Knowledgebase of Scientific Committee on Consumer Safety (SCCS) opinions facilitates Next Generation Risk Assessment of cosmetics

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Introduction

There are currently no validated animal-free replacement methods to assess repeated dose toxicity. This poses serious problems for developing new chemical compounds across various sectors, mainly in the cosmetic industry, where animal testing is fully banned in the EU. However, since biological responses in an animal cannot be reflected using single non-animal methods, it is necessary to use Integrated Approaches to Testing and Assessment (IATA) that rely on an integrated analysis of existing information coupled with the generation of new information using non-testing (e.g. grouping and read-across) and testing methods (e.g. *in vitro*). The appraisal of all existing information in an iterative approach is considered a core pillar in the search for animal-free risk assessment of chemicals [1].

AIMs

This study aimed at developing a user-friendly knowledgebase (KB) using semantic technology [2] in which existing toxicological data of cosmetic ingredients retrieved from SCCS opinions is gathered and maintained to assist non-animal systemic toxicity assessment. To add more weight to the toxicological evidence, Structural-Activity Relationship (SAR) data supported through the OECD QSAR Toolbox has been implemented in the KB. The use of the KB as hazard identifier tool and guidance for further *in vitro* testing is showcased in the field of hepatotoxicity.

Workflow & Objectives

SCCS opinions 2009-2019 Annexes II, III, IV, V, and VI of the European Cosmetics Directive

→ Design of a machine-understandable data structure conform to OECD guidelines
Automatic generation of SMILES

→ Collection of the common attributes facilitating data mapping

→ **TOXIN DB**
The training dataset is described in RDF

→ **1. Store and manage the data published by SCCS in machine-readable format**

Enrich the data by linkage to other datasets
TOXic Process Ontology (TXPO)
Reactome Pathway Database
MeSH-NCBI

→ OECD QSAR Toolbox API implemented to the database

→ **TOXIN KB**
the structural alerts retrieved within the applicability domain of the toolbox

→ **2. Add weight to the toxicological evidence using structural alert from OECD QSAR Toolbox**
3. Semi-automatic data modelling of all the remaining opinions

Defining *in vivo*-based General Health Concern information cluster
e.g. Liver toxicity cluster defined as an alteration in Chol, TG, AST, Phos, Bili, GGT, ALP, hepatic fat, hepatocellular hypertrophy, and hepatic necrosis

→ **TOXIN KB**
groups cosmetics inducing similar type of *in vivo* animal toxicity

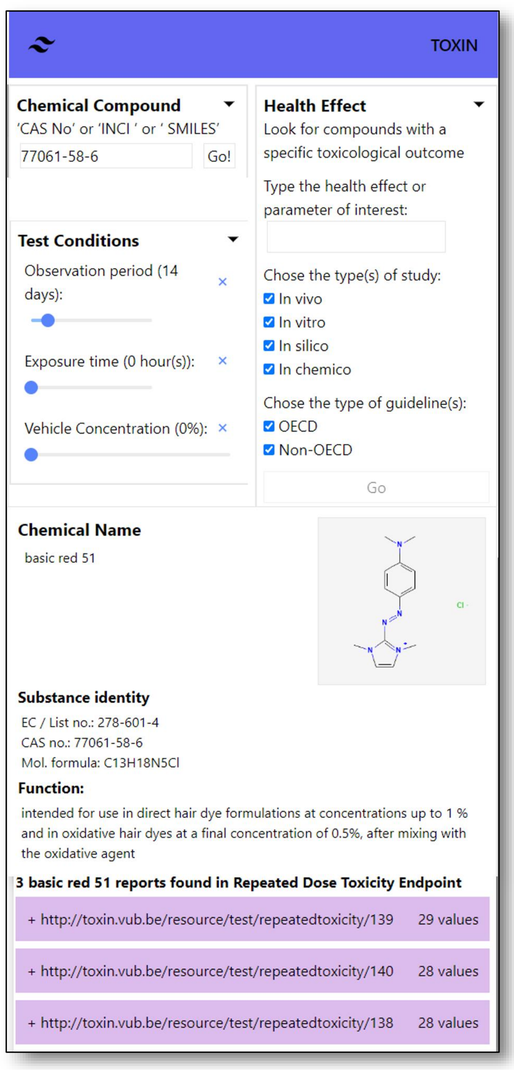
→ **4. Use TOXIN as a hazard identifier tool**

- ≅ 600 opinions published by SCCS
- Challenging compounds such as Triclosan has been reviewed a few times over the years. From 2009 to 2022, it has 4 submissions

