

DASS App v2.0: Implementing OECD Guideline No. 497 Updates

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Evaluation of Alternative Test Methods (NICEATM)**

**ASCCT-ESTIV Award Winners Series
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Outline

- Background
- DASS App v2.0 Updates
 - Integrated Testing Strategy
 - 2 out of 3
- Demo

Background: The DASS App

- The National Toxicology Program's **DASS App** is an open-source web application for users to apply defined approaches (DA) for skin sensitization (DASS) to their own data.

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 [NIJCEATM Defined Approaches to Identify Potential Skin Sensitizers](#) webpage.

[User Guide](#) [Contact Us](#)
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[Source Code](#)
Last updated: 2024-Oct-27

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

To begin, select the DA to be implemented. 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.

2 out of 3 (2o3) [?](#)

Integrated Testing Strategy (ITS) [?](#)

Key Event 3/1 (KE 3/1) Sequential Testing Strategy (STS) [?](#)

2o3 Borderline Evaluation

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

[Confirm DA Selection](#)

Background: Defined Approaches (DA)

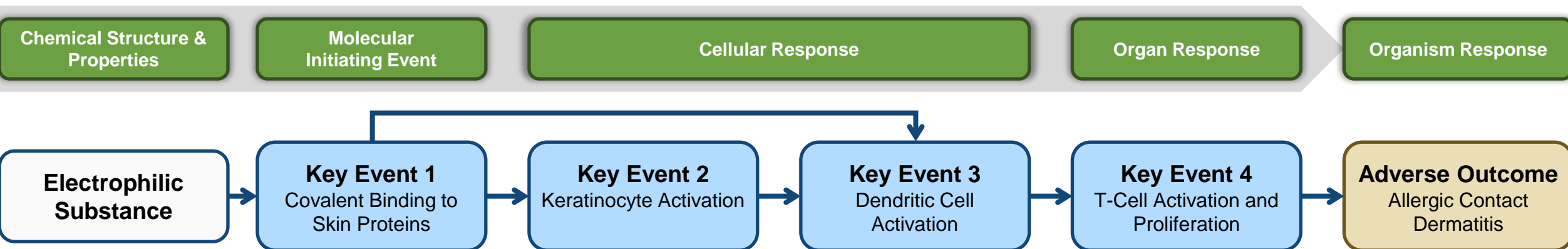
- A **defined approach** consists of
 - + a defined set of **information sources** (e.g., *in vitro/in chemico* data, *in silico* predictions)
 - + and a fixed **data interpretation procedure** (e.g., mathematical model, rule-based approach)
 - ↳ to derive a **prediction**
 - without the need for expert judgement.
- This removes subjectivity and allows DA predictions to be used under Mutual Acceptance of Data (MAD).

Background: Skin Sensitization

- Toxicological endpoint evaluated in hazard and risk assessment of chemicals.
- Initial exposures to a skin sensitizer activate the immune system such that subsequent exposures lead to **allergic contact dermatitis**.
- Traditionally evaluated with the *in vivo* murine local lymph node assay (LLNA) or the guinea pig maximization test.
- The **adverse outcome pathway (AOP) for skin sensitization initiated by covalent binding to proteins** has informed the development of defined approaches for predicting skin sensitization using non-animal assays (*in vitro/in chemico*).

