# Non Animal Testing Methods (NAM's Approach) For Skin Sensitisation

HIRNA PANDYA

GLOBAL REGULATORY TOXICOLOGIST

UPL LIMITED

# NAM's Approach

## Why Alternatives?

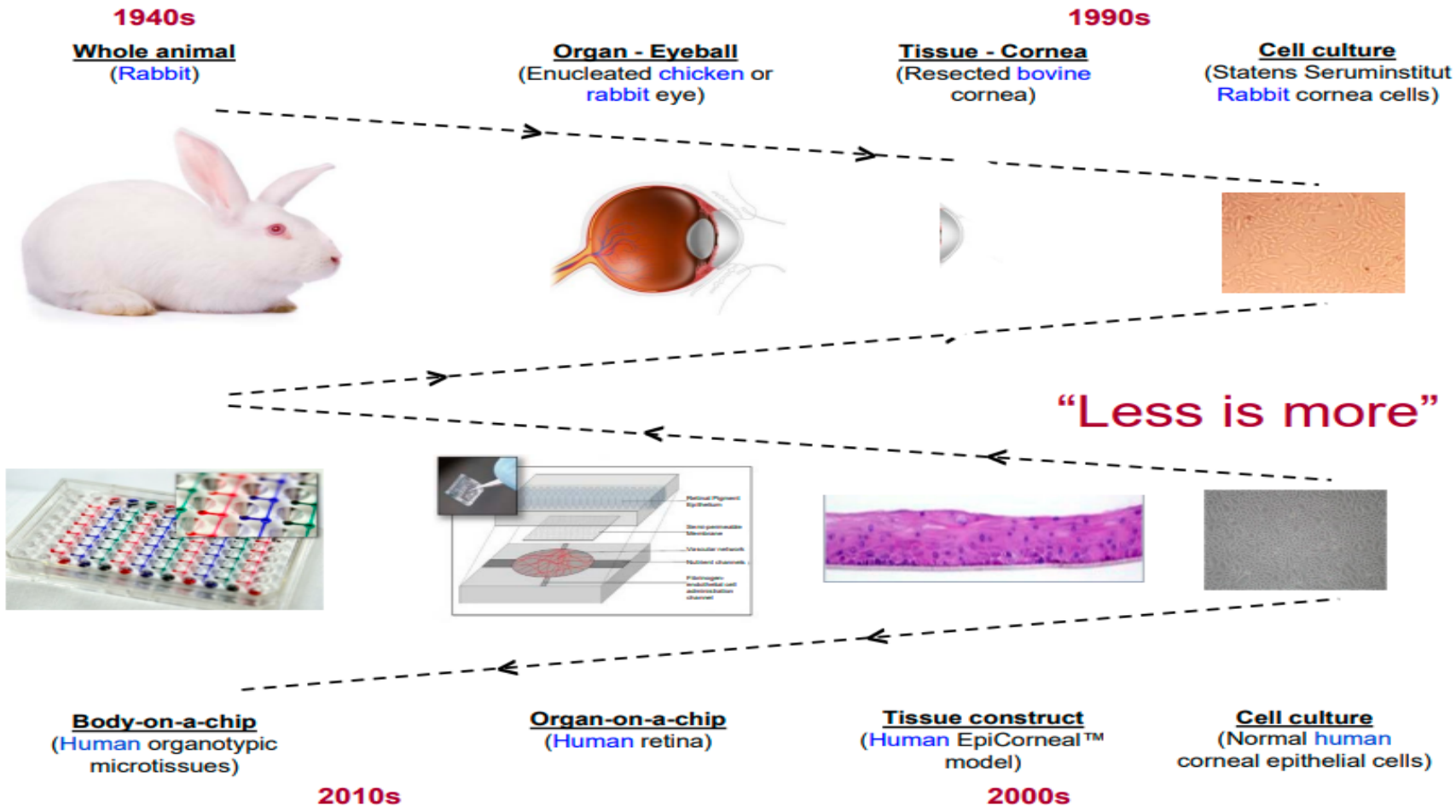
- ✓ Principles of 3R, save animals, cost effective, ethical, time saving and more efficient method available for testing and regulatory submission.

