



A Deep-Dive into the Application of Defined Approaches for Skin Sensitization in OECD GL 497 – the 203 and the ITS

ICCS BPG Skin Sensitization: Safety Assessment Using NAMs for Substances in Cosmetics and Personal Care Products Webinar

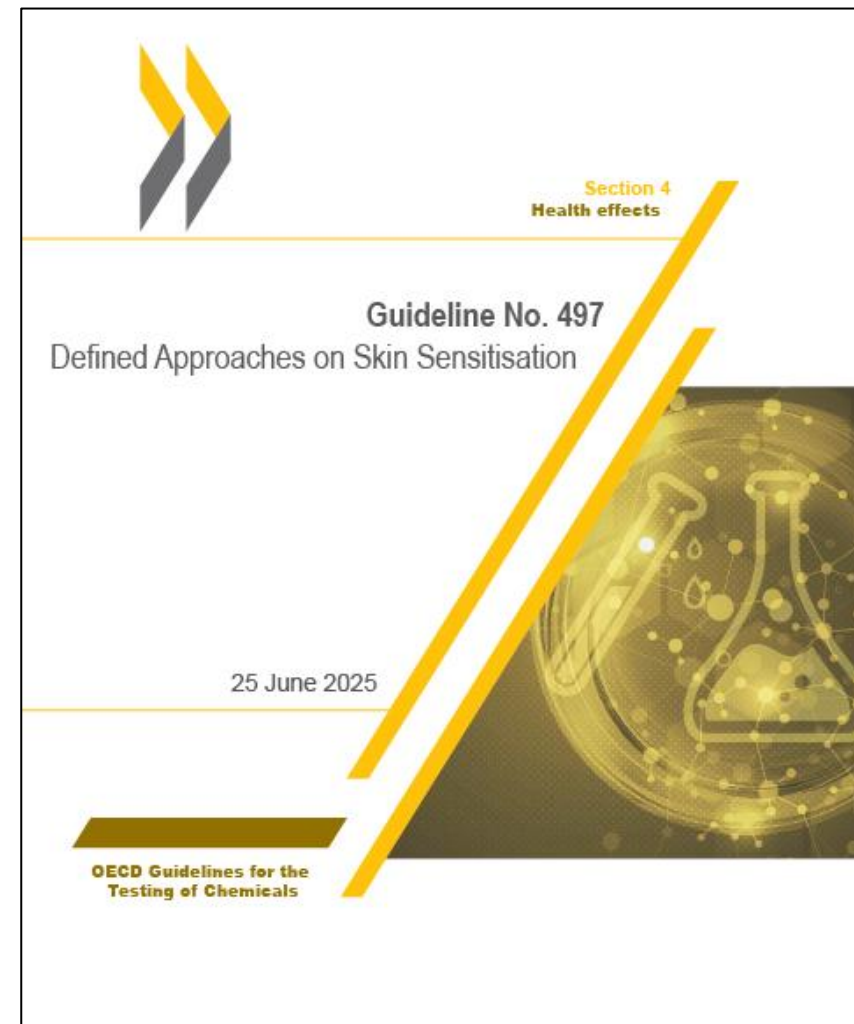
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Procter & Gamble Services NV**

ICCS

INTERNATIONAL
COLLABORATION ON
COSMETICS SAFETY

OECD Guideline 497

- Combination of several *in chemico/in vitro* and *in silico* information sources in Defined Approaches (DA)
- Hazard (Part I) and/or potency (Part II) of potential skin sensitizer or derivation of a Point of Departure (PoD) (Part III)
- Equivalent or better accuracy than *in vivo* LLNA
- Currently 3 DAs included in the Guideline
 - **The 2 out of 3 DA (2o3)**
 - **The Integrated Testing Strategy (ITS)**
 - SARA ICE
- Coming 2026: Regression model



Choice of DA

Table 7. Defined Approaches for Skin Sensitization using *In Chemico*, *In Vitro*, and *In Silico* Tools

Defined Approach	Problem Formulation Addressed	Input			
		KE1: ADRA or DPRA	KE2: KeratinoSens™, EpiSensA, or LuSens	KE3: GARD™Skin, h-CLAT, IL-8 Luc, or U-SENS™	<i>In Silico</i> : Derek Nexus, OECD QSAR Toolbox
2o3	Hazard (GHS 1 vs GHS NC)	X	X	X	--
ITS	Hazard (GHS 1 vs GHS NC) Potency Category(GHS 1A vs. GHS 1B)	X	--	X	X
		Input			
		KE1: DPRA, kDPRA	KE2: KeratinoSens™	KE3: h-CLAT, U-SENS™	<i>In Silico</i>
SARA-ICE°	Point of Departure (ED ₀₁ *)	X	X	X	N/A

°LLNA and HPPT data also may be used in SARA-ICE. Further information may be reviewed in OECD (2025).

