Transitioning from animal testing to non-animal methods reflecting human biology

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3Rs In Toxicology: Promises and difficulties

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SWEDISH SOCIETY OF TOXICOLOGY
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Today's presentation

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This presentation will:

- Introduce skin sensitization with focus on key mechanisms.
- Review available *in vivo* / *in vitro* methods that can be used to assess skin sensitization.
- Introduce the GARD technology – first and only harmonised OECD TG based on genomics and machine learning.
- Discuss future challenges and gaps that remains to be address by novel assays to ultimately REPLACE animal testing.

Introduction - skin sensitization

What is skin sensitization, and why do we need to test for it?

Skin sensitization is clinically manifested as Allergic Contact Dermatitis (ACD)

- Skin sensitization is an adverse hypersensitivity reaction.
- Skin sensitization is a chronic condition. Elicitation of symptoms can only be avoided by preventing exposure.
- It is estimated that 20% of the population in EU is sensitized to at least one compound.
- Common sensitizers and sources of exposure includes:
  - Metals (Nickel) present in jewelry
  - Fragrances present in cosmetics, toiletries, deodorants etc.
  - Epoxy resins present in adhesives
  - PPD present in henna dyes or hair colors.

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Introduction – Testing for Skin Sensitization

The paradigm shift to replace animal models

- Testing for skin sensitization has traditionally been performed using animal models (GPMT, LLNA)

Figure from Human Society International

- **Regulatory drivers:**
  - Ban on animal testing for cosmetic products and its raw materials (Cosmetics Regulation 1223/2009).
  - Under REACH, registrants can only carry out animal tests as a last resort.

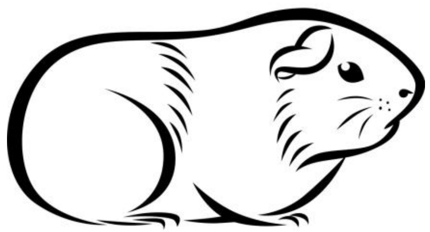
- **Scientific and technological drivers**
  - Not always predictive of human situation
  - Improved understanding of molecular mechanisms and the generation of Adverse Outcome Pathways (AOPs) enable development of relevant in vitro assays.

Introduction – Testing for Skin Sensitization

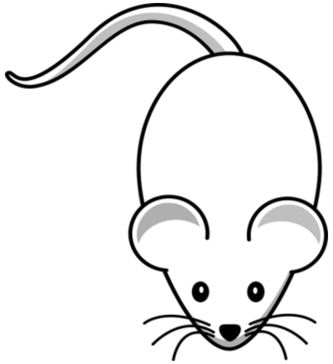
From *In vivo* methods to New Approach Methods

REDUCTION
REFINEMENT

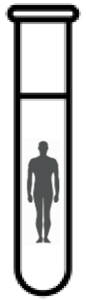
REPLACEMENT



Guinea Pig Assays
OECD TG 406



Local Lymph Node Assay
OECD TG 429



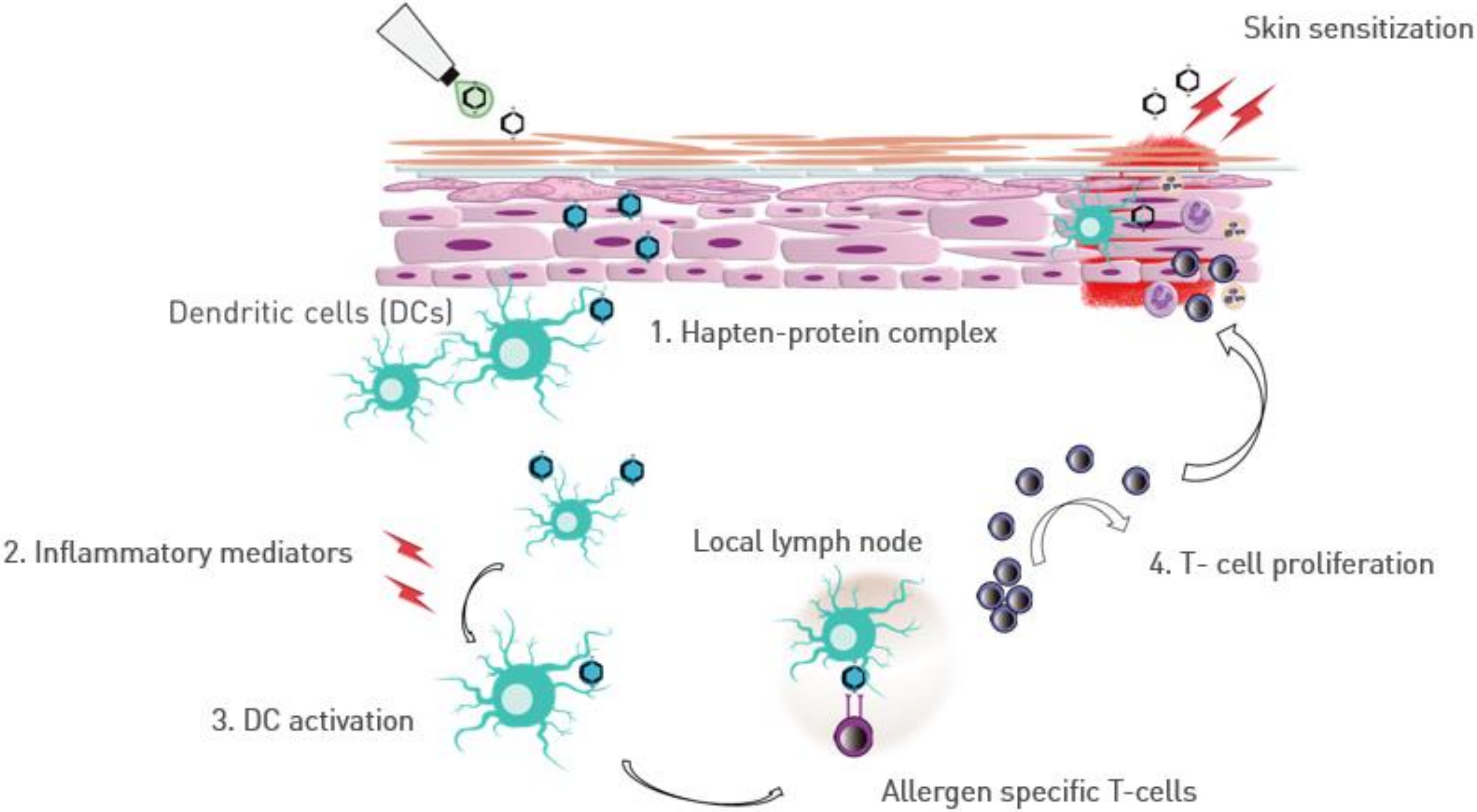
Mechanistically based NAMs
OECD TG 442 C,D,E