## Why do we need Alternative Toxicity testing ?

- ✓ Alternative methods are not only replacement strategy they also help to screen more number of chemicals without use of animals at initial stage as well as to accomplish the regulatory goal of classification, labelling, transport of chemicals and occupational safety. Not all in vitro methods are completely developed but many are in progress along with OECD & regulatory authorities to help industries achieve this task in order to save time, cost and effort required for *in vivo* testing methods.

# NAM's Approach

**The reductionist approach for *in vitro* testing**

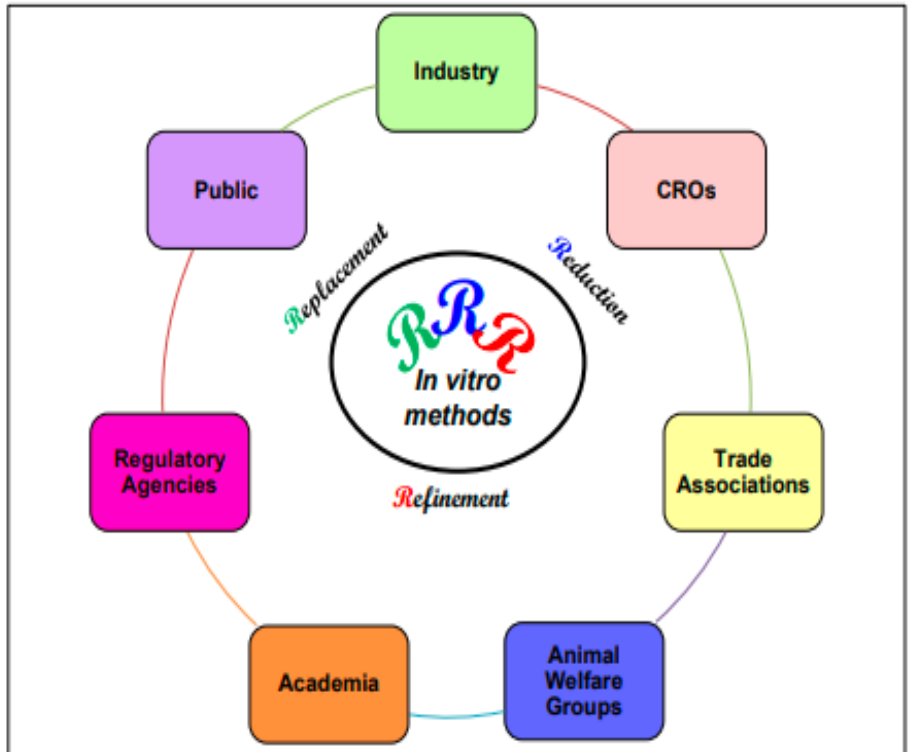




G.-E. Costin and H. A. Raabe. *In vitro* toxicology models. In: The Role of the Study Director in Non-clinical Studies. Pharmaceuticals, Chemicals, Medical Devices, and Pesticides. (Eds. William Brock, Barbara Mounho and Lijie Fu), John Wiley and Sons (2014).

G.-E. Costin. Advances in science: next generation of lab tools, models and testing platforms used in predictive toxicology. *Molecular Life* 2017; 1(1), 22-8, doi: 10.26600/MolLife.1.1.3.2017. Available at: http://molecular-life.org/wp-content/uploads/2017/07/Advances-science-next-generation-lab-tools-models-testing-platforms-used-predictive-toxicology.pdf.

