Skin Sensitization Next Generation Risk Assessment Framework and Case Study

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Expert Panel for Cosmetic Ingredient Safety
160th Meeting, March 7-8, 2022

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Cosmetic Industry needs Next Generation Risk Assessment (NGRA) Approaches for Skin Allergy that …

- are protective for consumers
- do not require the generation of animal data
- allow to set appropriate human induction thresholds
- are exposure-led and use novel exposure scenarios
- are robust and transparent
- are fit for purpose and use the weight of evidence

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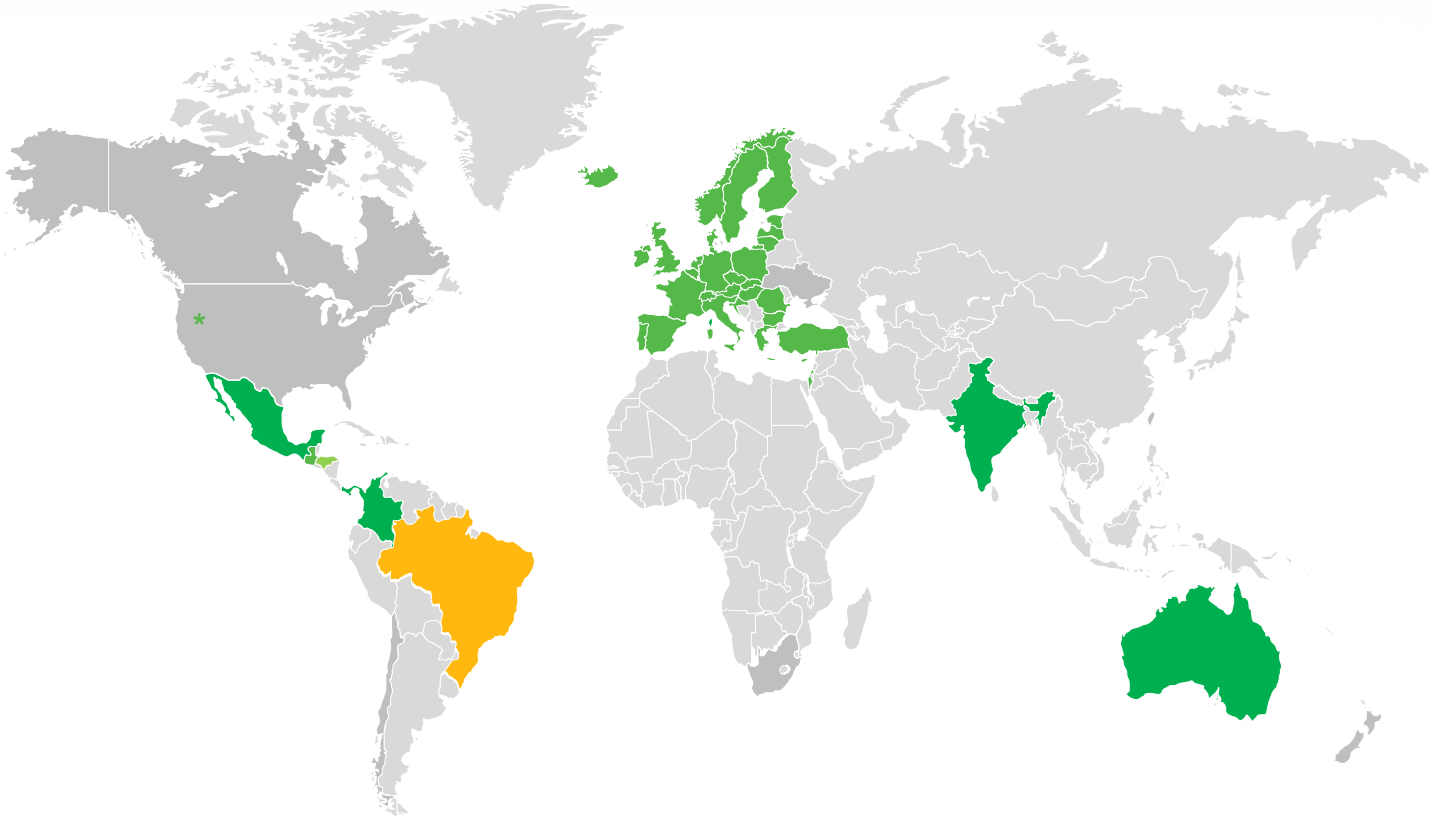
Animal Testing Bans for Cosmetic Ingredients Now Extends Beyond Europe