Omics and machine learning based NAMs
OECD TG 442E

1970s — 1990s — ~2010 → 2022

Introduction - skin sensitization

Molecular mechanisms of skin sensitization



Introduction – Testing for Skin Sensitization

NAM-based OECD Test Guidelines are mapped to the AOP

Keratinocytes

Dendritic cells

T-cells

Key Event 1
- Covalent binding to proteins

Key Event 2
- Pro-inflammatory signalling
- ARE-dependent pathways

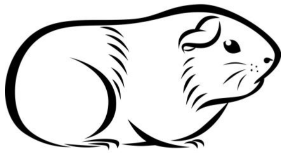
Key Event 3
- Activation of Dendritic cells

Key Event 4
- Proliferation of T-cells

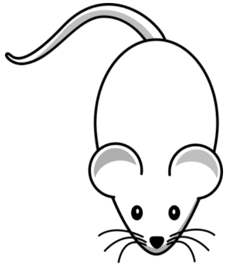
OECD 442 C
- DPRA
- ADRA
- kDPRA

OECD 442 D
- KeratinoSens™
- LuSens

OECD 442 E
- h-CLAT
- U-SENS
- IL-8 LUC
- GARD® (Genomic Allergen Rapid Detection™)



Guinea Pig Assays (OECD TG 406)



LLNA - Local Lymph Node Assay (OECD TG 429)

AOP - Adverse Outcome Pathway
NAM - New Approach Methods (KE 1-3)

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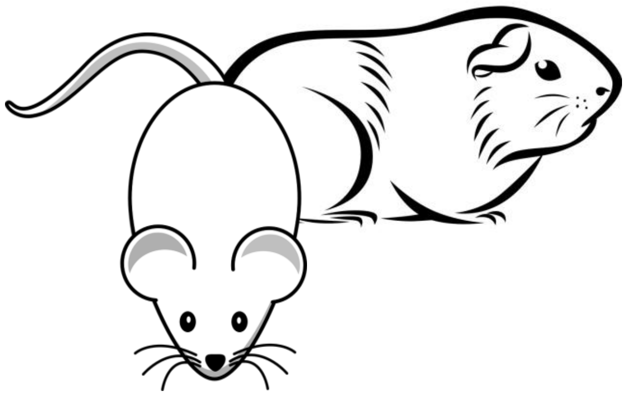
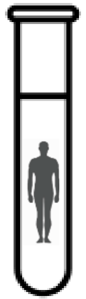
Introduction – Testing for Skin Sensitization

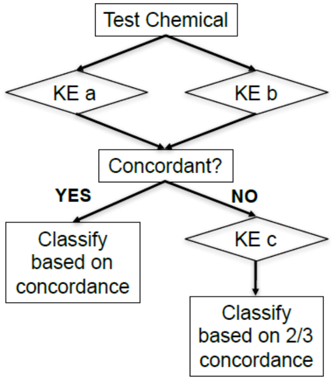
Defined Approaches to replace animal studies

Traditional testing:
In vivo

NAMs are combined into Defined Approaches to replace animal studies.







Score	h-CLAT MIT	DPRA depletion	DEREK
3	≤10 µg/mL	≥42.47%	-
2	>10, ≤150 µg/mL	≥22.62, <42.47%	-
1	>150, ≤5000 µg/mL	≥6.376, <22.62%	Alert
0	not calculated	<6.376%	No alert

Potency: Total Battery Score

Strong (1A):	**6-7**
Weak (1B):	**2-5**
Not classified:	**0-1**

Local Lymph Node Assay
(OECD TG 429)
Guinea Pig Assays
(OECD TG 406)