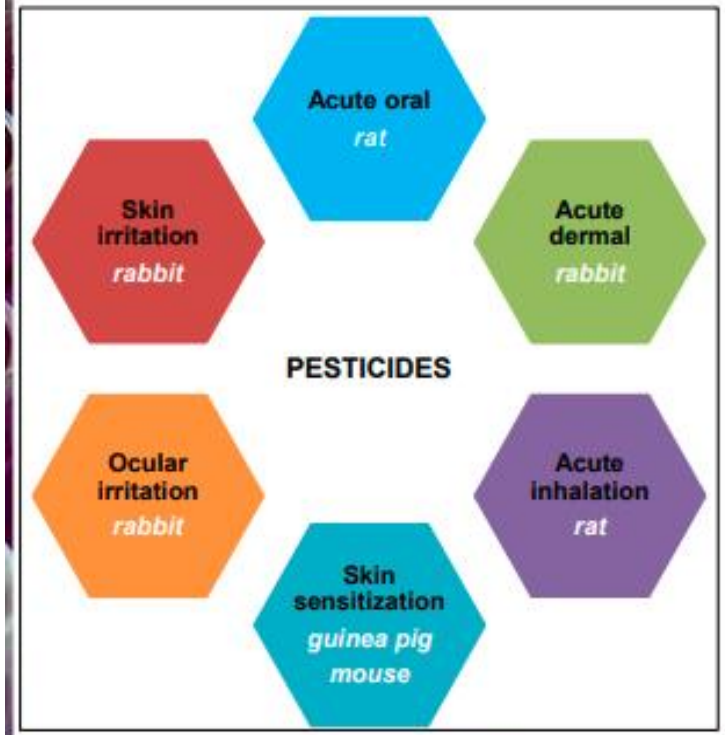
# Drivers of *In Vitro* Method Development




Ongoing evolution on so many levels

- Improve scientific basis for testing using human derived test models
- Reduce the number of animals for testing
- Increase predictivity
- Reduce time, price
- Harmonize requirements and prediction models

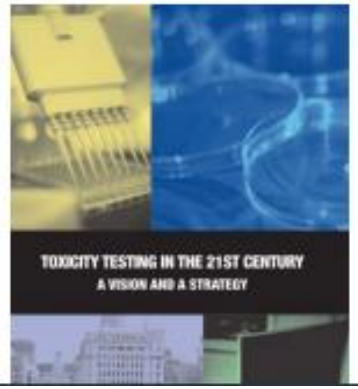
# Beyond the Traditional 6-Pack Acute Toxicity Testing




**Toxicity Categories**

| Study | Category I | Category II | Category III | Category IV |
|---|---|---|---|---|
| Acute Oral | Up to and including 50 mg/kg | >50 through 500 mg/kg | >500 through 5000 mg/kg | >5000 mg/kg |
| Acute Dermal | Up to and including 200 mg/kg | >200 through 2000 mg/kg | >2000 through 5000 mg/kg | >5000 mg/kg |
| Acute Inhalation | Up to and including 0.05 mg/liter | >0.05 through 0.5 mg/liter | >0.5 through 2 mg/liter | >2 mg/liter |
| Eye Irritation | Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days | Corneal involvement or irritation clearing in 8-21 days | Corneal involvement or irritation clearing in 7 days or less | Minimal effects clearing in less than 24 hours |
| Skin irritation | Corrosive (tissue destruction into the dermis and/or scarring) | Severe irritation at 72 hours (severe erythema or edema) | Moderate irritation at 72 hours (moderate erythema) | Mild or slight irritation (no irritation or slight erythema) |

| Study | Study results | Study results |
|---|---|---|
| Dermal Sensitization | Product is a sensitizer or is positive for sensitization | Product is not a sensitizer or is negative for sensitization |