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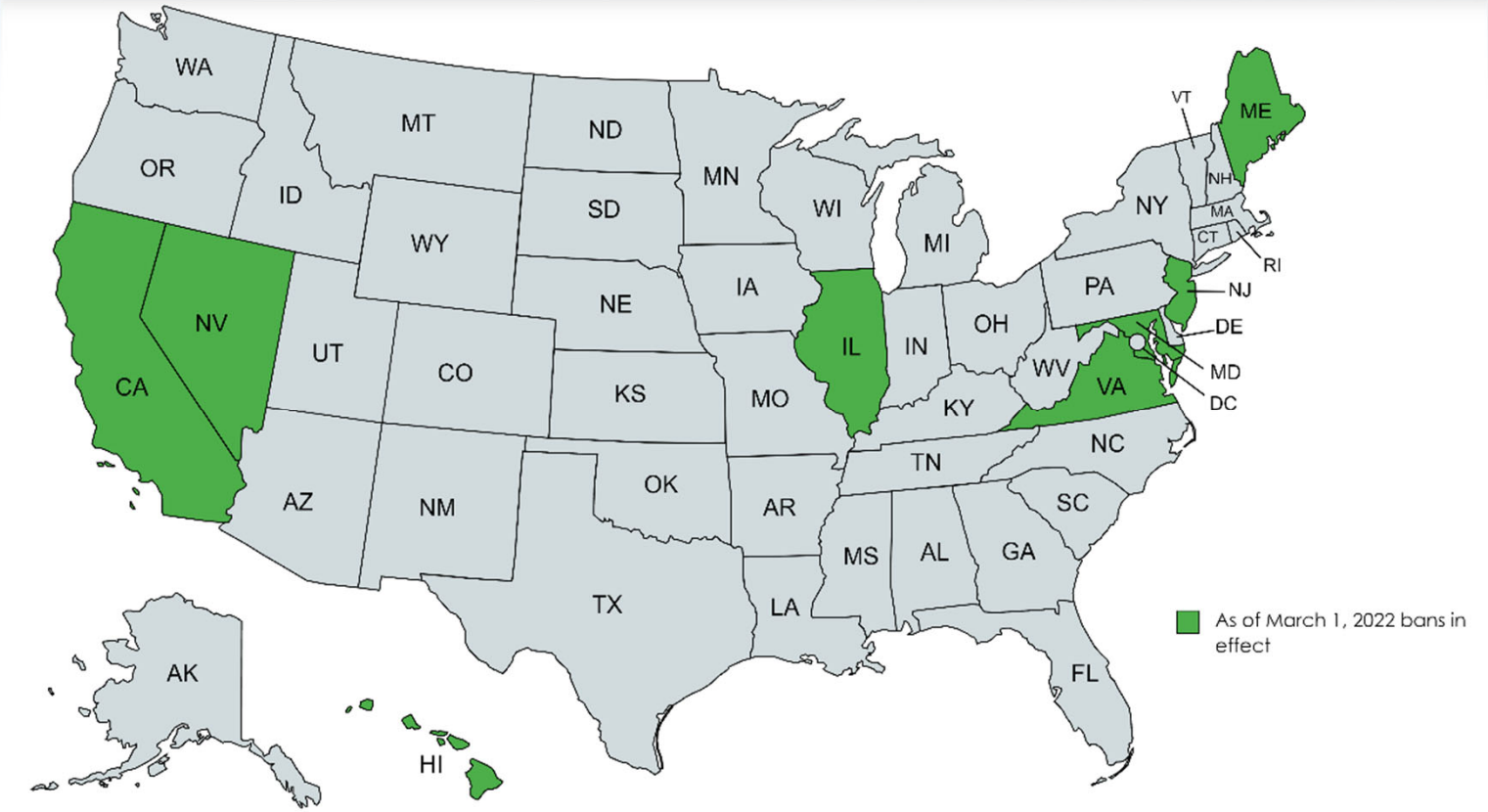
Animal Testing Bans for Cosmetic Ingredients in USA



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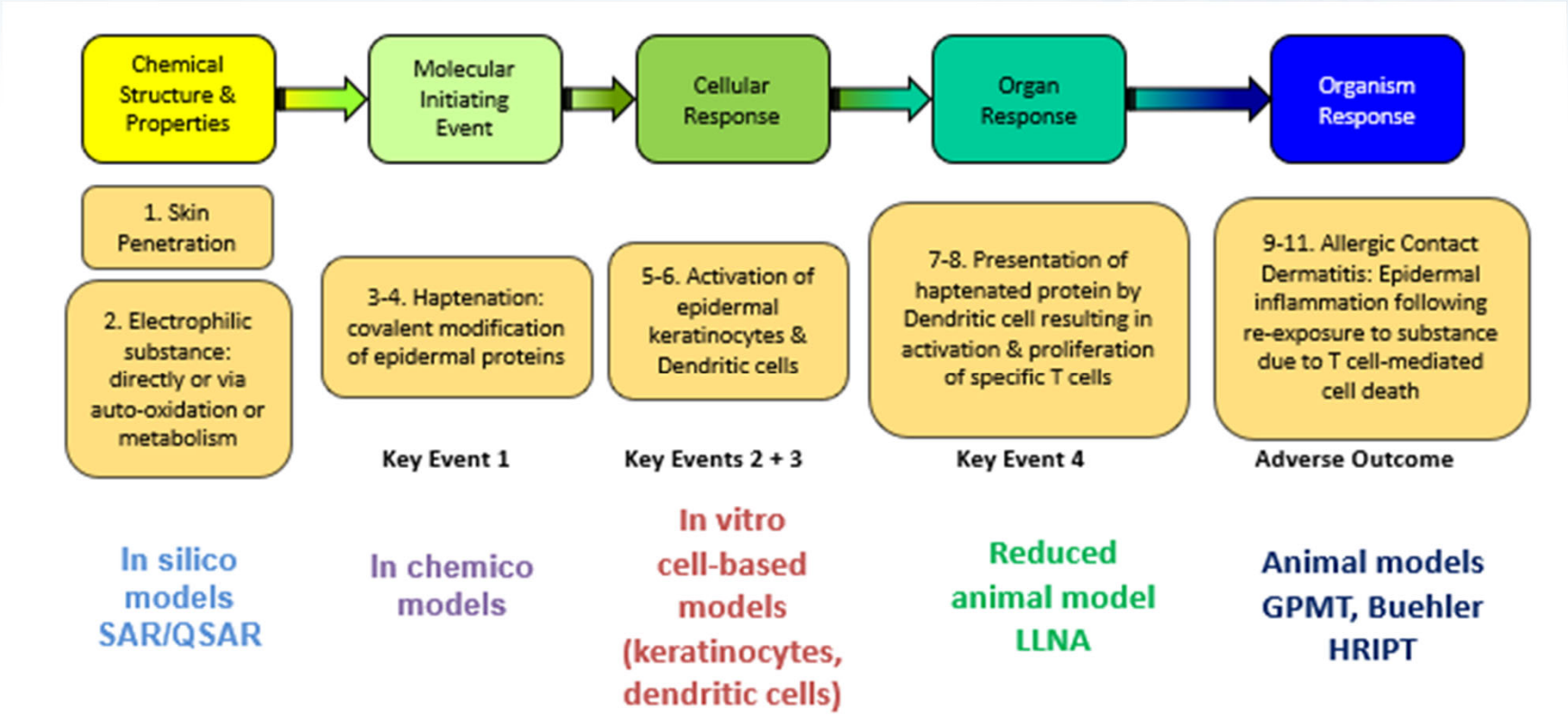
Adverse Outcome Pathway and Predictive Testing