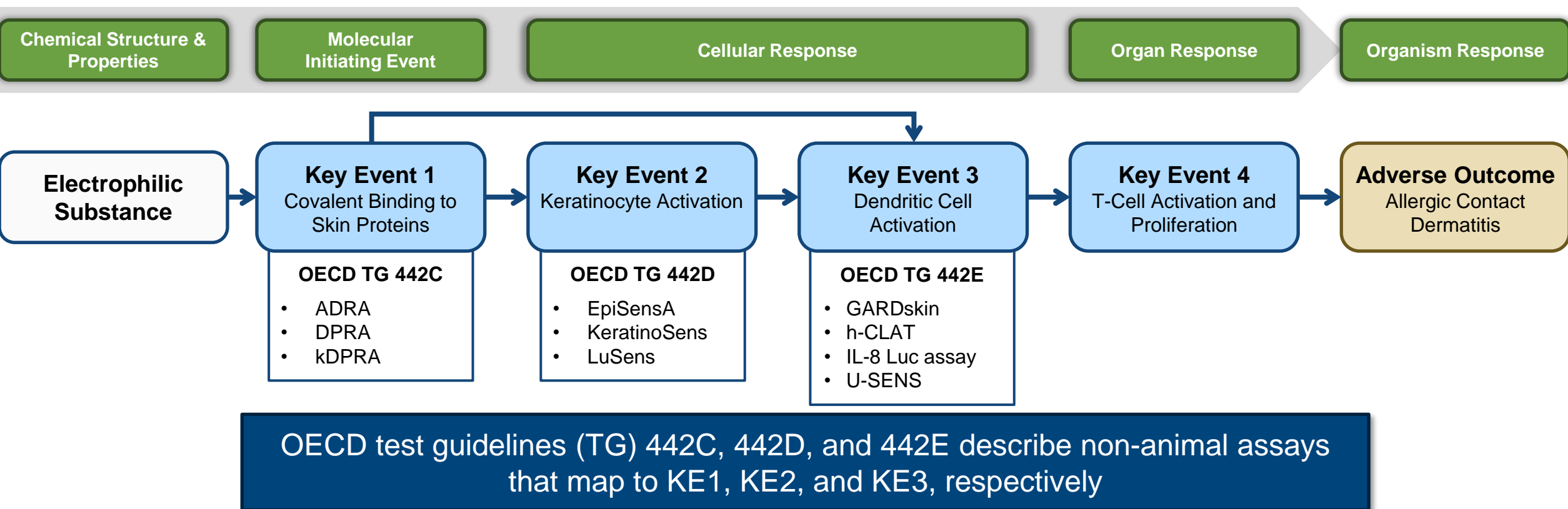
Background: Skin Sensitization

The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins



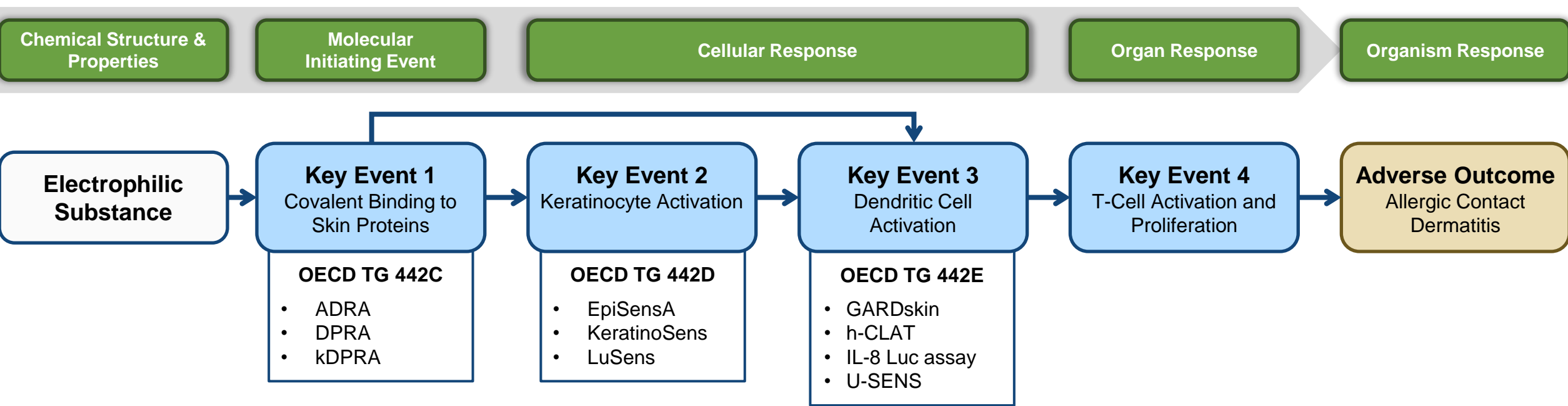
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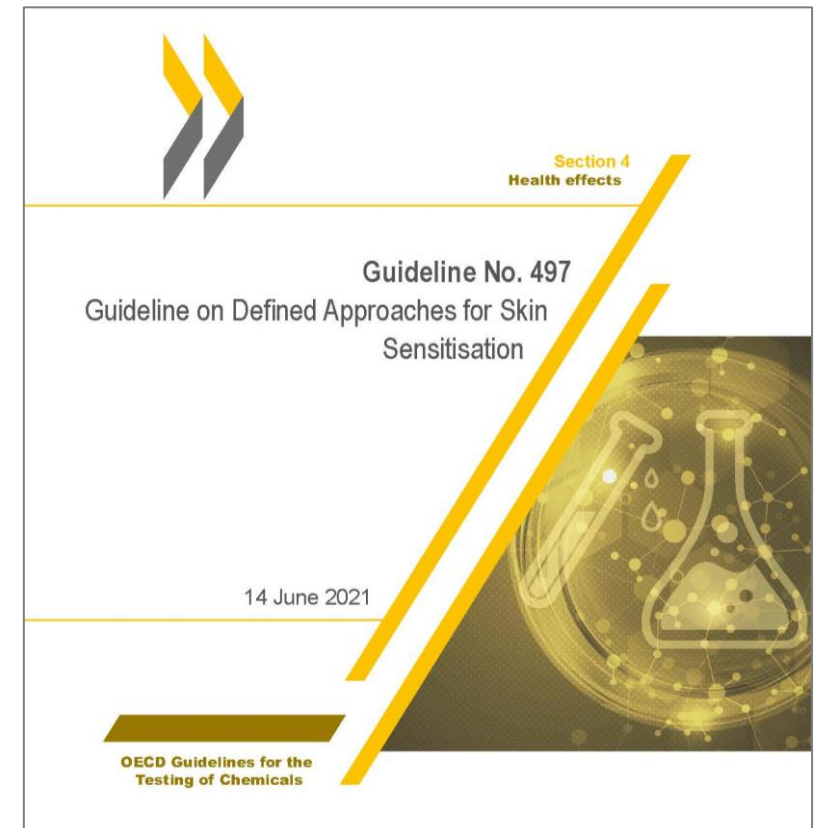