## The modern *in vitro* toxicology perspective

PROCESS FOR ESTABLISHING & IMPLEMENTING ALTERNATIVE APPROACHES TO TRADITIONAL *IN VIVO* ACUTE TOXICITY STUDIES

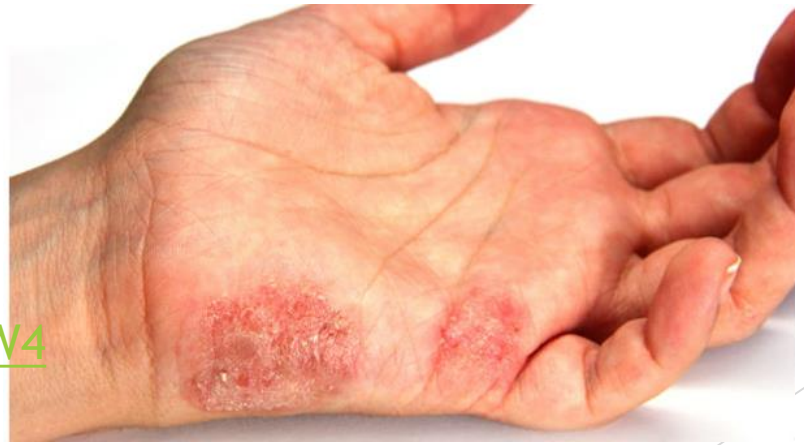
12/15/2014
Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington DC, 20460

USE OF AN ALTERNATE TESTING FRAMEWORK FOR CLASSIFICATION OF EYE IRRITATION POTENTIAL OF EPA PESTICIDE PRODUCTS

3-2-2015

Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington DC, 20460

# Skin Sensitisation



https://www.youtube.com/watch?v=n9z-h2XafW4

https://www.youtube.com/watch?v=80i5aIBw2f0

# Traditional Method: In Vivo Testing

- Study Objective: to identify substances with the potential to induce skin sensitisation (an allergic response)

- Method: OECD 406/OPPTS 870.2600 or OECD 429

Applicability to humans?

- Endpoint: sensitizer or non-sensitizer; used for classification purposes to alert users of potential hazards – implications on the target market (professionals vs amateurs) and risk assessment

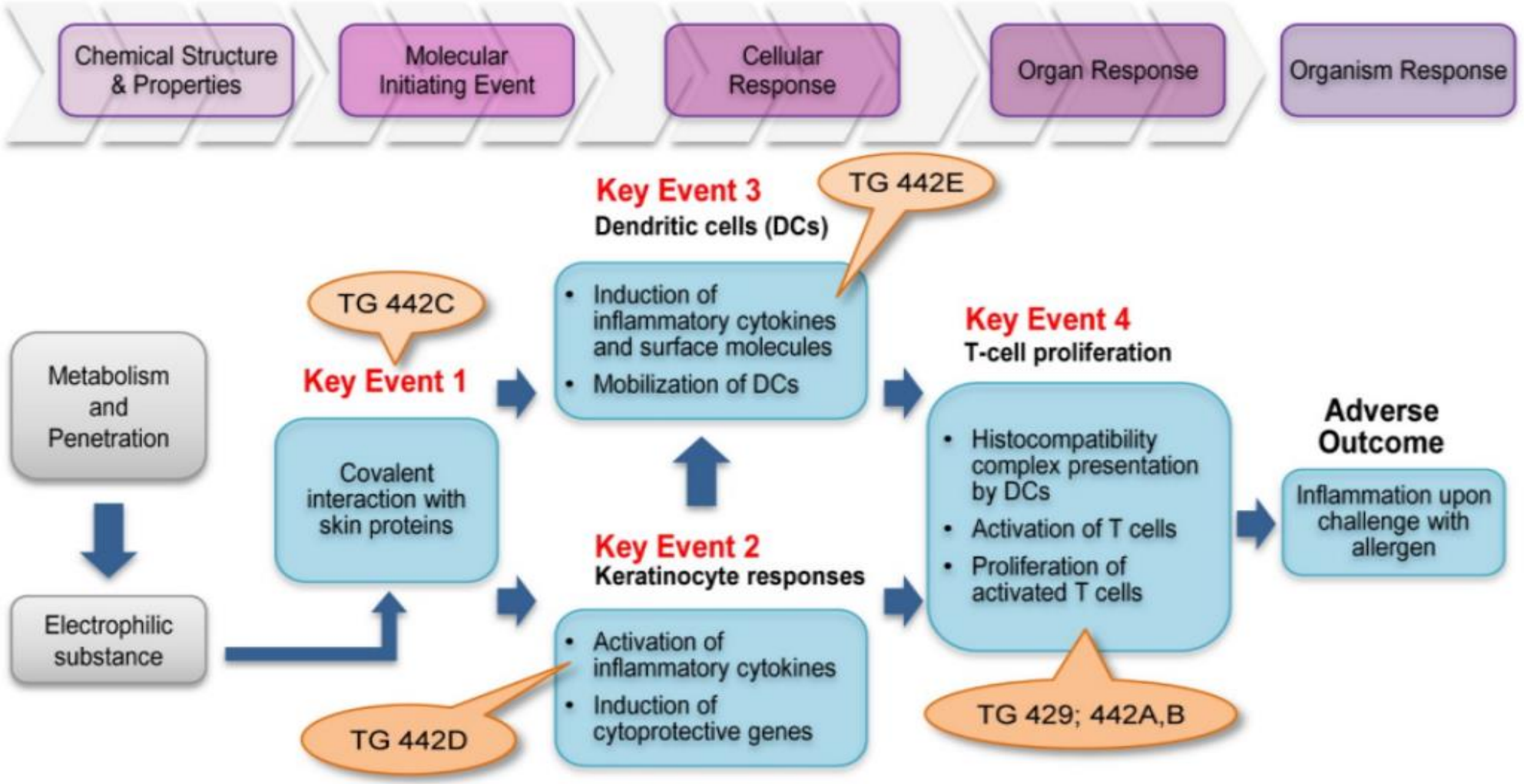
- Classification: CLP Regulation (EC) No. 1272/2008 and GHS
  - 3 categories: Skin sensitisation (Category 1, 1A, 1B) 'May cause an allergic skin reaction'

