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, US, [2]Inotiv, Inc., RTP, NC, US, [3]Humane Society International, Washington, DC, US, [4]NIH/NIEHS/DTT/NICEATM, Research Triangle Park, NC, United States, [5]OECD, [6]Constant Consulting

Background and Purpose

There are now internationally accepted defined approaches (DAs) that combine results from multiple *in vitro, in chemico*, and/or *in silico* methods for regulatory decision-making for skin sensitization assessments. These guidelines support Mutual Acceptance of Data by fixing data interpretation and assessment steps, thereby avoiding the need for expert judgement or subjective decisions for a particular regulatory context of use (e.g., hazard classification and labeling). The first Organisation for Economic Cooperation and Development (OECD) DA for Skin Sensitization (DASS) guideline relies upon multiple fixed information sources: varying combinations of the Direct Peptide Reactivity Assay (DPRA), the KeratinoSens assay, and/or the Human Cell Line Activation Test (h-CLAT), and *in silico* data from either the OECD QSAR Toolbox or the Lhasa Derek.

The future inclusion of new test methods into an OECD guideline necessitates the development of performance standards, which includes reference chemical lists and minimum criteria for performance (sensitivity, specificity, accuracy). We utilized data from the existing guideline as well as the proposed new methods for inclusion to develop these performance standards.

Methods

We identified several skin sensitization methods that align to the key events (KE) associated with the adverse outcome pathway (AOP) for skin sensitization and are under consideration as alternate information sources ("me-too" methods) within the DASS. These included the Amino acid Derivative Reactivity Assay (ADRA), the LuSens assay, Genomic Allergen Rapid Detection (GARD)Skin assay, the U937 Cell Line Activation Test (U-SENS), and InterLeukin-8 Reporter Gene (IL-8 Luc) Assay, all of which have been approved within their respective test guidelines, as well as several *in silico* models (iSafeRat, Leadscope Model Applier, StopTox). Utilizing pre-existing data for each of these methods, a list of chemicals to be used as performance standards reference chemicals was developed from the DASS reference chemical list (OECD TG 497, Annex 2). For the development of the reference chemical list, in addition to pre-existing data, an emphasis was placed on coverage of key chemical reactivity domains, representative United Nations Globally Harmonized System of Classification and Labelling of Chemicals sub-categories, and physico-chemical properties, to provide a balanced reference set relative to the larger DASS chemical list.

Results

The curated list of proposed Performance Standards Reference Chemicals consists of 40 chemicals spanning a range of chemical reactivity domains and physico-chemical properties. Of

these, 14 are required, with the developer given the option to choose an additional seven chemicals. The 21 total chemicals are utilized to conduct performance analysis of the DA(s) using the substitute assay for the corresponding KE-based information source. In silico "me-too" models will be assessed against the larger dataset, given the ease of running multiple predictions. The list was designed in such a way as to identify the strengths and limitations of an assay relative to its predictivity around different physico-chemical properties, reactivity domains, and potencies. This approach is supported by applications of the "me-too" concept in previous test guidelines as well as mechanistic alignment between assays that provide information on a particular KE in the AOP for dermal sensitization. The prediction models and scoring values required for application of a test method to a particular DA in the current guideline were also adapted for each method.

Conclusion

While there is a large dataset for the original assays used as information sources in the DASS guideline, the development of a flexible subset of reference chemicals allows the performance of a new *in chemico/in vitro* methods to be assessed without spending excess time and resources on a large amount of testing.

This work will help to guide test method/in silico developers in demonstrating the utility of a particular approach to fit within the DASS guideline and further expand the capabilities to safely assess the sensitization potential of chemical ingredients. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.