Non-animal skin sensitization testing under REACH

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1 Introduction

With the recent updates of the REACH regulation, via Commission Regulation 2016/863 and 2016/1688, further replacement of *in vivo* testing is now required. For skin sensitization, OECD test guidelines for *in vitro* and *in chemico* testing have recently been accepted or are still under development. Nevertheless, the non-*in vivo* testing strategy has already been included in the regulation (effective from 11 October 2016). Where before one *in vivo* test, preferably the LLNA, was performed, now a comprehensive testing strategy has been implemented, as no single *in vitro* test fulfils the requirements for the toxicity endpoint skin sensitization for either REACH or for Classification and Labelling according to UN GHS.

With the amended requirements for skin sensitizing substances, determination of skin sensitizing potency (Cat 1A *versus* 1B) is required, in order to identify extreme sensitizers needing a specific concentration limit for use in mixtures. There is currently no standardized way to assess potency with *in vitro* methods.

Charles River Laboratories proposes tiered strategies for both mono-constituents and for UVCB's, and discusses the challenges to get reliable information on skin sensitization potential, including potency, from these tests strategies to be able to perform *in vivo* studies only as a last resort.

2 AOP skin sensitization

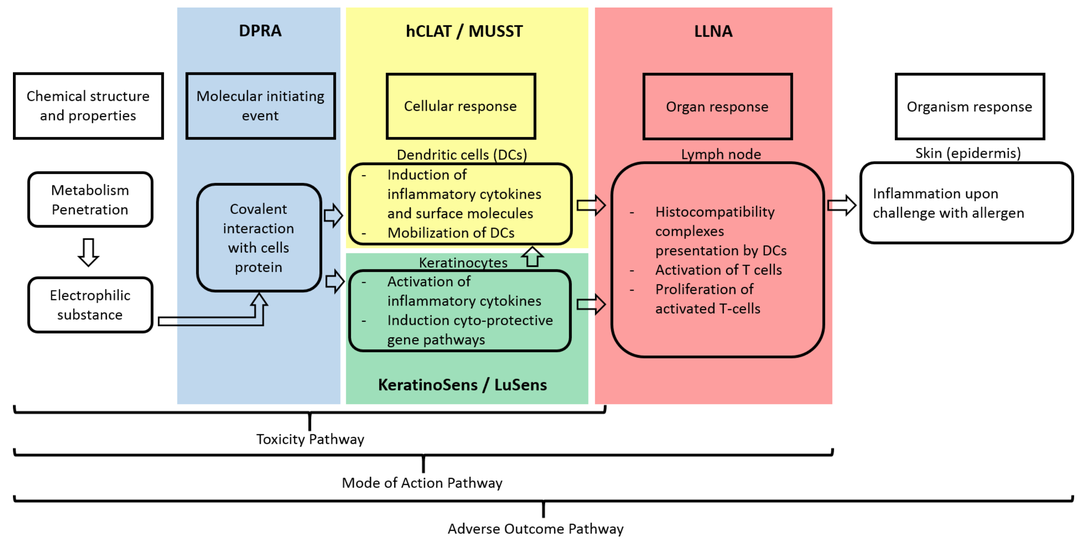


Figure 1: Skin sensitization Adverse Outcome Pathway

3 *In silico*, *in chemico* and *in vitro* skin sensitization tests

QSAR Model DEREK NEXUS (Knowledge-based):
No alerts fired may be extrapolated to a negative prediction.
Database includes information on metabolism and on potency (EC3).
Together with the study results, this information may give more body to the weight of evidence (WoE).

Table 1: Test system(s) per key event

Key Event	Non-*in vivo* test system
Key event 1: covalent binding to proteins	**DPRA**: OECD 442C (2015) OECD Guideline for the Testing of Chemicals: *In Chemico* Skin Sensitization: Direct Peptide Reactivity Assay (DPRA)
Key event 2: keratinocyte inflammatory responses	**KeratinoSens™**: OECD 442D (2015)) OECD Guideline for the Testing of Chemicals: *In Vitro* Skin Sensitization: ARE-Nrf2 Luciferase Test Method **LuSens™**: based on the ARE-Nrf2 Luciferase Test Method
Key event 3: activation of dendritic cells	**h-CLAT**: OECD 442E (2016) OECD Guideline for the Testing of Chemicals: *In Vitro* Skin Sensitization: human Cell line Activation Test (h-CLAT). **U-SENS™(MUSST)**: Draft OECD Guideline *In Vitro* Skin Sensitization U937 Skin Sensitization Test (U-SENS™)
Key event 4: T-cell proliferation	-
Adverse outcome	Contact hypersensitivity (allergic contact dermatitis in humans)