| *Animal test results for sub-category 1A* | |
|---|---|
| *Assay* | *Criteria* |
| *Local lymph node assay* | *EC3 value ≤ 2 %* |
| *Guinea pig maximisation test* | *≥ 30 % responding at ≤ 0,1 % intradermal induction dose or*<br>*≥ 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose* |
| *Buehler assay* | *≥ 15 % responding at ≤ 0,2 % topical induction dose or*<br>*≥ 60 % responding at > 0,2 % to ≤ 20 % topical induction dose* |

| *Animal test results for sub-category 1B* | |
|---|---|
| *Assay* | *Criteria* |
| *Local lymph node assay* | *EC3 value > 2 %* |
| *Guinea pig maximisation test* | *≥ 30 % to < 60 % responding at > 0,1 % to ≤ 1 % intradermal induction dose or*<br>*≥ 30 % responding at > 1 % intradermal induction dose* |
| *Buehler assay* | *≥ 15 % to < 60 % responding at > 0,2 % to ≤ 20 % topical induction dose or*<br>*≥ 15 % responding at > 20 % topical induction dose* |

# NAMS: Defined or Targeted Approach

**Figure 1. The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins (Adapted from Strickland et al. 2018)**



**Key Event 1** – First key molecular initiation event involves the covalent interaction (or haptenation) of the allergen with skin proteins

**Method:** OECD 442C: *In Chemico* Skin Sensitisation (Direct Peptide Reactivity Assay - DPRA)

**Objective:** Skin sensitisers are generally electrophilic and react with the nucleophilic moieties of proteins. The DPRA measures the depletion of two peptides containing either cysteine or lysine residues due to covalent binding to identify substances with the potential to induce skin sensitisation (an allergic response)

**Endpoint:** Positive result when a test chemical induces mean peptide depletion of cysteine- and lysine-containing peptides above 6.38%

**Key Event 2 -** Keratinocyte activation causing the release of inflammatory signals and changes in gene expression

**Method:** OECD 442D: *In Vitro* Skin Sensitisation (ARE-Nrf2 luciferase test method/ KeratinoSens™ assay and LuSens )

**Objective:** To measure a substance's ability to activate cytokines and induce cytoprotective genes in keratinocytes, which can activate AER-dependent genes leading to an inflammatory response

