Non *in vivo* skin sensitization strategy under REACh in practice: hurdles that need attention to minimize the need for *in vivo* testing

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1 Introduction

Since October 2016 *in vivo testing* for skin sensitization has been replaced by non-animal tests for the registration of industrial chemicals under REACh (Commission Regulation (EU) 2016/1688 and 2017/706. A comprehensive testing strategy has been implemented, as no single non *in vivo* test fulfils the requirements for the toxicity endpoint skin sensitization. *In vivo* testing for skin sensitizing properties is permitted as a last resort only.
With the amended requirements, skin sensitizing potency needs to be determined for skin sensitizing substances (Cat 1A *versus* 1B), 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 the available and accepted non *in vivo* methods.

Charles River Laboratories proposes tiered strategies for both mono/multi-constituents and for UVCB's/metal containing substances, and presents the results from 33 chemicals which have been tested for their skin sensitizing properties with non *in vivo* tests. Results obtained with the currently available and regulatory accepted non *in vivo* tests do not always permit a definite conclusion on skin sensitizing properties; moreover, as the present non *in vivo* tests do not include information on skin sensitizing potency, *in vivo* testing is still needed for a substantial number of substances.

2 AOP skin sensitization and tests

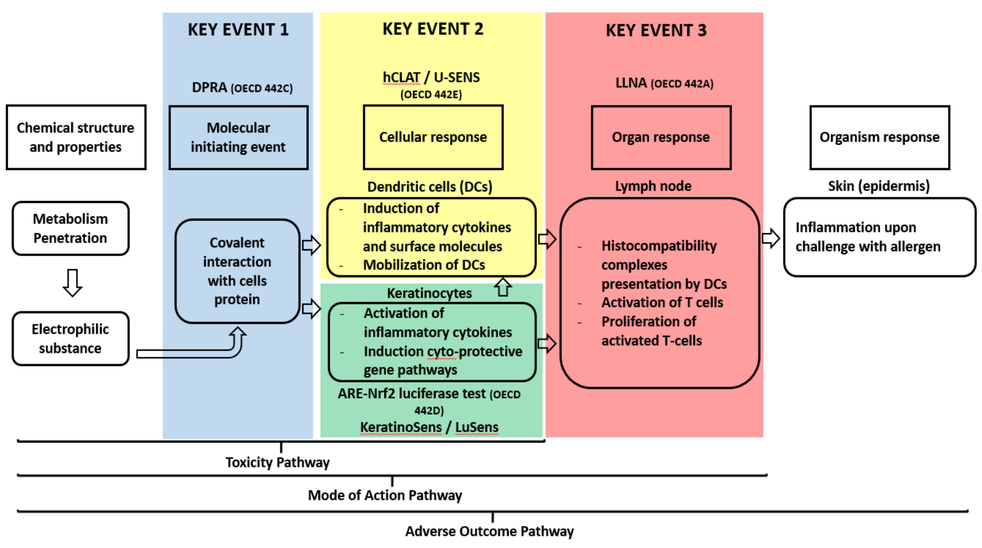
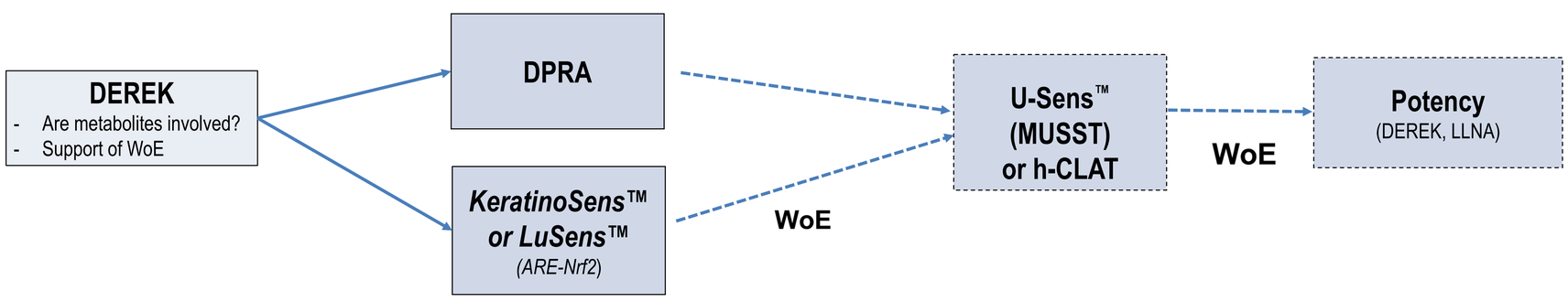


