National Institute of Environmental Health Sciences
Division of Translational Toxicology

Application of Defined Approaches for Skin Sensitization for Chemicals of Federal Agency Interest

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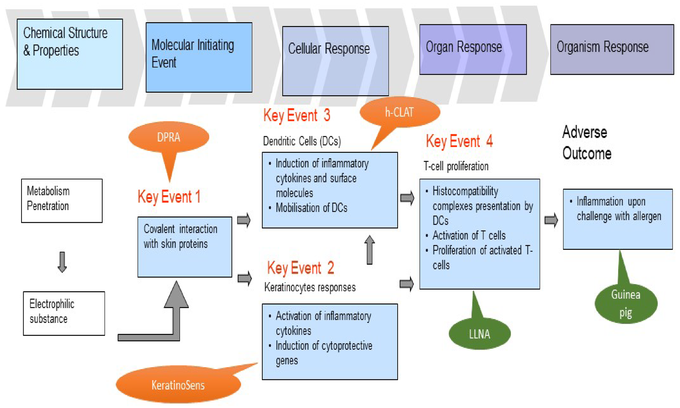
Introduction

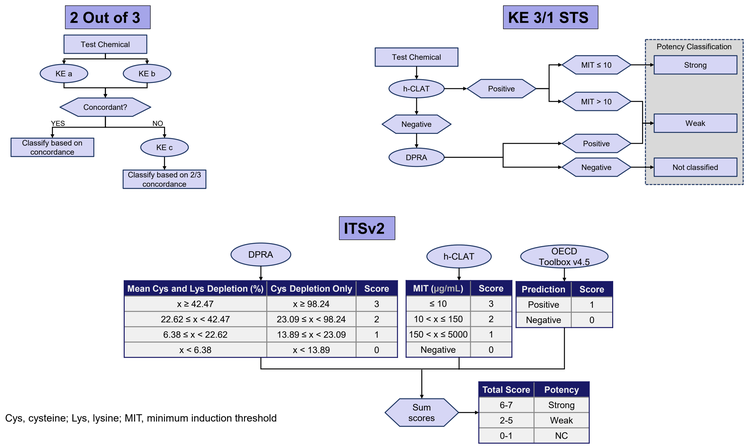
- Multiple U.S. federal agencies require the assessment of skin sensitization potential for their chemical evaluation and management programs.
- Although these agencies have historically relied on skin sensitization data from animal testing, several new approach methodologies (NAMs) have been internationally adopted as test guidelines.
- While none of these methods are considered complete replacements for animal tests, one approach to improve performance is to combine the results of NAMs that represent multiple key events of the adverse outcome pathway for skin sensitization (**Figure 1**) using defined approaches (DAs). However, the DAs for regulatory use described in Organisation for Economic Co-operation and Development Guideline 497 (OECD 2021) have been evaluated using primarily chemicals that are relevant to the cosmetics industry.
- This project evaluated three different DAs: 2 out of 3 (2o3) (OECD 2021), Integrated Testing Strategy (ITSv2) (OECD 2021a) and Key Event 3/1 Sequential Testing Strategy (KE 3/1 STS) (EPA 2018) (**Figure 2**). Substances relevant to programs within several federal agencies were tested in the following NAMs for skin sensitization for use in the DAs:
  - Direct Peptide Reactivity Assay (DPRA; OECD 2022a)
  - KeratinoSens™ assay (KS; OECD 2022b)
  - human Cell Line Activation Test (h-CLAT; OECD 2022c)
- NAM data were generated for 185 substances nominated by the National Toxicology Program (NTP), the U.S. Environmental Protection Agency (EPA), and the Consumer Product Safety Commission (CPSC). These substances also had in vivo local lymph node assay (LLNA) results.
- The skin sensitization hazard (i.e., sensitizer/nonsensitizer) and/or potency classification (i.e., Strong or GHS 1A; Weak or GHS 1B; NC = Not Classified, nonsensitizer) results for each NAM and DA were pooled by agency and office and compared, along with in vivo LLNA outcomes (**Figures 3-4**).

Figure 1. Adverse Outcome Pathway for Skin Sensitization



From OECD (2014)

Figure 2. DAs used to Assess Hazard and/or Potency Classification



Mean Cys and Lys Depletion (%)	Cys Depletion Only	Score
x ≥ 42.47	x ≥ 98.24	3
22.62 ≤ x < 42.47	23.09 ≤ x < 98.24	2
6.38 ≤ x < 22.62	13.89 ≤ x < 23.09	1
x < 6.38	x < 13.89	0