OECD test guidelines (TG) 442C, 442D, and 442E describe non-animal assays that map to KE1, KE2, and KE3, respectively

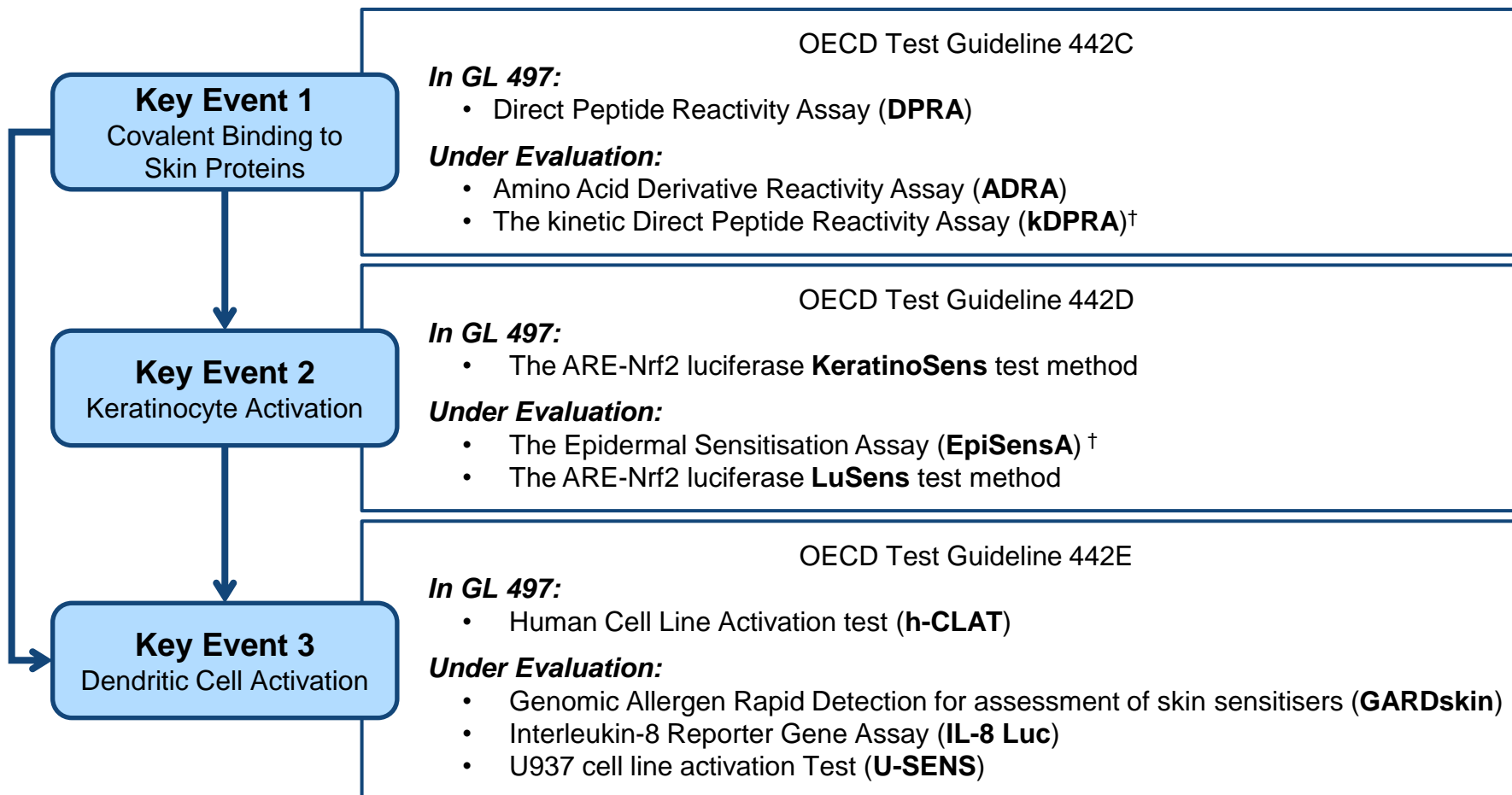
These assays have been used together in **defined approaches** to derive human-relevant hazard and/or potency predictions for potential skin sensitizers.

Background: Defined Approaches for Skin Sensitization (DASS)