Figure 1: Skin sensitization Adverse Outcome Pathway based on (ENV/JM/MONO(2012)10/PART1 and OECD tests

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

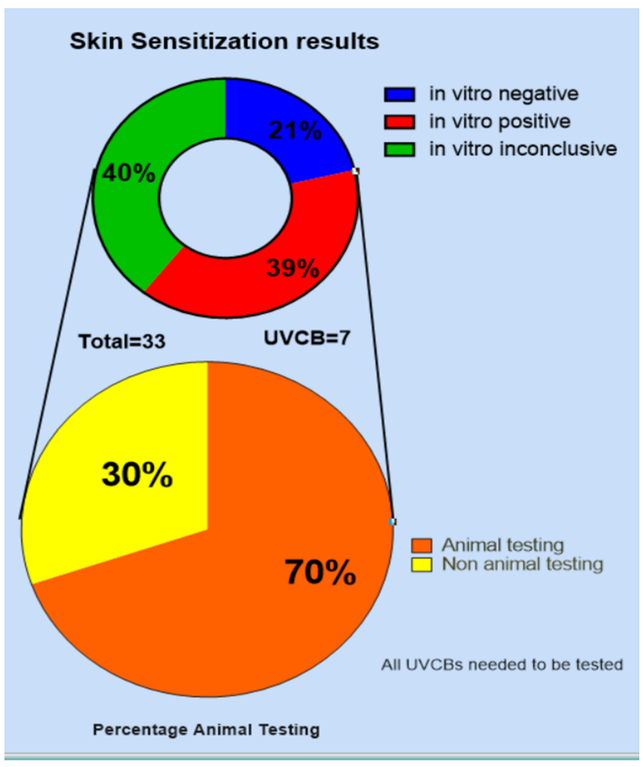
3 Proposed *Test Strategy for a) Mono/multi-constituents and for b) UVCBs/'metals'*

a



b **UVCBs, 'metals':** tests are available for key event 2 (ARE-Nrf2) and key event 3 U-SENS™ (MUSST)/h-CLAT, WoE and potency

4 Results



Test results
A significant number of tests gave inconclusive results (testing up to and including highest required test concentration not possible).

For UVCBs and 'metals' only two non *in vivo* tests are available yet, resulting generally in *in vivo* testing

In the absence of reliable information on skin sensitizing potency, positive substances still need to be tested *in vivo*.

5 Considerations and Conclusion

Hurdles that need attention:

- Low solubility of substance results in inconclusive results, as not tested up to and including the highest concentration required according to Guidelines
- High partition coefficient of substance (result is comparable to low solubility)
- High molecular weight (no dermal absorption)
- UVCB or 'metal': DPRA and DEREK not possible.; only two acceptable non *in vivo* tests available (two out of three tests not possible)
- The currently available non-animal test methods have no or limited metabolic capacity; this may result in false negative results
- Results key event 1 not crucial for overall outcome (a)
- No *in vitro* potency test is available yet that is acceptable for regulatory and classification purposes.

At this moment, non *in vivo* tests for skin sensitization cannot replace the LLNA completely (due to equivocal results, lack of potency), nor is it accepted yet by all other legal frameworks. Further development/refinement of alternative testing as well as reliable and acceptable *in vitro* skin sensitizing potency tests are required. Currently two potency tests are being validated*: GARDskin/SENS-IS*, which potentially could replace animal testing significantly.

(a) Barentsen HM, Jonis SU, Pelgrom SMGJ (*in prep*)
REACH alternative testing strategy for skin sensitization in practice; fact or fiction?