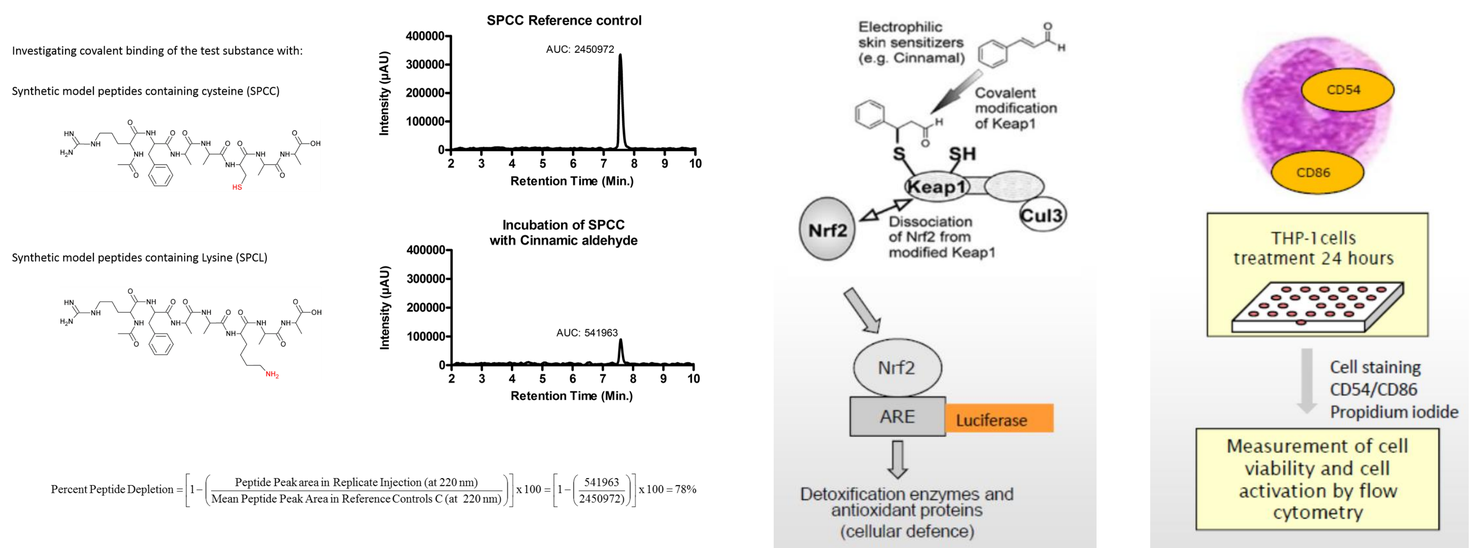
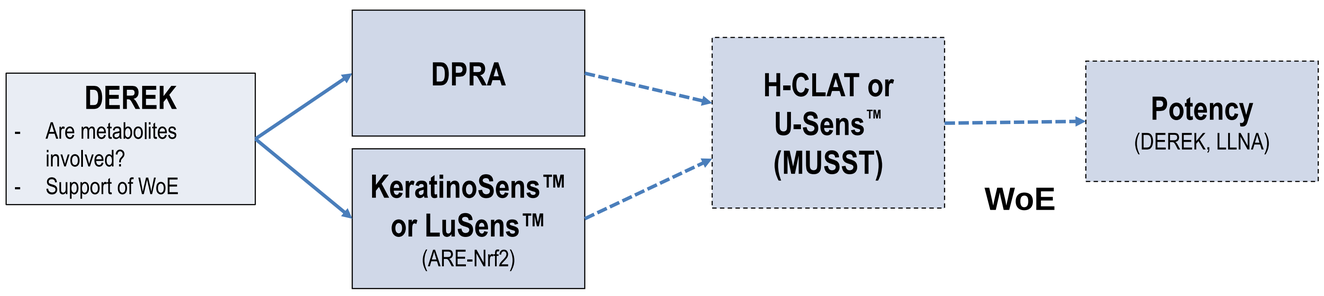


Figure 2: Principle of the DPRA, KeratinoSens™ and h-CLAT

4 Proposed Test Strategy for Mono-constituents (non-UVCB)



UVCB, metals: two test available for event 2 (ARE-Nrf2) and event 3 h-CLAT/U-SENS™ (MUSST), WoE and potency

DEREK: negative or positive
All tests can result in negative, positive or equivocal (*e.g* not tested up to and including 2000 µM due to solubility)

Test results non-UVCB
42 possible combinations of test results together with DEREK:
10 non-sensitizer
10 sensitizer (potency from DEREK, otherwise testing required *in vivo*)
22 equivocal (WoE/*in vivo* testing required)

Test results UVCB, metals
9 possible combinations of test results:
1 non-sensitizer
1 sensitizer (potency testing required)
7 equivocal (WoE/*in vivo* testing required)

5 Considerations and Conclusion

Considerations for improvement and development:

- Log Pow>3.5: *in vitro* testing may result equivocal.
- *In chemico* and/or *in vitro* tests results can be equivocal due to limited solubility of the test substance (not tested up to and including the highest concentration required).
- The currently available non-animal test methods have no or limited metabolic capacity; this may result in false negative results.
- UVCB or metal: DPRA and DEREK not possible.
- No *in vitro* potency test is available yet that is acceptable for regulatory and classification purposes.

In conclusion, a strategic battery of *in chemico/in vitro* tests, together with an appropriate *in silico* model, can be combined using a WoE approach to fulfil the requirements for the endpoint skin sensitization for REACH. At this moment, *in vitro* tests for skin sensitization cannot replace the LLNA completely (equivocal results, potency), nor is it accepted yet by other legal frameworks. Further development/refinement of *in vitro* tests as well as reliable and acceptable *in vitro* skin sensitizing potency tests are required. The complex test strategy for skin sensitization, together with the long time needed to develop reliable and acceptable *in vitro* studies, demonstrates the laborious way to go for replacement of *in vivo* studies.