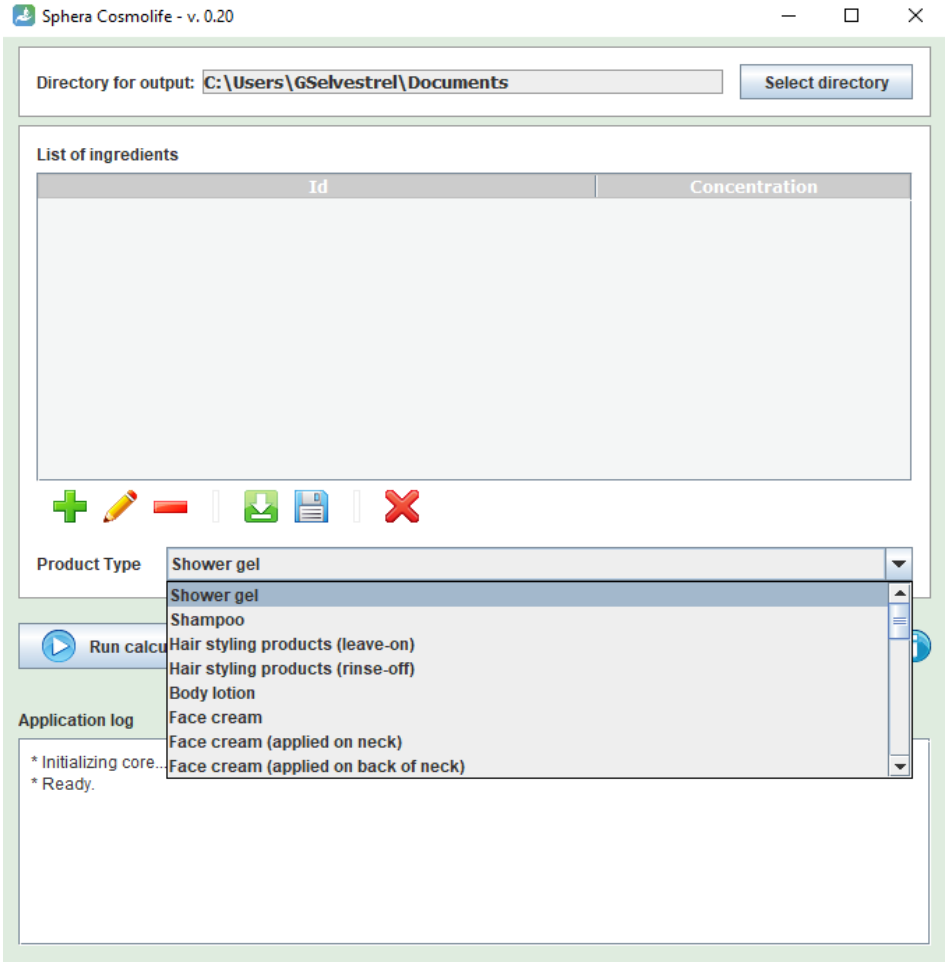
2. Identification of possible substitutes

Application to 6 case studies

LIFE VERMEER project - Case studies

- *Food Contact Materials*
- *Biocides*
- *Oil fractions*
- *Solvents*
- *Dispersants*
- *Cosmetics*

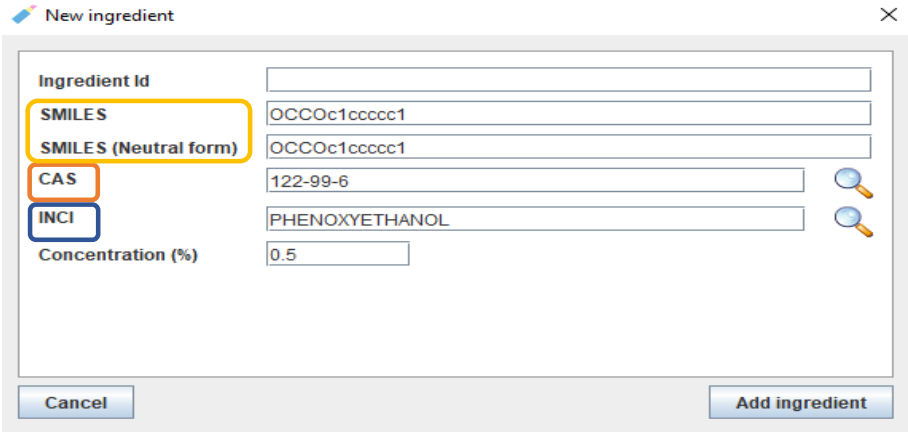
VERMEER-Cosmolife - input



- The user is asked to provide the information regarding the ingredient, its concentration and the product type