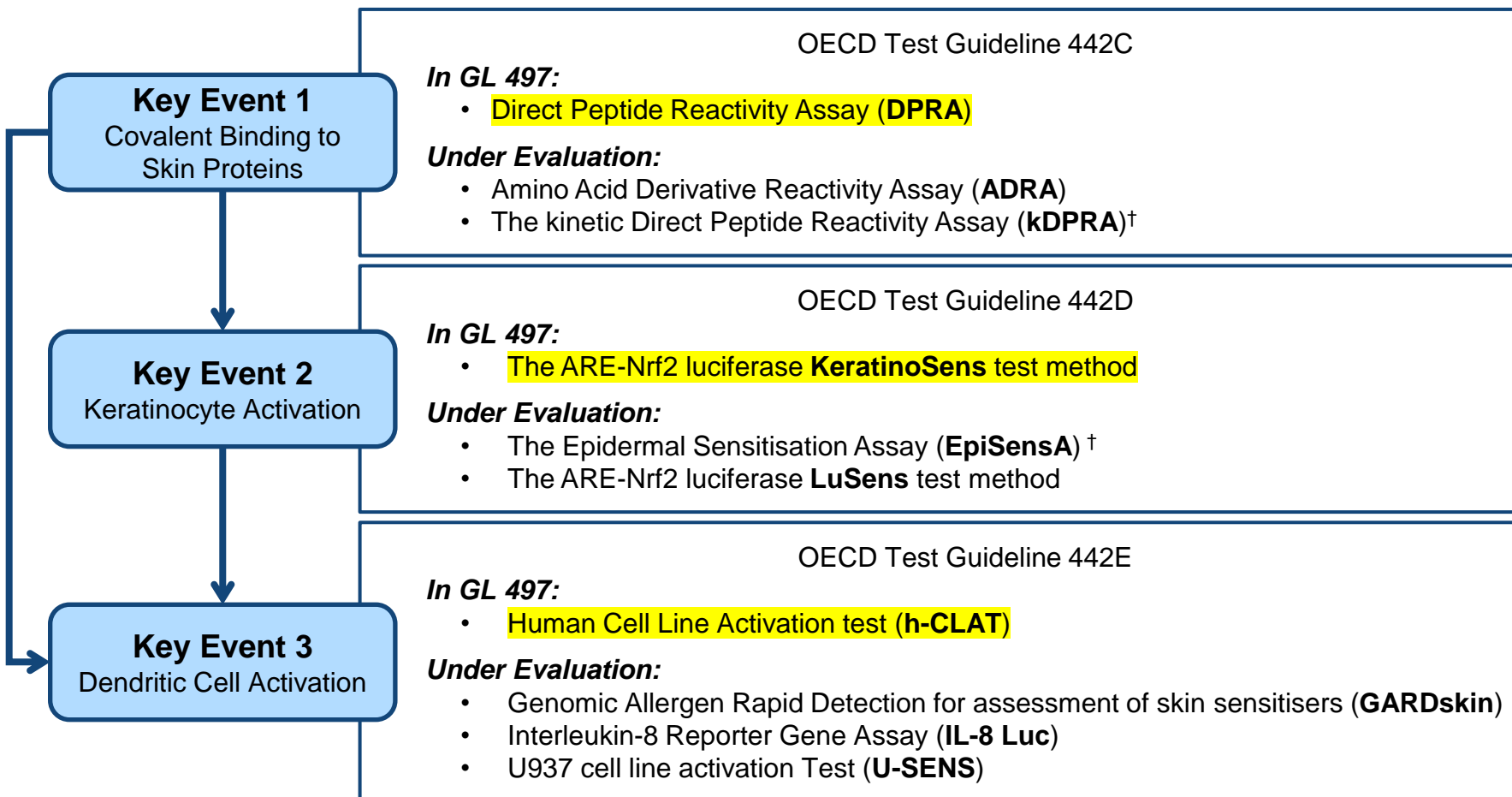
- The **OECD Guideline on Defined Approaches for Skin Sensitisation** was published in June 2021.
- This guideline (**GL 497**) formalizes the combination of several *in vitro*, *in chemico* and *in silico* information sources in a DA.
- The DAs covered in this groundbreaking guideline provide skin sensitization predictions with equivalent or better accuracy than the *in vivo* LLNA when compared against human data.
- The DAs included in GL 497 and available in the DASS App are:
 - The 2 out of 3 (2o3)
 - The Integrated Testing Strategy (ITS)



Background: Defined Approaches for Skin Sensitization (DASS)