Modified version of flow diagram from “The Adverse Outcome Pathway for Skin Sensitisation”, OECD report 2012

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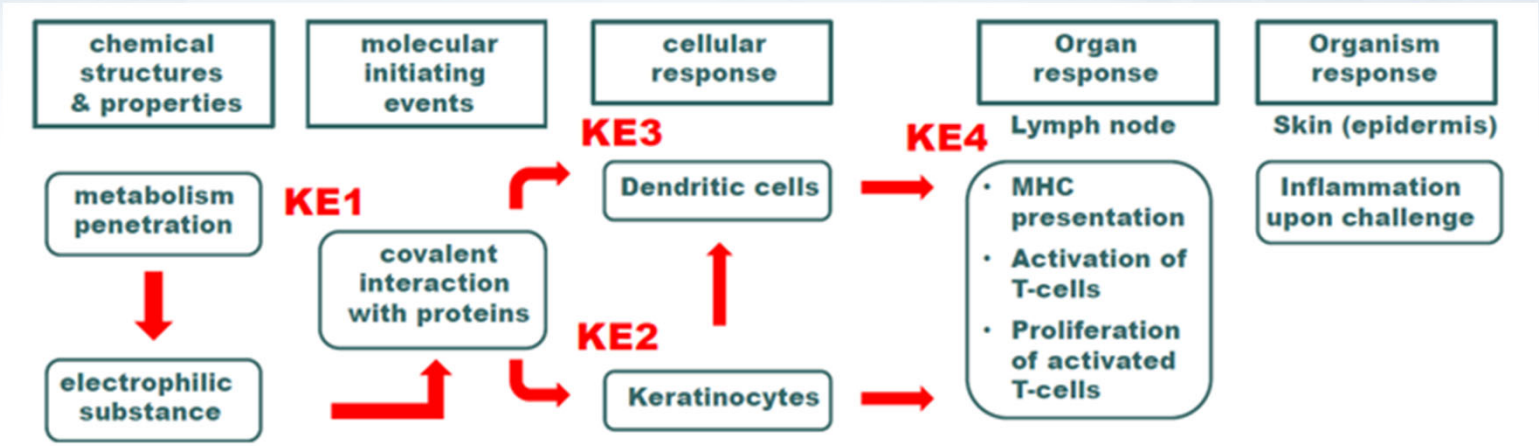
Adverse Outcome Pathway and Predictive Testing



DPRA Direct Peptide Reactivity Assay
ADRA *Amino acid Derivative Reactivity Assay*

Are-Nrf2 Luciferase test methods: **Keratinosens™** **LuSens**

h-CLAT Human Cell line Activation Test
U-SENS™ U937 Cell line activation test
IL-8-Luc Interleukin-8 Reporter Gene Assay

OECD 442C
In chemico (KE1)

OECD 442D
In vitro (KE2)

OECD 442E
In vitro (KE3)

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Summary of available *in chemico/in vitro* skin sensitization test methods and defined approaches (European Chemicals Agency, October 2021)

Latest update	AOP key event measured	Test method	Validation status, regulatory acceptance	OECD test guideline	Outcome according to the test method/guideline
2021	Key Event 1 (peptide /protein binding	DPRA	Validated and regulatory acceptance	OECD TG 442C	SS or NS with complementary information
2021		ADRA	Validated and regulatory acceptance	OCD TG 442C	SS or NS with complementary information
2021		kDPRA	Validated and regulatory acceptance	OECD TG 442C	Cat 1A or Cat 1B/NS
2018	Key Event 2 (Keratinocyte response)	Keratinosens™	Validated and regulatory acceptance	OECD TG 442D	SS or NS with complementary information
		LuSens	Validated/under regulatory review	OECD TG 442D	SS or NS with complementary information

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Summary of available *in chemico/in vitro* skin sensitization test methods and defined approaches (European Chemicals Agency, October 2021)

Latest update	AOP key event measured	Test method	Validation status, regulatory acceptance	OECD test guideline	Outcome according to the test method/guideline
2018	Key Event 3 (Monocytic / dendritic cell response)	h-CLAT	Validated and regulatory acceptance	OECD TG 442E	SS or NS with complementary information
2018		U-SENS™	Validated and regulatory acceptance	OECD TG 442E	SS or NS with complementary information
2018		IL-8 Luc	Validated and regulatory acceptance	OECD TG 442E	SS or NS with complementary information
2021	Defined approach	2 out of 3	Validated and regulatory acceptance	OECD TG 497	SS or NS
2021		ITS v1 or v2	Validated and regulatory acceptance	OECD TG 497	SS (Cat 1A or 1B) or NS

Abbreviations: SS = skin sensitiser, NS = non-sensitiser, Cat 1A = extreme/strong sensitiser according to CLP, Cat 1B = moderate sensitiser according to CLP.

Note: In all cases, the most recent version of the test guideline should be used.

All of the methods above have been validated by an international validation body before adoption by the OECD or EU.

