THREE STEPS TO SELECT ANALOGUES FOR SKIN SENSITIZATION PREDICTION USING READ-ACROSS: AN ILLUSTRATIVE CASE STUDY WITH VANILLIN

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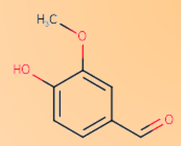
1 INTRODUCTION

The Next Generation Risk Assessment (NGRA) framework for the evaluation of the skin sensitization potential of ingredients uses an exposure-led weight of evidence (WoE) approach which includes new approach methodologies (NAMs) [1,2]. As previously illustrated, read-across may be a key component to increase confidence in the hazard characterisation for the skin sensitization NGRA[3]. However, it requires an explicit description of the analogue identification process as well as a transparent justification of the final analogue selection.

Target chemical: Vanillin

NAME: VANILLIN
CAS number: 121-33-5

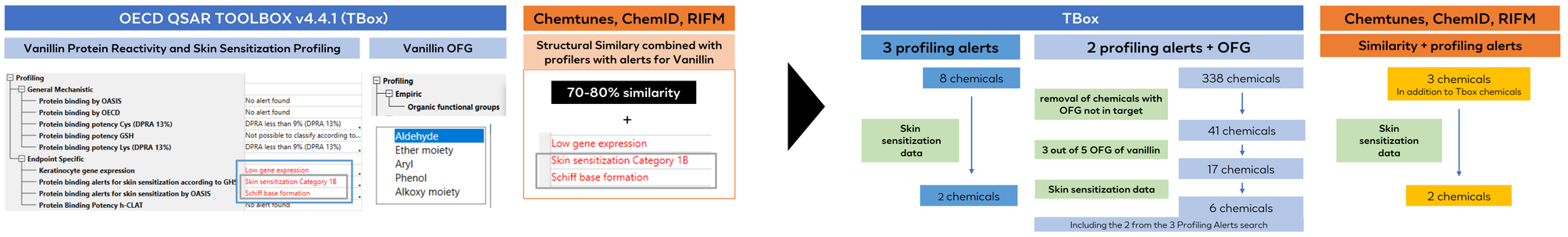
STRUCTURE

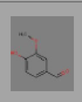


2 WORKFLOW

3 RESULTS & DISCUSSION

1 CHEMICAL SEARCH & SKIN SENSITIZATION INFORMATION



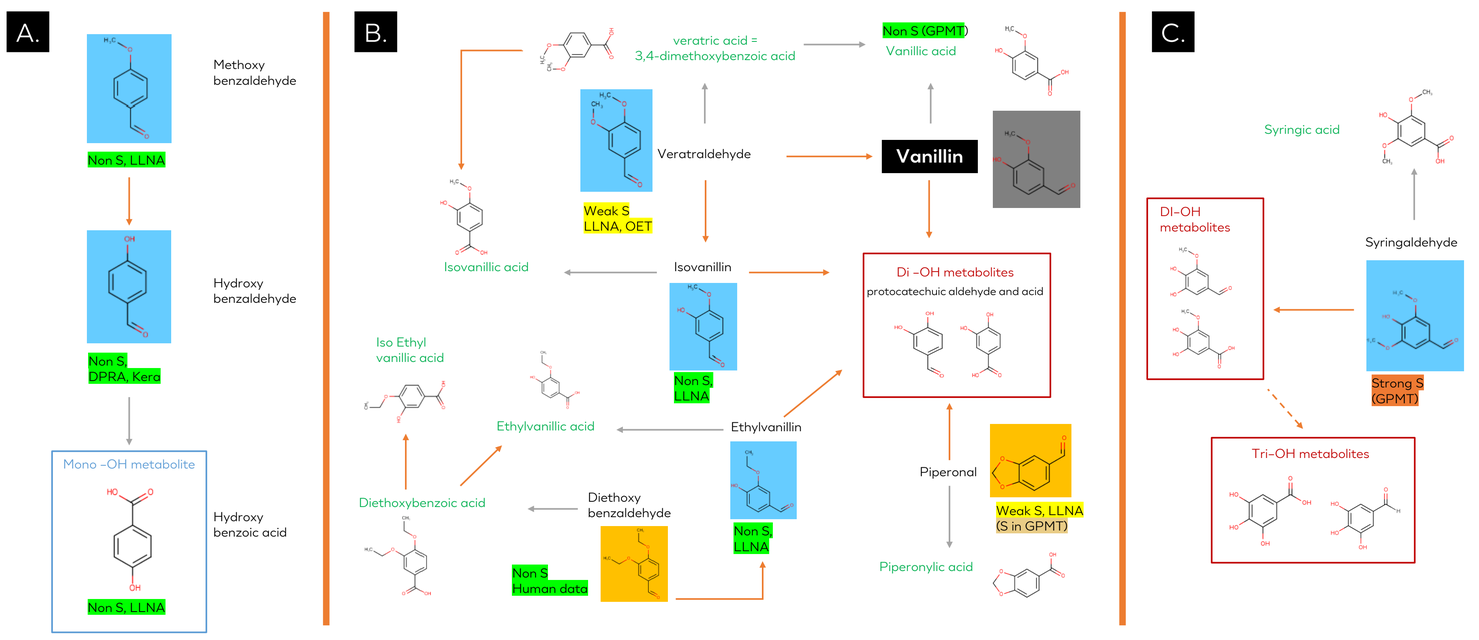
CHEMICAL SEARCH	Chemicals	CAS Number	121-33-5	120-14-9	123-11-5	134-96-3	123-08-0	121-32-4	621-59-0	120-57-0	2029-94-9
		Common Chemical Name	VANILLIN	VERATRALDEHYDE	METHOXY BENZALDEHYDE	SYRINGALDEHYDE	p-HYDROXY BENZALDEHYDE	ETHYLVANILLIN	ISOVANILLIN	PIPERONAL	DIETHOXY BENZALDEHYDE
		SMILES	COc1ccc(C=O)cc1O	COc1ccc(C=O)cc1OC	COc1ccc(C=O)cc1	COc1cc(C=O)cc(OC)c1O	Oc1ccc(C=O)cc1	CCOc1cc(C=O)ccc1O	COc1ccc(C=O)cc1O	O=Cc1ccc2OCOc2c1	CCOc1ccc(C=O)cc1OCC
		Structure									