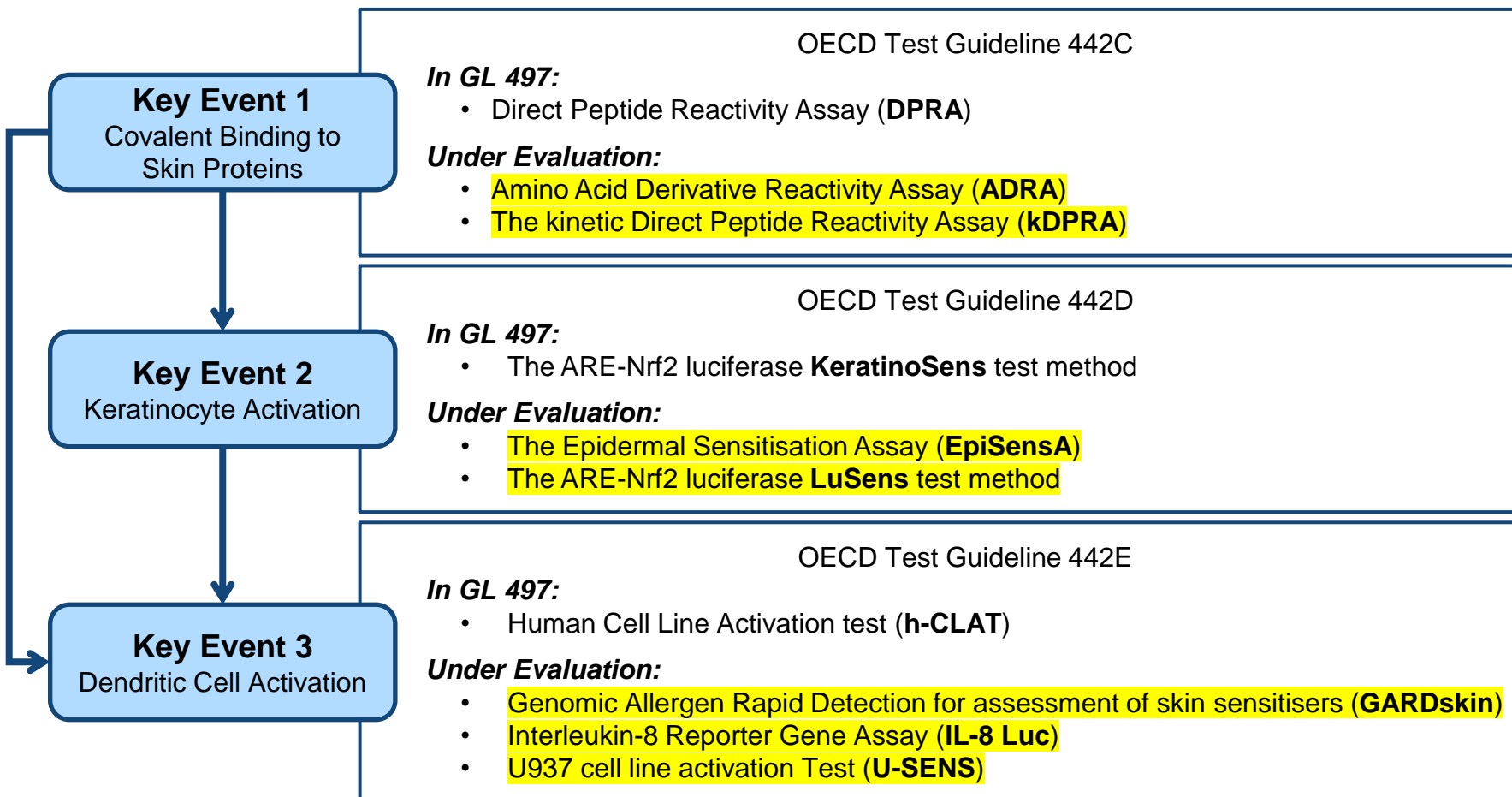


Background: Defined Approaches for Skin Sensitization (DASS)



The defined sets of information sources for the 2o3 and ITS DAs include DPRA, KeratinoSens, and/or h-CLAT.

Background: Defined Approaches for Skin Sensitization (DASS)



The defined sets of information sources for the 2o3 and ITS DAs include DPRA, KeratiNoSens, and/or h-CLAT.

Additional projects are underway at the OECD to further improve the utility and applicability domain of the DASS, including the **evaluation of alternate information sources.**

Background: The DASS App

- The DASS App provides a point-and-click interface to apply the DAs in GL 497.
 - **Information Source**
 - Externally, the user runs the assays and models from the DA's required information sources for their chemicals of interest.
 - The user compiles the data and uploads it into the DASS App.
 - **Data Interpretation Procedure**
 - The app applies the selected DA's data interpretation procedure to the user-uploaded data.

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.

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2 out of 3 (2o3) ⓘ
 Integrated Testing Strategy (ITS) ⓘ
 Key Event 3/1 (KE 3/1) Sequential Testing Strategy (STS) ⓘ

2o3 Borderline Evaluation

Flag borderline assay results prior to applying DA 2o3 (Requires data from individual runs) ⓘ

[Confirm DA Selection](#)

Background: DASS App v2.0 Updates

- DASS App v1 implemented the ITS and 2o3 DAs as described in GL 497.
- The latest update to the DASS App (v2.0) includes:
 - **Additional options for assays currently under evaluation for the ITS and 2o3 DAs.**
 - **Evaluation of borderline results from assay run data for use in the 2o3 DA.**
 - Reference data from the Integrated Chemical Environment (ICE) for comparison with user's results.
 - Interactive visualizations of user uploaded quantitative data to contextualize results.
 - Newly designed graphical user interface.



National Institute of
Environmental Health Sciences
Division of Translational Toxicology

Access the DASS App
<https://ntp.niehs.nih.gov/go/952311>

DASS App v2.0 Updates

Integrated Testing Strategy

Integrated Testing Strategy

Information Sources

	In GL 497	Under Evaluation
KE1 assay	<ul style="list-style-type: none"> DPPRA 	<ul style="list-style-type: none"> ADRA
KE3 assay	<ul style="list-style-type: none"> h-CLAT 	<ul style="list-style-type: none"> GARDskin IL8-Luc assay U-SENS
<i>In silico</i> prediction	<ul style="list-style-type: none"> Derek Nexus OECD QSAR Toolbox 	<ul style="list-style-type: none"> iSafeRat Leadscope Model Applier SStopTox

Data Interpretation Procedure

1. Score individual results using ITS scoring rules.
2. Sum individual scores.
3. Translate combined score to GHS potency category using ITS scoring rules.