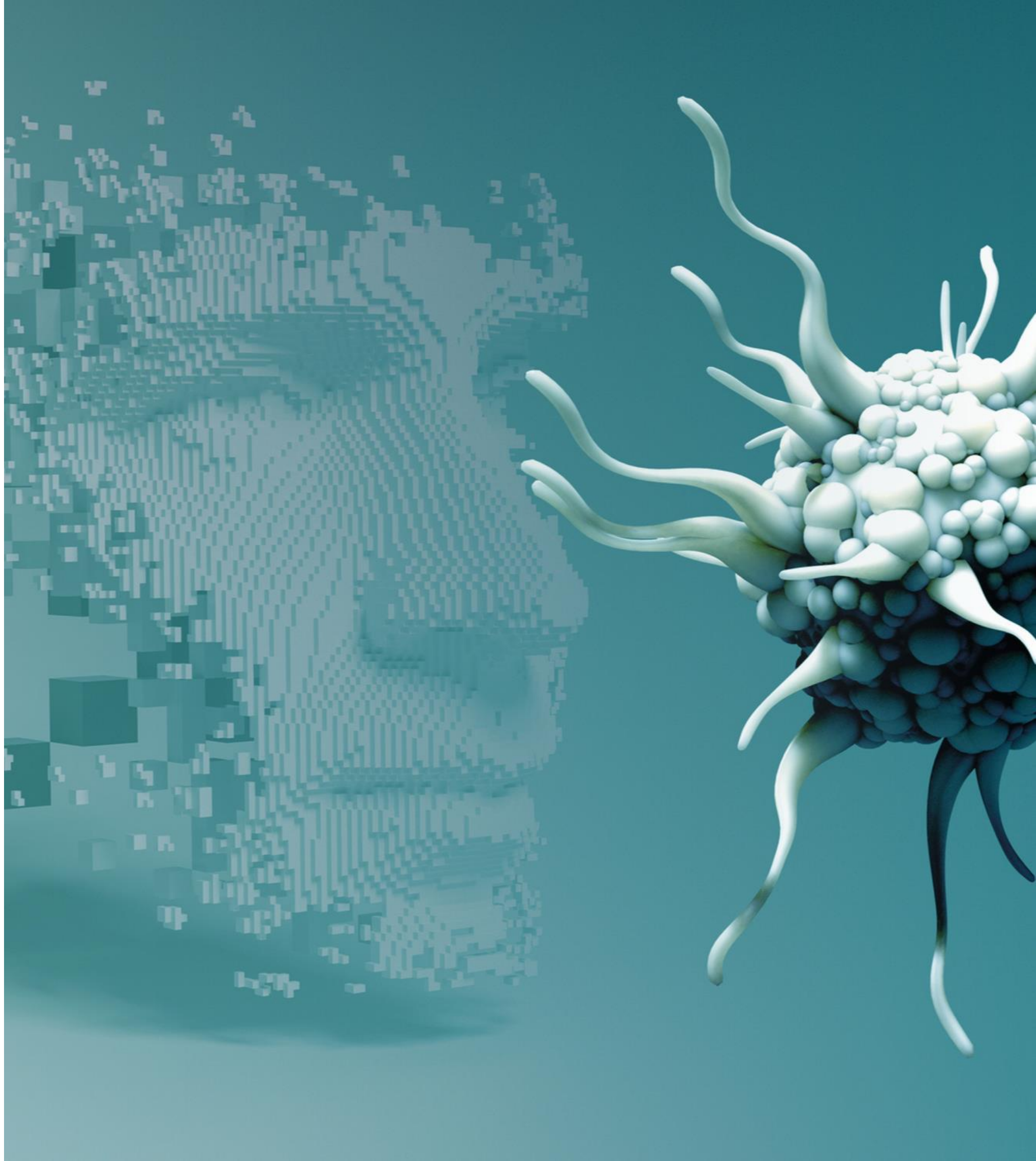
OECD TG 442C
OECD 442D
OECD TG 442E

OECD TG 497 on Defined Approaches for Skin Sensitization.
Hazard: 2 out of 3
GHS potency: ITSv1, ITSv2

GARD®

Genomic Allergen Rapid Detection™

Key technological features: **Genomics and machine learning**

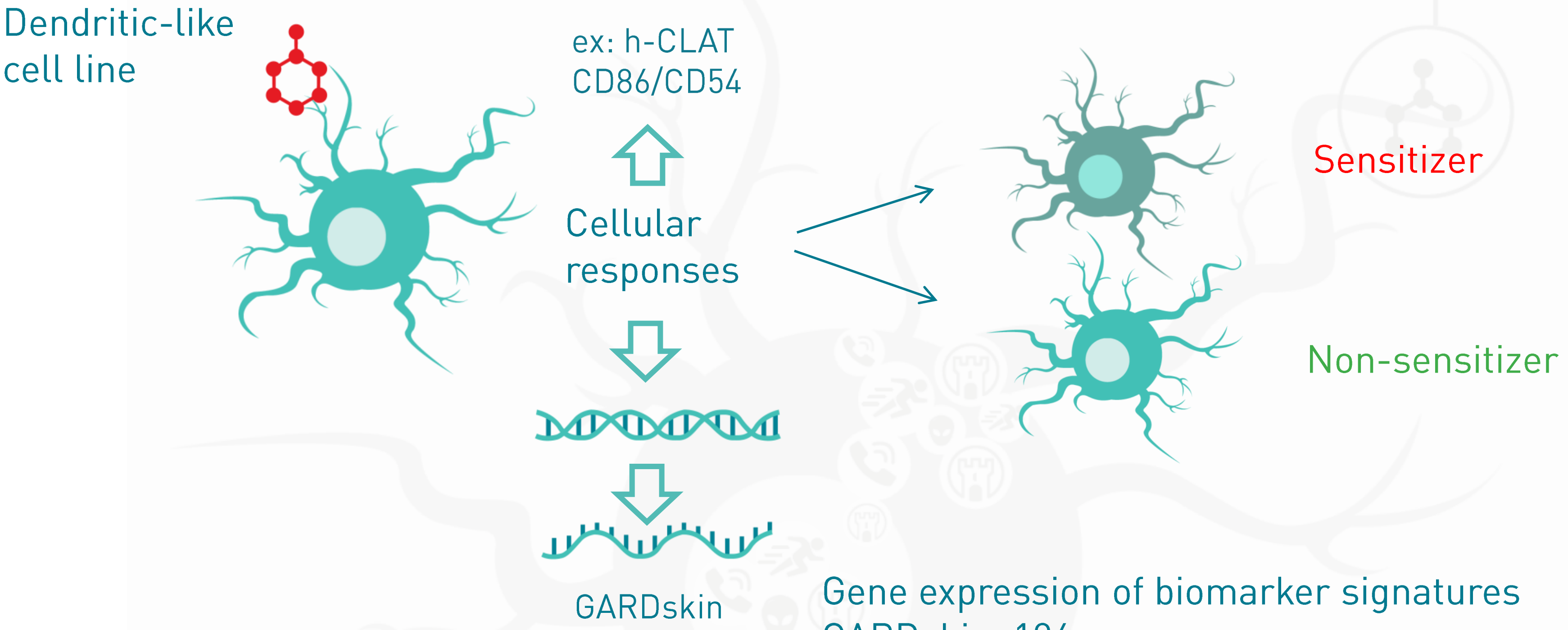


"This is the first harmonised method that generates and interprets genomic data for a regulatory endpoint"
- OECD Test Guidelines for chemicals

