XCellR8
Redefining testing

Skin sensitisation and preservatives

How *in vitro* tests can contribute to more human-relevant safety evaluations

in-cosmetics webcast, 1st April 2020

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Skin sensitisation – why testing is so important

- 1-3% (~7.5 million people) of the European population suffer from contact allergy to a cosmetic ingredient.
- Skin sensitization to a cosmetic ingredient is a **permanent condition**
- *In vitro* tests provide an ethical alternative to human trials
- Preservatives and fragrances are the most common causes of skin sensitization in cosmetic products
- This is true for both natural and synthetic ingredients - **natural does not mean safe!**

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Skin sensitisation and preservatives

- Preservatives are tightly regulated in Europe:
  - Annex V of Cosmetics Regulation EC1223/2009 list 57 approved preservatives and maximum allowable concentration. Last updated 23rd January 2020.
- Allergic reactions to preservatives still persist:
  - e.g. restrictions on MI (methylisothiazolinone) “not very effective” in lowering skin sensitisation risk: reduced incidence only from 0.7% to 0.5%.
- Widespread use of historical animal data for safety assessment / SDS - simplistic approach:
  - Limited relevance to humans.
  - Fails to consider ingredient interactions in a final formulation.
  - Fails to consider aggregate exposure from multiple products.

* Ezendam *et al* (2018). Food Chem Toxicol **112**: 242-250

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The *in vitro* solution

- Historical animal data has failed to protect consumers from skin sensitisation to preservatives in cosmetics.
- *In vivo* human patch tests for skin sensitisation have ethical issues (sensitisation is a permanent condition).
- Human-relevant data is needed to improve human safety.
- The technology is available – *in vitro* tests meet these needs.
- Greater uptake of the new tests by industry is needed as due diligence to ensure the safest products are on the market, rather than rely on the bare minimum of historical safety data from outdated animal tests.

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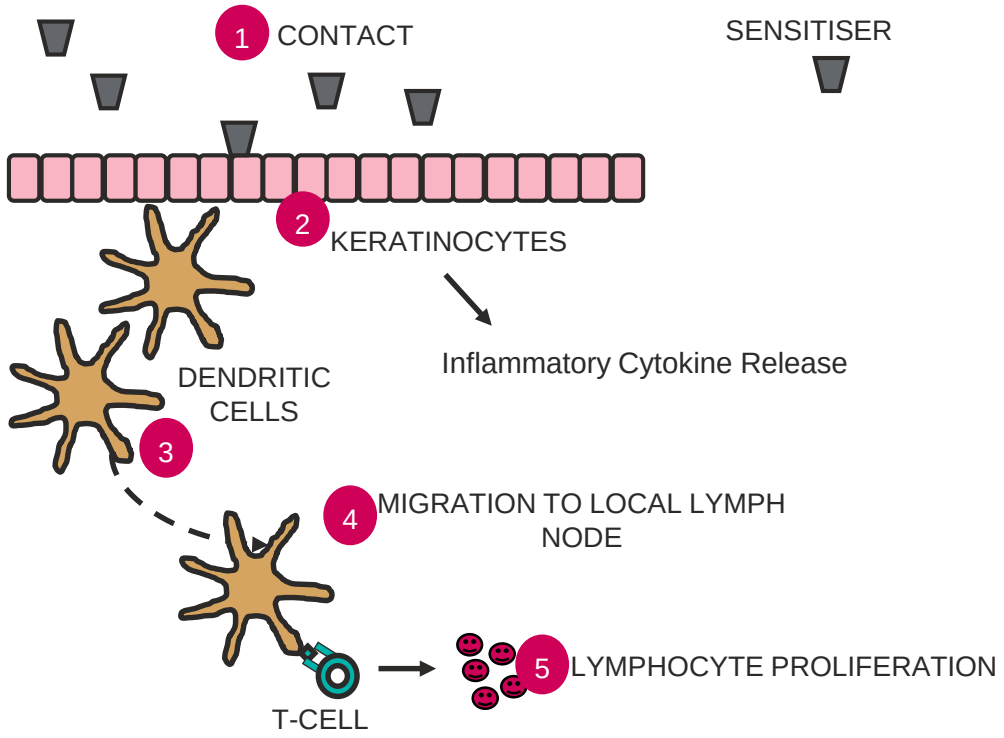
Skin sensitisation leading to allergy - adverse outcome pathway (AOP)



KEY EVENTS IN SKIN SENSITISATION AND RELATED TESTS

1. Contact
   (Direct Peptide Reactivity Assay – **DPRA**)
2. Release of Pro-Inflammatory Cytokines by Keratinocytes (**KeratinoSens™**)
3. Dendritic Cell Activation/Maturation
   (human Cell Line Activation Test – **h-CLAT**)
4. Migration
5. T-cell Proliferation
   (Local Lymph Node Assay - LLNA)

A truly global effort! – test developed in Switzerland (Givaudan), US (P&G) and Japan (Kao / Shiseido)

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Current regulatory guidance favours "2 out of 3" approach

In vitro skin sensitisation testing

WHY 2 OUT OF 3?