DASS App v2.0: Integrated Testing Strategy

Information Sources

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<i>In silico</i> prediction	<ul style="list-style-type: none"> Derek Nexus OECD QSAR Toolbox 	<ul style="list-style-type: none"> iSafeRat Leadscope Model Applier SStopTox

Added in DASS App v2.0

Data Interpretation Procedure

1. Score individual results using ITS scoring rules.
2. Sum individual scores.
3. Translate combined score to GHS potency category using ITS scoring rules.

DASS App v2.0: Integrated Testing Strategy

- Unique scoring thresholds were defined for each of the assays under evaluation.
- These thresholds were added to DASS App v2.0.

KE1 Assay

Score	ADRA		DPRA	
	Mean NAC & NAL Depletion (%)	NAC Depletion (%)	Mean Cys & Lys Depletion (%)	Cys Depletion (%)
3	$x \geq 46.4$	$x \geq 67.4$	$x \geq 42.47$	$x \geq 98.24$
2	$15.5 \leq x < 46.4$	$17.5 \leq x < 67.4$	$22.62 \leq x < 42.47$	$23.09 \leq x < 98.24$
1	$4.9 \leq x < 15.5$	$5.6 \leq x < 17.5$	$6.38 \leq x < 22.62$	$13.89 \leq x < 23.09$
0	$x < 4.9$	$x < 5.6$	$x < 6.38$	$x < 13.89$

KE3 Assay

Score	GARDskin	h-CLAT	U-SENS
	Input Conc (μM)	MIT ($\mu\text{g/mL}$)	EC150 ($\mu\text{g/mL}$)
3	$x \leq 13.03$	$x \leq 10$	$x \leq 3$
2	$13.03 < x \leq 56.44$	$10 < x \leq 150$	$3 < x \leq 35$
1	$x > 56.44$	$150 < x \leq 5000$	$35 < x < 200$
0	Negative	Negative	Negative

DASS App v2.0: Integrated Testing Strategy

- Results include a summary of user selections, individual ITS scores, and the hazard and potency predictions.

Required Endpoint	Selection	Flagged
DA	ITS	
KE1 Assay	ADRA	
KE3 Assay	GARDskin	
KE1 Mean Depletion	ADRA_mean_dep	FALSE
KE3 Quantiative Value	GARDskin_input_conc	FALSE
In Silico Call	Derek_prediction	FALSE
In Silico Applicability Domain	Derek_ad	FALSE

ITS.ke1_score	ITS.ke3_score	ITS.insil_score	ITS.total_score	ITS.hazard	ITS.potency
3	1	1	5	Positive	1B
0	NA	0	0	Inconclusive	Inconclusive
NA	NA	1	1	NA	NA
2	NA	1	3	Positive	Inconclusive
1	NA	0	1	Inconclusive	Inconclusive
0	0	1	1	Negative	NC
3	3	1	7	Positive	1A
0	NA	1	1	Inconclusive	Inconclusive
3	3	1	7	Positive	1A
1	2	1	4	Positive	1B
0	2	1	3	Positive	1B
1	1	1	3	Positive	1B
0	NA	1	1	Inconclusive	Inconclusive



DASS App v2.0 Updates

2 out of 3

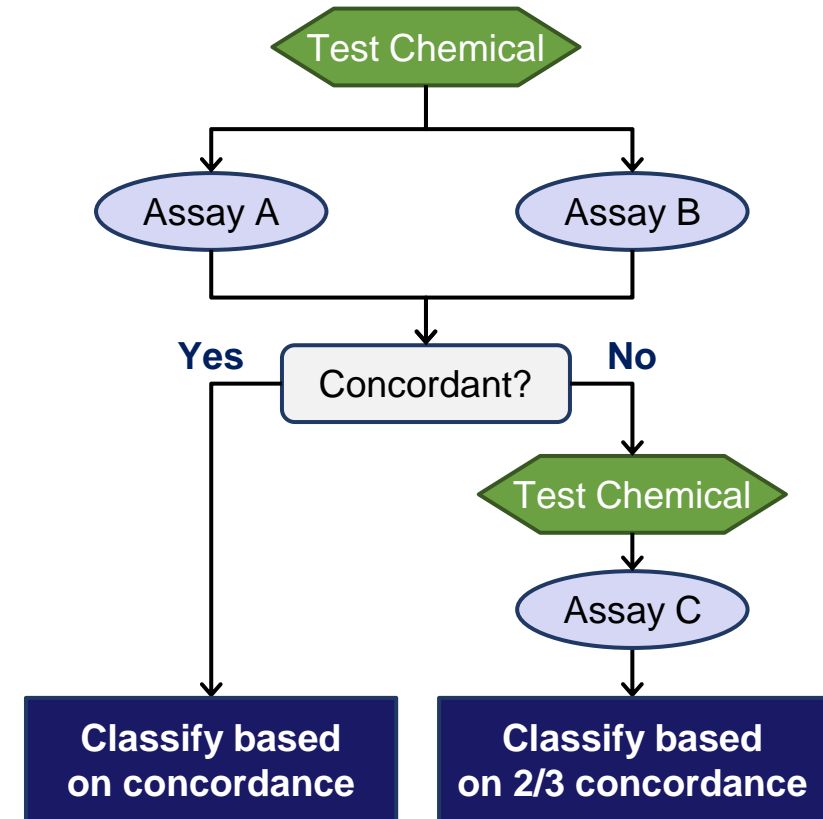
2 out of 3

Information Sources

	In GL 497	Under Evaluation
KE1 assay	<ul style="list-style-type: none"> • DPRA 	<ul style="list-style-type: none"> • ADRA
KE2 assay	<ul style="list-style-type: none"> • KeratinoSens 	<ul style="list-style-type: none"> • LuSens
KE3 assay	<ul style="list-style-type: none"> • h-CLAT 	<ul style="list-style-type: none"> • GARDskin • U-SENS