**Endpoint:** Positive result = test chemical induces >1.5-fold or 50% increase luciferase activity (at viabilities > 70%) when compared to the vehicle control

**Key Event 3** – Dendritic cell activation (responsible for initiating an immune response) following exposure to the antigen

**Method:** OECD 442E *In Vitro* Skin Sensitisation (h-CLAT, U-SENS, or the IL-8 Luc assay)

**Fourth OECD GARD Test recently introduced Sep 22**

**Objective:** determine if a test substance binds to and activates local dendritic cells which would lead to the stimulation of an immune response required for sensitisation of the skin

(a). Quantify the change in the expression of cell surface marker(s) due to the activation of monocytes and dendritic cells following exposure to sensitisers (increase expression of CD54 and CD86 surface markers); or

(b). Measure the changes in Interleukin-8 (IL-8) expression, a cytokine associated with the activation of dendritic cells.

**Endpoint:**The relative fluorescence or luminescence intensity of the treated cells compared to solvent/vehicle control are calculated and used in the prediction model, to support the discrimination between sensitisers and non-sensitisers.

**Key Event 4:** Proliferation of antigen specific T cells, leading to sensitisation

**Method:** *In vivo* OECD 429 LLNA – there are currently no validated non-animal methods available to assess the ability of a substance to activate Key Event 4

**Objective:** assess induction response **-** sensitisers induce primary proliferation of lymphocytes in the auricular lymph nodes that drain the site of chemical application. This proliferation is proportional to the dose applied and provides a measurement of sensitisation.

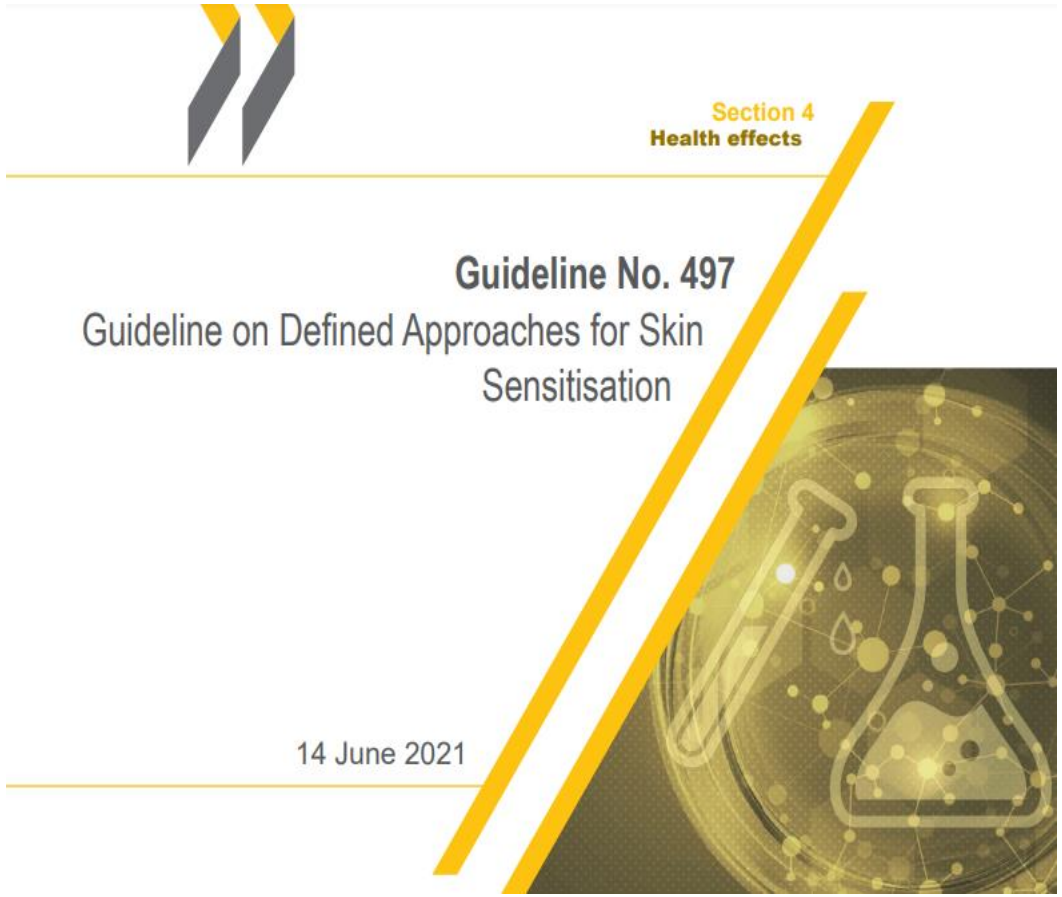
**Endpoint:** discriminate between sensitisers and non-sensitisers, sub-categorise and determine potency