In vitro tests replacing a complex *in vivo* pathway;
Advanced mechanistic data on human response.

- DPRA (OECD TG 442c)
- KeratinoSens™ (OECD TG 442d)
- h-CLAT (OECD TG 442e)

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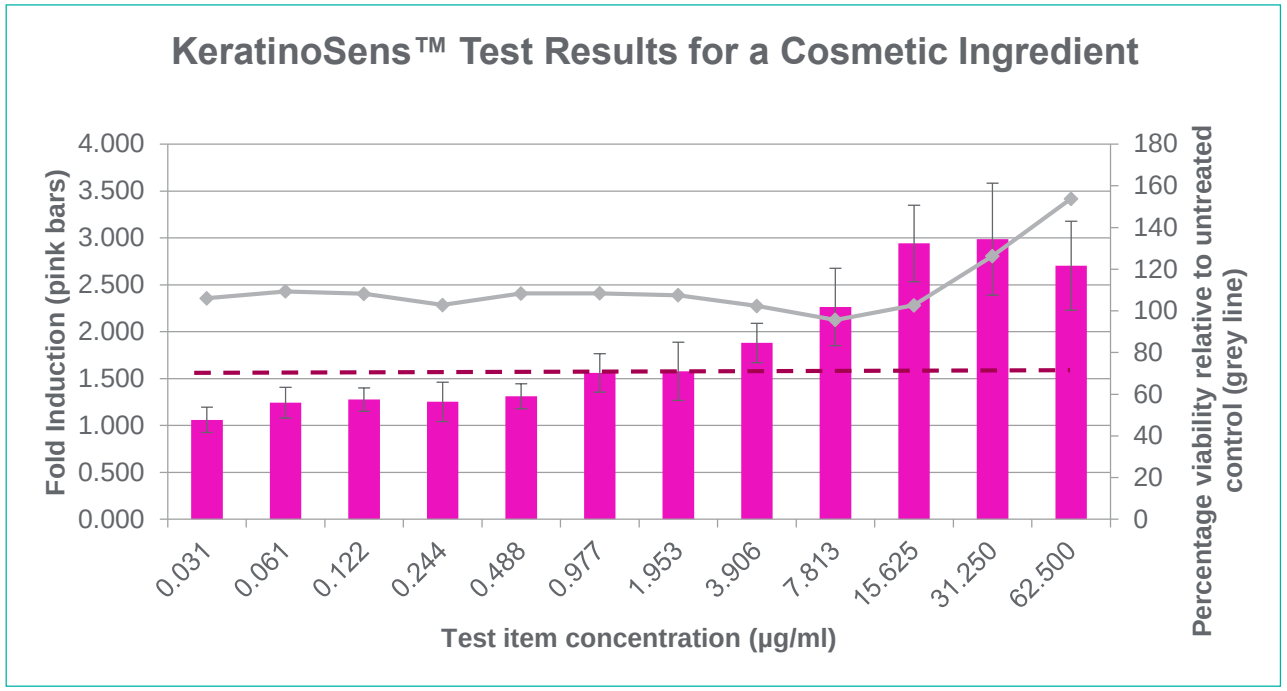
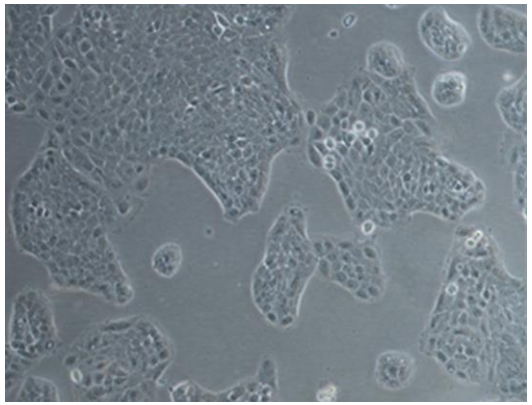
Skin sensitisation test results for a cosmetic ingredient





KeratinoSens™ cells in animal-product-free medium (human serum)