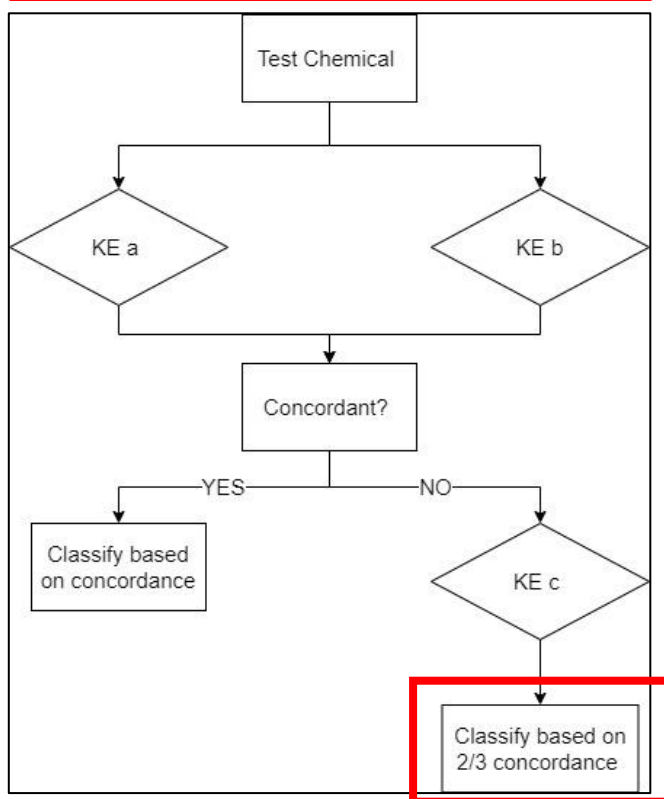
*ED₀₁ is the estimate of a dermal dose at which there is a 1% chance of inducing sensitization using a human predictive patch test.

- Hazard, Classification, Potency, Point of Departure (PoD)
- Hypothesis
- Availability of information sources



GL497 Part I/ II: 2 Defined Approaches

2 out of 3 Hazard (GHS 1 vs. NC)



ITS Hazard (GHS 1 vs. NC) or GHS 1A, 1B, NC

Information Source Type	KE1 (OECD TG 442C)		KE3 (OECD TG 442E)		<i>In silico</i>			
Information Sources Permitted	ADRA, DPRA		GARD®skin, h-CLAT, U-SENS™		Derek Nexus, OECD QSAR Toolbox			
Score	ADRA mean NAC & NAL % depletion	ADRA NAC % depletion*	DPRA mean Cys & Lys % depletion	DPRA Cys % depletion†	GARD®Skin (µg/mL)	h-CLAT MIT (µg/mL)	U-SENS™ EC150 (µg/mL)	Please refer to individual protocol to apply score
3	≥46.4	≥67.4	≥42.47	≥98.24	≤13.03	≤10	≤3	
2	≥15.5, <46.4	≥17.5, <67.4	≥22.62, <42.47	≥23.09, <98.24	>13.03, ≤56.44	>10, ≤150	>3, ≤35	
1	≥4.9, <15.5	≥5.6, <17.5	≥6.38, <22.62	≥13.89, <23.09	>56.44	>150, ≤5000	>35, ≤200	Positive
0	<4.9	<5.6	<6.38	<13.89	-	not calculated	not calculated / >200	Negative