- The software allows to add single or multiple ingredients

- Ingredients can be entered using **INCI**, **CAS** or **SMILES**



VERMEER-Cosmolife - summary

The software provides a preliminary output table with a summary of the hazard and exposure features of the ingredients

Processed product

Product type: **Body lotion**

Ingredients:

	Ingredient Id	CAS	INCI	Conc. %	Annex	Mutagenicity	Skin Sensitization	Dermal abs.	MoS	TTC
Details	DISODIUM EDTA	139-33-3	DISODIUM EDTA	0.05	-	NON-Mutagen (EXPERIMENTAL value)	Sensitizer (low reliability)	10%	162337.66	0.0015 mg/kg bw/day
Details	GLYCERIN	56-81-5	GLYCERIN	5.0	-	NON-Mutagen (EXPERIMENTAL value)	NON-Sensitizer (EXPERIMENTAL value)	80%	52.37	0.03 mg/kg bw/day
Details	POTASSIUM BENZOATE	582-25-2	POTASSIUM BENZOATE	2.0	V	NON-Mutagen (EXPERIMENTAL value)	NON-Sensitizer (EXPERIMENTAL value)	80%	14.03	0.03 mg/kg bw/day
Details	EUGENOL	97-53-0	EUGENOL	0.001	III	NON-Mutagen (EXPERIMENTAL value)	Sensitizer (EXPERIMENTAL value)	80%	233360.39	0.03 mg/kg bw/day
Details	GERANIOL	106-24-1	GERANIOL	0.001	III	NON-Mutagen (EXPERIMENTAL value)	Sensitizer (EXPERIMENTAL value)	40%	158441.56	0.03 mg/kg bw/day

VERMEER-Cosmolife – internal exposure

The software calculates the SED following 3 different approaches:

Absorption=100%
(Oral/inhalation)

Absorption=50%
(Dermal; defined by SCCS)

Kroes Approach
(Refined approach)

1. Implementation in VEGA of two models that allow predicting **Kp (Skin Permeability Coefficient)**

Example: Potts and Guy: $\boldsymbol{logKp = 0,71log(K_{O/W}) - 0.0061MW - 2.7}$ **(cm/h)**

2. Once predicted Kp, we obtain **Jmax (Maximum flux)**

$$\boldsymbol{Jmax = Kp * Cwater,sat}\ \textbf{(mg/cm²/h)}$$

3. Based on Jmax, Kroes proposed three default exposure values:

%A =
- **10%** if Jmax <= 0.1 µg/cm²/h
- **40%** if 0.1 < Jmax <= 10 µg/cm²/h
- **80%** if Jmax > 10 µg/cm²/h

VERMEER-Cosmolife - hazard

The software includes the HAZARD IDENTIFICATION, considering different toxicological endpoints, including NOAEL

4. Hazard identification

Mutagenicity - Ames test (Janus workflow prediction)	NON-Mutagen (EXPERIMENTAL value)
In vitro micronucleus assay (IRFMN model prediction)	Inactive (EXPERIMENTAL value)
Chromosomal aberration test (Coral model prediction)	Inactive (good reliability)
Skin sensitization (Consensus of Caesar and JRC models)	NON-Sensitizer (EXPERIMENTAL value)
Skin sensitization (Caesar model prediction)	NON-Sensitizer (good reliability)
Skin sensitization (IRFMN/JRC model prediction)	NON-Sensitizer (EXPERIMENTAL value)

Output Example

Other endpoints, such as Carcinogenicity, Reproductive/Developmental toxicity, Endocrine Disruptors, Skin Irritation and Eye Irritation and Acute Toxicity will be included soon

VERMEER-Cosmolife - MoS

The software provides the Risk Characterization, considering the process proposed within the SCCS Notes of Guidance.