Overall “2 out of 3” classification: Sensitiser

% cysteine depletion	% lysine depletion	Mean % peptide depletion*	Reactivity class	Classification
32.3%	11.3%	21.8%	Low	Sensitiser

DPRA RESULTS FOR A COSMETIC INGREDIENT

*Cut-off for non-sensitiser: 6.38%

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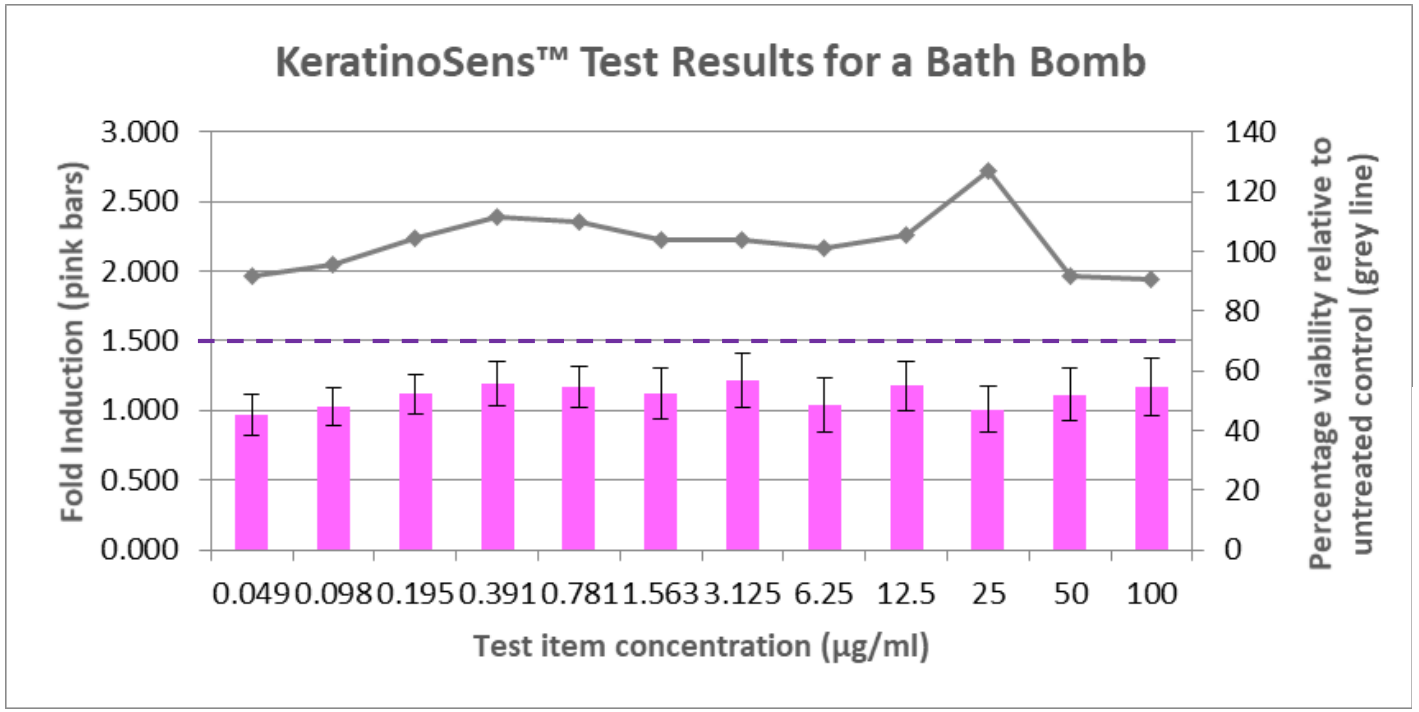
Skin sensitisation test results for a bath bomb (finished product)



DPRA RESULTS FOR A BATH BOMB

% Cysteine Depletion	% Lysine Depletion	Mean % Peptide Depletion	Reactivity Class	Classification
2.4%	0.0%	1.2%	Minimal	Non-Sensitiser

Sample skin sensitisation test results for a bath bomb. None of the concentrations caused >1.5-fold induction of the response in the KeratinoSens™ test, and peptide depletion in the DPRA was categorised as "Minimal". The bath bomb was therefore classified as a non-sensitiser using the "2 out of 3" approach. The results were used as supporting information for safety assessment purposes and to contribute to an internal database for ongoing product monitoring and development.