Potency	Total Battery Score
UN GHS 1A	6-7
UN GHS 1B	2-5
Not classified	0-1



Information Sources for 2o3 and ITS

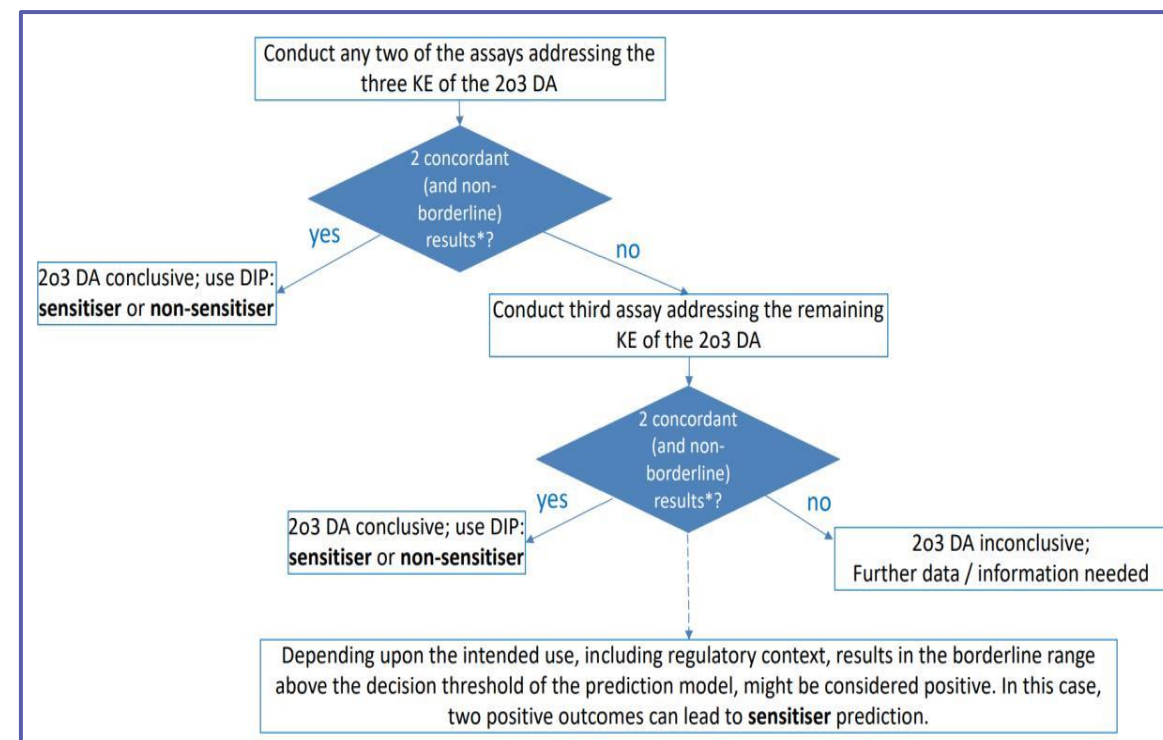
Addressing AOP Key Event	2o3	ITS
KE1	ADRA, DPRA	ADRA, DPRA
KE2	Keratinosens™, LuSens, EpiSensA	
KE3	GARD™skin, h-CLAT, IL-8, U-SENS	GARD™skin, h-CLAT, U-SENS