Direct Peptide Reactivity Assay (DPRA) OECD 442C

- AOP Molecular Initiating Event
- Key Event 1 – Haptenation: Covalent modification of epidermal proteins
- In chemico method used for supporting the discrimination between skin sensitizing chemicals and non-sensitizing chemicals
- Quantifies the reactivity of chemicals towards model synthetic peptides containing either lysine or cysteine
- Endpoint is % depletion of peptide
- ECVAM DB-ALM Protocol 154: https://jeodpp.jrc.ec.europa.eu/ftp/jrc-opendata/EURL-ECVAM/datasets/DBALM/LATEST/online/DBALM_docs/154_P_%20Direct%20Peptide%20Reactivity%20Assay.pdf

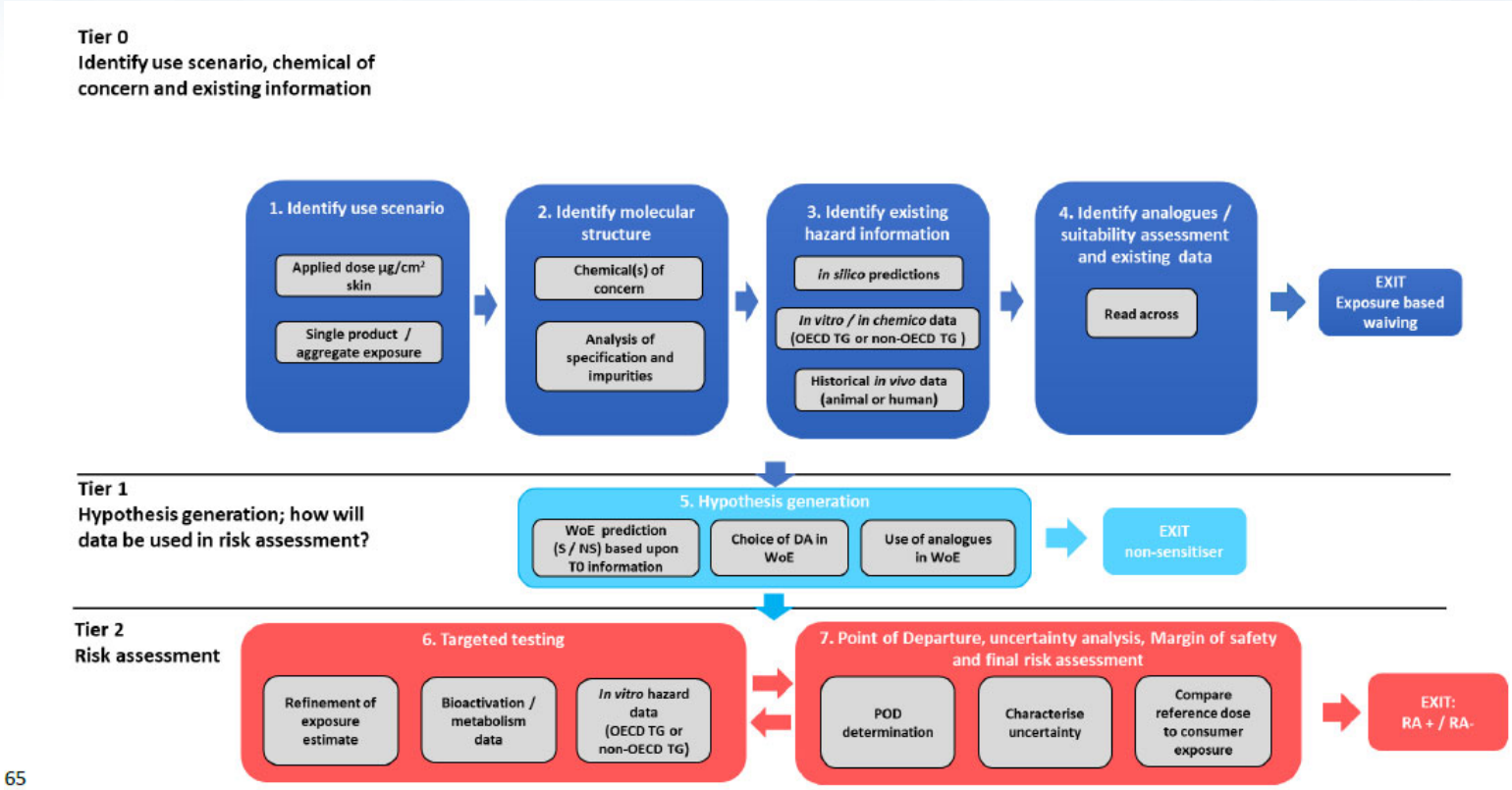
KeratinoSens™ (ARE-Nrf2 Luciferase) OECD 442D

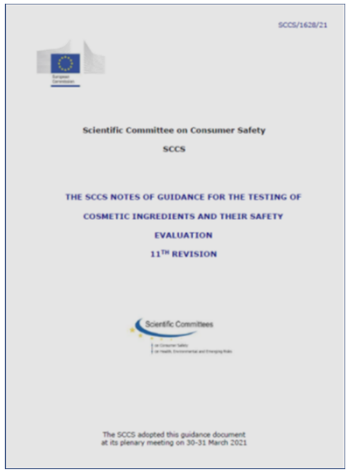
- AOP Cellular Response
- Key Event 2 – Activation of epidermal keratinocytes
- The release of pro-inflammatory cytokines and the induction of cyto-protective pathways in keratinocytes is the second key event in skin sensitization
- In vitro method designed to support the discrimination between skin sensitizing chemicals and non-sensitizing chemicals
- Quantifies luciferase gene induction as a measure of the activation of the Keap1-Nrf2-antioxidant/electrophile response element (ARE)-dependent pathway in an immortalized adherent cell line derived from HaCaT human keratinocytes transfected with a selectable plasmid
- Endpoints EC 1.5, EC2 and EC3 (concentration needed for luciferase induction), IC30 and IC50 (concentration needed to reduce viability), and I_{max} (maximum fold induction of the luciferase gene over solvent control)
- ECVAM DB-ALM Protocol 155: https://jeodpp.jrc.ec.europa.eu/ftp/jrc-opendata/EURL-ECVAM/datasets/DBALM/LATEST/online/DBALM_docs/155_P_%20KeratinoSens.pdf

h-CLAT (Human Cell Line Activation Test) OECD 442E

- AOP Cellular Response
- Key Event 3 – Activation of monocytic/Dendritic cells
- The activation process in which DC change from antigen processing to antigen presenting cells is considered a key event in skin sensitization
  - Activation involves the modulation of the expression of cell surface phenotypic markers (e.g., CD54, CD80, CD86 and major histocompatibility complex class II)
- In vitro method designed to support the discrimination between skin sensitizing chemicals and non-sensitizing chemicals
- Quantifies expression of CD86 and CD54 on human monocytic leukemia cell line THP-1, used as a surrogate for human DC
- Endpoints = relative fluorescence intensity of CD86 and CD54 on cell surface by flow cytometry
- ECVAM DB-ALM Protocol 158: https://jeodpp.jrc.ec.europa.eu/ftp/jrc-opendata/EURL-ECVAM/datasets/DBALM/LATEST/online/DBALM_docs/158_P_human%20Cell%20Line%20Activation%20Test.pdf

