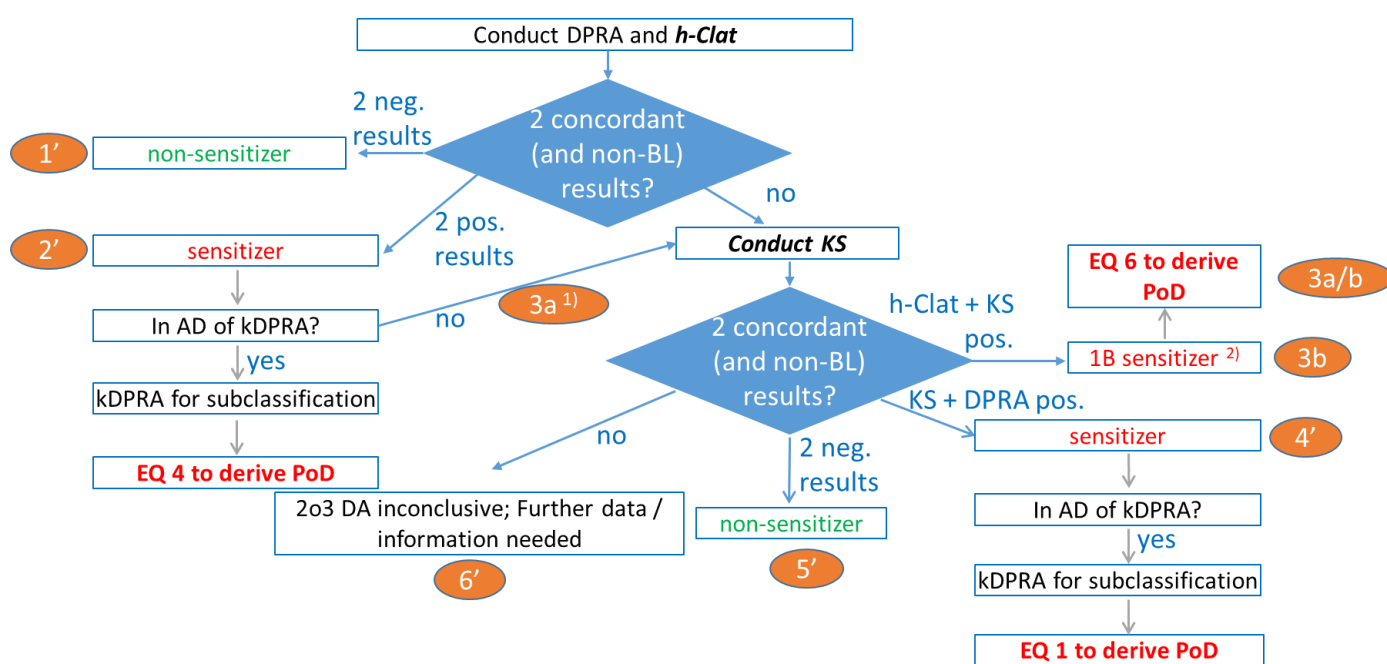*Natsch and Gerberick:*

Integrated Skin Sensitization Assessment Based on OECD Methods (II): Hazard and Potency by Combining Kinetic Peptide Reactivity and the "2 out of 3" Defined Approach

Supplementary Data: ESM2

1   An alternative testing sequence starting with h-CLAT instead of KeratinoSens

1) Chemicals ouside of the applicability domain of the kDPRA according to APPENDIX III, ANNEX 1 of OECD TG 442C can be assessed based on h-Clat and KeratinoSens data if potency information is required
2) Chemicals negative in DPRA and kDPRA, but positive in h-Clat and KS are normally not 1A sensitizers based on kDPRA and based on DA ITS. Chemicals assessed with EQ 6 because outside of AD of kDPRA (scenario 3a) are not considered 1B chemicals directly, unless DPRA is negative

Fig. ESM2-1: An alternative testing sequence includes GHS sub-classification and PoD determination in the 2o3 DA starting with the h-CLAT
The numbers in orange bubbles indicate the different scenarios indicated in ESM2.

2   Analysis of prediction accuracy compared to the OECD-curated data if available instead of published databases

Tab. ESM2-1: Chemicals rated positive by the 2o3 DA (n = 116)[a] assessed for PoD using different testing sequences
Data are compared to LLNA EC3 values from the curated OECD database where available, else historically published values are used.

Approach	Fold-misprediction[b] (Geomean)	Fold-misprediction[b] (Median)	Chemicals > 5-fold under-predicted[c] (n, %)	Chemicals > 10-fold under-predicted (n, %)	Chemicals > 5-fold over-predicted[c] (n, %)	Chemicals > 10-fold over-predicted (n, %)
According to Figure 1	3.7	3.0	15 (13%)	7 (6%)	24 (21%)	10 (9%)
Performing h-CLAT and DPRA first	3.7	3.2	16 (14%)	6 (5%)	23 (20%)	11 (9%)
Using all evidence	3.3	2.6	18 (16%)	5 (4%)	15 (13%)	6 (5%)

[a] Different from the parallel analysis (Natsch, 2022) comparing the different equations on all chemicals including negatives, this analysis is focused on the subset of chemicals rated positive in the 2o3 DA and assessed with different testing sequences. [b] The ratio between the higher and the lower values of the measured and predicted EC3 value. Predicted EC3 > 100% were set to 100%. [c] Under-predicted chemicals are those for which the measured LLNA EC3 is lower than the predicted EC3; over-predicted chemicals are those with measured LLNA EC3 higher than the predicted value.

For the analysis according to Figure 1, Tables ESM3-1 and ESM3-2 list the individual chemicals which are mispredicted by a factor > 5-fold when the analysis is done vs. the published, historical LLNA database. When analyzing against the OECD-curated LLNA values, with the very few exceptions listed below, the same mispredictions are obtained:

− *Chemicals > 5-fold overpredicted by the calculated PoD:* There is one additional chemical that appears overpredicted vs the OECD database: 2-ethyl-hexyl-acrylate, CAS 103-11-7. However, the OECD DB contains a citation error and lists an LLNA EC3 of 37.4 %, citing Dearman et al. 2007. However, that value is for ethyl acrylate and not 2-ethyl-hexyl acrylate. ECHA also lists a second LLNA value for 2-ethyl-hexyl acrylate of 18.9%. Taking this citation error into account, the same chemicals are > 5-fold overpredicted in both analyses.

− *Chemicals > 5-fold underpredicted by the calculated PoD:* Few differences in Table ESM2-2 below are observed when comparing with the OECD database:

Tab. ESM2-2: Chemicals > 5-fold mispredicted by either the OECD LLNA DB or the historical database but not by both

	LLNA published	LLNA OECD	EC3 predicted	Fold misprediction vs published	Fold misprediction vs OECD
1,4-Phenylenediamine	0.16	0.11	0.6	3.6	5.3
3-Dimethyl-amino-1-propylamine	2.2	3.5	11.3	5.1	3.2
Ethylene-diamine	2.2	NC (negative in 2 of 3 LLNA studies) [1]	15.3	7.0	Not applicable
1,4-Hydroquinone	0.1	0.19	0.8	8.5	4.5

[1] While ethylendiamine was widely considered a positive reference chemical vs LLNA data, OECD review indicates that it is negative in 2 of 3 studies.

Reference

Natsch, A. and Gerberick, G. F. (2022). Integrated assessment of skin sensitization based on OECD validated methods (I): Deriving a point of departure for risk assessment. *ALTEX 39*, 636-646. doi:10.14573/altex.2201141