National Institute of Environmental Health Sciences
Division of Translational Toxicology

Advancing Flexibility for Defined Approach Guidelines: Developing Performance Standards to Incorporate Alternate Information Sources into the Defined Approach for Skin Sensitization Guideline

K. Sullivan[1], E. Reinke[2], D.S. Macmillan[3*], H. Raabe[1], D. Germolec[4], D. Allen[2*], E. Choi[5], J. Filipovska[6], N. Kleinstreuer[4]

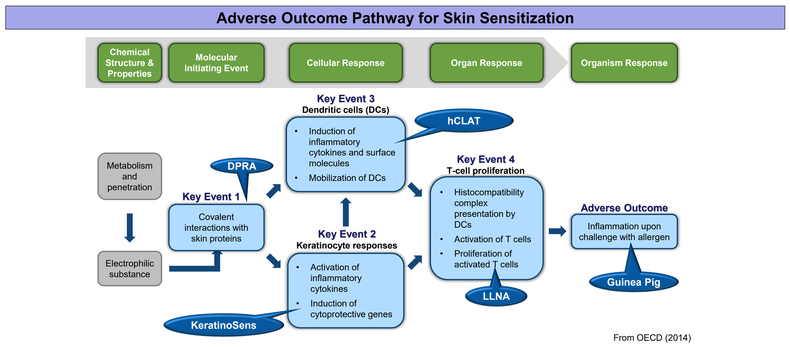
[1]Institute for In Vitro Sciences, Gaithersburg, MD; [2]Inotiv, RTP, NC; [3]Humane Society International, Washington, DC; [4]NIH/NIEHS/DTT/NICEATM, RTP, NC; [5]Organisation for Economic Co-operation and Development (OECD), Paris, France; [6]Independent Researcher and Consultant, Ohrid, North Macedonia

Abstract 4576
Poster P411

Introduction

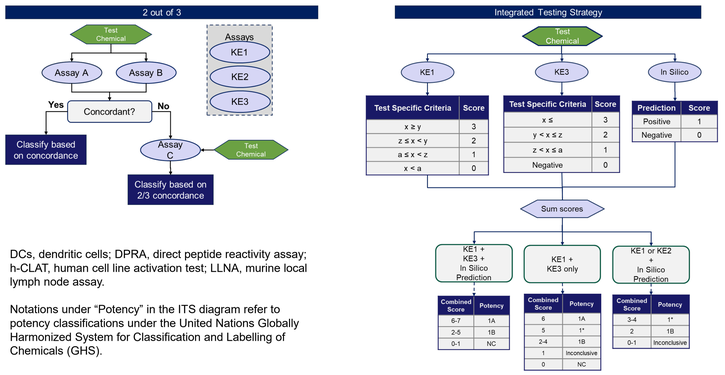
- Skin sensitization (also known as allergic contact dermatitis, ACD) occurs following repeated skin contact with an allergen.
- The key biological events underlying skin sensitization initiated by covalent binding to proteins have been summarized as an adverse outcome pathway (AOP) by the Organisation for Economic Co-operation and Development (OECD 2014). The AOP begins with a molecular initiating event, leading to intermediate key events (KE), and terminating with the adverse effect, ACD (**Figure 1**).
- OECD has accepted mechanistically based in chemico and in vitro assays addressing the first three KEs of the skin sensitization AOP (OECD 2022, 2023c, 2023d). These assays have in turn been incorporated into defined approaches (DAs).
- A DA consists of a defined set of information sources (e.g., in silico predictions, in chemico or in vitro data) used in a fixed data interpretation procedure (e.g., a mathematical model or rule-based approach) to provide predictions without the need for expert judgment.
- Several skin sensitization DAs were published in 2021 by OECD under Guideline No. 497 (OECD 2023a; **Figure 1**), each achieving equivalent or better predictions of the human response than the relevant animal test(s) (Kleinstreuer et al. 2018).
  - 2 out of 3 (2o3): uses in chemico (KE1) and in vitro (KE2/KE3) data to predict hazard.
  - Integrated testing strategy (ITS): uses in chemico (KE1) and in vitro (KE3) data and in silico predictions to predict hazard and potency.
- Performance standards (PS) provide a consistent means to evaluate "me-too" methods: proposed methods that have a similar mechanistic basis and applicability domain as accepted methods. PS are incorporated into several OECD guidelines to improve flexibility and access to a variety of equivalent approaches.
- In 2022, the OECD Expert Group on Skin Sensitization began considering the addition to Guideline 497 of KE-based test methods included in OECD TG 442C/D/E as alternate information sources in the DAs. This project is also investigating the substitution of the in silico information sources used in the ITS DA with other in silico models.
- In order to facilitate ongoing and future efforts for Guideline 497, development of PS for the defined approaches for skin sensitization is necessary. This poster presents the proposed PS for Guideline 497.