Next Generation Risk Assessment (NGRA) Framework for Skin Sensitisation




SCCS/1628/21

Scientific Committee on Consumer Safety
SCCS

THE SCCS NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION
11TH REVISION

Scientific Committees

The SCCS adopted this guidance document at its plenary meeting on 30-31 March 2021

SCCS 11th NoG 2021
(SCCS/1628/21)

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Gilmour & Kern et al, RTP, 116, 2020 https://doi.org/10.1016/j.yrtph.2020.104721

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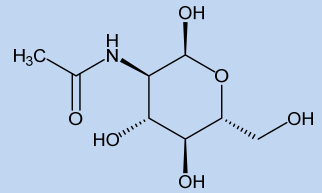
Skin Sensitization Risk Assessment – Acetyl glucosamine

Tier 0 - Step 1: Identify use scenario

Acetyl glucosamine
(CAS 10036-64-3)

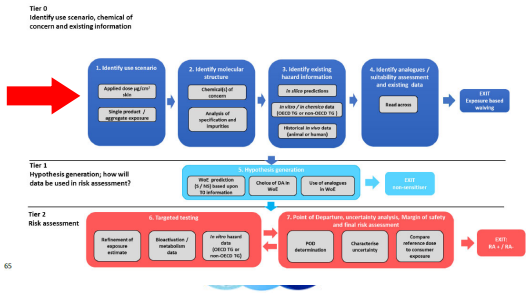


1 (CAS RN 10036-64-3)

Product	Product applied (g/day)	Use level (%)	Skin retention	Skin surface (cm^2)	Consum. Exp. Level ($\mu g/cm^2/d$)
Face cream	1.54	5	1	565	136

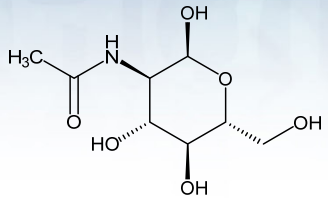
Ref.: SCCS Notes of Guidance (SCCS/1628/21) from 2021. Section 3-3.4 (external exposure)

- How is the chemical used?
- Are the assumptions conservative?
- Is the exposure very low to consider an “exposure based waiving” approach?



Skin Sensitization Risk Assessment – Acetyl glucosamine

Tier 0 - Step 2: Identify molecular structure

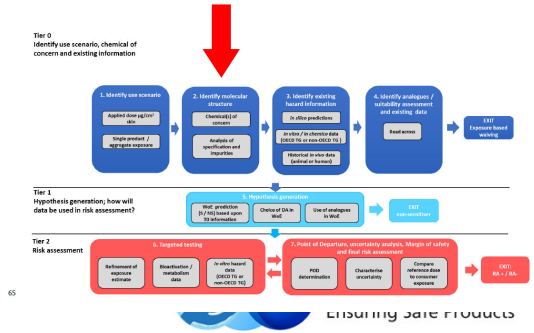


1 (CAS RN 10036-64-3)

Physical-chemical properties, specification and impurities

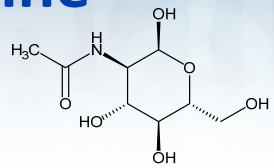
MW	Log P	Boil. Pt	Vap pressure	H_2O Solubility pH7 [M]	LogD (pH7)	Protein Binding [%]	Fraction ionized [%]	Purity
221.2	-1.63	460.4	6.35E-12	4,43	-2.48	22.48	0.859	99.42%

- Do the phys-chem properties point towards applicability domain problems?
- Which phys-chem properties are needed for a defined approach?
- Are they based on *in silico* predictions or experimental data?



Skin Sensitization Risk Assessment – Acetyl glucosamine

Tier 0 - Step 3: Identify existing information

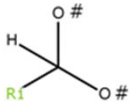


1 (CAS RN 10036-64-3)

TIMES-SS		DEREK Nexus	In vitro [OECD]			Bio-availability
Parent	Metabol.		DPRA	KeratinoS	h-CLAT	
Out of Domain	Out of Domain	Neg./ Alert	**NS** Mean depletion of cyst. and lysine 1%	**NEG** KEC1.5: >2000 KEC3: >2000 IC50: >2000	**NEG** EC150: >10000 EC200: >10000 CV75: >10000	Good bioavailability, readily penetrates human skin

DEREK alert: alkyl aldehyde precursor. Sugars are excluded due to stability of 5- or 6-member rings. Glucosamine is an amino sugar synthesized from glucose and glutamine. ToxTree - no alerts.

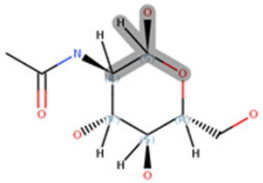
Alert Description Image

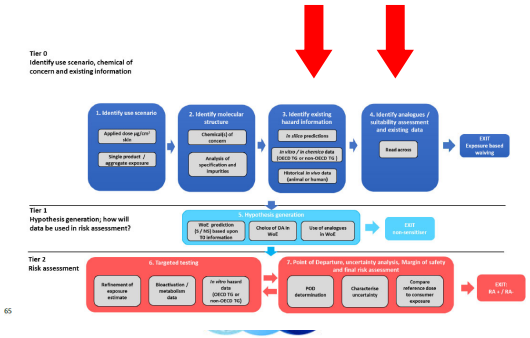


R1 = H, C (cannot be multiply bonded unless to a beta-disubstituted alkene)