The GARD® technology platform – how it works

Transcriptomic read-out of the biological response

Biological system: Dendritic-like cell line (KE3)
Readout: Gene expression (genes and toxicity pathways)



Gene expression of biomarker signatures
GARDskin: 196 genes.

Full transparency: Identities of genes being measured available in peer-reviewed scientific literature.

See for example: Johansson et al. (2011) A genomic biomarker signature can predict skin sensitizers using a cell-based in vitro alternative to animal tests. BMC Genomics.

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The GARD® technology platform – how it works

Genes cover mechanistically relevant toxicity pathways

196 genes

Key Event 1

Covalent binding to proteins

Captures events downstream of **KE1**

Metabolic activity & identifies pre/pro haptens

ALDH
NAT-1
CYP - Cytochrome p-450

Key Event 2 (Keratinocytes)

>Pro-inflammatory signalling
>Cytoprotective signalling

> Keap1-Nrf2-ARE pathway & AHR signalling

NQO1
HMOX1
Thioredoxin reductase I

> Pro-Inflammatory cytokines mediating e.g. TNFα, INFγ, IL-8

FAS
MAP2KI
COX20

> Inflammasome

NLRP
PSTPIP1

Key Event 3 (Dendritic cells)

>Antigen recognition
>DC activation
>DC maturation
>DC migration

> DC migration & maturation

CD86

MAPK- activation
PKA- and GPCR- mediated signalling

> Antigen recognition & Innate immune activation

TLR-4
TLR-6
RXRA – retinoic X receptor
NLRP
PSTPIP1

> Self-defence mechanisms

C3a/C5a-activation pathways

Key Event 4 (T-cells)

Activation/ proliferation of T-cell

Covers the 3 Key steps for T-cell activation:

Antigen presentation
Co-stimulation
Cytokine secretion

Biological relevance established by experts during ESAC review: "Many of the identified transcription pathways, such as oxidative stress, immune responses, dendritic cell activation and cytokine responses are in line with mechanisms described under key events of the skin sensitization AOP" – *ESAC opinion*

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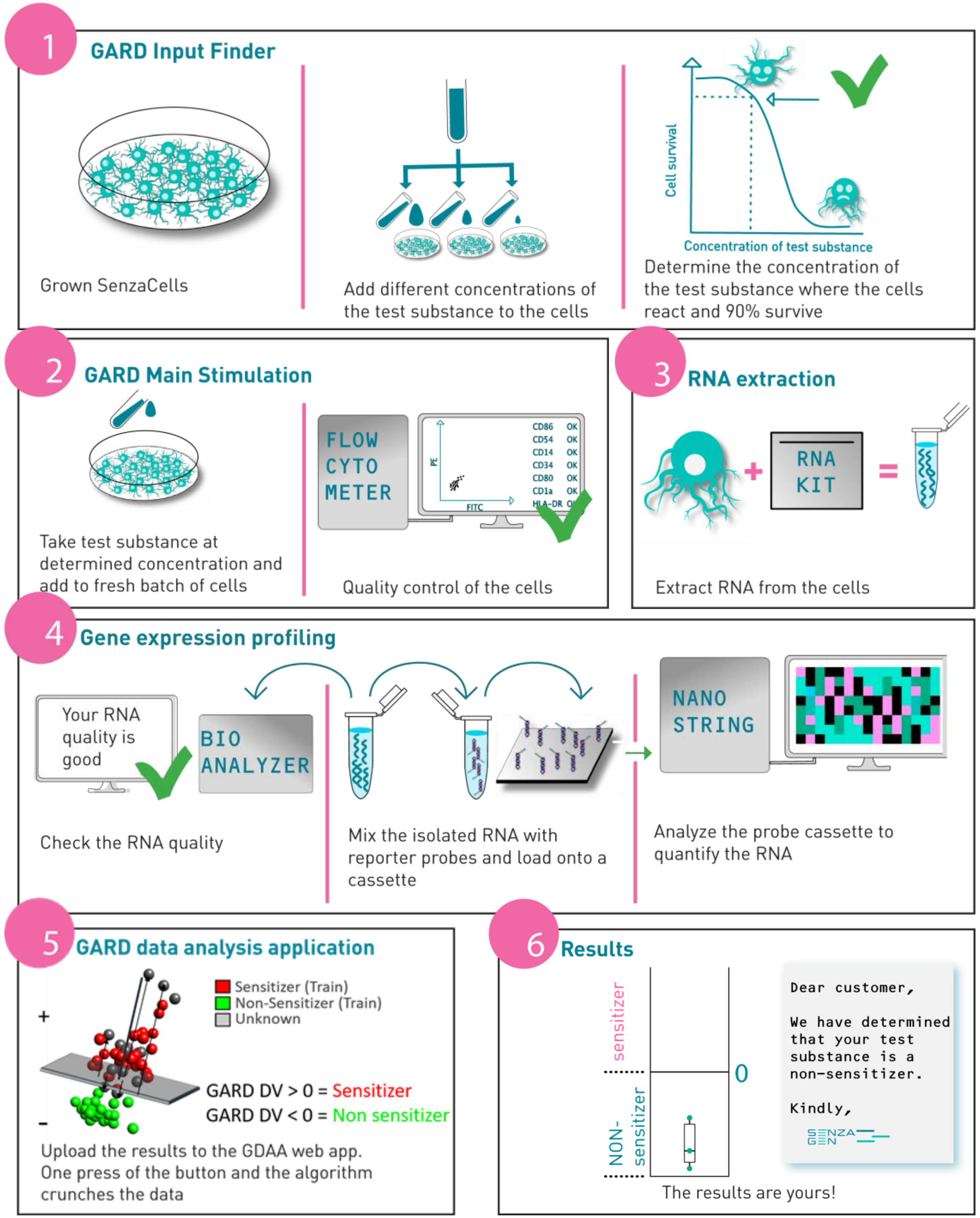
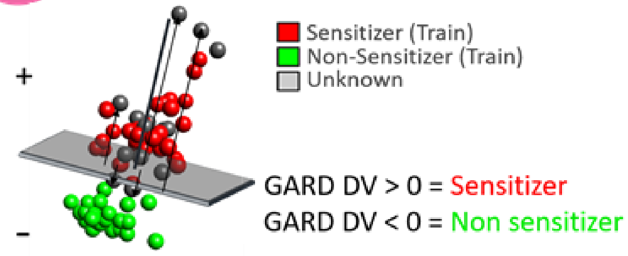
How to GARD® your products in 6 Steps