<i>In silico</i> tool		Derek Nexus, OECD Toolbox

- Each test method – following respective OECD TG for decision making
- Need to consider applicability domains
- Derek Nexus (version dependent v6.1.0)
- DASS workflow in OECD Toolbox



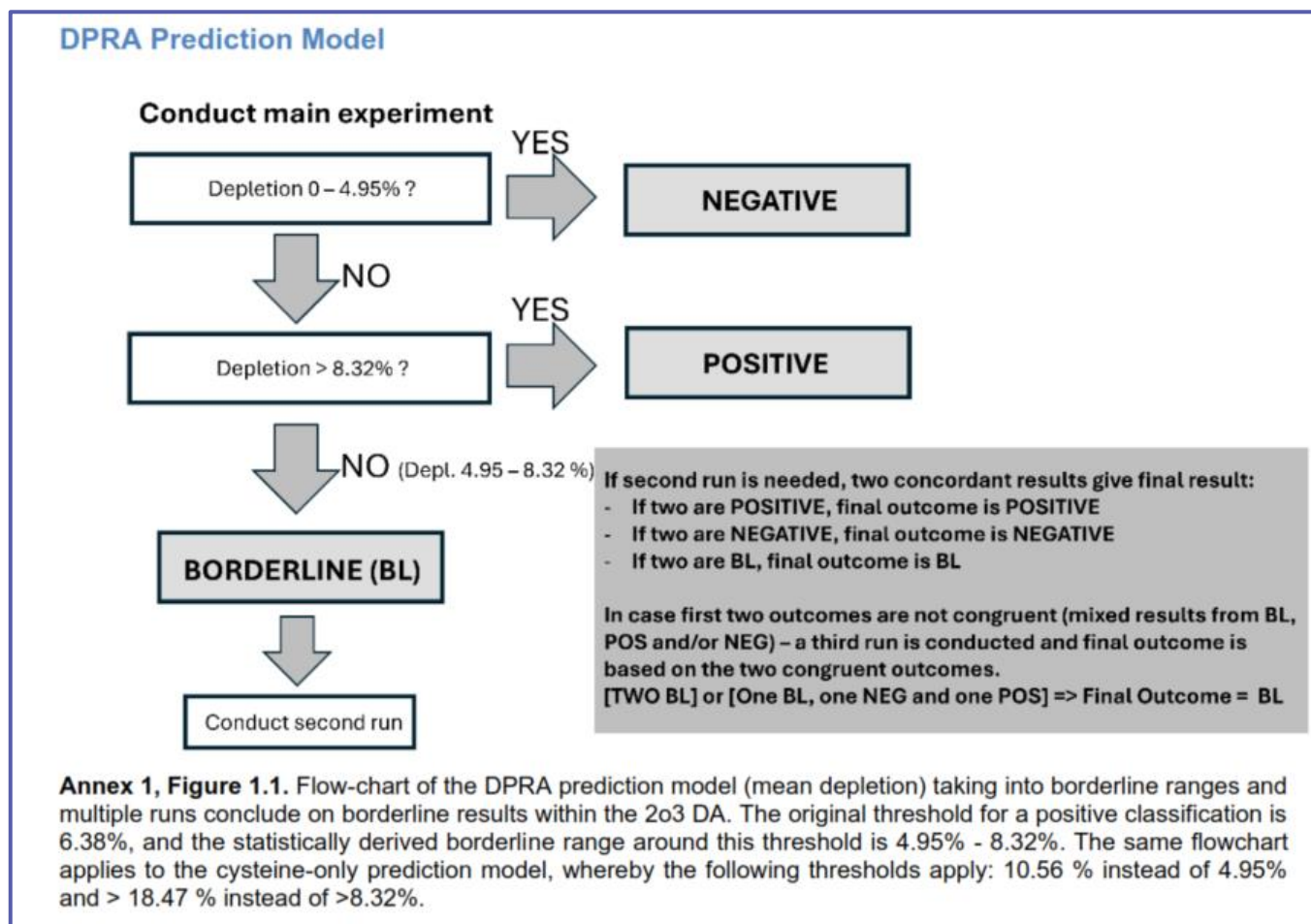
The 2 out of 3 Defined Approach (2o3)

