MechoA (Mechanism of Action) SAR Model and Skin Sensitisation Screening: 3-methoxyphenol, 4-methoxyphenol and 1,4-dimethoxybenzene case study.

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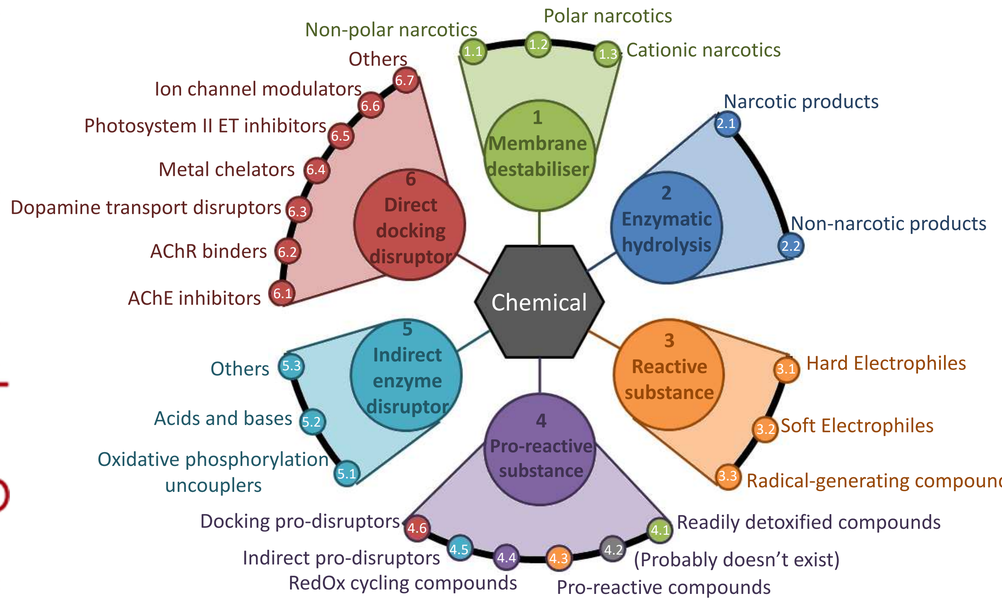
Replacing Experimentation



Scheme 1. MechoA scheme *in-silico* model (freeware)

Introduction

The Molecular Initiating Event (MIE) of the skin sensitisation Adverse Outcome Pathway (AOP, Figure 1, below), is the covalent binding of an electrophilic substance to nucleophilic centres of epidermal proteins, such as, cysteine (-SH) or lysine residues (-NH_2). The hapten-protein complex formed starts the signaling pathway that results in cellular, organ and organism response, *i.e.*, the clinical signs of erythema and edema. Therefore, structural alerts for electrophilic substances are an important aspect in predicting skin sensitisation. Mechanisms of action scheme (MechoA, Scheme 1) is an *in-silico* model that can predict, among other things[1] if a substance is likely to be reactive (MechoA 3 group: electrophilic substances) or pro-reactive, *i.e.* if it will be metabolized into a reactive substance (MechoA 4.3: metabolized into MechoA 3 group substances). In this case study, the skin sensitisation outcome of three structurally similar substances **1**, **2** and **3** is explained *via* their MechoAs and compared to the profilers for skin sensitisation and protein binding in the software Toxtree[2].

Skin Sensitisation Adverse Outcome Pathway and Mechanism of Action (MechoA)

