A Defined Approach to Skin Sensitization Using Derek Nexus and Non-Animal Assays

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Introduction

Skin sensitisation initiated by covalent binding to proteins is one of the best understood adverse outcome pathways (AOP)[1]. The AOP consists of 4 key events and a number of assays have been designed to measure one or more of these (Figure 1) and have corresponding OECD Test Guidelines[2-4]. However, it is generally accepted that combinations of information sources (e.g. *in chemico/in vitro assays/in silico* results/read-across), known as defined approaches, are needed to cover the whole AOP and be an adequate replacement of the current *in vivo* assays (LLNA/GPMT) used to predict skin sensitisation. Here, we present a defined approach using the guided integration of *in silico* (Derek Nexus) and *in chemico/in vitro* assay data using exclusion criteria.

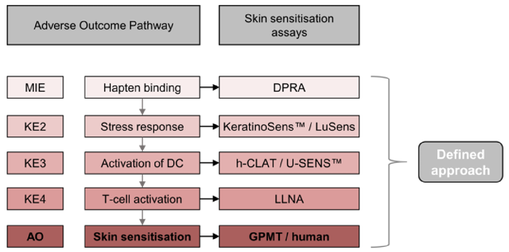

Figure 1. The skin sensitisation AOP describing the 4 key events which lead to the adverse outcome (AO)[1]. MIE = Molecular Initiating Event. KE = Key event. AO = Adverse outcome.

Materials and Methods

A dataset for evaluation of the defined approach was collated from two published data sources (Figure 2)[5-6]. Chemicals with ambiguous structures, e.g. undefined mixtures/polymers, and chemicals with discordant *in vitro/in vivo* data (upon combination) were removed from the dataset and not considered for further analyses (Table 1).

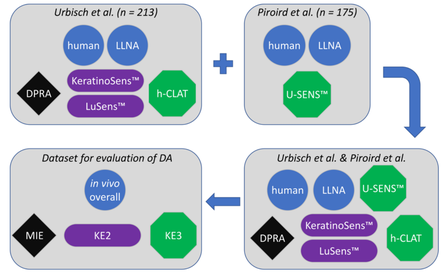

Figure 2. Combination of two published datasets to be used to evaluate the defined approach[5-6].

Key Event	Assay	+	-	Total	Discordant data	Final total
MIE	DPRA	120	72	192	n/a	192
2	KeratinoSens™	122	62	184	5	195
	LuSens	45	32	77		
3	h-CLAT	113	50	163	25	186
	U-SENS™	130	39	169		
4	LLNA	173	60	233	human call taken if both available	240
AO	Human	83	46	129		

Table 1. Grouping of in chemico and in vitro data into their corresponding key events and grouping of human and LLNA data into an in vivo call.

Establishing exclusion criteria

Exclusion criteria		Derek	MIE	KE2	KE3	Comment
Metabolism	Prohapten	✓	✗	✓	✓	For chemicals inducing skin sensitisation after metabolic activation (prohaptens) the assay measuring the MIE (DPRA) is de-prioritised as it lacks metabolic competency[2].
logP	> 3.5	✓	✓	✓	✗	Chemicals with a logP > 3.5 and a logP > 5 are de-prioritised in assays measuring KE3 (h-CLAT/U-SENS™) and KE2 (KeratinoSens™/LuSens), respectively, as more lipophilic chemicals may lack high solubility in these cell-based assays[3-4].
	> 5	✓	✓	✗	✗	
Lysine reactive	Exclusive	✓	✓	✗	✓	KE2 assays are designed to replicate the Nrf2-ARE pathway which is associated with skin proteins binding to cysteine residues[7-8], therefore, exclusively lysine-reactive chemicals may not be reliably predicted (acylating agents and related chemicals).
Likelihood	Equivocal	✗	N/A			Derek alerts with a likelihood of equivocal indicate that there is less evidence of skin sensitisation potential than other likelihoods (e.g. certain). Consequently, an equivocal *in silico* prediction is de-prioritised.
Negative prediction	Misclassified features	✗	N/A			Non-alerting chemicals which gave a negative prediction of 'non-sensitiser with misclassified features' or 'non-sensitiser with unclassified features' were de-prioritised as these are associated with higher uncertainty.
	Unclassified features	✗	N/A			

Table 2. Summary of exclusion criteria used in the defined approach. MIE = Molecular Initiating Event. KE = Key event.

