Testing of Regulatory-Relevant Chemicals for Skin and Respiratory Sensitization Hazard

V.J. Johnson[1], E.N. Reinke[2], T. LaPratt[2], J. Strickland[2], K.T. To[2], O. Larne[3], H. Johansson[3], A. Forreryd[3], D.G. Allen[2], N.C. Kleinstreuer[4], D. R. Germolec[4]

[1]Burleson Research Technologies, Inc., Morrisville, NC, United States; [2]Inotiv, RTP, NC, United States; [3]SenzaGen AB, Lund, Sweden; [4]NIH/NIEHS/DTT/NICEATM, RTP, NC, United States

Background and Purpose

Identifying sensitization hazards is important for comprehensive safety assessments of chemicals to which humans are exposed via the dermal or inhalation route. Several new approach methodologies (NAMs) have been adopted as test guidelines for identifying potential skin sensitizers by the Organization for Economic Cooperation and Development (OECD). One of these, the GARD®skin dermal sensitization assay, uses a genomic biomarker profile to identify potential skin sensitizers. GARDskin is included in OECD Test Guideline 442E describing in vitro methods for skin sensitization and is under evaluation for addition to Guideline 497, Defined Approaches for Skin Sensitization. In this project, we tested chemicals nominated by several U.S. federal agencies in the GARDskin assay, both as a stand-alone method and as an information source within several accepted defined approaches for skin sensitization and assessed the concordance with reference data. While several non-animal approaches have been accepted to identify skin sensitizers, no such methods have been accepted to evaluate respiratory sensitization potential. The lack of accepted methods leaves a data gap in safety assessments; evaluation of non-animal methods targeted to respiratory sensitization will help fill this data gap. Thus, we also tested a variety of nominated chemicals in the GARD®air respiratory sensitization assay, which utilizes a biomarker profile specific to respiratory sensitization that differs from the GARDskin biomarker profile.

Methods

Test chemicals were nominated by several U.S. federal agencies for assessment in either GARDskin (31 chemicals) or GARDair (100 chemicals with known respiratory hazards). The chemicals assessed in GARDskin were selected as "challenging to test" chemicals. Reference data on these chemicals were compiled from the local lymph node assay (LLNA), human predictive patch test (HPPT), and three non-animal skin sensitization test methods: the direct peptide reactivity assay, KeratinoSens assay, and human cell line activation test (h-CLAT). Since no test guideline for respiratory sensitization exists, these data were also used as a reference for comparison to the GARDair data, along with case reports of respiratory sensitization from occupational exposures. Nominated chemicals were tested according to the applicable GARD protocol. Predictions of either dermal or respiratory hazard were made based on test outcomes that rely on gene expression analysis of a defined biomarker gene set for each method. These gene expression patterns are analyzed in a pattern recognition and machine learning application to produce a hazard (yes/no) output.

Results

When compared to LLNA reference data for the 31 difficult-to-test chemicals, the hazard predictions from the GARDskin assay demonstrate a sensitivity of 86%, specificity of 42%, and accuracy of 64%. By comparison, performance metrics for hazard prediction for the three other non-animal skin sensitization assays ranged from 40-54% for sensitivity, 22-47% for specificity,

and 31-50% for accuracy. Seven chemicals in the GARDskin data set also had HPPT data. For this limited set of chemicals, the sensitivity was 60%, specificity was 100%, and accuracy was 71%.

GARDskin was also assessed as a drop-in replacement for the h-CLAT in two defined approaches (DA) for skin sensitization hazard. DAs using GARDskin were found to have better performance than the classic defined approaches using h-CLAT (2 of 3 and the Key Event 3/1 Sequential Testing Strategy). Testing of nominated chemicals in the GARDair assay is ongoing.

Conclusions:

This study will demonstrate the utility of the GARD platform for assessing dermal and respiratory sensitization hazard for chemicals of federal agency interest, including several that are known to be difficult to test using NAMs. The GARDskin assay performed better than the other non-animal skin sensitization assays when compared to the LLNA. GARDskin showed better performance for a "challenging" chemical set in the 2 of 3 DA over the traditional application using h-CLAT for key event 3 of the adverse outcome pathway for dermal sensitization. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C and HHSN273201400017C.

Keywords: Alternatives to Animal Testing; Sensitization; Chemical allergy; New Approach Methodologies