Phase 1 Validation of the Electrophilic Allergen Screening Assay

J. Strickland[*], J. Gordon[**], J. Hettick[***], B. Law[***], E. J. Petersen[****], N. Solomotis[*****], J. Truax[*], R. Uhl[**], J. Yourick[*****], D. Allen[*], N. Kleinstreuer[******]

[*]ILS, Research Triangle Park, NC, USA

[**]CPSC, Rockville, MD, USA

[***]CDC/NIOSH/HELD, Morgantown, WV, USA

[****]NIST, Gaithersburg, MD, USA

[*****]FDA/CFSAN, Laurel, MD, USA

[******]NIH/NIEHS/DNTP/NICEATM, Research Triangle Park, NC, USA

Covalent binding of an electrophilic chemical to a nucleophilic binding site on skin protein is a known molecular initiating event in the skin sensitization adverse outcome pathway. The electrophilic allergen screening assay (EASA) assesses this event by measuring depletion of up to two probe chemicals displaced by a test chemical that binds to the probe(s). Probe depletion is detected using absorbance or fluorescence measurements. A test chemical is positive if the depletion of any probe is ≥30%, and negative if probe depletion is either <10% in the absorbance or <15% in the fluorescence test. Confirmatory testing is conducted when results fall between the criteria for positive and negative outcomes. NICEATM is coordinating a multi-laboratory validation study to characterize the usefulness and limitations of the EASA for skin sensitization hazard classification. Phase 1 of this study involved testing 10 coded chemicals three times in three laboratories to demonstrate reproducibility. Classifications as sensitizers or nonsensitizers were reproducible within each laboratory for 100% (10/10) of the chemicals. Among laboratories, classifications were reproducible for 90% (9/10) of the chemicals. The classifications of 70% (7/10) of the chemicals were accurate with respect to LLNA classifications in two laboratories; one laboratory's results yielded accuracy of 60% (6/10). All misclassifications were false negatives. Phase 1 data were considered adequate to progress to Phase 2. In this phase, we will develop a 96-well format to increase throughput and comprehensively assess test method accuracy. *This was funded with U.S. federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C*.