O atoms marked # cannot be attached to any additional heteroatoms

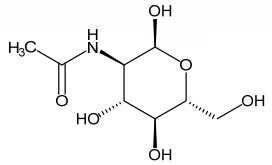
Match with query compound



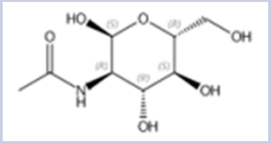
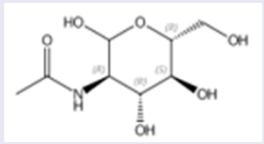
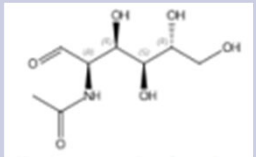


Skin Sensitization Risk Assessment – Acetyl glucosamine

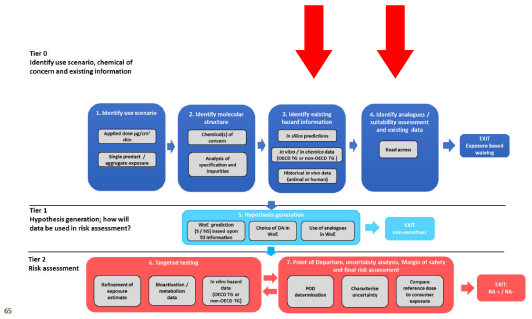
Tier 0 - Step 4: Identify analogues /suitability assessment and existing data



1 (CAS RN 10036-64-3)

	CAS#	Sensitization Data	NAM data
	10036-64-3	HRIPT (3) HMT (2)	Available (DPRA NS + h-Clat neg, KeratinoSens neg)
	72-87-7	Not available	Not available
	7512-17-6	Not available	Not available

(Wu et al. 2011, Blackburn et al. 2011, Blackburn and Stuard 2014)



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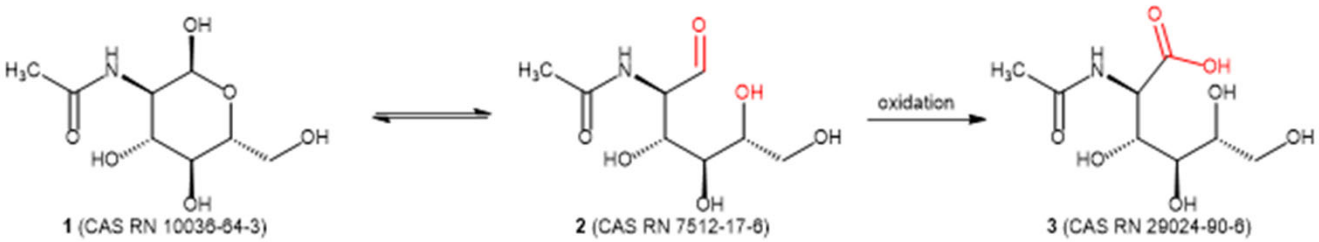
Skin Sensitization Risk Assessment – Acetyl glucosamine

Tier 0 - Step 4: Identify analogues /suitability assessment and existing data

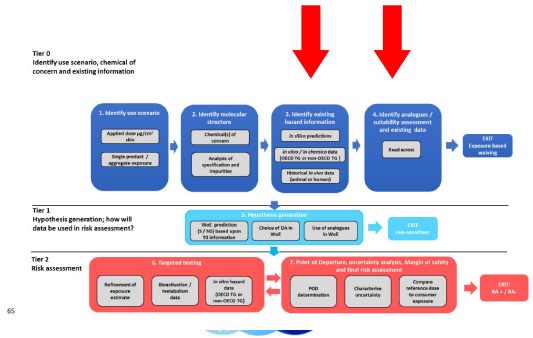
Metabolism Information Prediction



1 (CAS RN 10036-64-3)

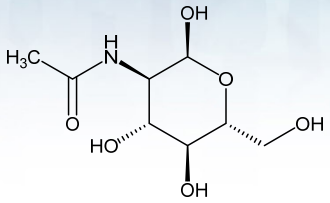
2 (CAS RN 7512-17-6)

3 (CAS RN 29024-90-6)



Skin Sensitization Risk Assessment – Acetyl glucosamine

Tier 1 – Step 5: Hypothesis generation

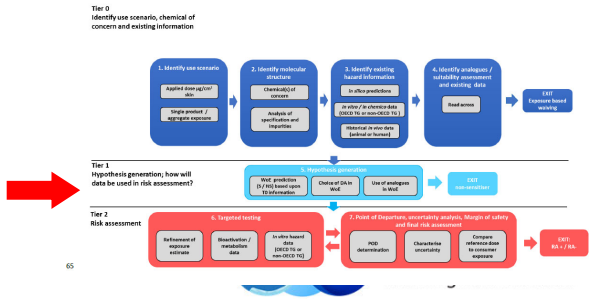


1 (CAS RN 10036-64-3)

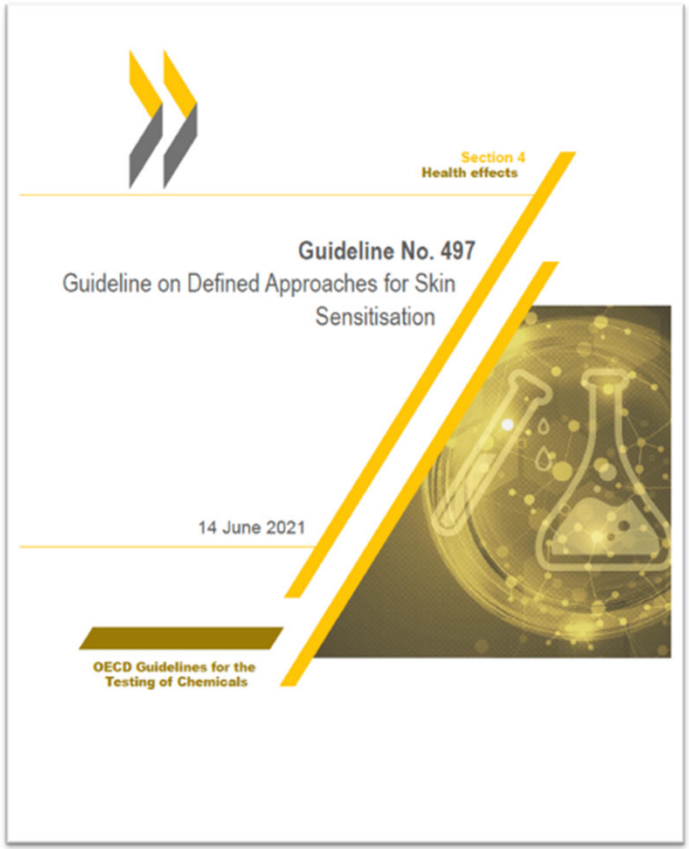
- Dermal penetration, readily penetrates ➔ Bioavailable
- *In silico* prediction for Acetyl glucosamine ➔ Non-sensitizer