- **Hazard prediction** based on concordant results from up to 3 assays (different KE)
- If 2 assays **are concordant** and NOT borderline, then the DA is conclusive
 - Positive + **Positive** + either = Positive (**sensitizer**)
 - Negative + Negative + either = Negative (**non-sensitizer**)
- If the first 2 assays **are discordant** then third assay is conducted & majority 2o3 call is taken
 - Positive + Negative + Positive = Positive (**sensitizer**)
 - Positive + Negative + Negative = Negative (**non-sensitizer**)
- If **borderline** (thresholds in the guideline) then **more information may be needed**.



2o3 Borderline Results

- Each test method – different prediction model that might require multiple runs
- Methodologies to derive borderline ranges are available in GL497 (or publications)
- Example: DPRA



The Integrated Testing Strategy (ITS)

- **Hazard prediction** (UN GHS 1/ NC) and **Potency prediction** (UN GHS 1A and 1B)
- Score applied to results from KE1 and KE3 information sources and one *in silico* prediction

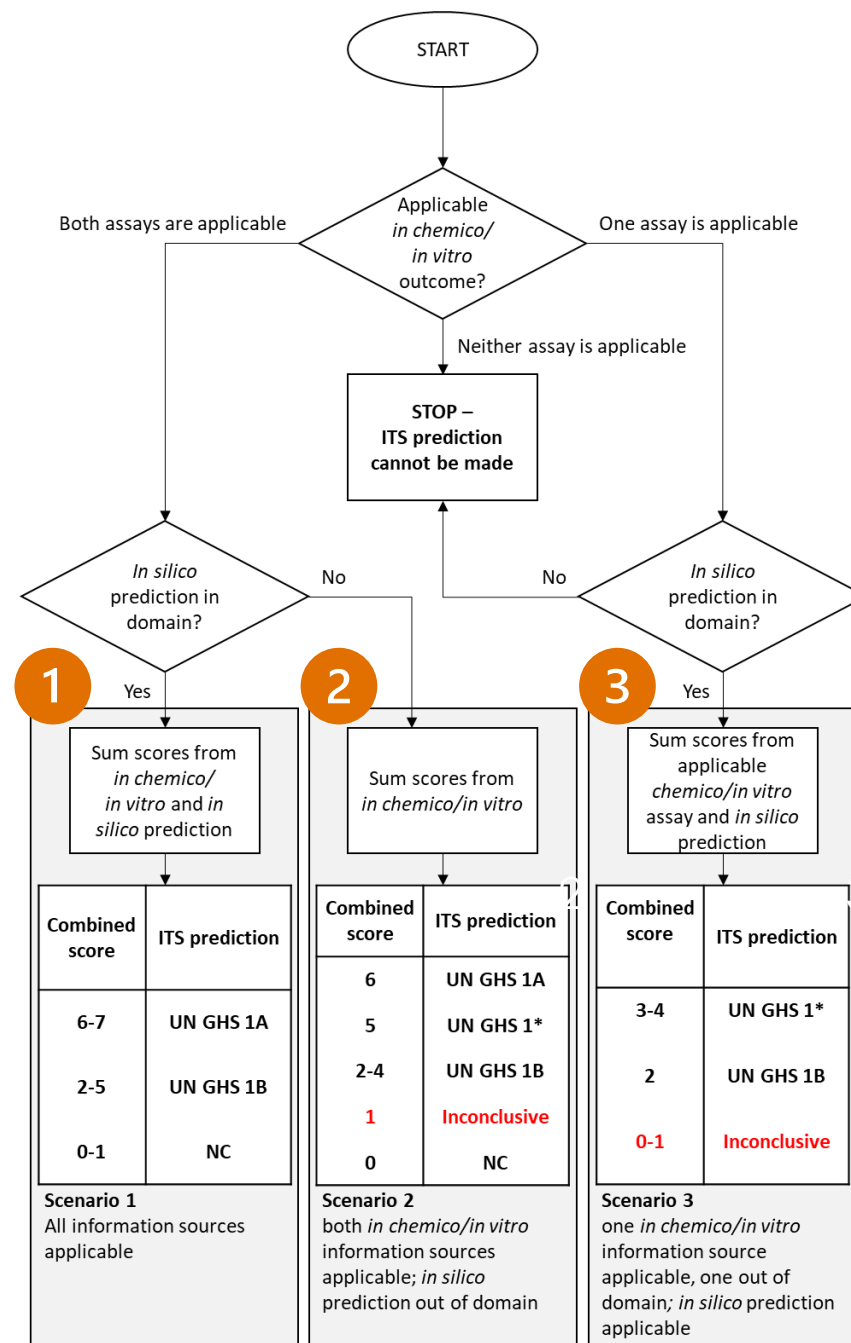
Information Source Type	KE1 (OECD TG 442C)				KE3 (OECD TG 442E)			<i>In silico</i>
Information Sources Permitted	ADRA, DPRA				GARD®skin, h-CLAT, U-SENS™			Derek Nexus, OECD QSAR Toolbox
Score	ADRA mean NAC & NAL % depletion	ADRA NAC % depletion*	DPRA mean Cys & Lys % depletion	DPRA Cys % depletion†	GARD®Skin (µg/mL)	h-CLAT MIT (µg/mL)	U-SENS™ EC150 (µg/mL)	Please refer to individual protocol to apply score
3	≥46.4	≥67.4	≥42.47	≥98.24	≤13.03	≤10	≤3	
2	≥15.5, <46.4	≥17.5, <67.4	≥22.62, <42.47	≥23.09, <98.24	>13.03, ≤56.44	>10, ≤150	>3, ≤35	
1	≥4.9, <15.5	≥5.6, <17.5	≥6.38, <22.62	≥13.89, <23.09	>56.44	>150, ≤5000	>35, ≤200	Positive
0	<4.9	<5.6	<6.38	<13.89	-	not calculated	not calculated / >200	Negative

Potency	Total Battery Score
UN GHS 1A	6-7
UN GHS 1B	2-5
Not classified	0-1



ITS Overview

- 3 different workflows
- Standard ITS (1) - when all information sources are in domain **1**
- Partial information sources (2, 3) – one component of the ITS is missing
 - 2** *In silico* prediction out of domain
 - 3** One of the *in chemico/in vitro* assays out of domain
- Inconclusive predictions can be considered in a weight of evidence approach or within an Integrated Approach to Testing and Assessment together with other data



Use of Derek or OECD Toolbox for ITS

Derek

- Version dependent (v6.1.0)
- License required to get a login to webapp
- <https://www.skingsensda-itsv1.lhasacloud.org>

The screenshot shows the Derek software interface for a skin sensitisation prediction. The chemical structure is 4-chloro-2-nitrobenzamide. The interface includes a toolbar, a chemical structure editor, and a results table.

Chemical Structure: NC(=O)c1ccc(Cl)cc1[N+](=O)[O-]

Test Results:

Method	Result	Score
DPRA	Positive	3
h-CLAT	Positive	3
Derek	Positive	1
Hazard prediction	Sensitiser	7
Potency prediction	UN GHS 1A	

OECD TB

- Freely available software (v.4.5)
 - <https://qsartoolbox.org>
- Automated DASS workflow

The screenshot shows the QSAR Toolbox software interface. The main window displays a workflow editor with a list of documents and a dialog box for selecting a workflow.