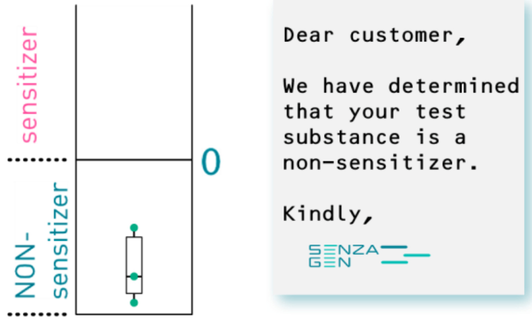
How to GARD® your products in 6 Steps

5 **GARD data analysis application**



Upload the results to the GDAA web app. One press of the button and the algorithm crunches the data

6 **Results**



The results are yours!

Importantly: All genes contributes to a final classification, but with different weights

Prediction algorithm:

$$DV = b + \sum_{i=1}^{n} w_i x_i$$

n: number of variables (n for GARDskin:196)
b: constant (SVM intercept)
W_i: weight for variable i
X_i: Normalized gene expression data for variable i

Prediction model:
Mean DV ≥ 0 : Skin sensitiser (UN GHS category 1)
Mean DV < 0 : Non-sensitiser.

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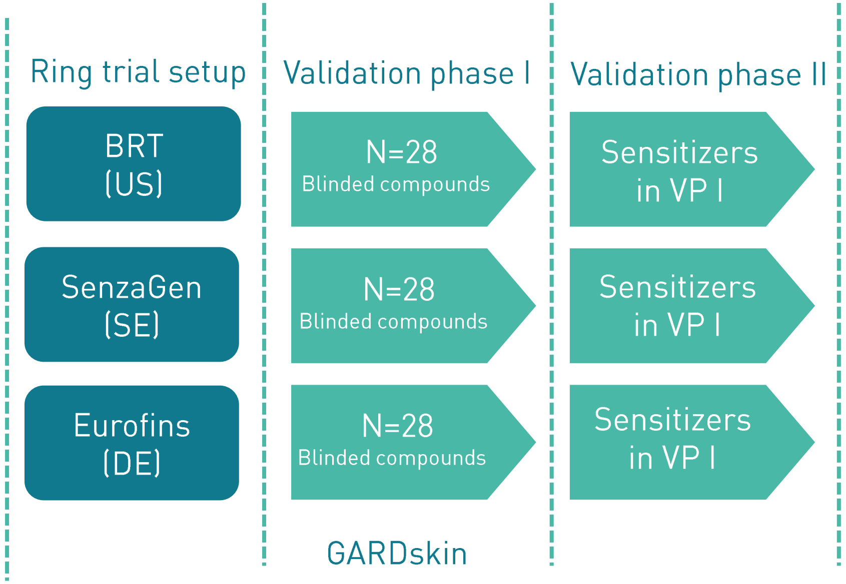
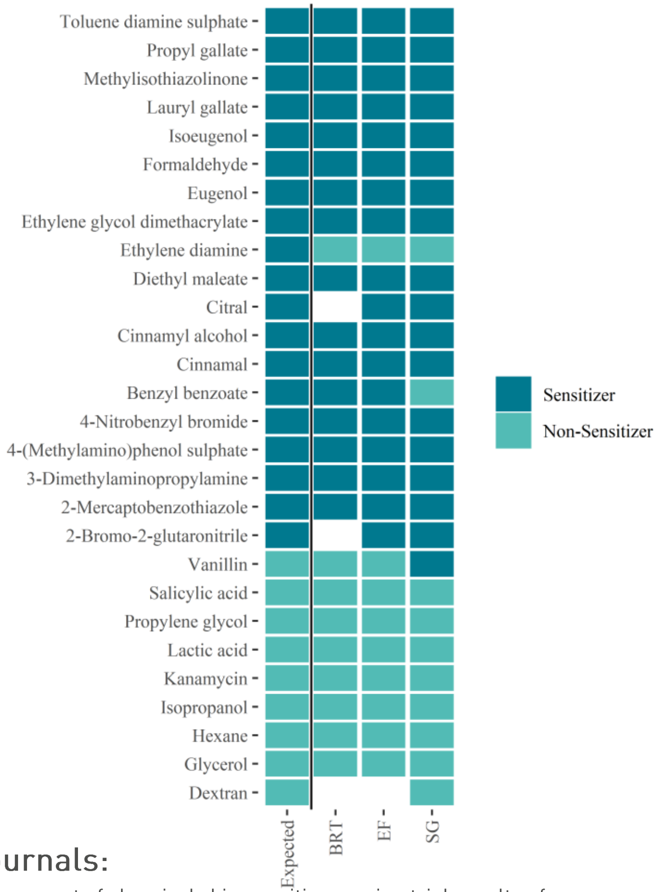
The OECD approval of GARD®skin