- NAM data (DPRA, KeratinoSens, h-CLAT) ➔ Non-sensitizer
- Analogues/isomers, no data ➔ No data

Acetyl glucosamine is probably NOT a skin sensitizer
➔ Apply DA ('s) with existing data
➔ Use of OECD 497 2 out of 3, ITS1 and ITS2 as DA
➔ Use of BN-ITS as DA



OECD GL 497 Guideline on Defined Approaches to Skin Sensitisation



Defined Approaches …

- are designed to address pre-defined endpoint/prediction
- are from defined information sources
- the sequence is defined and next steps are rule-based
- are fixed data interpretation procedures
- provide clear regulatory conclusions

Defined approaches remove expert judgement and are not flexible, which makes them suitable for harmonization.

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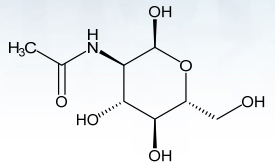
Summary of the DAs Included in OECD GL 497

DA/Method	Information Sources	Capability (Hazard and/or Potency)	Hazard Performance vs. LLNA N~168	Hazard Performance vs. Human N~63	GHS Potency Performance vs. LLNA (Accuracy)	GHS Potency Performance vs. Human (Accuracy)
2o3 DA	DPRA, KeratinoSens™, h-CLAT	Hazard	84% BA, 82% Sens, 85% Spec	88% BA, 89% Sens, 88% Spec	-	-
ITSv1 DA	DPRA, h-CLAT, DEREK Nexus v6.1.0	Hazard, Potency (GHS)	81% BA, 92% Sens, 70% Spec	69% BA, 93% Sens, 44% Spec	70% NC, 71% 1B, 74% 1A	44% NC, 77% 1B, 65% 1A
ITSv2 DA	DPRA, h-CLAT, OECD QSAR Toolbox v4.5	Hazard, Potency (GHS)	80% BA, 93% Sens, 67% Spec	69% BA, 94% Sens, 44% Spec	67% NC, 72% 1B, 72% 1A	44% NC, 80% 1B, 67% 1A
LLNA (provided for comparison)	*in vivo*	Hazard, Potency	-	58% BA, 94% Sens, 22% Spec	-	25% NC, 74% 1B, 56% 1A

*BA = Balanced Accuracy, average of Sensitivity and Specificity

Skin Sensitization Risk Assessment – Acetyl glucosamine

Tier 2 - Step 7: Define NS/S and/or POD using BN-ITS



1 (CAS RN 10036-64-3)

- Predicts a skin sensitization potency (even when data are missing)
- Expressed as probability distribution of LLNA pEC3, 4 potency classes: nonsensitizers (NS), weak (W), moderate (M), and strong-extreme (S) sensitizers.

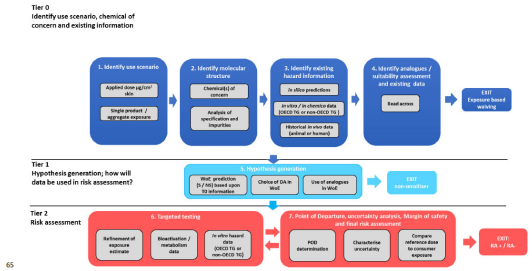
Status	pEC3 Category		Bayes Factors				Probabilities				Strength of Evidence
Success	Class	Category	1	2	3	4	1	2	3	4	
TRUE	C1	Non-sensitizer	42.94	0.15	0.03	0.00	0.9489	0.0399	0.0111	0.0000	Strong

P(LLNA=NS, W, M, S| evidence)

↓

EC3% (50th or any other percentile)

- Can be used:
  - For classification and labeling under the GHS C&L scheme
  - To set POD for QRA
  - For Non-Sensitizers we do not set a POD
  - For the development of testing strategy if data are missing. Measures progress by uncertainty reduction.



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Skin Sensitization Risk Assessment – Acetyl glucosamine

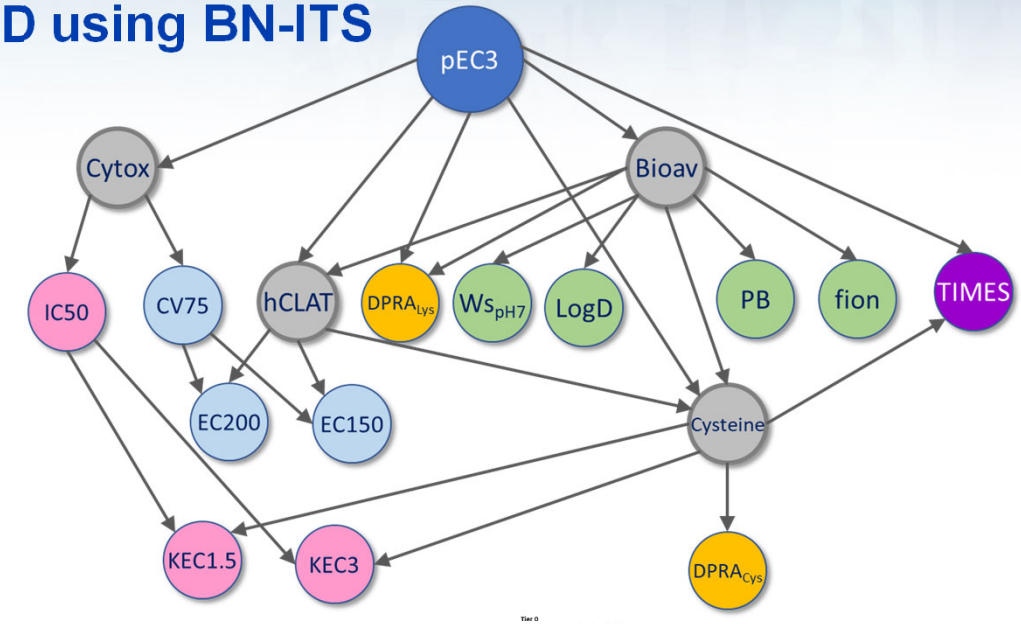
Tier 2 - Step 7: Define NS/S and/or POD using BN-ITS