Workflow Selection Dialog:

Choices

Select workflow


- EC3 from LLNA assay
- Fish, LC50(EC50) at 96h for Pimephales promelas (mortality)
- EC3 from LLNA or Skin sensitisation from GPMT assays
- EC3 from LLNA or Skin sensitization from GPMT assays for defined approaches (SS AW for DASS)
- Skin sensitisation from GPMT assay
- Demo workflow

Buttons: Run on server, Run, View, Cancel



Automation via DASS App

An official website of the United States government [Here's how you know](#) Calendar & Events | News & Media | Get Involved | Support

 **National Toxicology Program**
U.S. Department of Health and Human Services

Search the NTP Website **SEARCH**

Research ▾ | Data & Resources | Publications | Who We Are ▾

Home > NICEATM: Alternative Methods > Test Method Evaluations > Identification of Skin Sensitizers > Defined Approaches to Identify Potential Skin Sensitizers > **DASS App**

<https://ntp.niehs.nih.gov/go/952311>

DASS App

Web App for Using Defined Approaches to Predict Skin Sensitization Hazard and Potency [REPORT A PROBLEM](#)

The DASS App

The DASS App applies defined approaches on skin sensitization (DASS) to predict skin sensitization hazard (sensitizer or non-sensitizer) and potency (based on UN GHS categories). The defined approaches (DA) generate predictions by integrating data from in vitro assays that represent key events in the Adverse Outcome Pathway for Skin Sensitization and in silico hazard predictions.

For more information about DASS and their regulatory applications, visit the [NICEATM Defined Approaches to Identify Potential Skin Sensitizers](#) webpage.

- [User Guide](#)
- [Contact Us](#)
- [Manuscript](#)
- [Launch App in New Window](#)

[Source Code](#)
Last updated: 2025-May-01

Select Defined Approach | Upload Data | Select Data Columns | Review Selection | Results | Compare

To begin, select the DA to be implemented from the choices below. Click on the information buttons next to the DA names to view a description of the DA and the test methods required to implement the DA.

Defined Approaches

- 2 out of 3 (2o3) [?](#)
- Integrated Testing Strategy (ITS) [?](#)
- Key Event 3/1 Sequential Testing Strategy (KE 3/1 STS) [?](#)

2o3 Borderline Evaluation

Flag borderline assay results prior to applying DA 2o3 (Requires data from individual runs) [?](#)

Confirm DA Selection

<https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/skin-sens/da/dass-app>



Case Studies: 2o3 and ITS

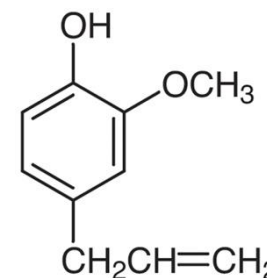
- Hazard (GHS 1/NC)
- Classification (GHS 1A/1B/NC)
- Semi quantitative PoD derivation using ITS



Case Study: 2o3 for Hazard Identification

Is Eugenol a skin sensitizer?

Answer the question using the 2o3 DA



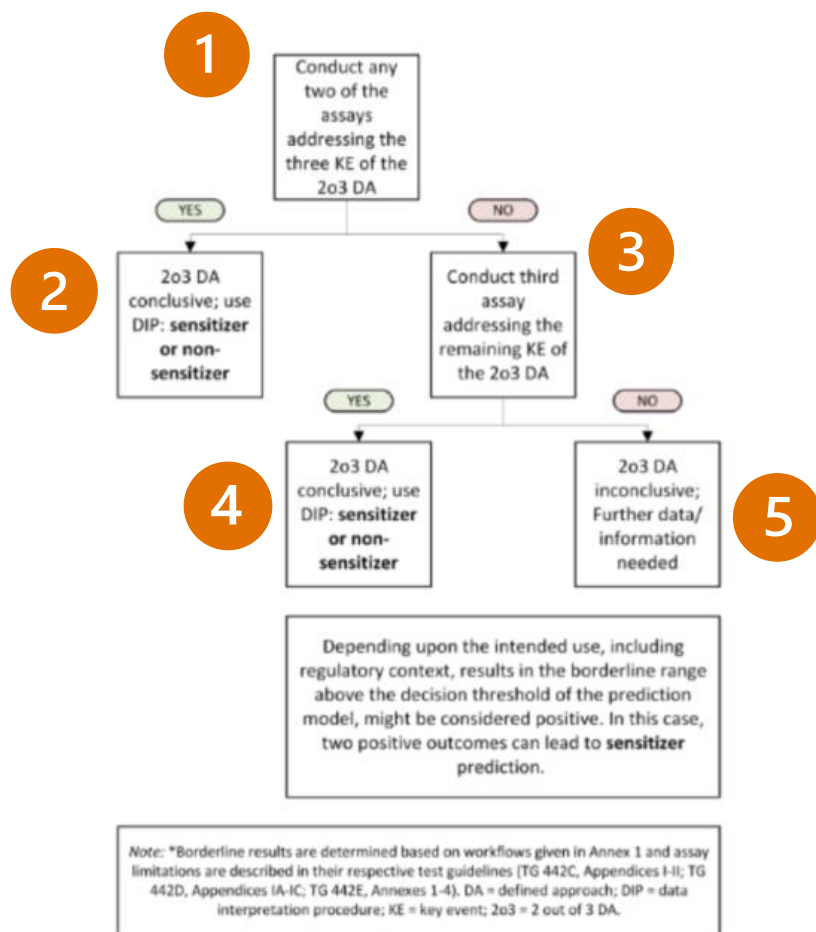
Available information:

