Generation of novel *in chemico/in vitro* skin sensitisation data to evaluate the human relevance of defined approaches

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Introduction

Skin sensitisation, leading to allergic contact dermatitis, is a common occupational health issue traditionally assessed using *in vivo* assays like the murine local lymph node assay (LLNA). However, political and ethical pressure has led to increased use of non-animal alternatives such as *in chemico* and *in vitro* assays designed to measure specific key events in the skin sensitisation adverse outcome pathway (Figure 1)[1].

Several assays are now validated by the Organisation for Economic Co-operation and Development (OECD)[2-4] but individual results from these assays are not sufficient to assign a given chemical as a sensitiser - as such results from multiple information sources (assays, *in silico* models, physicochemical parameters) are often combined in what is known as a defined approach (DA)[5].

DAs are often built upon animal data and consequently can be less predictive of human sensitisation potential. Furthermore, this can be difficult to assess as the amount of publicly available *in chemico / in vitro* data with corresponding human data is sparse. As such, a collaborative project was devised whereby chemicals lacking *in chemico / in vitro* data but with human potency data (and LLNA data, where available) would be identified and *in chemico / in vitro* data generated. This data would then be used to (1) assess the performance of the assays against human and LLNA data (if available) (2) assess against several well-known DAs[6-8] and (3) investigate the relationship between *in chemico / in vitro* data and human potency.

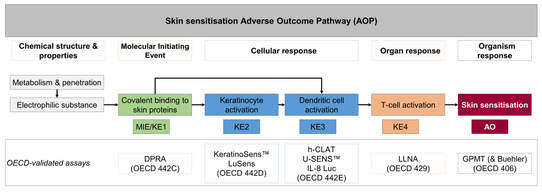


Figure 1. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins (adapted from OECD 2012).[1]

Prioritisation of chemicals

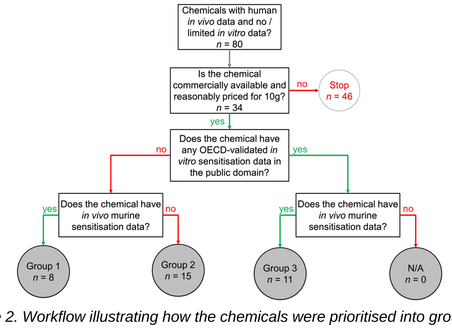


Figure 2. Workflow illustrating how the chemicals were prioritised into groups.

Newly generated *in vitro* data and DA results

The 8 chemicals assigned into Group 1 were purchased, assigned a unique identification code by Lhasa Limited and tested blind by Covance Laboratories Limited in the DPRA, KeratinoSens™, and h-CLAT assays (Table 1). Selected data from each assay, the corresponding *in vivo* data, and the DA result when using this data is shown below. These data were then used to assess the performance of each assay individually, and when used within a DA.

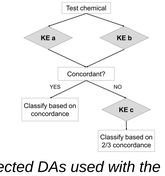
	***In vivo* data**		***In chemico / in vitro / in silico* data**				**DA**			
Code	**Human (category)**	**LLNA (EC3 %)**	**DPRA (mean depletion %)**	**KeratinoSens™ (EC1.5 µM)**	**h-CLAT (MIT µg/mL)**	**Derek Nexus**	**BASF 2/3**	**Kao ITS**	**Kao STS**	**Lhasa DA**
LL-001	1	0.4	50.0	0.98	0.38		*	1A	1A	1A
LL-002	5	>10	1.12	133.37	507.43		*	NC	1B	NC
LL-003	4	65.9	0	184.66	850.33		*	NC	1B	*
LL-006	4	19.2	0.26	N/A	315		*	NC	1B	*
LL-018	1	8.6	3.57	58.34	2061.41		*	1B	1B	NC
LL-019	5	60	9.93	42.96	161.72		*	1B	1B	1B
LL-027	3	>10	1.69	N/A	578.69		*	NC	1B	NC
LL-029	6	>25	3.81	N/A	N/A		*	NC	NC	NC

*Table 1. Newly generated in chemico / in vitro data for Group 1 chemicals and corresponding in vivo and DA information. Red box = the chemical is considered to be a skin sensitiser by the relevant assay/DA. Green box = non-sensitiser. Grey box = no prediction possible. 1A = strong sensitiser, 1B = weak sensitiser, NC = not classified. * = no potency prediction possible.*

Performance when used in selected DAs

Defined Approaches

BASF 2/3

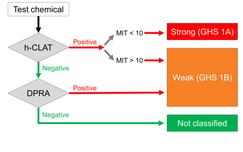


Kao ITS

Score	h-CLAT MIT (µg/ml)	DPRA depletion (%)	Derek Nexus
3	< 10	≥ 42.47	-
2	> 10 ≤ 150	≥ 22.62 < 42.47	-
1	> 150 ≤ 5000	≥ 6.376 < 22.62	Positive (alert fired)
0	Not calculated	< 6.376	Negative (no alert)

Total	Potency prediction
6-7	Strong (GHS 1A)
2-5	Weak (GHS 1B)
0-1	Not classified

Kao STS



Lhasa DA

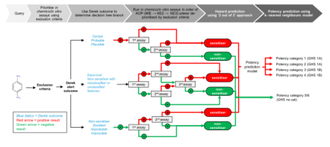


Figure 3. Selected DAs used with the newly generated in chemico/in vitro data.