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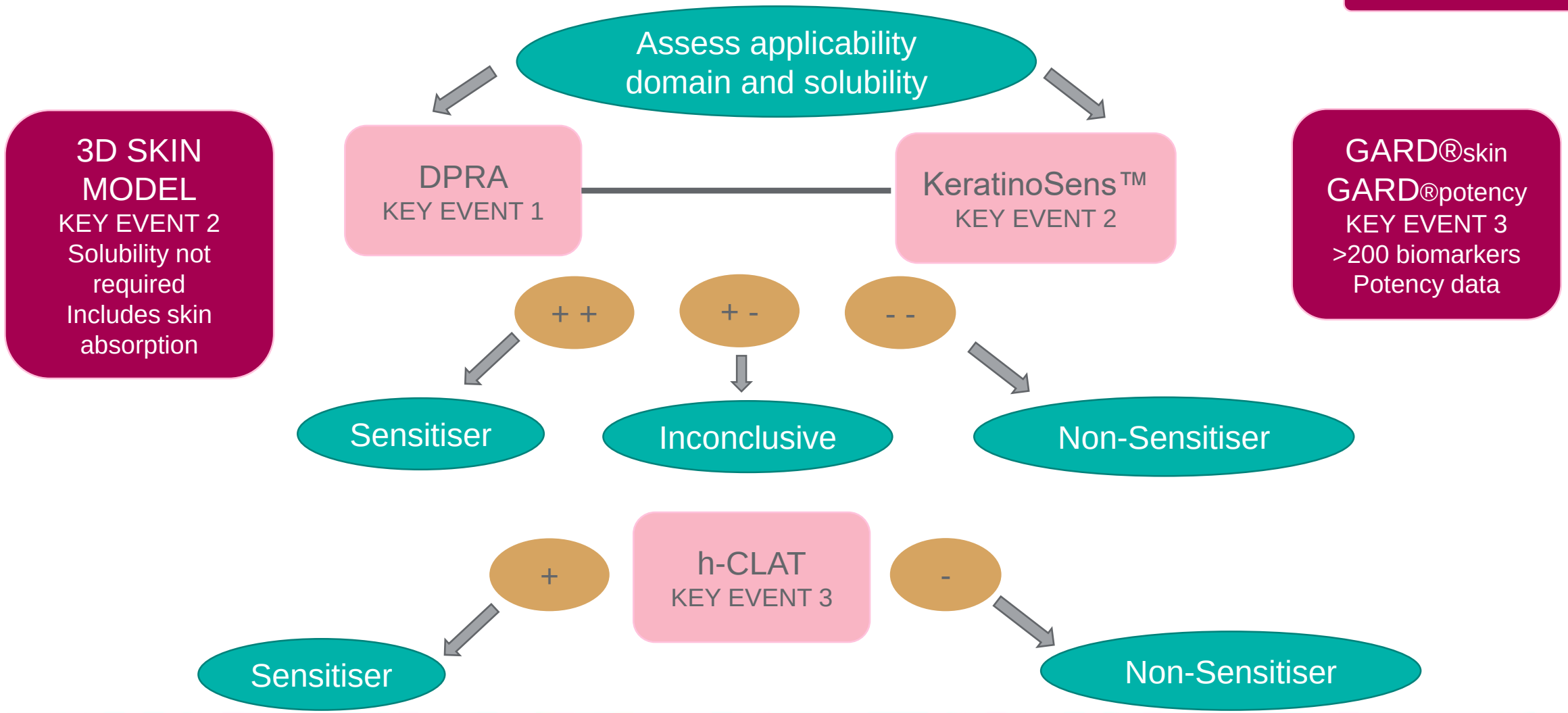
In vitro skin sensitisation testing – decision tree

KEY:
REGULATORY
NON-REGULATORY



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What is truly animal-free testing?

- All *in vitro* tests are not equal in terms of “animal-free” status
- Most *in vitro* methods use animal components
  - Fetal bovine serum
  - Tissue extracts
  - Antibodies
- Reasons are largely historical
- Truly animal-free testing needs to be animal-*product*-free
- Driven by:
  - **Science:** greater human relevance and higher reproducibility.
  - **Ethics:** consumer and industry demand for sustainable, ethical products (*and* ethical testing), eg vegan products require vegan-compliant testing