- Eugenol CASRN 97-53-0
- MW 164.2
- No technical difficulties
- In domain of assays
- No borderline results

	DPRA	Keratinosens™
Measured data	Depletion Cys (%) 9.2 Depletion Lys (%) 19.2	EC1.5 (μM) >2000 IC50 (μM) 1505.7
Final call (based on TG)	Positive	Negative



Case Study: 2o3 for Hazard Identification



Follow DA procedure as outlined in BPG

1. Review data from 2 assays: e.g. DPRA and Keratinosens™
2. Results not conclusive – no decision possible
3. Need for third assay – find new data

Figure 9. Data Interpretation Procedure for 2o3 Defined Approach for Hazard Classification (adapted from OECD 2025)



Case Study: 2o3 for Hazard Identification

- New data for Eugenol – h-CLAT

	DPRA	Keratinosens™	h-CLAT
Measured data	Depletion Cys (%) 9.2 Depletion Lys (%) 19.2 Mean Depletion (%) 14.2	EC1.5 (μM) >2000 IC50 (μM) 1505.7	EC150 (μg/ml) 64.4 EC200 (μg/ml) 137.2 CV75 (μg/ml) 143.2 MIT (μg/ml) 64.4
Final call (based on TG)	Positive	Negative	Positive

Follow DA procedure as outlined in BPG

1. DPRA and Keratinosens™ – Positive and negative
2. Results not conclusive – no decision possible
3. Need for third assay – find new data
4. h-CLAT – positive – now conclusive decision: 2 out of 3 are positive
5. **Eugenol = Skin sensitizer. No need for further data**



Case Study: ITS for Hazard or Potency (GHS)

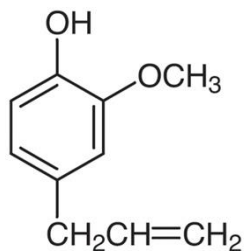
Is Eugenol a skin sensitizer?

Can you determine a GHS hazard classification?

Answer the question using the ITS DA.

Available information

- Eugenol CAS# 97-53-0
- MW 164.2
- No technical difficulties
- In domain
- No borderline results



	DPRA	Keratinosens™	h-CLAT	OECD TB
Measured data	Depletion Cys (%) 9.2 Depletion Lys (%) 19.2 Mean Depletion (%) 14.2	EC1.5 (μM) >2000 IC50 (μM) 1505.7	EC150 (μg/ml) 64.4 EC200 (μg/ml) 137.2 CV75 (μg/ml) 143.2 MIT (μg/ml) 64.4	
Final call (based on TG)	Positive	Negative	Positive	Positive



Case Study: ITS for Hazard

Table 9. Scores associated with assay results for ITS, as presented in OECD GL 497 (2025)

Information Source Type	KE1 (OECD TG 442C)				KE3 (OECD TG 442E)			<i>In silico</i>
	ADRA mean NAC & NAL % depletion	ADRA NAC % depletion*	DPra mean Cys & Lys % depletion	DPra Cys % depletion†	GARD™ _{skin} (µM)	h-CLAT MIT (µg/mL)	U-SENS™ EC150 (µg/mL)	
Score								Derek Nexus, OECD QSAR Toolbox
3	≥46.4	≥67.4	≥42.47	≥98.24	≤13.03	≤10	≤3	
2	≥15.5, <46.4	≥17.5, <67.4	≥22.62, <42.47	≥23.09, <98.24	>13.03, ≤56.44	>10, ≤150	>3, ≤35	
1	≥4.9, <15.5	≥5.6, <17.5	≥6.38, <22.62	≥13.89, <23.09	>56.44	>150, ≤5000	>35, <200	Positive
0	<4.9	<5.6	<6.38	<13.89	-	not calculated	not calculated / ≥200	Negative
Hazard	Total Score from ITS DA							
Sensitizer	2-7							
Non-sensitizer	0-1							

Abbreviations: ADRA: Amino acid Derivative Reactivity Assay; NAC: N-(2-(1-naphthyl) acetyl)-L-cysteine, NAL: α-N-(2-(1-naphthyl) acetyl)-L-lysine; DPRA: Direct Peptide Reactivity Assay; Cys: cysteine, Lys: Lysine; GARD: Genomic Allergen Rapid Detection; h-CLAT: Human Cell Line Activation Test; MIT: minimum induction threshold; U-SENS: U-937 cell line activation test; EC: effective concentration.

