charles river

Summary

A tiered strategy for both mono-constituents and for UVCBs can provide reliable information on skin sensitization potential, including potency, meaning that *in vivo* studies are performed only as a last resort

SAFETY ASSESSMENT

Non-animal Skin Sensitization Testing

OECD test guidelines for *in vitro* and *in chemico* testing

The implementation of non-animal alternatives for skin sensitization testing has been driven by legislative changes, the 3Rs, and animal welfare considerations. With the 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). Whereas before, one *in vivo* test, preferably the LLNA, was performed, now a comprehensive testing strategy has been implemented, as no single non *in vivo* 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 under REACH, in order to identify extreme sensitizers needing a specific concentration limit for use in mixtures. There is currently no standardized (accepted) way to assess potency with *in vitro* methods.

Based on the regulations, the team at Charles River has developed a tiered approach for both mono and multi-constituents and for Substance of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs), and have generated reliable data on skin sensitization potential, including potency, from these tests strategies[1,2]. Consequently, *in vivo* studies need only be performed as a last resort.

Adverse Outcome Pathway (AOP) Testing

Skin sensitization Adverse Outcome Pathway (AOP) testing comprises tests to address different key events in the AOP: peptide binding, antioxidant response element (ARE), mediated gene expression, and dendritic cell activation. AOP-based testing is now specifically requested for REACH, EPA and EU Cosmetic Directive submissions but, due to the complexity of the AOP, no single *in vitro* test is acceptable for meeting the requirements for the endpoint skin sensitization and is acceptable and capable of fully classifying substances into UN GHS categories (Cat 1A and 1B).

A number of tests are performed in a tiered testing strategy utilizing *in silico* (e.g., DEREK), *in chemico* (DPRA, OECD 442C) and *in vitro* assays (including ARE-Nrf2 Luciferase Test, OECD 442D; U-SENS™, OECD 442E). These tests are anticipated to essentially replace *in vivo* tests such as LLNA (OECD 429). Using this strategic battery of three tests (see Fig 1), along with an appropriate *in silico* model, can support a Weight of Evidence (WoE) approach, such as the 2 out of 3 model proposed by Bauch *et al*. (2012)[3].

Additional *in vitro* tests that include potency assessment (e.g.,GARD, SENS-IS) are currently in the validation process at ECVAM. These tests may, in the near future, complement the test battery.

QSAR Model DEREK NEXUS (Knowledge-based)

If no alerts are fired, this may be extrapolated to a negative prediction. The database includes information on metabolism, potency (EC3) when an alert is present, and together with the study results, this information may give more body to the WoE and can be considered for classification.

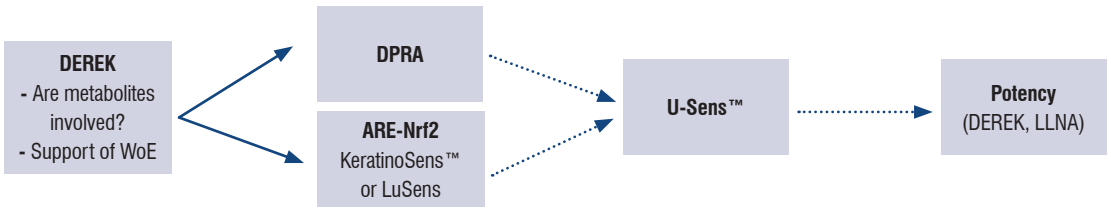
Test strategy for UVCB and/or metals: DEREK and DPRA are not applicable; only two tests available, one for event 2 (ARE-Nrf2) and one for event 3 (U-Sens); WoE and potency.

In silico prediction can play a role by considering (false) negative *in vitro* tests, as DEREK can provide information on relevant metabolites (metabolic activation is generally lacking in the *in vitro* tests). Also in case of equivocal and positive test results, information from DEREK can provide relevant information on potency before considering an *in vivo* test (LLNA).

References:

1. Non-animal skin sensitization testing under REACH. S.M.G.J. Pelgrom, W.M.A. Westerink, J.C.W. Rijk, J.L. Vinall, J.B. Welch, C.S. Roper, J.J.C. Paulussen. Charles River Laboratories
2. Skin sensitization testing strategy and in-house fit-for-purpose validations at Charles River. J.L. Vinall, C.S. Roper, J.J.C. Paulussen, J.C.W. Rijk, W.M.A. Westerink, J.B. Welch. Charles River Laboratories
3. Putting the parts together: combining *in vitro* methods to test for skin sensitizing potentials. Bauch *et al*. (2012). Regul Toxicol Pharmacol;63(3); 489-504

Fig. 1 Proposed Test Strategy for Mono-constituents



DPRA	ARE-Nrf2	Conclusion
Negative	Negative	**Non-Sensitizer:** WoE approach
Positive	Positive	**Sensitizer:** Potency evaluation required
Positive	Negative	U-Sens™ 2 out of 3 approach to determine sensitizer/non-sensitizer
Negative	Positive	
Equivocal*	Equivocal*	If sensitizer: further potency evaluation may be required

* If one or both tests gives equivocal results

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