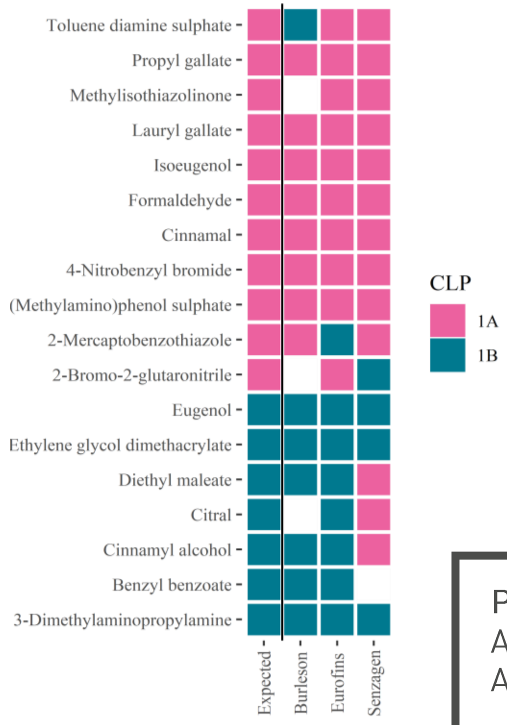
Machine learning and omics arrive in the field of regulatory toxicology



GARDskin predictions



GARDpotency predictions



Performance statistics:

Accuracy (Hazard):	94%
Accuracy (potency):	86%
WLR	82.1- 88.9%
BLR	92%

Validation studies published in peer-reviewed scientific journals:

GARDskin: Published in Johansson et al. (2019), Validation of the GARD™skin assay for assessment of chemical skin sensitizers - ring trial results of predictive performance and reproducibility. *Toxicological Sciences.*

GARDpotency: Published in Gradin et al. (2020), The GARD™potency Assay for Potency-Associated Subclassification of Chemical Skin Sensitizers - Rationale, Method Development and Ring Trial Results of Predictive Performance and Reproducibility. *Toxicological Sciences.*

The OECD approval of GARD®skin

Machine learning and omics arrive in the field of regulatory toxicology

Regulatory breakthrough

- GARD®skin represents a landmark opinion – first ever harmonised TG based in genomics & machine learning.
- Bringing disruptive technologies into the guidelines are challenging:
  - The expert input from EURL ECVAM, the OECD secretariate, the OECD expert group for skin sensitization and the Swedish national coordinator of the Test Guidelines programme (kemikalieinspektionen) has been extremely useful.
  - The validation of GARDskin sets a new standard for the validation of similar next generation models in the future.

Genomics and Machine Learning arrive in the field of Regulatory Toxicology

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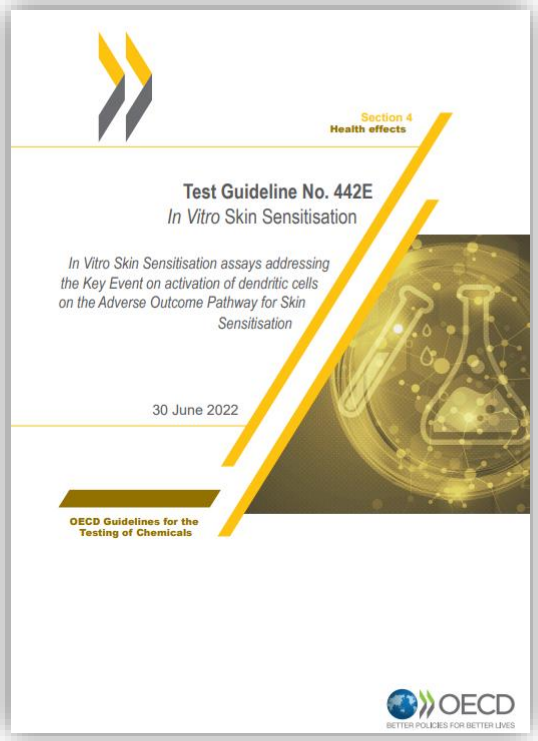
The OECD approval of GARD®skin

Adopted into Test Guideline 442E for *in vitro* skin sensitization

OECD Test Guideline No. 442 E - *In Vitro* Skin Sensitisation

Assays addressing the KE on activation of DCs on the AOP for Skin Sensitisation



- Test methods in OECD TG 442E can be used indiscriminately to address countries requirements for test results on KE3
- Data from individual assays supports the discrimination between skin sensitisers and non-sensitisers within an IATA.
- Dependent on regulatory context, positive results from test methods in TG 442E may be used on their own to classify a chemical into UN GHS category 1.

For an overview of regulatory information requirements:

Daniel et al. (2018) International regulatory requirements for skin sensitization testing. Regulatory Toxicology and Pharmacology.

Remaining challenges

Data gaps and limitations to be addressed by novel in vitro methods

Applicability domain (AD)

- OECD TGs validated using a narrow subset of the chemical space.
- OECD TGs validated for monoconstituents. Limited data available for complex mixtures.

Quantitative assessment of relative sensitizing potency

- Quantitative assessment of skin sensitizing potency on a continuous scale for use in QRA and to establish a threshold dose.

Biocompatibility testing of medical devices

- Requires assay compatibility to both polar and non-polar extraction vehicles (ISO-10993-12).
- Assay must be sensitive to detect potential sensitizers in a complex extract.