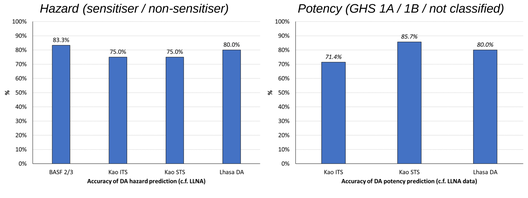


*Figure 4. Accuracy of each DA - compared against **LLNA** data*

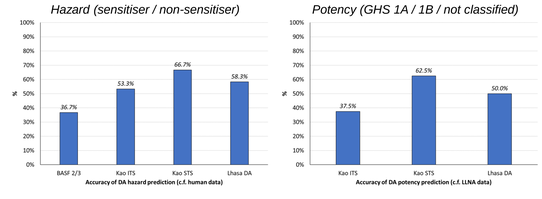


*Figure 5. Accuracy of each DA - compared against **human** data*

The newly generated data was used in several previously published DAs[6-8] (Figure 3) and all were more accurate when predicting LLNA data compared to human data. This could be due to the DAs being developed using mainly LLNA data - however, this could also be down to the small dataset (n = 8) where one or two mispredictions have a large impact on performance metrics - as well as the conflicting *in vivo* results meaning 3 chemicals (LL-002, LL-019, and LL-027) can only be correct in one species.

The BASF 2/3 gave the highest prediction of skin sensitization hazard (83%) when compared against LLNA data although all other DAs assessed were correct for at least 75%. Conversely, the Kao STS was most predictive for the human outcome (67%), closely followed by the Lhasa DA (58%) and the Kao ITS (53%) whereas the BASF 2/3 did not perform quite as well (37%). For prediction of potency, the Kao STS was the most accurate (LLNA, 86%; Human, 63%), followed by the Lhasa DA (LLNA, 80%; Human, 50%), and then the Kao ITS (LLNA, 71%; Human, 38%).

Performance of *in vitro* assays vs *in vivo* data

Generally, the newly generated assay data were less predictive of LLNA (Figure 6) and human data (Figure 7) than when compared against a larger set of *in vivo* data published previously by Cosmetics Europe. The sensitivity increased for h-CLAT but not the DPRA/KeratinoSens™ in both cases.

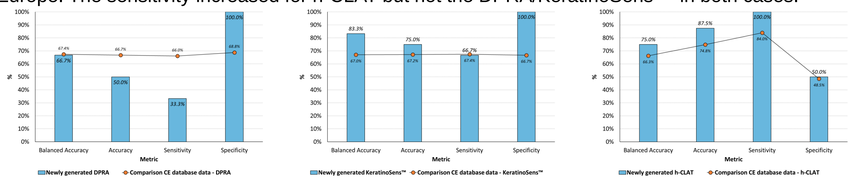


Figure 6. Performance of newly generated data (blue bars) and a larger, previously published Cosmetics Europe (CE) dataset (orange circles) for each assay when compared against LLNA data.

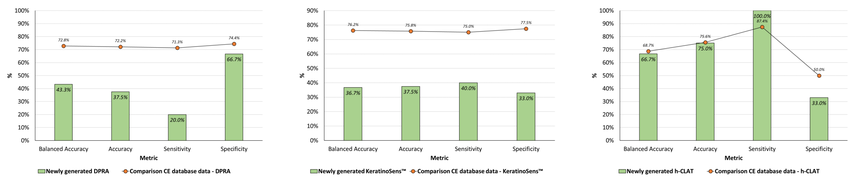


Figure 7. Performance of newly generated data (green bars) and a larger Cosmetics Europe (CE) dataset (orange circles) for each assay when compared against human data.

Conclusions and further work

- DPRA, KeratinoSens™, and h-CLAT data has been generated for 8 chemicals with publicly available LLNA and human data.
- Individual assay results as well as DAs utilising these data were more predictive of LLNA data than human data.
- The 4 DAs were reasonably accurate for predicting skin sensitization hazard (between 75%-83% compared to LLNA and between 37%-67% compared to human data) and potency (between 71%-86% compared to LLNA and between 38%-63% compared against human data)
- Overall, the Kao STS provided the highest accuracy for both hazard and potency, followed by the Lhasa DA - however, the small dataset must be considered before definitive conclusions are drawn.
- An additional 15 chemicals (Group 2) are being tested.
- Additional analysis including applicability domain exploration and investigating possible links to human potency will be carried out once the data is finalised.
- A manuscript detailing the full data and analysis is in preparation.

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. (2015). 3. OECD. Key Event Based Test Guideline 442D (2018). 4. OECD. Key Event Based Test Guideline 442E (2018). 5. OECD. Guidance Document on the Reporting of Defined Approaches and Individual Information Sources to be Used within IATA for Skin Sensitisation. (2017). 6. Urbisch, D. *et al*. Regul. Toxicol. Pharmacol. 71, 337–351 (2015). 7. Takenouchi, O. *et al*. J. Appl. Toxicol. 35, 1318–1332 (2015). 8. Macmillan, D. S. & Chilton, M. L.. Regul. Toxicol. Pharmacol. 101, 35-47 (2019).