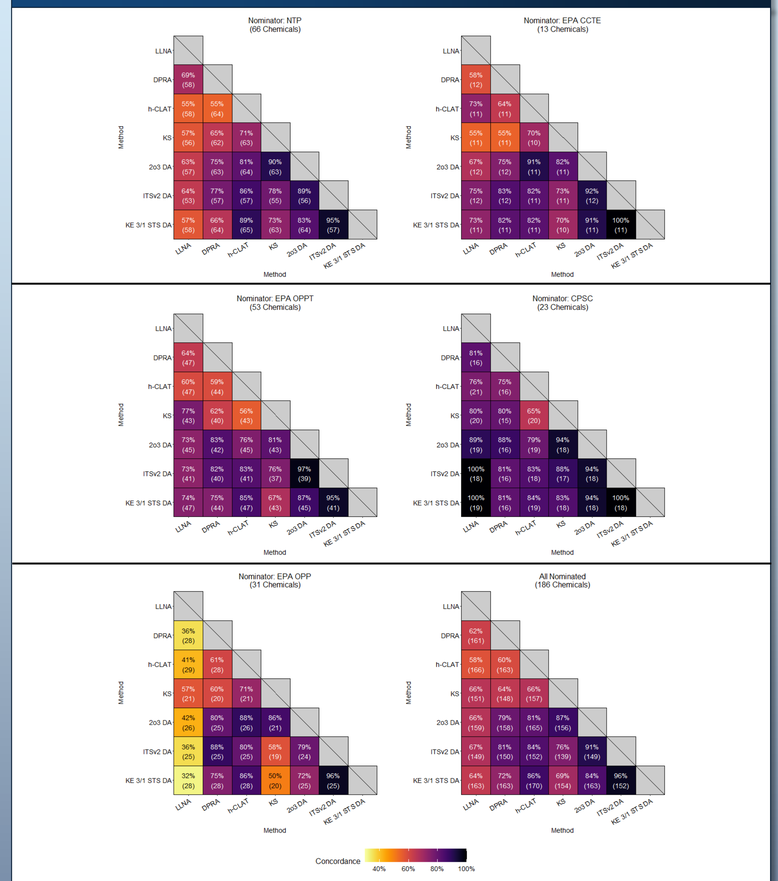
MIT (µg/mL)	Score
≤ 10	3
10 < x ≤ 150	2
150 < x ≤ 5000	1
Negative	0

Prediction	Score
Positive	1
Negative	0

Total Score	Potency
6-7	Strong
2-5	Weak
0-1	NC

Cys, cysteine; Lys, lysine; MIT, minimum induction threshold

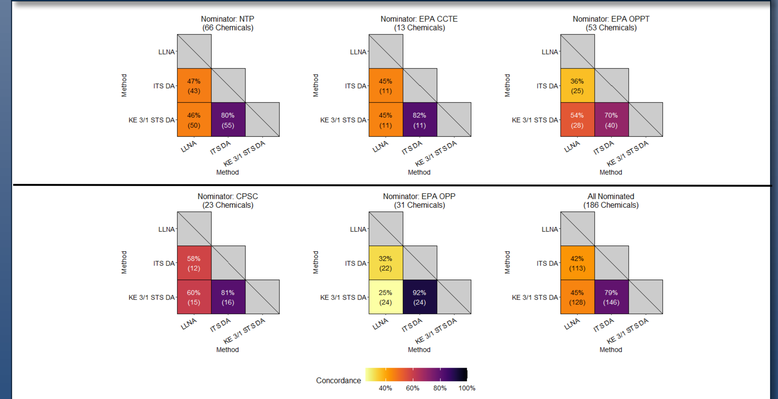
Figure 3. Concordance of the DAs for Hazard Classification



- Percent concordance was calculated based on total number of chemicals that shared a prediction (positive or negative) over the total number of chemicals shared between the assay or DA.
- Numbers in parentheses indicate the numbers of chemicals predicted by both comparators.

CCTE, Center for Computational Toxicology and Exposure; OPP, Office of Pesticide Programs; OPPT, Office of Pollution Prevention and Toxics

Figure 4. Concordance of the DAs for Potency Classification



- Percent concordance was calculated based on total number of chemicals that shared a prediction (positive or negative) over the total number of chemicals shared between the assay or DA.
- Numbers in parentheses indicate the numbers of chemicals predicted by both comparator assays.

CCTE, Center for Computational Toxicology and Exposure; OPP, Office of Pesticide Programs; OPPT, Office of Pollution Prevention and Toxics

Results

- Some data sets had skewed distributions of positive and negative (for hazard) or 1A, 1B, and negative (for potency) reference data. For example, the CPSC data set had no negative substances and the EPA OPP data set had only two positive substances.
- For hazard classification, concordance between assays was higher among NAMs than between NAMs and LLNA (**Figure 3**).
  - Highest hazard concordance noted for comparisons involving ITSv2 whereas the lowest hazard concordance for all methods was seen for comparisons involving the LLNA.
  - The lowest hazard concordance for individual assays or DAs was for substances nominated by EPA OPP. The heterogeneity or limited solubility of several of these substances made them incompatible with certain test systems.
  - The highest hazard concordance among the DAs was for the substances nominated by EPA CCTE.
- Concordance for potency classification was highest between the KE 3/1 STS DA and the ITSv2 DA (**Figure 4**).
  - The overall potency concordance between the DAs and the LLNA was highest with CPSC nominations, however EPA OPP nominations had the highest potency concordance between the two DAs of all the nominated sets.
  - Among the nominator groups, the potency concordance with the LLNA was the lowest for the EPA OPP nominations, likely due to the heterogeneity/insolubility issues noted above.

Conclusions

- Some substances of agency interest are not compatible with in vitro test systems that require dissolution or homogeneous solutions of test substance.
- Results from in vitro testing and application of DAs may provide a useful alternative to animal testing for skin sensitization hazard and potency classification of substances relevant to a wide range of federal agency programs.
- Additional evaluations are necessary to further characterize the applicability of NAMs to skin sensitization assessments for a broad range of chemicals and products.

References

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