Figure 1. AOP for Skin Sensitization and 2o3 and ITS DAs



From OECD (2014)

Defined Approaches published in OECD Guideline No. 497



DCs, dendritic cells; DPRA, direct peptide reactivity assay; h-CLAT, human cell line activation test; LLNA, murine local lymph node assay.

Notations under "Potency" in the ITS diagram refer to potency classifications under the United Nations Globally Harmonized System for Classification and Labelling of Chemicals (GHS).

Table 1. "Me-Too" Methods Under Consideration

Method type	KE 1	KE 2	KE 3	In silico model
Original	Direct peptide reactivity assay (DPRA)	KeratinoSens™	Human cell line activation test (h-CLAT)	Derek Nexus
				OECD QSAR Toolbox
Alternate method	Amino acid derivative reactivity assay (ADRA)	LuSens™	IL-8 Luc	iSafeRat
			U-SENS™	Leadscope Model Applier
			GARD®skin	StopTox

Development of Performance Standards, Reference Chemicals, Borderline Ranges, and Decision Scores

- It is typical to have around 20 PS reference chemicals to evaluate the performance of a new "me-too" method.
  - A proposed addition for the in chemico and in vitro information sources is that a set of 14 required "core" chemicals plus 7 additional chemicals, totaling 21 chemicals from the reference list be used.
- The proposed PS reference substance list for in chemico or in vitro methods is a flexible list of 40 chemicals selected from the original 196 reference chemicals in Annex 2 of the Supporting Document to the Guideline on DAs for Skin Sensitisation (OECD 2023b; **Table 2**). These chemicals were chosen to represent a range of physicochemical properties, potencies, and chemical reactivity domains and are available on the open market.
- In silico methods will be expected to provide predictions for all reference chemicals.
- For each new test method under consideration for addition into the 2o3 DA, borderline ranges are calculated by adapting the prediction model developed for the original 2o3 information sources.
- For each new information source (test method or in silico tool) under consideration for addition into the ITS DA, a scoring rubric was created. The scoring was determined using a similar protocol to the original methods/in silico tools.
- Performance criteria are being developed for the new information sources that will require specific balanced accuracy minimums and are dependent on the reference benchmark species (mouse or human) and are under evaluation by the OECD Expert Group.
- **Table 3** shows the range of performance values for the different permutations of the DAs based on a common set of 21 chemicals, indicated as a "Y" in **Table 2**. These chemicals were selected becausee all test methods under evaluation had data on these chemicals from the Reference Chemical list

Table 2. Proposed Reference Chemical List

Chemical Name	CASRN	PS chem. used for evaluation	Human GHS	LLNA GHS	2o3 pred. (hazard)	ITS pred. (GHS)
Benzylidene acetone*	122-57-6	Y	1A	1	1	1A
Cinnamaldehyde*	104-55-2	Y	1A	1A	1	1A
Diethyl maleate	141-05-9	N	1A	1B	1	1A
DNCB*	97-00-7	Y	1A	1A	1	1A
Formaldehyde*	50-00-0	Y	1A	1	1	1A
Tetrachlorosalicylanilide*	1154-59-2	Y	1A	1A	1	1A/1B
Aniline*	62-53-3	Y	1B	1B	NC	1B
Chlorpromazine	50-53-3	N	1B	1	NC	1B
Cinnamic alcohol	104-54-1	Y	1B	1B	1	1B
Dibenzoyl peroxide	94-36-0	N	1B	1A	NC	1B
Eugenol	97-53-0	Y	1B	1B	1	1B
Farnesol*	4602-84-0	Y	1B	1B	1	1B
Isoeugenol*	97-54-1	Y	1B	1A	1	1B
2-Mercaptobenzothiazole*	149-30-4	Y	1B	1A	1	1A
Neomycin sulfate*	1405-10-3	Y	1B	N/A	N/A	1B
Penicillin G	61-33-6	N	1B	1B	1	1B
Phenyl benzoate	93-99-2	N	1B	N/A	1	1B
Sulfanilamide	63-74-1	N	1B	NC	NC	NC
4-aminobenzoic acid*	150-13-0	Y	NC	N/A	NC	NC
Citronellol	106-22-9	N	NC	1B	1	1B
Hexyl salicylate*	6259-76-3	Y	NC	1	NC	1B
Hydrocortisone*	50-23-7	Y	NC	N/A	1	1B
n-Hexane	110-54-3	Y	NC	NC	NC	NC
Propylene glycol*	57-55-6	Y	NC	NC	NC	NC
α-Hexylcinnamaldehyde	101-86-0	N	N/A	1B	NC	NC
p-Benzoquinone	106-51-4	N	N/A	1A	1	1A
Bromothalonil	35691-65-7	Y	N/A	1A	1	1A
Butan-1-ol	71-36-3	Y	N/A	NC	NC	NC
Citral	5392-40-5	N	N/A	1B	1	1A
EGDMA	97-90-5	Y	N/A	1B	1	1B
Ethylene diamine (free base)	107-15-3	N	N/A	1B	1	1B
Glycerol	56-81-5	N	N/A	NC	NC	NC
4-Hydroxybenzoic acid	99-96-7	N	N/A	NC	NC	NC
Isopropanol	67-63-0	N	N/A	NC	NC	NC
Lactic acid	50-21-5	N	N/A	NC	NC	NC
4-Nitrobenzyl bromide	100-11-8	N	N/A	1A	1	1A
Propyl gallate	121-79-9	Y	N/A	1A	1	1A
Resorcinol	108-46-3	N	N/A	1B	NC	1B
Salicylic acid	69-72-7	N	N/A	1B	NC	NC
Vanillin	121-33-5	N	N/A	NC	NC	NC