For cosmetics, the focus is on systemic effects and a MoS (Margin of Safety) is calculated, according to the formula:

$$MoS = \frac{POD\ (Point\ of\ Departure)}{SED(\ Systemic\ Exposure\ Dose)}$$

MoS > 100 

Output Example

6. Risk characterization

MoS - Margin of Safety with 100% absorption	101.46
MoS - Margin of Safety with 50% absorption	202.92
MoS - Margin of Safety with 10% absorption (from Kroes thresholds)	1014.61

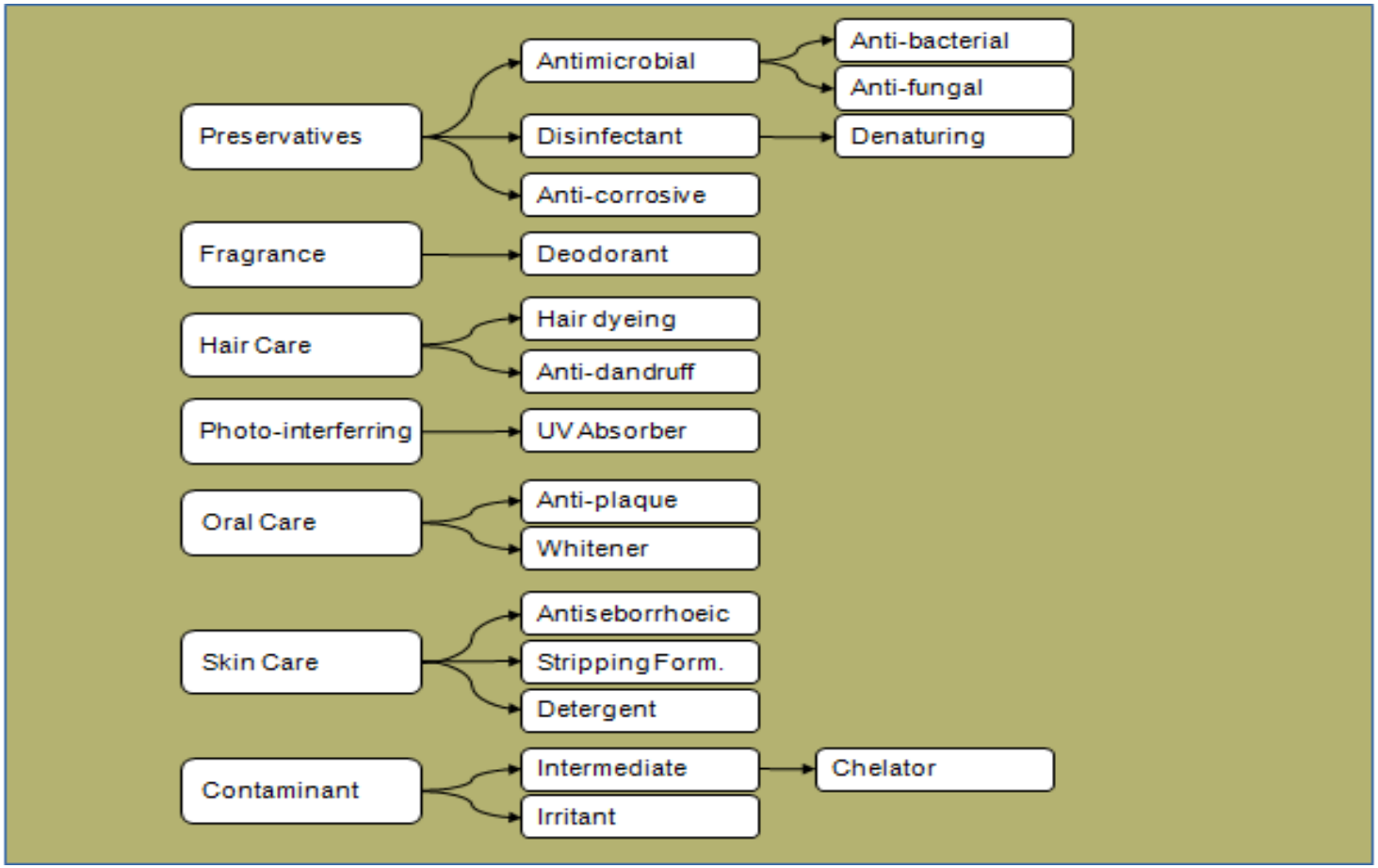
ToxEraser – planning substitution

Systematic retrieval of information, concerning:

- **safety**
- **functional uses**
- **similarity (read-across)**

ToxEraser – functional use

Cosmetics according to their functional uses: a hierarchical ontology



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Unique sw environment

Chemical features and tools associated to different aspects?

Need of a single conceptual scheme:

- *Functional properties* } Industry
- Phys-chem properties
- Toxicological properties
- Environmental properties

} Same for authorities and industries

Conclusions

- Global challenges, broad view
- Multitask methodologies
- Set up priorities
- Players: politics, economy, science
- Joining efforts to get higher targets

- In silico models for

1. *new paradigm: holistic*
2. *prioritization*
3. *models for the most relevant endpoints*
4. *safer substances*