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Case #1: GARDskin Applicability Domain

Extending the applicability domain – difficult-to-test chemicals

Lipophilic compounds

Chemicals that are difficult to dissolve in the standard test solutions water and DMSO.

Indirectly acting haptens

Chemicals that require metabolic activation to become skin sensitizers.

Metals and metal salts

Chemicals that lack data to demonstrate applicability

Complex mixtures

Chemicals that are often with unknown molecular weight. May also be associated with high cytotoxicity or solubility issues.

Publications in collaboration with Lubrizol, Johnson Matthey and Corteva

Forreryd, A., Gradin, R., Humfrey, C., Sweet, L. and Johansson, H. (2022). Exploration of the GARD™ skin applicability domain: Indirectly acting haptens, hydrophobic substances and UVCBs. *ALTEX*

Forreryd, A., Gradin, R., Rajapakse, N., Deag, E. and Johansson, H. (2022). The GARD™skin assay: Investigation of the applicability domain for metals. Manuscript in review.

Corvaro, M., Henriquez J., Settivari, R., Mattson, U.T., Forreryd, A., Gradin, R., Johansson, H. and Gehen, S. (2022). GARD™skin and GARD™potency: a proof-of-concept study to investigate the applicability domain for agrochemical formulations. Manuscript submitted.

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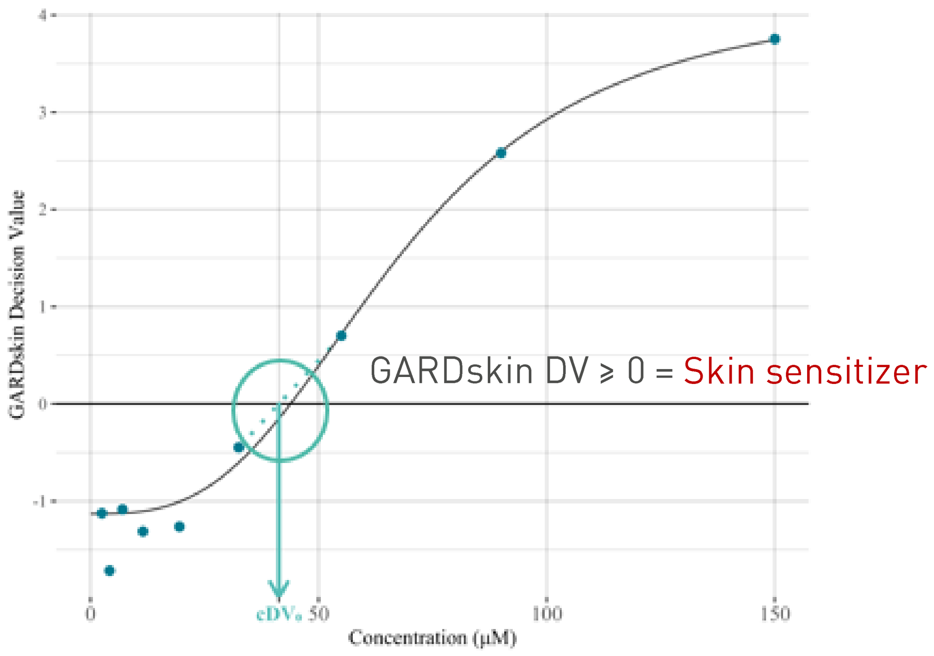
Case study #2: GARDskin Dose-Response

Quantitative assessment of skin sensitizing potency on a continuous scale

- Perform the GARDskin assay in a titrated range of concentrations ($n \geq 6$).
- Apply standard GARDskin protocol to generate a decision value (DV) for each concentration.
- From the resulting dose-response curve: Estimate cDV_0 (lowest concentration required to induce a positive classification ($DV \geq 0$)).



	GARD	LLNA
Response value	DV	SI
Binary Threshold	DV = 0	SI = 3
Readout	cDV_0 (DV_0 Concentration)	EC3 Concentration

SCIENTIFIC REPORTS nature Gradin, R., Forreryd, A., Mattson, U., Jerre, A., Johansson, H. (2021) Quantitative assessment of sensitizing potency using a dose-response adaptation of GARDskin. Nature Scientific Reports

Case study #2: GARDskin Dose-Response

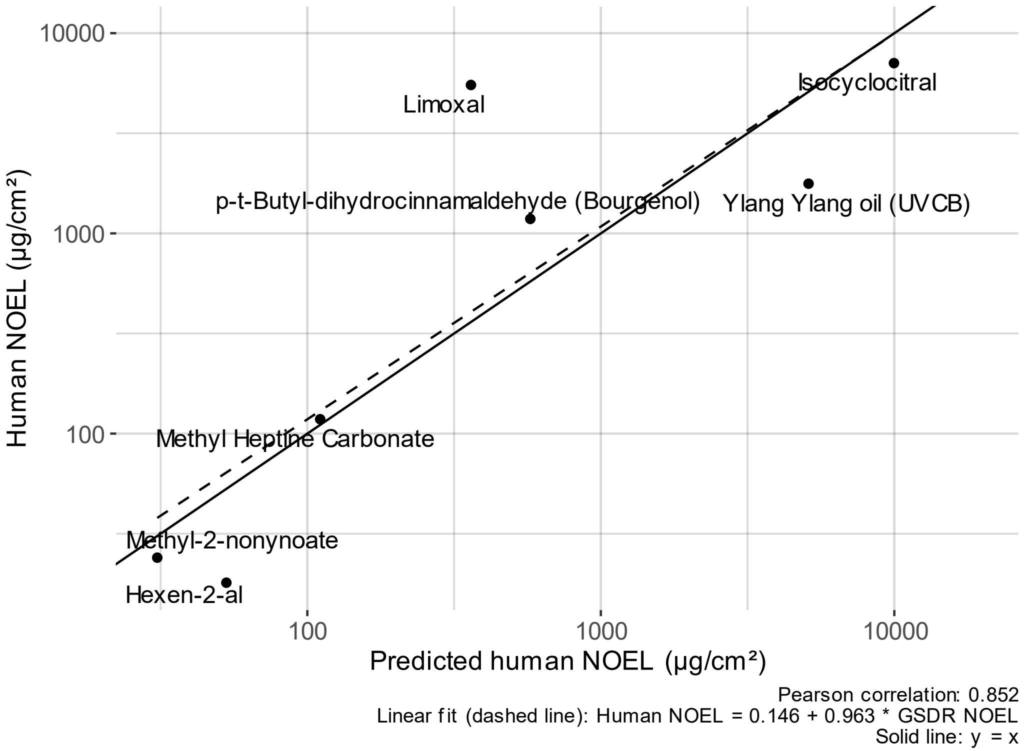
Quantitative assessment of skin sensitizing potency on a continuous scale