Results

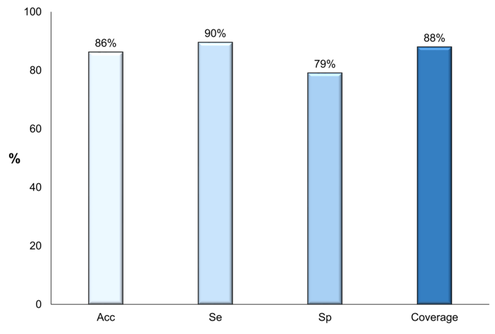

Figure 5. Performance of defined approach hazard prediction vs in vivo call. 86% of chemicals are correctly predicted as sensitisers or non-sensitisers by the defined approach. The sensitivity and specificity are 90% and 79%, respectively. The coverage is 88% as the defined approach can make hazard predictions for 211/240 chemicals in the dataset.

Non-predictions are possible in cases where there is a lack of in chemico/in vitro data for a specific chemical and/or where data exist but are outside the applicability domain of the assay/model based on the exclusion criteria.

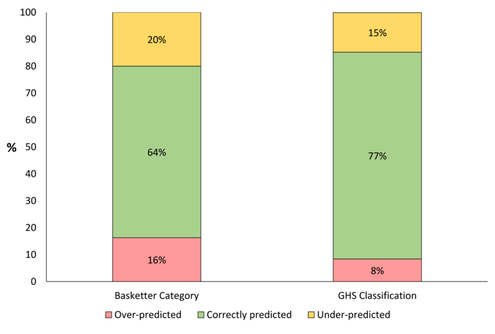

Figure 6. Performance of defined approach potency prediction vs in vivo call. The correct Basketter classification (1-6) is predicted for 64%, and the correct GHS classification (1A, 1B, 2) for 77% of the dataset. Of the chemicals classified as sensitiser/non-sensitiser in the hazard prediction, 90% have a corresponding potency prediction (191/211 chemicals).

Non-predictions are possible where an alerting chemical contains insufficient LLNA (EC3) or human data in the potency prediction model (e.g. < 3 nearest neighbours) or where a non-alerting chemical is classified as a sensitiser based solely on in chemico/in vitro data.

Decision tree based defined approach

Chemical of interest → Prioritise *in chemico/in vitro* assays using exclusion criteria → Use Derek outcome to determine decision tree branch → Run *in chemico/in vitro* assays in order of AOP (MIE → KE2 → KE3) unless de-prioritised by exclusion criteria → Hazard prediction using '2 out of 3' approach → Potency prediction using *k*-nearest neighbours model