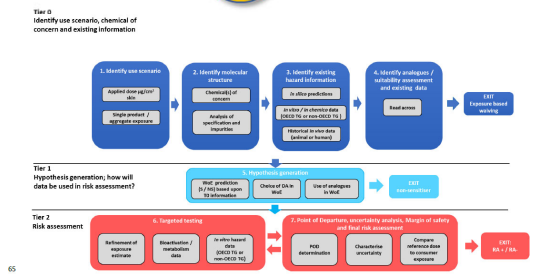
Input Parameters

- Phys Chem properties
- In silico potency prediction considering metabolism and potential for auto-oxidation (TIMES-SS)
- **KE1:** Cys and Lys binding in DPRA
- **KE2:** KEC1.5, KEC3 and IC50 in KeratinoSens™
- **KE3:** EC150, EC200 and CV75 in h-CLAT

Output

- **Probability distribution in each potency class**
- **Bayes factor (BF) to evaluate confidence**





Jaworska et al. Arch. Toxicol. 2015, Kern et al. Manuscript in preparation in 2021

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Skin Sensitization Risk Assessment – Acetyl glucosamine Weight of Evidence and Conclusions

In vivo animal data:

- none

In silico data:

- DEREK alert for skin sensitization (aldehyde precursor), however sugars are excluded.
- ToxTree = no alerts.
- TIMES prediction out of domain.

In chemico and in vitro data:

- DPRA average depletion rate 1% = Non-Sensitizer
- KeratinoSens IC50 > 2000 uM = Negative
- h-CLAT viability >50% = Negative

Defined Approaches:

- **Bayesian Net ITS = strong evidence (BF>40) non-sensitizer**
- *OECD 497 2 out of 3 = non-sensitizer*
- *OECD 497 ITS1 (DEREK) = non-sensitizer*
- *OECD 497 ITS2 (OECD Toolbox) = non-sensitizer*

Acetyl glucosamine is concluded with strong evidence to be a non-sensitizer.

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Skin Sensitization Risk Assessment – Acetyl glucosamine
Supportive Clinical Data

- Repeated insult patch test performed in 108 subjects using a mask containing 0.005% Acetyl Glucosamine; non sensitizing; Anonymous 2018; submitted February 19, 2021 (data1_Glucosamine_122021) Estimated exposure under patch = 2.5 ug/cm^2.
- Maximization assay performed in 25 subjects using a leave-on product containing 0.25% Glucosamine HCl; non sensitizing; Anonymous 2007; submitted February 19, 2021 (data1_Glucosamine_122021) Estimated exposure under patch = 55.6 ug/cm^2.
- Maximization assay performed in 25 subjects using a product containing 0.01% Glucosamine; non-sensitizing; Anonymous 2005; submitted February 19, 2021 (data1_Glucosamine_122021) Estimated exposure under patch = 1.25 ug/cm^2.
- Repeated insult patch test performed in 51 subjects using a leave-on product containing 0.005% Glucosamine HCl; Anonymous 2012; submitted February 29, 2021 (data2_Glucosamine_122021) Estimated exposure under patch = 1.25 – 1.90 ug/cm^2.
- Repeated insult patch test performed in 105 subjects using a liquid foundation containing 2% Acetyl Glucosamine; non-sensitizing; Anonymous 2011; submitted February 11, 2022 (*data_Glucosamine_032022*; TKL Research 2011). Estimated exposure under patch = 1,000 ug/cm^2.

- HRIPT and HMT glucosamine testing concentrations of 1.25 – 1,000 ug/cm^2 exceed the maximum estimated usage concentration of 136 ug/cm^2 by 7-fold, confirming the lack of sensitization at the maximum consumer exposure level. Therefore, this data confirms the conclusion of the Next Generation Risk Assessment for Acetyl glucosamine.

Clinical data confirms that Acetyl glucosamine is a non-sensitizer.

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Additional Resources and Case Studies

- October 2020 PCPC Virtual Science Symposium: Petra Kern - Assessing Skin Sensitization Potential of Cosmetic Ingredients with New Approach Methodologies.
- Kimber, I (2021). The activity of methacrylate esters in skin sensitization test methods II. A review of complementary and additional analyses. Regulatory Toxicology and Pharmacology 119: 104821. https://doi.org/10.1016/j.yrtph.2020.104821
- Reynolds, G, Reynolds, J, Gilmour, N, Cubberley, R, Spriggs, S, Aptula, A, Przybylak, K, Windebank, S, Maxwell, G and Baltazar, MT (2021). A hypothetical skin sensitization next generation risk assessment for coumarin in cosmetic products. Regulatory Toxicology and Pharmacology 127: 105075. https://doi.org/10.1016/j.yrtph.2021.105075
- Lee, I, Na, M, Lavelle, M and Api, AM (2022). Derivation of the no expected sensitization induction level for dermal quantitative risk assessment of fragrance ingredients using a weight of evidence approach. Food and Chemical Toxicology 159: 112705. https://doi.org/10.1016/j.fct.2021.112705

P&G Colleagues who helped with the case study

- Lori Reinsalu
- ElLantae Byrd
- Gang Yan

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