Data Interpretation Procedure

Use the consensus assay outcomes (positive/negative) for hazard identification



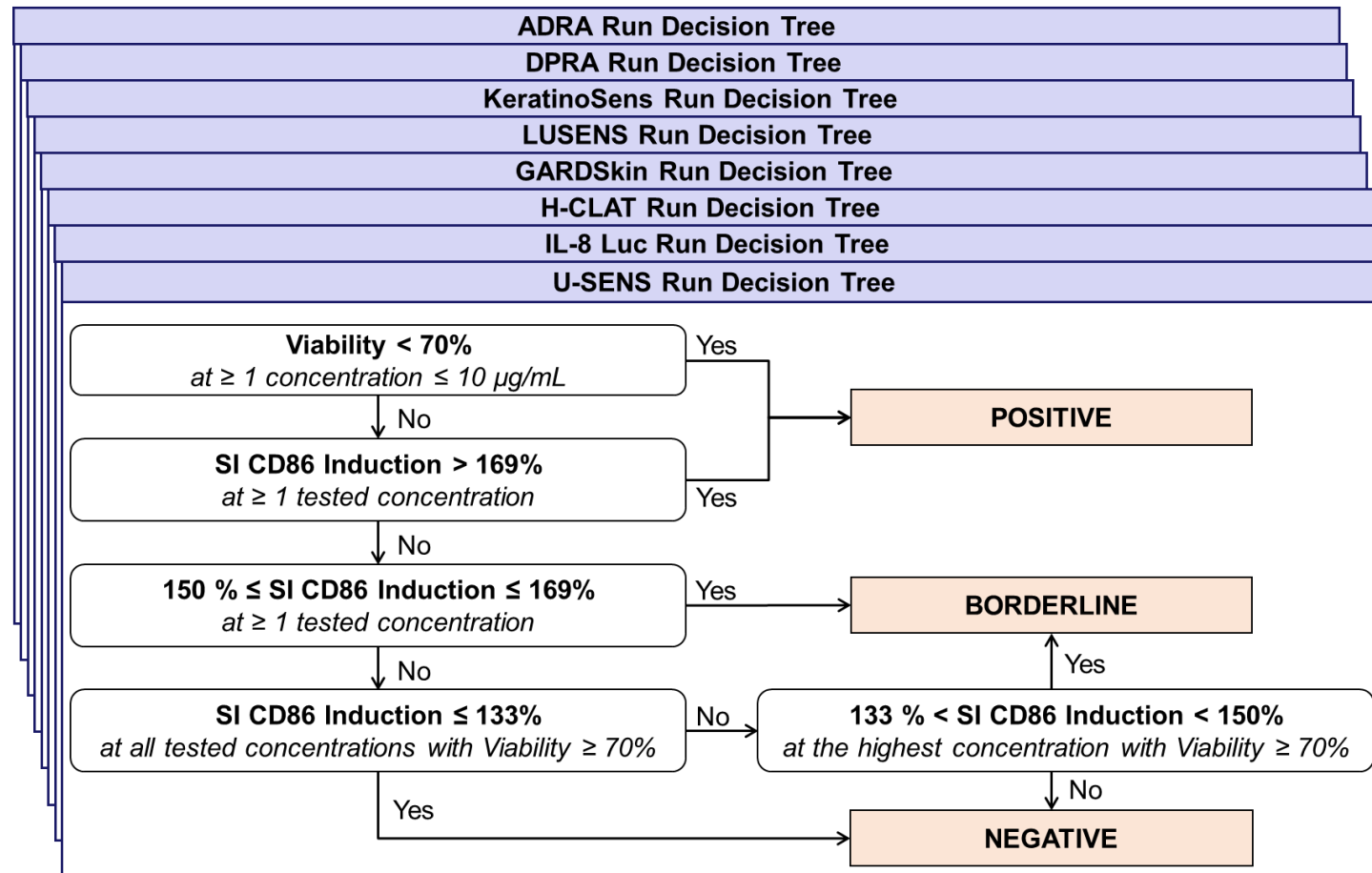
Borderline Results in the 2o3

- For each of the individual assays, the 2o3 inputs are derived by translating continuous data to binary classifications (positive/negative) using cut-off values.
- Results that are close to classification cut-offs increase uncertainty of the 2o3 prediction.
- GL 497 describes borderline ranges and decision trees for DPRA, KeratinoSens, and h-CLAT assay outcomes.