# Genomic Allergen Rapid Detection ASSAY OECD 442E

- The GARD™ assay can be performed to predict the ability of chemicals to induce skin sensitisation based on the analysis of relative expression levels of a biomarker signature of 196 genes.
- The test method uses SenzaCell, a human myeloid leukemia cell line. This cell line works as an *in vitro* model of human dendritic cells and chemical stimulation of the cells can be assessed.
- Based on a derived decision value (DV) from a Support Vector Machine (SVM) model chemicals can be predicted to be sensitizers or non-sensitizers. In combination with other complementary information within an Integrated Approach to Testing and Assessment (IATA) or as a stand-alone method, the GARD™ Assay can be used as a reliable *in vitro* method to assess skin sensitising potential of chemicals.
- **The GARD™ method mimics the immune system by using human dendritic cells (3rd molecular Key Event). It predicts the ability of chemical compounds to induce skin sensitisation by measuring changes in the genomic profile of the cells after chemical treatment.“**

- **“ONE SIZE MAY NOT FIT ALL”**

# NAMS: Defined or Targeted Approach




Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing

DRAFT FOR PUBLIC COMMENT
April 4, 2018

EPA's Office of Chemical Safety and Pollution Prevention:

Office of Pesticide Programs
Office of Pollution Prevention and Toxics

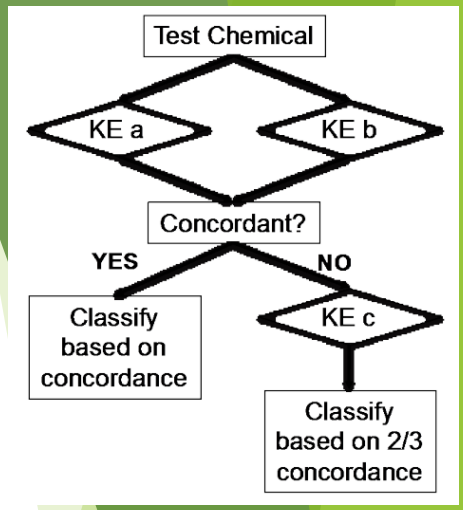
## Defined approaches that are acceptable to the LLNA for regulatory submission:

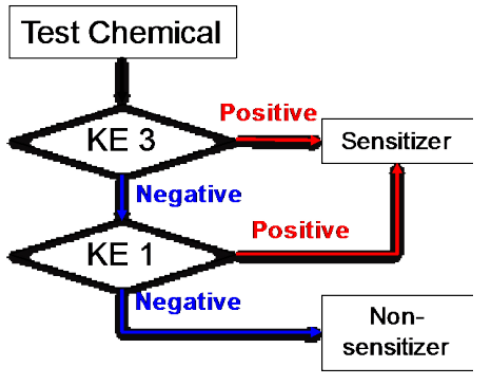
1. Option 1: AOP "2 out of 3" to predict skin sensitization hazard by sequential testing, in an undefined order, in up to three internationally accepted non-animal methods that map to KEs 1-3 of the AOP.