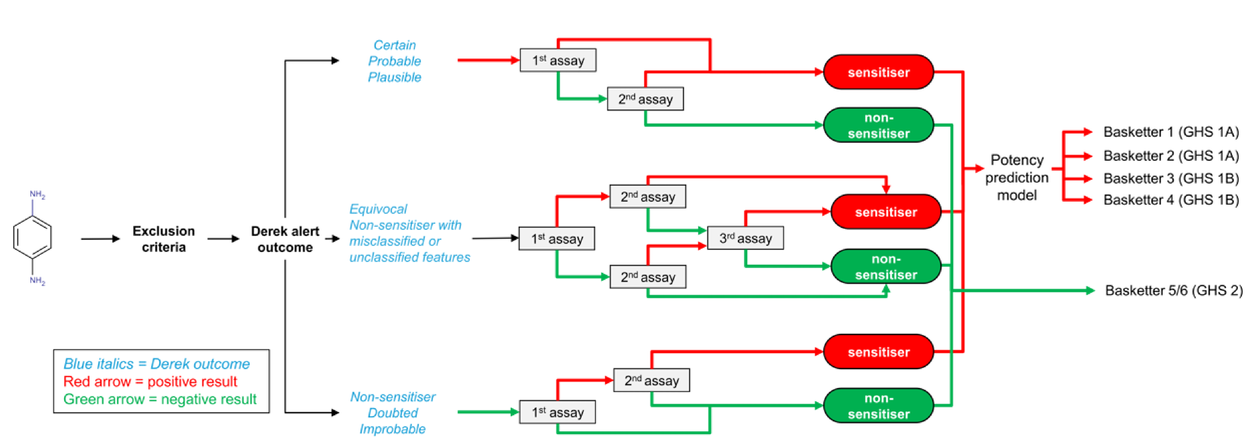

Figure 4. To begin, Derek evaluates the metabolism, lipophilicity, and lysine reactivity of an alerting chemical (only logP calculated for non-alerting chemicals) and in chemico/in vitro assays are de-prioritised according to the exclusion criteria. Next, the Derek prediction is used to dictate which branch of the decision tree is to be followed. The required number of assays are now run in order of KE in the AOP (MIE → KE2 → KE3), unless de-prioritised in the previous step, until two concordant results are obtained. When the chemical is predicted to be a non-sensitiser, the GHS classification is assigned as 2, and the Basketter classification as category 5/6. When the chemical is predicted to be a sensitiser it is then assigned a Basketter category (1-4) and GHS classification (1a-1B) based on the output of the alert-based k-nearest neighbours potency prediction model[9], built upon a dataset of over 700 chemicals with publicly available human and/or LLNA data.

Conclusions

A decision tree defined approach has been designed using exclusion criteria based on known limitations of *in chemico/in vitro* assays covering key events 1-3 and an *in silico* model (Derek) covering the whole skin sensitisation AOP. The hypothesis proposed was that excluding less reliable assays and/or *in silico* outcomes for specific chemicals would produce a rational and reasonable defined approach for the prediction of skin sensitisation potential.

The defined approach correctly predicts the hazard classification (sensitiser/non-sensitiser) for 86% of chemicals where a prediction could be made (*n* = 211) and correctly identifies the Basketter potency category and GHS classification for 64% and 77% of chemicals where a potency prediction could be made (*n* = 191).

As some of the chemicals in the dataset described in this paper may have been used in the development of the *in chemico/in vitro* assays, further work will be focussed on the validation of the defined approach using a true test set of new *in chemico/in vitro* data - Lhasa Limited are actively exploring and welcoming avenues and collaborators where this can be achieved.

References: 1. OECD. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins Part 1: Scientific Evidence. 2012. 2. OECD. Test No. 442C: *In Chemico* Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA). 2015. 3. OECD. Test No. 442D *In Vitro* Skin Sensitisation assays addressing the Key Event on: Keratinocyte activation (draft). 2017. 4. OECD. Test No. 442E: *In Vitro* Skin Sensitisation assays addressing the Key Event on: Activation of dendritic cells. 2017. 5. Urbisch *et al*. Assessing skin sensitization hazard in mice and men using non-animal test methods. *Regul Toxicol Pharmacol*. 2015, 71, 337-351. 6. Piroird *et al*. The Myeloid U937 Skin Sensitization Test (U-SENS) addresses the activation of dendritic cell event in the adverse outcome pathway for skin sensitization. *Toxicol Vitr*. 2015, 29, 901-916. 7. Natsch A. The KeratinoSens™ Assay for Skin Sensitization Screening. In: *Alternatives for Dermal Toxicity Testing*. 2017, 235-248. 8. Ramirez *et al*. LuSens: a keratinocyte based ARE reporter gene assay for use in integrated testing strategies for skin sensitization hazard identification. *Toxicol In Vitro*. 2014, 28, 1482-1497. 9. Canipa *et al*. A quantitative *in silico* model for predicting skin sensitization using a nearest neighbours approach within expert-derived structure-activity alert spaces. *J Appl Toxicol*. 2017, 37, 985-995.