Results

Advanced search function: gather existing *in vivo* information (objective 1)

- 30 cosmetic ingredients from Annexes potentially toxic to the liver identified in the training dataset



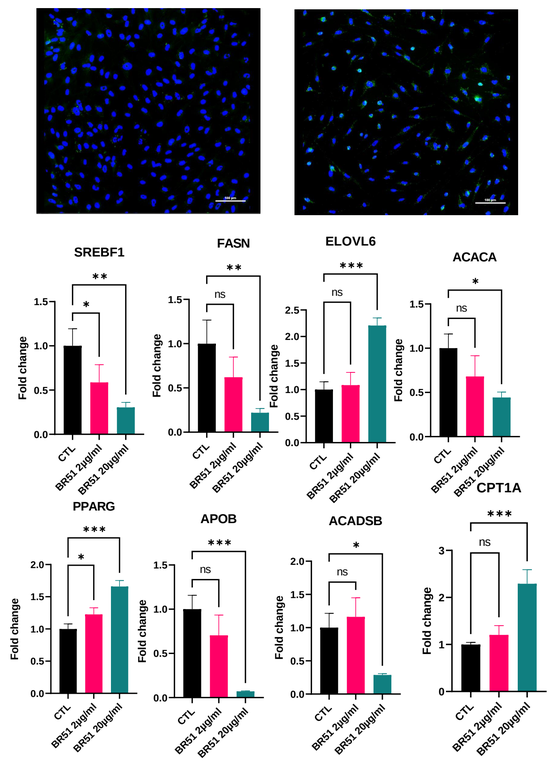
In silico prediction for hepatotoxicity: weight-of-evidence assessment (objectives 2 & 3)

- HESS (Hazard Evaluation Support System): 9 out of 30 ingredients carrying hepatotoxic structural alert identified by OECD QSAR Toolbox
- VEGA (IRFMN model): 2 out of 30 toxic predictions
- HC Yellow 13 is identified as hepatotoxic with both *in silico* tools
- With both algorithms, the majority of compounds can't be categorized

	Effects											
	In vivo										*In silico*	
Cosmetic compound	Chol	TG	AST	Phos	Hepatic fat	Hepatocellular hypertrophy	Bilirubin	GGT	ALP	Hepatic necrosis	OECDToolbox - HESS (RDT)	VEGA- IRFM (hepatotoxicity)
5-Amino-6-chloro-o-cresol	increase of						increase of				Energy metabolism dysfunction alert	Unknown
Acetylated vetiver oil—AVO = vetiveryl acetate	increase of										not categorized	Unknown
Basic brown 17	increase of	increase of					increase of	increase of			not categorized	Non toxic
Basic red 51	increase of	increase of		increase of / decrease of			decrease of	increase of	increase of	increase of	not categorized	Unknown
Basic violet 2	increase of										not categorized	Unknown
Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane			increase of								not categorized	Unknown
Butylphenyl methylpropional (p-BMHCA)					increase of						Chlorphentermine (Hepatotoxicity) Alert	Unknown
Cetylpyridinium chloride			increase of								not categorized	Toxic
Decamethylcyclopentasiloxane D5					increase of						not categorized	Unknown
EcoG +	increase of								increase of		not categorized	Unknown
HC blue 15	increase of	increase of		increase of		increase of					Renal toxicity alert	Non toxic
HC Yellow 13	increase of										Flutamide (Hepatotoxicity) Alert	Toxic
Hydroxyethyl-2-nitro-p-toluidine			increase of				increase of		increase of		not categorized	Unknown
Hydroxyethyl-3,4-methylenedioxyaniline HCl	increase of			increase of		increase of	increase of				not categorized	Unknown
Hydroxyethyl-p-phenylenediamine sulphate	increase of										Anilines (Hepatotoxicity) Rank C +Hemolytic anemia with methemoglobinemia alert	Unknown
Hydroxypropyl p-phenylenediamine and its dihydrochloride salt (A165)	increase of		increase of				increase of	increase of			Anilines (Hepatotoxicity) Rank C +Hemolytic anemia with methemoglobinemia alert	Unknown
Methylimidazoliumpropyl p-phenylenediamine HCl (A166)			increase of								not categorized	Unknown
N,N'-bis-(2-hydroxyethyl)-2-nitro-p-phenylenediamine	increase of	increase of									not categorized	Unknown
N-Methyl-2-pyrrolidone	increase of								increase of		not categorized	Unknown
o-Aminophenol			increase of								Hemolytic anemia with methemoglobinemia alert+ Rank B; Renal Toxicity Alerts	Unknown
Phenoxyethanol	decrease of	increase of							increase of		Anilines (Hepatotoxicity) Rank C +Hemolytic anemia with	Unknown
Toluene-2,5-diamine (sulphate)		increase of									Anilines (Hepatotoxicity) Rank C + Hemolytic anemia with methemoglobinemia alert	Unknown
1,5-Naphthalenediol							increase of		decrease of		3-Methylcholantrene (Hepatotoxicity) Alert; 2-Acetylaminofluorene (Hepatotoxicity) Alert; α-Naphthyl-isothiocyanate (Hepatotoxicity) Alert; Mefenamic Acid (Hepatotoxicity) Alert; N-hydroxy-2-acetylaminofluorene (Hepatotoxicity) Alert; Sulfasalazine (Renal toxicity) Alert + Renal toxicity alert	Unknown
2,6-Dihydroxyethylaminotoluene							increase of				Not categorized	Unknown
2,7-Naphthalenediol							increase of	increase of		increase of	β-Naphthylisothiocyanate (Hepatotoxicity), Acetaminophen (Hepatotoxicity) Alert	Unknown
Citric acid (and) silver citrate									increase of		Energy metabolism dysfunction	Non toxic
Diethylene glycol monoethyl ether									increase of		Renal toxicity alert	Unknown
Methoxypropylamino cyclohexenylidene							increase of				Aliphatic nitriles (Hepatotoxicity) Rank B	Unknown
Triclosan			increase of					increase of	increase of	increase of	Renal toxicity alert	Non toxic
Disperse Blck 9			increase of					increase of	increase of		Renal Toxicity Alert	Unknown

