NTP Approaches to Assessment of Dermal Hypersensitivity

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Allergic diseases are the result of aberrant immune responses, termed hypersensitivity responses, directed against antigens that are innocuous in most individuals. Hypersensitivity responses consist of an induction phase where the immune system is “primed” to respond inappropriately to an antigen, and an elicitation phase where subsequent exposure to the same antigen results in a vigorous and accelerated response manifested as allergic disease. Allergic skin disease occurs in approximately 12% of children in the U.S. and is also a common consequence of occupational exposure to chemicals. Guinea pigs were traditionally used to test the sensitizing potential of chemicals, but animal costs, sensitivity issues, and subjectivity of the assays endpoint led to the development of alternative tests. The mouse Local Lymph Node Assay is currently the in vivo method of choice for determining skin sensitizing potential as it provides a marked refinement and reduction in animal use compared to guinea pig assays without a loss of accuracy (Basketter et al., 2002; Dean et al., 2001; Gerberick et al., 2007). Assessment of the potential to induce skin sensitization is a regulatory requirement for industrial chemicals, pesticides, and cosmetics and has been at the center of concerted efforts to replace animal testing in recent years.

To date, there has been a great deal of progress in using in vitro models to assess chemical sensitization, and in particular, dermal hypersensitivity and irritancy. The OECD has published an adverse outcome pathway (AOP) for skin sensitization linking molecular initiating events and cellular and tissue effects in the sensitization process to specific adverse outcomes (OECD 2012 a, b). For skin sensitization, these key events include: 1) covalent interaction with skin proteins, 2) activation of inflammatory cytokines and induction of cytoprotective genes, 3) induction of inflammatory cytokine and surface molecules and mobilization of dendritic cells, and 4) activation of T cells. Several in vitro testing methods for assessment of hypersensitivity have been validated and have associated OECD test guidelines, or are in the process of validation and international interlaboratory ring-trials. Examples of validated in vitro methods include the direct peptide reactivity assay (DPRA), an in chemico test that measures the ability of a substance to form a hapten–protein complex (Gerberick et al. 2004, Gerberick et al. 2007, OECD 2015a), the KeratinoSens® assay that assesses the activation of the Nrf2 pathway in keratinocytes, indicating a substances’ ability to induce cytoprotective responses and release of cytokines by keratinocytes (Emter et al. 2010, OECD 2015b), and the human cell line activation test (h-CLAT) that measures the ability of a substance to activate and mobilize dendritic cells in the skin (Alepee et al. 2015, Ashikaga et al. 2006, OECD 2016, Piroird et al. 2015).

It is clear from these efforts that no individual in vitro test can yet recapitulate the hypersensitivity immune response in its entirety. Therefore, the NTP, in coordination with the ICCVAM skin sensitization working group (SSWG), used machine learning approaches to assess combinations of various non-animal methodologies to develop predictive models. Models were developed with the initial goal of classifying substances as sensitizers or non-sensitizers without requiring animal data, and a further goal of separating strong and weak sensitizers. These
machine learning analyses suggest that various integrated approaches of training models using DPRA, KeratinoSens, h-CLAT, read-across, and logP data were more accurate in identifying potential skin sensitizers than in vitro, in chemico, or in silico methods by themselves, and can show up to 92% accuracy in predicting known human skin sensitizers (Strickland et al., 2017; Zang et al., 2017).

To further investigate the utility of these approaches, NICETAM solicited chemical nominations from ICCVAM agencies for which there were existing LLNA data but limited or no in vitro evaluation. Through the NTP Immunotoxicology contract with Burleson Research Technologies, the chemicals are being screened using the LuSens assay (Ramirez et al. 2014, a non-proprietary method similar to the KeratinoSens® assay), the DPRA, and the h-CLAT. A total of 266 chemicals and chemical formulations were nominated; 135 have been shipped to the contract lab and NTP is attempting to procure or has already obtained those remaining. The list of chemicals includes pesticides, formulations, excipients, personal care product ingredients, and industrial agents. These in vitro data will be used to understand the applicability domain of the individual test methods, and will be combined with the other physicochemical property and read-across features to run the published models on this new set of chemicals and compare the predictions with the animal data.

References:


Gerberick GF, Ryan CA, Dearman RJ, Kimber I. 2007. Local lymph node assay (LLNA) for detection of sensitization capacity of chemicals. Methods 41:54-60.


