Incorporating Adverse Outcome Pathway Knowledge in Skin Sensitization Testing Methods and Integrated Testing and Decision Strategies

William S. Stokes, National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, Division of the National Toxicology Program, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC, USA

Allergic contact dermatitis (ACD) resulting from exposures to chemical contact allergens is a serious occupational and public health issue. Accordingly, regulatory authorities require testing to identify chemicals and products that may cause skin sensitization (SS), and require labeling to warn consumers and workers of SS hazards. Guinea pigs (GP) have been used for decades to evaluate chemicals for SS potential, using the ACD clinical manifestations of erythema and edema during the elicitation phase as the positive endpoint. More recently, the murine local lymph node assay (LLNA) has been adopted internationally as a valid substitute for the GP test. The LLNA measures an earlier mode of action (MOA) event during the induction phase: proliferation of lymphocytes in the draining lymph node for the skin area where the chemical is applied. The MOA-based LLNA provides numerous advantages compared to the GP tests, including using 60 per cent fewer animals, avoidance of all pain and distress, and 75% less time to complete.

More recently, other upstream key events in the pathway leading to induction of SS have been identified, including the key molecular initiating event of covalent binding of the chemical with protein, and specific cellular responses in keratinocytes and dendritic cells. These key events are collectively referred to as an Adverse Outcome Pathway (AOP). Several in vitro test methods that measure key AOP events have been developed, including the direct peptide reactivity assay (DPRA), Keratinosens^{SM}(KS), and h-CLAT (HC). NICEATM subsequently evaluated the SS predictivity of four testing strategies that incorporate AOP-based test methods. In the first strategy, individual methods were evaluated. In the second strategy, the three methods were used in a battery approach, where chemicals with positive results in two or more assays were considered SS. The 3rd strategy used a decision tree analysis based on structural reactivity (SR) (Safford, 2011) and the three individual methods. In the last strategy, an integrated and testing decision strategy (ITDS) informed by the decision tree analysis was constructed using SR, DPRA, and the reduced LLNA (rLLNA). Substances positive in both SR and DPRA were classified as sensitizers without animal testing, and substances that were SR- or SR+/DPRA- were tested in the rLLNA.

Among the 3 individual test methods, DPRA had the highest sensitivity (89%) and specificity (78%) for the set of 67 chemicals. DPRA alone was still more predictive than the 3-test battery approach. The decision tree strategy had the best performance using only SR and DPRA, but performance was no better than DPRA alone. The ITDS yielded 100%(119/119) sensitivity and 87% (34/39) specificity, while using 78% fewer animals. Regulatory implementation of this initial ITDS could significantly and immediately reduce animal while identifying SS hazards at the same rate as the standard LLNA. Future planned improvements in this AOP-based ITDS are expected to further reduce and eventually replace animal use while continuing to support regulatory decisions on skin sensitization hazards.