	Analogue source	Tbox	Target chemical	X	X	X	X	X	X		
		ChemID		X				X	X		
		Chemtunes		X		X		X	X	X	X
		RIFM		X	X	X		X	X	X	X
	Profilers with alert for vanillin	Keratinocyte gene expression	Low gene expression >> Vaniline derivatives	Not possible to classify according to these rules		Low gene expression >> Vaniline derivatives	Not possible to classify according to these rules	Low gene expression >> Vaniline derivatives	Not possible to classify according to these rules		
		Protein binding alerts for skin sensitization according to GHS	Skin sensitization Category 1B >> Aldehydes	Skin sensitization Category 1B >> Aldehydes							
		Protein binding alerts for skin sensitization by OASIS	Schiff base formation with carbonyl compounds >> Aldehydes	Schiff base formation with carbonyl compounds >> Aldehydes							
	Structural features	Organic functional group (OFG)	Aldehyde Alkoxy moiety Aryl Ether moiety Phenol	Aldehyde Alkoxy moiety Aryl Ether moiety	Aldehyde Alkoxy moiety Aryl Ether moiety	Aldehyde Alkoxy moiety Aryl Ether moiety Phenol	Aldehyde Aryl Phenol	Aldehyde Alkoxy moiety Aryl Ether moiety Phenol	Aldehyde Alkoxy moiety Aryl Ether moiety Phenol	Aldehyde Aryl Benzodioxole	Aldehyde Alkoxy moiety Aryl Ether moiety
	Skin sensitization	Conclusion		Weak sensitizer LLNA Open epicutoneous test	Non sensitizer LLNA Keratinosens	Strong sensitizer GPMT	Non sensitizer DPRA+Keratinosens	Non sensitizer LLNA	Non sensitizer LLNA	Sensitizer/Weak sensitiser GPMT / LLNA	No evidence of skin sensitization human repeated patch test

