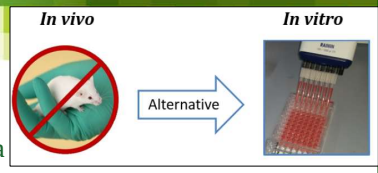
Understanding *in vitro/in chemico* skin sensitization testing methods and application in hazard classification

Mahesh Rachamalla, Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK, S7N 5E2, Canada



Introduction

Over the last decade, phenomenal development has been made in the development of non-animal tests to assess contact hypersensitivity.

Recent advances in adverse outcome pathway (AOP) framework yielded several validated non-animal tests for determination of sensitisation potential.

REACH regulation via commission regulation 2016/863 and 2016/1688 replacement of skin sensitisation tests were identified. OECD guidelines for *in vitro* skin sensitisation and *in chemico* guidelines were developed[1].

Other in vitro tests t are under validation for skin sensitization potential determination.

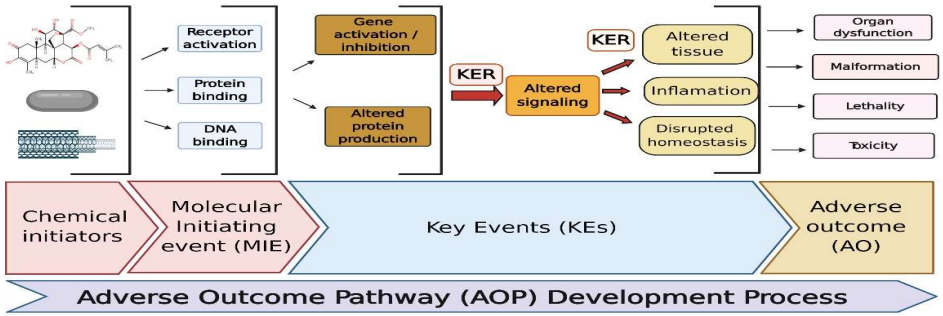
Benefits of alternative testing

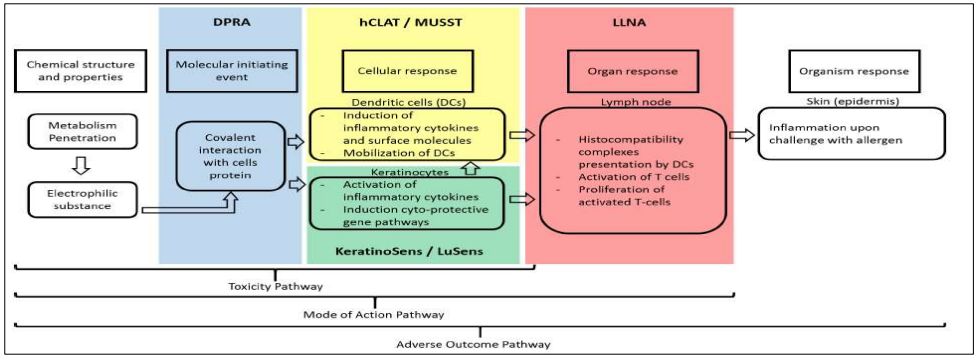
Easy to perform and interpret the data even for non toxicologist.

By 2035 US EPA to eliminate all animal tests, it will be good approach to develop in vitro test methods for future.

In vitro methods will give mechanistic data as well to understand the pathway of sensitisation which was not able to determine in existing in vivo methods[2,3].

Adverse outcome pathways (AOP) and its role in alternative animal model development





Test method	Key event	Hazard outcome	Hazard classification
DPRA: OECD 442C (2015): in chemico skin sensitization test Direct peptide reactivity assay (DPRA)	Covalent binding with proteins	Skin sensitizer or Not sensitizer with complementary information	Skin sensitizer category 1
KeratinoSens: OECD 442D (2015): In Vitro skin-sensitization: ARE-Nrf2 luciferase test method **LuSens: based on the ARE-Nrf2 Luciferase test method.**	Keratinocyte inflammatory response	Skin sensitizer or Not sensitizer with complementary information	Skin sensitizer category 1
h-CLAT: OECD 442E (2016): In vitro skin sensitization: human cell line activation test (h-CLAT) **U-SENS (MUSST): U937 skin sensitization test (U-SENS)**	Activation of dendritic cells	Skin sensitizer or Not sensitizer with complementary information	Skin sensitizer category 1

Current challenges

So many *In chemico / in vitro* tests are being currently in development or validation steps using AOP conceptual frameworks.

However, using alternative tests for every chemical multiple tests need to be conducted because no single test can give you the complete results like in vivo testing.

There is no complete classification criteria established only using in vitro testing methods, currently sometimes in vivo testing is recommended based on the chemical properties.

These tests results should be integrated, normally under a weight-of-evidence approach.

Alternative animal testing sometimes may provide false negative results which needs to be evaluated with care.

Despite progress in the field, there is still much to be achieved and the best target for potency prediction is still an open question.

In vitro potency estimation is still a challenge and further work is necessary. This will also require a better understanding of the basic mechanisms determining the *in vivo* potency before an effective *in vitro* strategy can be put in place.

Key references

1. Corsini E, Roggen EL, Galbiati V, Gibbs S. Alternative approach for potency assessment: In vitro methods. *Cosmetics*. 2016;3(1):1-9. doi:10.3390/cosmetics3010007
2. European Chemicals Agency. How to use new or revised in vitro test methods to address skin sensitisation. 2018;(February):1-11.
3. World Health Organization, WHO, WHO Expert Committe on Biological Standardization, WHO, Chocarro L. FP. Skin Sensitization in Chemical Risk Assessment. *WHO Tech Rep Ser No67*. 2008;1004(July):1-91