Background

- Collaboration with International Flavors & Fragrances – presented at SOT 2022.
- Blinded testing of 12 materials (incl. a UVCB and a multiconstituent).
- GARDskin Dose-response cDV_0 values used to predict LLNA EC and Human NOEL.

Results

- GARDskin Dose-Response predicted Human NOEL values correlated extremely well with reference data.
- NESIL – No Expected Sensitization Induction Level is the point of departure for QRA.
- More data was recently presented at the ASCCT meeting – poster available on requests.



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Case study #3: Medical device testing

In vitro skin sensitization testing of medical devices/solid materials (ISO 10993-10)

Biocompatibility testing of medical devices

- Requires assay compatibility to both polar and non-polar extraction vehicles (ISO 10993-12).
- Assay must be sensitive to detect potential sensitizers in a complex extract.

Adaption of protocols

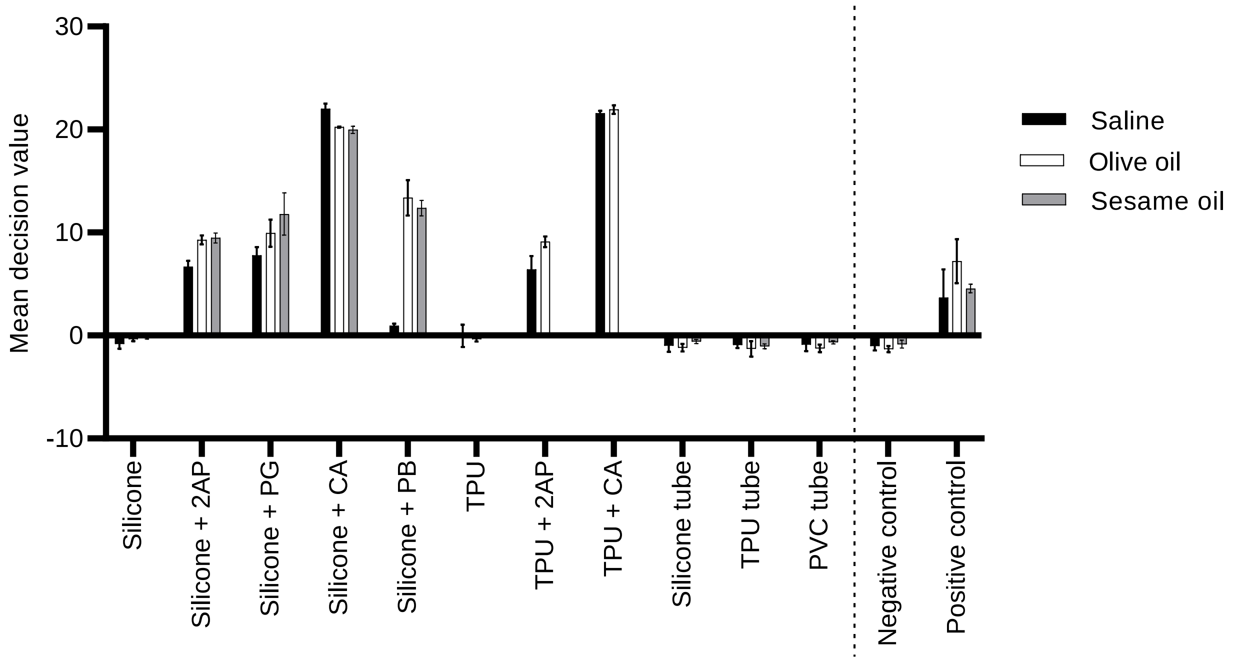
- Protocols adapted to polar and non-polar solvents.
- OECD TGs not compatible with non-polar vehicles.

Proof of concept study

- Polymers (Silicon/TPU) spiked with sensitizers.
- Tubes (Silicone, TPU and PVC) – neg controls.
- Extractions in saline, olive oil and sesame oil.

Results

- Protocols adapted for testing in polar/non-polar vehicles. All materials correctly classified.



Jenvert R, et et al. Evaluation of the Applicability of GARDskin to Predict Skin Sensitizers in Leachables from Medical Device Materials. Manuscript in preparation.

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Summary & acknowledgements

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- REPLACEMENT of animal studies for the endpoint of skin sensitization has been very succesful.
- Several NAM-based approaches have been adopted into as OECD TGs, and when combined into DAs, they often outperform traditional animal assays.
- Novel assays and approaches are still needed to address remaining challenges – Proof of concept data have been provided as case studies in this presentation.

KEMI Kemikalieinspektionen
SWEDISH SOCIETY OF TOXICOLOGY SINCE 1969
OECD BETTER POLICIES FOR BETTER LIVES
EURL ECVAM European Union Reference Laboratory for Alternatives to Animal Testing

Thanks for your attention!

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