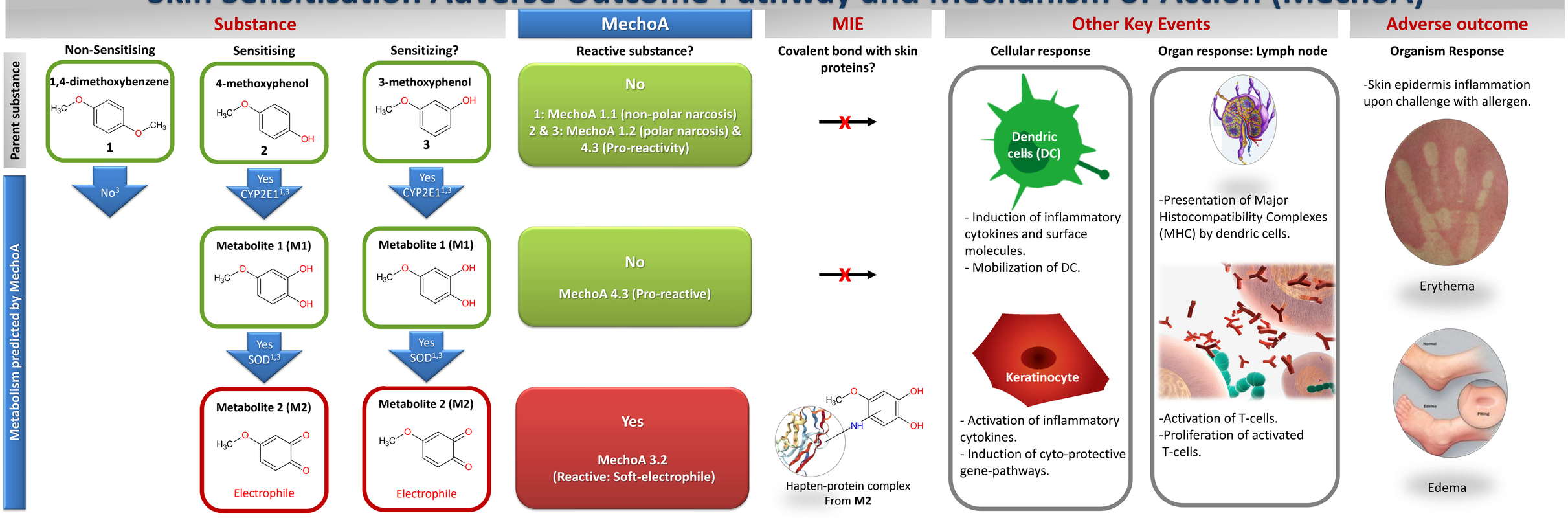


Figure 1. MechoAs for substances 1, 2 and 3 within the skin sensitisation AOP

How to explain the differences in skin sensitisation for these structurally similar substances?

Parent substances **1**, **2** and **3** are all predicted by the MechoA scheme as non-reactive substances (not as MechoA 3 which would be reactive parent substances expected to sensitize, Figure 1, MIE). The first key event necessary for skin sensitisation is electrophilic reactivity of the substance or its metabolites with skin proteins. Since no electrophilic reactivity is detected for the parent substances, covalent protein binding is not expected. So, why the differences in skin sensitisation in *in-vivo* studies for these structurally similar substances? (Table 1) – The answer is "due to their metabolites".

Substance **1** does **not** fall into the reactive or pro-reactive categories of the MechoA scheme model (MechoAs 3 or 4.3). Therefore, it is not expected to bind to proteins, and no skin sensitisation should occur. This result is consistent with the observed *in-vivo* results and Toxtree's skin sensitisation profiler prediction (Table 1). However, Toxtree's protein binding profiler does give an alert for this substance as a Michael acceptor, with no further information[2].

Substances **2** and **3 do** fall into the pro-reactive category of the MechoA scheme model: MechoA 4.3, which predicts their metabolization into a common metabolite[3]: **M1** (4-methoxycatechol) and **M2** (o-quinone) by CYP2E1 and SOD enzymes respectively (Figure 1).

The **M2** metabolite does fall into the reactive electrophile category: MechoA 3.2. Therefore, **M2** is expected to bind to proteins (MIE, Figure 1). This is in agreement with Toxtree's predictions for protein binding profiler for both substances. However, in this case, Toxtree's skin sensitisation profiler gives an alert for **2**, but not for **3.**

The prediction for **2** is reliable by comparison with the *in-vivo* result for the same substance. In the absence of *in-vivo* data for **3**, the skin sensitisation non-alert by Toxtree cannot be validated. Nonetheless, based on **2** and **3** sharing the same electrophilic metabolite (**M2**) which is expected to bind to proteins, it is more likely for **3** to also be a skin sensitizer.

Chemical Name And Structure	1,4-dimethoxybenzene (1)	4-methoxyphenol (2)	3-methoxyphenol (3)
Model	**Outcome**		
MechoA**	Not reactive	Reactive metabolite	Reactive metabolite
Toxtree: Protein binding	Alert	Alert	Alert
Toxtree skin sensitisation reactivity domains	No Alert	Alert	No Alert
***in-vivo**[4,5]**	Non-SS	SS	No data

Table 1. Comparison of skin sensitisation predictions by Toxtree and the MechoA scheme model
**In-vivo* validated study, similar to guinea pig maximization test (GPMT).
**Predictions based on the reactivity of parent substance and/or metabolites.

Conclusions and Perspectives

The MechoA *in-silico* model can be used in the comprehensive evaluation of both *in-vivo* skin sensitisation data and (Q)SAR predictions based on protein binding. For example, the mechanistic insight given by MechoA scheme model for **2** and **3** raises concern for the no-alert given by Toxtree skin sensitisation profiler for **3**. It is necessary to run other (Q)SARs for this endpoint and substance in a weight of evidence (WoE) approach. In this way, MechoAs can also help in the decision making for experimental testing if it were necessary to strengthen available results or if it were required by the regulation.

The MechoA scheme model is therefore a useful complementary tool to the skin sensitisation prediction models providing further comprehensive information about the mechanisms of actions of the parent substances, metabolites and potential hapten identification. Furthermore, it is known that electrophilic substances are capable of DNA binding. Therefore, MechoAs can also be a valuable tool in the evaluation of substance mutagenicity.

References

(1) Bauer, Franklin J., et al. (2018).
(2) Enoch, S.J. et al (2008 and 2011)
(3) Moridani, M. Y, et al. (2003) & Picardo, M. (1987).
(4) https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/16005/7/5/2
(5) https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/2003/7/5/2.
(6) MechoA scheme model has been implemented into a user-friendly interface, that only requires the SMILES code of the substance as input, and is freely available on-line at https://isaferat.kreatis.eu/.

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