Generating additional AOP-based mechanistic *in vitro* data for liver steatosis

- Basic Red 51 (BR51) tested at sub-cytotoxic concentrations *in vitro* on human skin stem cell-derived hepatocyte-like cell (hSKP-HPC)
- Fluorostaining of fatty acid with Bodipy after 24h shows an accumulation of fat
- The results of RT-qPCR after 24h exposure suggest this effect is probably due to a downregulation of the expression of APOB
- The expression of other key genes involved in lipid metabolism has significantly been modified such as PPARG



CONCLUSION

- We developed a tool (TOXIN) based on semantic technology that facilitates data extraction from SCCS opinions.
- Using TOXIN, we retrieved 30 cosmetic ingredients with potential hepatotoxic effects in laboratory animals.
- Integration of OECD QSAR Toolbox in TOXIN improves the weight of evidence regarding hepatotoxicity. To confirm the *in silico* predictions of the OECD Toolbox, we used the VEGA platform.
- Based on the results of the tool and in the context of NGRA, we further evaluated BR51 *in vitro* using human stem cell-derived hepatocyte-like cells. We found indications that this compound also induced the accumulation of intracellular lipid suggesting steatogenic potential.

References: 1. Dent MP, Vaillancourt E, Thomas RS, Carmichael PL, Ouedraogo G, Kojima H, et al. Paving the way for application of Next Generation Risk Assessment to safety decision-making for cosmetic ingredients. Regulatory Toxicology and Pharmacology. 2021;125:105026.
2. Sanctorum A, Riggio J, Maushagen J, Sepehri S, Arnesdotter E, Delagrange M, et al. End-user engineering of ontology-based knowledge bases. Behaviour & Information Technology. 2022;41(9):1811–29.

Abbreviations: DB: DataBase, KB: KnowledgeBase, RDF: Resource Description Framework, API: Application Programming Interface, Chol: Cholesterol, TG: Triglyceride, AST: Aspartate TransAminase, Phos: Phospholipid, Bili: Bilirubin, GGT: Gamma-glutamyl Transferase, ALP: Alkaline Phosphatase, APOB: Apolipoprotein B, PPARG: Peroxisome proliferator- activated receptor gamma, SREBF1: Sterol regulatory element-binding transcription factor 1, FASN: Fatty acid synthase, ELOVL6: ELOVL Fatty Acid Elongase 6, ACACA: Acetyl-CoA Carboxylase Alpha, CPT1A: Carnitine palmitoyltransferase 1A, ACADSB: Short/branched chain acyl-CoA dehydrogenase

Statistics: the analysis was done with ordinary one-way ANOVA with Šidák's multiple comparison test. A p-value of 0.05 is used.

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