Assay	Endpoint	Cut-off	Borderline Range
DPRA	Mean peptide depletion (%)	6.38	4.95 – 8.32
	Cysteine-only depletion (%)	1.89	10.56 – 18.47
KeratinoSens	Luciferase induction (fold-change)	15	1.35 – 1.67
H-CLAT	Relative fluorescence intensity CD54	200	157 – 255
	Relative fluorescence intensity CD56	150	122 – 184

DASS App v2.0: 2o3 Borderline Workflow

- DASS App v2.0 features a new workflow to evaluate assay run data to flag borderline results prior to applying the 2o3 workflow.
- The borderline workflow can be applied to assays described in GL 497 or assays under evaluation.

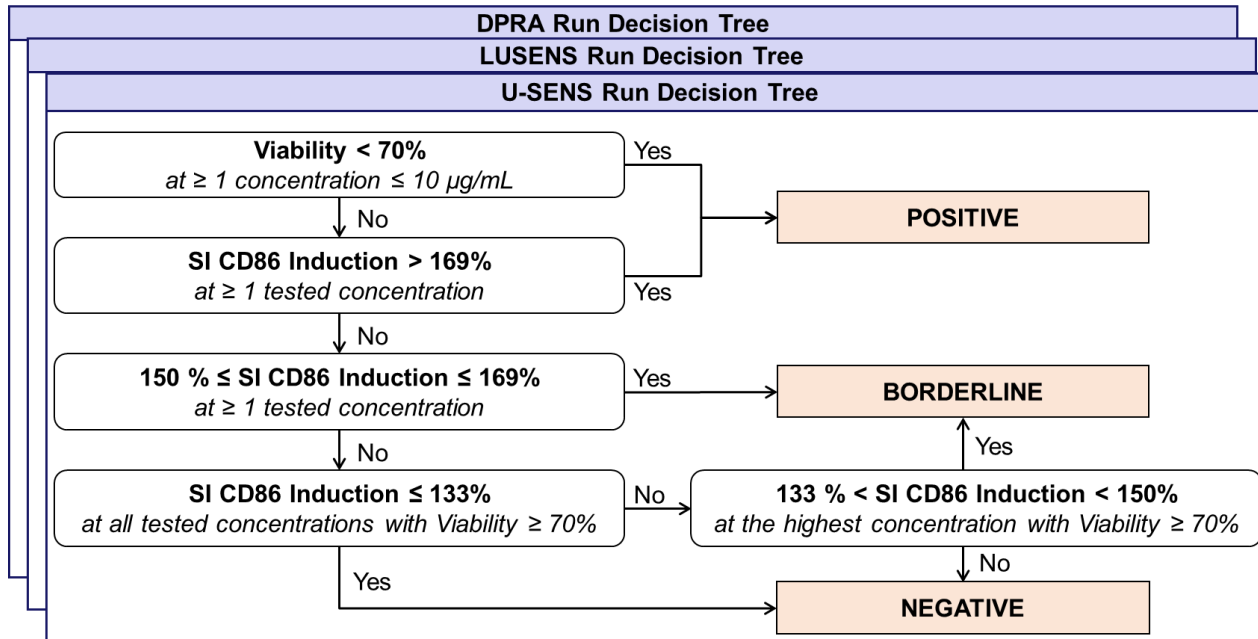


DASS App v2.0: 2o3 Borderline Workflow

User Data

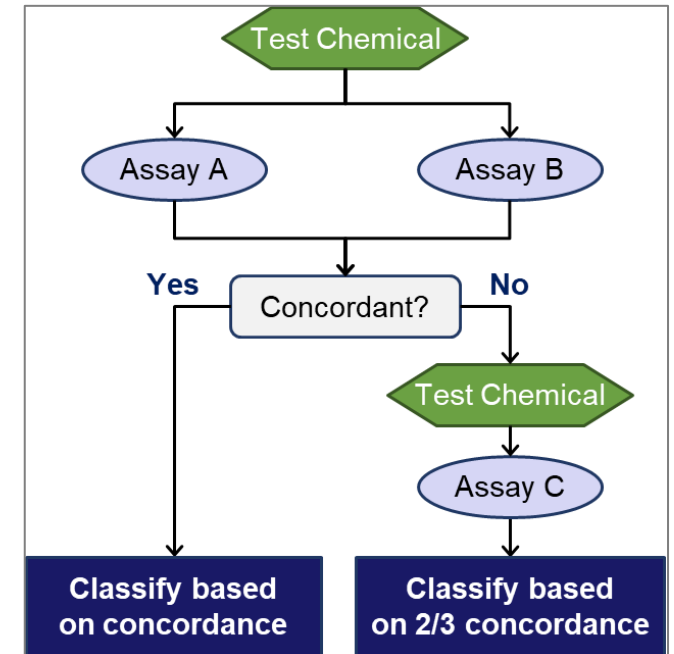


Borderline Evaluation



2o3

Data Interpretation Procedure



Summary

- The DASS App enables users to apply the 2o3 and ITS DAs as described in OECD GL 497.
- DASS App v2.0 introduced a new borderline evaluation workflow for the 2o3 DA.
- Users can now apply the ITS DA using alternate KE1 or KE3 assays.



Demo



Acknowledgments

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