For KE1, KE3, and in silico, only one assay/model selected from each is used for determining a score. *NAC-only depletion thresholds are used in the case of co-elution with the NAL peptide. †Cysteine-only depletion thresholds are used in the case of co-elution with the lysine peptide.

Follow DA as outlined in BPG

1. Review data from one KE1, one KE2 assay and one *in silico* tool
2. Assign scores based on assay results
3. Sum up scores (if > 1 = sensitizer)



Case Study: ITS for Hazard

	DPRA	Keratinosens™	h-CLAT	OECD TB
Measured data	Depletion Cys (%) 9.2 Depletion Lys (%) 19.2 Mean Depletion (%) 14.2	EC1.5 (μM) >2000 IC50 (μM) 1505.7	EC150 (μg/ml) 64.4 EC200 (μg/ml) 137.2 CV75 (μg/ml) 143.2 MIT (μg/ml) 64.4	
Final call (based on TG)	Positive	Negative	Positive	Positive
ITS score	1	0	2	1

Table 9. Scores associated with assay results for ITS, as presented in OECD GL 497 (2025)

Hazard	Total Score from ITS DA
Sensitizer	2-7
Non-sensitizer	0-1

Eugenol example

1. Review data from one KE1, one KE2 assay and one *in silico* tool
2. Assign scores based on assay/model results
3. Sum up scores (if > 1 = sensitizer)
4. Eugenol Total Score: 4
5. **Eugenol = Sensitizer**



Case Study: ITS for GHS Potency Classification

	DPRA	Keratinosens™	h-CLAT	OECD TB
Measured data	Depletion Cys (%) 9.2 Depletion Lys (%) 19.2 Mean Depletion (%) 14.2	EC1.5 (µM) >2000 IC50 (µM) 1505.7	EC150 (µg/ml) 64.4 EC200 (µg/ml) 137.2 CV75 (µg/ml) 143.2 MIT (µg/ml) 64.4	
Final call (based on TG)	Positive	Negative	Positive	Positive
ITS score	1	0	2	1

Table 10. ITS DA scores and associated UN GHS subcategorization, adapted from OECD GL 497 (2025)

UN GHS subcategorization	Total Score from ITS DA
UN GHS 1A	6-7
UN GHS 1B	2-5
Not Classified	0-1

Follow DA as outlined in BPG

1. Review data from one KE1, one KE2 assay and one *in silico* tool
2. Assign scores based on assay/model results
3. Sum up scores and use table in BPG (or GL 497) to identify GHS potency category
4. **Eugenol: Total ITS score 4 = UN GHS 1B**



Case Study: Semi Quantitative PoD Derivation

We know Eugenol is a skin sensitizer GHS 1B.

Can you determine a PoD for a risk assessment using ITS information?

Answer the question using the BPG

- GHS 1B = weak (moderate) skin sensitizer
- GHS 1B equals PoD > 500 $\mu\text{g}/\text{cm}^2$
 - Based on LLNA and HRIPT (GHS/CLP)
- PoD ranges for weak – moderate skin sensitizers range from 500 (250) to > 10000 (25000) $\mu\text{g}/\text{cm}^2$
- **Using a PoD of > 500 $\mu\text{g}/\text{cm}^2$ is conservative**

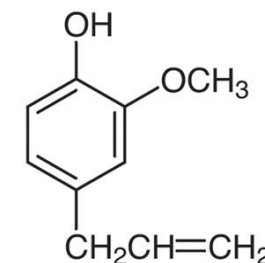


Table 11. Proposed Dose Ranges Associated with Skin Sensitization Potency, from Na et al. (2022)

Potency Category	Dose Range ($\mu\text{g}/\text{cm}^2$)	
	As proposed in Api et al. (2017)	As proposed in ECETOC 2003
Extreme	<25	<25
Strong	25-500	25-<250
Moderate	500-2500	250-<2500
Weak	>2500-10,000	2500-25,000
Very weak	>10,000	
Non sensitizer	--	--



THANK YOU!