All predictions are extracted from Annex 2 of Guideline 497, *indicates proposed "Core Chemical"

Table 3. Performance of New Methods With Proposed PS Chemicals

DA/Method	Information Sources *	Capability	Hazard Performance vs. LLNA	Hazard Performance vs. Human	Potency Performance vs. LLNA	Potency Performance vs. Human
2o3 DA	KE1: ADRA, DPRA KE2: KeratinoSens™, LuSens KE3: GARDskin, h-CLAT, IL-8 Luc, U-SENS™	Hazard	96-100% BA 92-100% Se 100% Sp	78-95% BA 89-100% Se 67-100% Sp	-	-
ITS DA	KE1: ADRA, DPRA, KE3: GARDskin, h-CLAT, U-SENS™ In silico: Derek Nexus, iSafeRat, Leadscope Model Applier, OECD QSAR Toolbox, StopTox	Hazard, Potency	97-100% BA 93-100% Se 100% Sp	71-88% BA 92-100% Se 50-75% Sp	95-100% NC 84-94% 1B 83-93% 1A	70-83% NC 73-83% 1B 85-95% 1A
LLNA (for comparison)	In vivo	Hazard, Potency	-	67% BA, 100% Se 33% Sp	-	100% NC 83% 1B 88% 1A

*One each per KE and/or in silico, where applicable.
Evaluated against a subset of up to 21 substances from the Proposed Reference Chemicals List (Table 2) e.g. fewer substances with potency data mean that the ITS DA potency performance may have been evaluated against less than 21.
BA = balanced accuracy, Se = sensitivity, Sp = specificity.

Significance of the PS for Guideline 497

- Several new methods are being evaluated for inclusion into OECD Guideline 497. These methods include assays that cover KE1 (ADRA), KE2 (LuSens), KE3 (GARDskin, IL-8 Luc, U-SENS™) as well as new in silico information sources (iSafeRat, Leadscope Model Applier, StopTox).
- Performance standards can expedite the inclusion of additional methods that address the same KEs into existing DAs.
- Each drop-in permutation of "me-too" information sources (e.g. using ADRA in place of DPRA in the 2o3) is under evaluation for performance in the DAs already published in Guideline 497, with the intention that the methods could be used in any combination.
- Once completed, the performance standards could also be used to evaluate new DAs.
- Efforts to include new DAs that predict points-of-departure for risk assessment in Guideline 497 are underway.

References

Kleinstreuer et al. 2018. Non-animal methods to predict skin sensitization (II): an assessment of defined Approaches. Critical reviews in Toxicology. https://doi.org/10.1080/10408444.2018.1429386.
OECD 2014. Guidance Document No. 168. https://doi.org/10.1787/9789264221444-en.
OECD 2023a. Guideline No. 497: Defined Approaches on Skin Sensitisation. https://doi.org/10.1787/b92879a4-en.
OECD 2023b. Supporting document to the Guideline (GL) on Defined Approaches (DAs) for Skin Sensitisation https://one.oecd.org/document/ENV/CBC/MONO(2021)11/En/pdf.
OECD 2023c. Test No. 442C: In Chemico Skin Sensitisation (DPRA). https://doi.org/10.1787/9789264229709-en.
OECD 2022. Test No. 442D: In Vitro Skin Sensitisation (KS). https://doi.org/10.1787/9789264229822-en.
OECD 2023d. Test No. 442E: In Vitro Skin Sensitisation (h-CLAT). https://doi.org/10.1787/9789264264359-en.

Acknowledgments

The Intramural Research Program of the National Institute of Environmental Health Sciences (NIEHS) supported this poster. Technical support was provided by Inotiv-RTP under NIEHS contract HHSN273201500010C and by Burleson Research Technologies, Inc., under NIEHS contract HHSN273201400017C.

*D.S. MacMillan and D. Allen are currently affiliated with the International Collaboration on Cosmetics Safety, New York, NY.