2 *IN SILICO* REACTIVITY, AUTOXIDATION SIMILARITY, PCP, SKIN ABSORPTION

EXTENDED PROFILING	Reactivity profiling	Protein Binding Potency h-CLAT	No alert found	No alert found							
		Protein binding by OASIS									
		Protein binding by OECD									
		Protein binding potency Cys (DPRA 13%)	DPRA less than 9% (DPRA 13%) >>Non-Conjugated monoaldehydes >> Vaniline derivatives	DPRA less than 9% (DPRA 13%) >>Non-Conjugated monoaldehydes >> Vaniline derivatives	DPRA less than 9% (DPRA 13%) >>Non-Conjugated monoaldehydes	DPRA less than 9% (DPRA 13%) >>Non-Conjugated monoaldehydes >> Vaniline derivatives	DPRA less than 9% (DPRA 13%) >>Non-Conjugated monoaldehydes	DPRA less than 9% (DPRA 13%) >>Non-Conjugated monoaldehydes >> Vaniline derivatives			
		Protein binding potency GSH	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)							
		Protein binding potency Lys (DPRA 13%)	DPRA less than 9% (DPRA 13%) >> Vaniline derivatives; Grey zone 9-21% (DPRA 13%) >> Non-alpha,beta-conjugated monoaldehydes	DPRA less than 9% (DPRA 13%) >> Vaniline derivatives; Grey zone 9-21% (DPRA 13%) >> Non-alpha,beta-conjugated monoaldehydes	Out of mechanistic domain	DPRA less than 9% (DPRA 13%) >> Vaniline derivatives	Out of mechanistic domain	DPRA less than 9% (DPRA 13%) >> Vaniline derivatives; Grey zone 9-21% (DPRA 13%) >> Non-alpha,beta-conjugated monoaldehydes	DPRA less than 9% (DPRA 13%) >> Vaniline derivatives; Grey zone 9-21% (DPRA 13%) >> Non-alpha,beta-conjugated monoaldehydes	DPRA less than 9% (DPRA 13%) >> Vaniline derivatives	DPRA less than 9% (DPRA 13%) >> Vaniline derivatives; Grey zone 9-21% (DPRA 13%) >> Non-alpha,beta-conjugated monoaldehydes
	Autoxidation		Aldehyde → Acid	Aldehyde → Acid							
	Similarity	Dice measure in Tbox		[70%,80%)	[60%,70%)	[60%,70%)	[60%,70%)	[70%,80%)	[90%,100%]	[60%,70%)	[50%, 60%)
	Physico chemical properties (CPC)	MW	152.1	166.1	136.1	182.1	122.1	166.1	152.1	150.1	194.2
		ClogP	1.5	1.8	1.8	1.5	1.4	2.1	1.5	1.8	2.9
		pKa Acid	7.8			7.8	7.7	7.9	9.2		
		Water Solubility Class	Slightly soluble	Slightly soluble	Slightly soluble	Very slightly soluble	Slightly soluble	Very slightly soluble	Slightly soluble	Slightly soluble	Very slightly soluble
		Volatility Class	Semi_volatile	Semi_volatile							
	Skin absorption	Living Skin * RF Class	Very high	Very high							

3 SKIN METABOLISM (*IN SILICO* & *IN VITRO* DATA): VANILLIN + 8 ANALOGUES

PHASE-I METABOLISM COMMONALITY BETWEEN TARGET AND ANALOGUES



Same pathways for the target and analogues:

⇨ Aldehyde oxidation (→) and/or dealkylation (→)

⇨ Di –OH metabolites with –CHO or –COOH: Reactive

⇨ Acids and Mono –OH metabolite with –COOH: Non-reactive

Categorization in 3 sub-groups:

A. 2 chemicals
   - Non-sensitizing metabolites

B. Target and 5 chemicals
   - Non-reactive or non-sensitizing acid metabolites
   - Identical reactive metabolites that could be further oxidized into acids (No sensitization data)

C. 1 chemical
   - Non-reactive acid metabolite
   - Different reactive metabolites (compared to group B) and possibly further oxidation in a reactive and sensitizer chemical

- Vanillin was readily absorbed through human skin and almost completely metabolized within 2 hours using fresh human skin explant
- Skin metabolites included vanillic acid, protocatechuic aldehyde and acid
- Also detected were the phase-II metabolites vanillin alcohol glucuronide and sulfate conjugates, protocatechuic aldehyde glucuronide and vanillin glucuronide
- No evidence for additional hydroxylation at he benzene ring of vanillin

NB: The presence of both a para -OH and a meta -OMe group only in syringaldehyde analogue like vanillin does not seem sufficient to convey strong sensitization potential as other chemicals with the identical substructure were found non sensitizer.

4 CONCLUSIONS

1. The eight chemicals retrieved using protein reactivity and OFG showed inconsistent skin sensitization information
2. No refinement of analogue selection was achieved with extended profiling based on additional reactivity profilers, PCP, similarity, skin absorption and autoxidation
3. Skin metabolism prediction together with metabolite reactivity profiling and existing *in vitro* skin metabolism data on vanillin enabled to allocate the eight analogues to three sub-groups (A, B, C) supporting the selection of sub-group B analogues for read-across
4. Based on this, vanillin was finally classified as a weak skin sensitizer.

L'ORÉAL RESEARCH & INNOVATION

REFERENCES

1. Gilmour & Kern et al. RTP 116, 2020
2. Assaf et al. RTP 123 (2021)
3. Gautier et al. RTP 117 (2020)
4. Genies et al. JAT 39 (2019) and 40 (2020)

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