NTP Approaches to Assessment of Dermal Hypersensitivity

Presenter: Dr. Dori Germolec, NIEHS/DNTP/Toxicology Branch

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 at 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:

Alépée N, Piroird C, Aujoulat M, Dreyfuss S, Hoffmann S, Hohenstein A, Meloni M, Nardelli L, Gerbeix C, Cotovio J. 2015. Prospective multicentre study of the U-SENS test method for skin sensitization testing. Toxicol In Vitro 30:373-82.

Ashikaga T, Yoshida Y, Hirota M, Yoneyama K, Itagaki H, Sakaguchi H, Miyazawa M, Ito Y, Suzuki H, Toyoda H. 2006. Development of an in vitro skin sensitization test using human cell lines: the human Cell Line Activation Test (h-CLAT). I. Optimization of the h-CLAT protocol. Toxicol In Vitro 20:767-773.

Basketter DA, Evans P, Fielder RJ, Gerberick GF, Dearman RJ, Kimber I. 2002. Local lymph node assay – validation, conduct and use in practice. Food Chem Toxicol. 40: 593-8.

Dean JH, Twerdok LE, Tice RR, Sailstad DM, Hattan DG, Stokes WS. 2001. Evaluation of the murine local lymph node assay. Conclusions and recommendations of an independent scientific peer review panel. Regul Toxicol Pharmacol 34:258-73.

Emter R, Ellis G, Natsch A. 2010. Performance of a novel keratinocyte-based reporter cell line to screen skin sensitizers in vitro. Toxicol Appl Pharmacol 245:281-290.

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.

Gerberick GF, Vassallo JD, Bailey RE, Chaney JG, Morrll SW, Lepoittevin JP. 2004. Development of a peptide reactivity assay for screening contact allergens. Toxicol Sci 81:332-43.

Gerberick GF, Vassallo JD, Foertsch LM, Price BB, Chaney JG, Lepoittevin JP. 2007. Quantification of chemical peptide reactivity for screening contact allergens: a classification tree model approach. Toxicol Sci 97:417-427.

OECD. 2012a. OECD Series on Testing and Assessment No. 168. The Adverse Outcome Pathway for Skin Sensitisation Initated by Covalent Binding to Proteins. Part 2: Use of the AOP to Develop Chemical Categories and Integrated Assessment and Testing Approaches. OECD Publishing: Paris.

OECD. 2012b. OECD Series on Testing and Assessment No. 168. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. Part 1: Scientific Assessment. OECD Publishing: Paris.

OECD. 2015a. Test No. 442C. In Chemico Skin Sensitization: Direct Peptide Reactivity Assay (DPRA). In OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. OECD Publsihing: Paris.

OECD. 2015b. Test No. 442D. In Vitro Skin Sensitisation: ARE-Nrf2 Luciferase Test Method. In OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. OECD Publishing: Paris.

OECD. 2016. OECD guideline for the testing of chemicals: human Cell Line Activation Test (h-CLAT). http://www.oecd-ilibrary.org/docserver/download/9716121e.pdf?expires=1472755387&id=id&accname=guest&checksum=094DA96195CA77C00F5FA1C0790057C3 [October 25, 2017]

Piroird C, Ovigne JM, Rousset F, Martinozzi-Teissier S, Gomes C, Cotovio J, Alépée N. 2015. The Myeloid U937 Skin Sentization Test (U-SENS) addresses the activation of dendritic cell event in the adverse outcome pathway for skin sensitization. Toxicol In Vitro 29: 901-16.

Ramirez T, Mehling A, Kolle SN, Wruck CJ, Teubner W, Eltze T, Aumann A, Urbisch D, van Ravenzwaay B, Landsiedel R. 2014. LuSens: a keratinocyte based ARE reporter gene assay for use in integrated testing strategies for skin sensitization hazard identification. Toxicol In Vitro 28: 1482-97.

Strickland J, Zang Q, Paris M, Lehmann DM, Allen D, Choksi N, Matheson J, Jacobs A, Casey W, Kleinstreuer N. 2017. Multivariate models for prediction of human skin sensitization hazard. J Appl Toxicol 37: 347-60.

Zang Q, Paris M, Lehmann DM, Bell S, Kleinstreuer N, Allen D, Matheson J, Jacobs A, Casey W, Strickland J. 2017. Prediction of skin sensitization potency using machine learning approaches.