- First assays are run for two KEs.
- If results are consistent then the chemical is categorized as positive or negative.
- If results are discordant, a third KE assay is run. Overall result based on two concordant findings
- Capabilities: Hazard only

2. Option 2 or 3: KE 3/1 sequential testing strategy (STS) is a simple decision tree that requires KE 1 (e.g., DPRA) and KE3 (e.g., h-CLAT, IL8-Luc, U-SENS) data as inputs and in silico (Derek Nexus/OECD QSAR)

- KE 3 assay conducted first; if the response is positive, the test substance is classified as a sensitizer.
- If a negative result is obtained from a KE3 assay, an assay for KE1 is conducted.
- A negative KE1 study confirms a non-sensitizer and a positive result for KE1 concludes sensitizer
- Capabilities: Hazard and potency



## Advantages

- Reduces reliance on animal testing
- Animal welfare benefits
- Time efficient and reproducible
- Information on the cellular and molecular events
- Support the discrimination between skin sensitisers and non-sensitisers
- Depending on the regulatory framework, positive results generated with these methods may be used on their own to classify a chemical into UN GHS/CLP Reg. Category 1

## Key Notes

- Substances may be outside the applicability domain of in silico models (may lead to inconclusive results)
- Cannot be used to sub-categorise skin sensitisers (i.e. Cat 1A or 1B), nor to predict potency. If considered necessary, the *in vivo* LLNA is used to determine sub-categorisation and potency.
- Care required when interpreting the results of test substances with Log Kow > 3.5 (OECD 442E)

# NAMS: Defined or Targeted Approach

Skin sensitization is example of combined approach with OECD tools

- Test Guidelines for evaluating skin sensitisation (https://doi.org/10.1787/20745788) – In chemico/ in vitro TG – In vivo TG
- AOP for skin sensitisation (https://aopkb.oecd.org/) – Provides a mechanistic basis including a molecular initiating event (MIE) and all key events (KE) leading to AO – Testable events
- Test Guidelines for evaluating skin sensitisation (https://doi.org/10.1787/20745788) – In chemico/ in vitro TG – In vivo TG

# In-Silico Models

- DEREK
- OECD QSAR Tool Box
- Automated workflow in OECD QSAR Tool Box for Skin Sensitisation prediction
- QSARs for skin sensitisation (https://www.qsartoolbox.org/) – The OECD Toolbox includes predictions based on profilers (e.g. protein binding) and also now includes the AOP

# ECHA Overview for all methods, limitations for risk assessment evaluation

- Testing for skin sensitisation must always start with in chemico/in vitro test methods when new testing is required. In vivo testing is only needed if in vitro methods are not suitable for the substance or if the results of the in vitro tests are not adequate for classification and risk assessment.
- https://echa.europa.eu/documents/10162/1128894/oecd_test_guidelines_skin_sensitisation_en.pdf/

# OECD Guidance Documents for Reference

- OECD document No. 256

GUIDANCE DOCUMENT ON THE REPORTING OF DEFINED APPROACHES AND INDIVIDUAL INFORMATION SOURCES TO BE USED WITHIN INTEGRATED APPROACHES TO TESTING AND ASSESSMENT (IATA) FOR SKIN SENSITISATION

- OECD document No. 255

Reporting of Defined Approaches to be Used within Integrated Approaches to Testing and Assessment.

# LINKS FOR REFERENCE:

- EPA:https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/adopting-21st-century-science-methodologies-metrics
- https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce
- FDA: https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda
- GHS: https://unece.org/about-ghs
- ICCVAM public forum: https://ntp.niehs.nih.gov/whatwestudy/niceatm/3rs-meetings/past-meetings/pubforum-2021/iccvamforum-2021.html
- OSHA: https://www.osha.gov/hazcom
- CPSC: https://www.cpsc.gov/Business--Manufacturing/Testing-Certification/Recommended-Procedures-Regarding-the-CPSCs-Policy-on-Animal-Testing#:~:text=The%20CPSC%20has%20codified%20its,1500%20(77%20FR%2073289)

# Thank You for Listening!

If you have any questions or want to find out more on a particular topic, please let us know.