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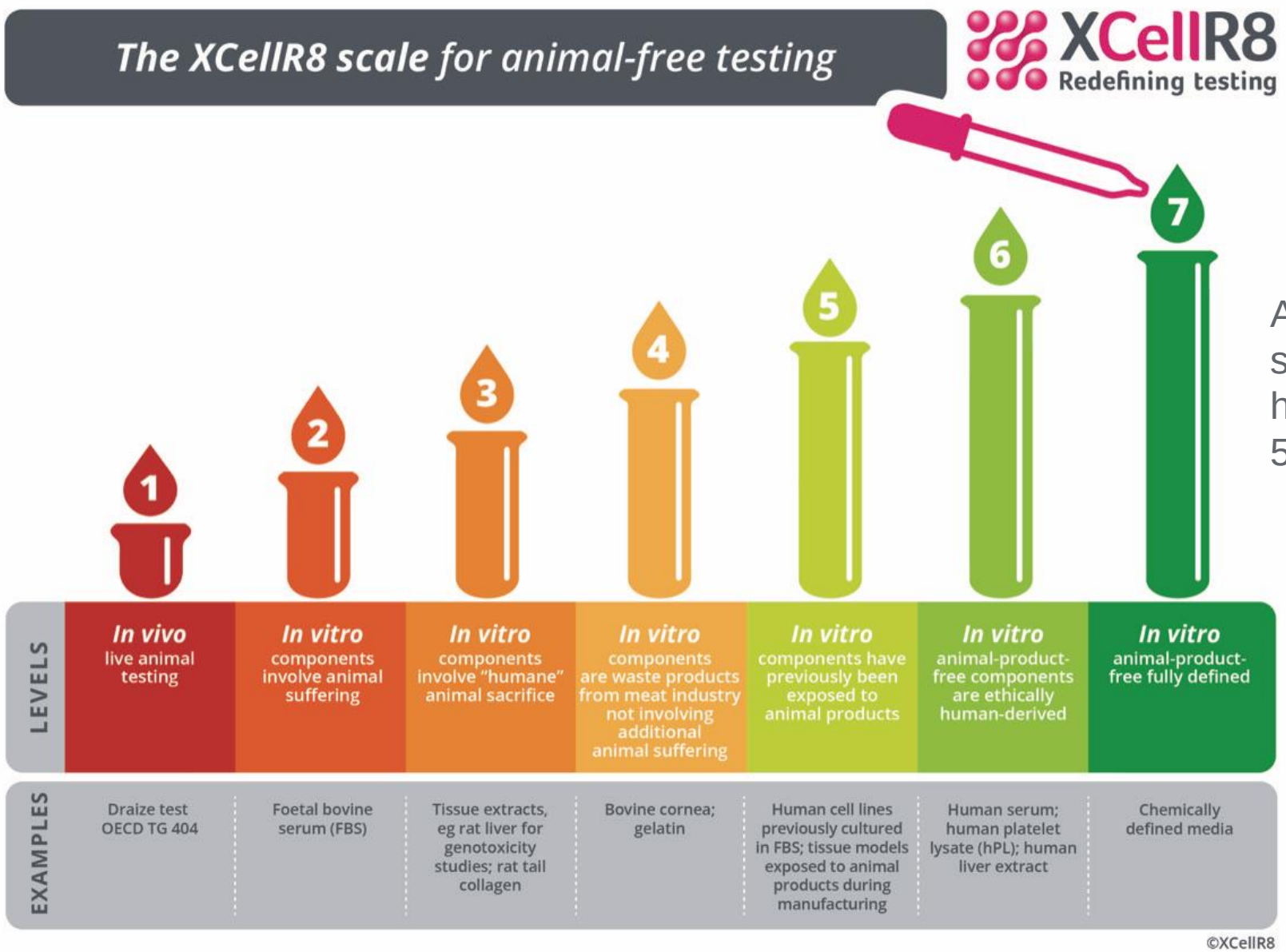

Adaptations of skin sensitisation tests have achieved Levels 5-7 (green zone)

Adaptation of KeratinoSens and h-CLAT tests to human serum shifted their animal-free status from Level 2 to Level 6

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Conclusions

- *In vitro* skin sensitisation tests provide regulatory safety data for Safety Data Sheets (SDS) with increased human relevance compared with historical animal data.
- Non-regulatory screens can complement regulatory tests to build a weight-of-evidence.
- Valuable to re-assess safe use limits of preservatives listed in Annex V of the EU Cosmetics Regulation.
  - What happens if human *in vitro vs.* animal test data don't match?
  - Animal tests were never validated but have become the benchmark – how reliable are they?
  - *In vitro* tests combined with any *in vivo* human information = highest relevance.
- Suitable to assess new preservative candidates for inclusion in Annex V.
- Suitable for testing many finished products.
- Animal-free status of the tests (Level 1-7) should be assessed for compliance with fully "cruelty-free" / vegan approaches.

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Thank you!

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