Allergic contact dermatitis presents a concern for developers of personal care, chemical, pharmaceutical, and medical device products. The development of non-animal methods to assess skin sensitization is a priority due to the EU cosmetics testing ban, the 2018 REACH deadline, and the goal of reducing animal use. Currently approved methods use either guinea pigs (GPMT) or mice (LLNA) to assess skin sensitization after the test substance has been intradermally injected or applied to their skin. Parameters such as redness, itchiness, scaling, and inflammation or increased cell count in lymph nodes are used to rate the skin sensitizing hazard or potency of the chemical. The SenCeeTox assay represents a method to assess skin sensitizing potentials and potency of chemicals in a tiered approach without the use of animals.

This study builds upon previous studies (McKim et al., 2010; McKim et al., 2012) showing that the in vitro SenCeeTox assay can correctly identify and categorize chemical sensitizers when used in-house. The aim of this project was to further validate the SenCeeTox assay by conducting an inter-laboratory validation at the Flemish Institute for Technological Research (VITO).

**Methods**

MatTek's three-dimensional human skin model, Epiderm™ was treated in triplicate with six concentrations of each blinded test article. The test articles evaluated were: metol, isoeugenol, 2,3-butanedione, 2-mercaptobenzothiazol, eugenol, 1-chloro-2,4-dinitrobenzene, glycerol, 2-hydroxyethylmethacrylate, 2-hydroxyethylacrylate, and lactic acid.

- **Following 24 hr exposure to the test articles, the following endpoints were measured:**
  1. Cytotoxicity was assessed by measuring lactate dehydrogenase (LDH) in tissue supernatant.
  2. The ability of each chemical to directly react with glutathione (GSH).
  3. Expression of seven genes controlled by the Nrf2/Keap1 complex and the AR are modulated in the EpiDerm® model.

**Advantageouslux Pathway**

- The cytotoxicity, GSH depletion, and potency of gene expression (lowest concentration that produces a significant increase, number of genes responding and the magnitude of induction) results were analyzed with a proprietary algorithm to generate an In Vitro Toxicity Index (IVTI) for each test article and predict each chemical’s likelihood of causing a human sensitization reaction.

**Nrf2/ARE Signaling Pathway**

**Results**

**Conclusions and Future Directions**

**SenCeeTox® Advantages**

- Provides potency categorization (one potency category).
- Assay is applicable for soluble compounds, insoluble compounds or finished products.
- MatTek’s Epiderm™, a 3-dimensional human skin model, allows topical application of the test material.
- Mechanistically based: measures key events in the AOP for skin sensitization including protein activity (GSH depletion) and increased expression of genes regulated by the ARE and XRE pathways in keratinocytes (see Fig. 1).
- Complies with current European Union, Indian, and Israeli requirements that cosmetics not be tested on animals.
- Completely replaces animal use and reduces time and cost as compared to animal testing.

**References**