

Further Evaluation of Chemicals and Mixtures for Skin Sensitization Potential and Potency Using a Reconstructed Human Epithelium (3D) Tissue Model and the IVSA

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ABSTRACT

Human skin models release IL-18 in response to a wide range of dermal sensitizing chemicals. Using a 3D skin model (epiCS[®]) in the *In Vitro* Sensitization Assay (IVSA), we measured IL-18 secretion as a biomarker of sensitization. In this test model we are able to achieve 90% accuracy when testing 20 test chemicals (11 sensitizers, 7 non-sensitizing irritants and 2 non-toxic materials). Further analysis of the data revealed that test chemical concentrations that induced a 2-fold increase in IL-18 secretion (Stimulation Index; SI-2) allowed for derivation of potency categorizations. The IL-18 SI-2 was proportional to the potency of the sensitizer. A limitation of all OECD-validated *in vitro* sensitization assays is that test substances that are mixtures are often not compatible with the test system. An advantage of the epiCS[®] test system is that the 3D tissues can be topically dosed, like skin, with a wide variety of substances, such as liquids, gels, powders, and waxes. Next, we explored the feasibility of expanding IVSA's applicability domain. We obtained sensitizing and non-sensitizing mixtures (as listed on their SDSs) from several commercial sources used in different industries for assessment. These included hair dyes, caulking material, adhesives, antimicrobial fuel additive and propolis (dietary supplement). A positive response (SI \geq 2) was detected for all the known sensitizing mixtures. In summary, the IVSA was able to correctly distinguish sensitizing chemicals and mixtures from non-sensitizing materials with high accuracy and sensitivity.

INTRODUCTION

The CDC estimates more than 13 million individuals in the US are likely exposed to chemicals that cause skin diseases. Many of these skin diseases are contact dermatitis, caused by exposure to allergic workplace chemicals, costing an estimated one billion dollars annually in healthcare and lost productivity (<http://www.cdc.gov/niosh/topics/skin/>). In ACD, keratinocytes in the skin are the first to contact and elicit a response to allergens during an exposure. The 3D human epidermis equivalent epiCS[®] is reconstructed from normal human primary epidermal keratinocytes. Keratinocytes have been shown to secrete a wide range of cytokines. Evidence demonstrates that cytokine IL-18 is an essential component of dermal sensitization. Most notable it has been shown using IL-18-deficient mice that IL-18 is not required for irritation contact dermatitis, but is required for an optimal ACD response (*Antonopoulos et al., 2008. Journal of Leukocyte Biology 83: 361-7*). An IL-18 endpoint has been used to predict sensitization in tissue models using filter paper applications (*Gibbs et al., 2013, Toxicology and Applied Pharmacology 272(2):529-41*). To identify sensitizing compounds, we measured IL-18 secretion from epiCS[®] after treatment with chemicals. Upon successfully testing individual chemicals, we next chose to test whether or not we could expand the applicability domain of the assay by testing commercially available mixtures.

MATERIALS AND METHODS

Sensitizers and irritants/non-sensitizers were topically applied directly to the stratum corneum of epiCS[®] tissues. The primary vehicles used were Ethanol and Acetone:Olive Oil (AOO). At 24 hours post-chemical application, media was sampled and analyzed by ELISA (MBL, Nagano, Japan) for secreted IL-18. IL-18 responses were measured as a **Stimulation Index (SI)**, a fold increase above vehicle control. Tissues were washed and then tissue viability was measured by the MTT assay. An **SI of 2.0** was chosen as a cut-off for a positive response. Mixtures were tested once. At least two independent experiments were performed for individual chemicals. The chemical concentration (%) that achieved an SI-2 with IL-18 in epiCS[®] tissues was calculated when possible. A chemical was considered positive if at least one concentration of the material tested positive in at least two independent experiments.

RESULTS

Table 1. Chemicals Tested in epiCS® IVSA

Chemicals Evaluated	IL-18 Result	IL-18 IVSA SI-2 TA% ^a
Sensitizers		
Citral	+	0.01
4-Nitrobenzylbromide	+	0.02
1-Chloro-2,4-Dinitrobenzene	+	0.03
p-Phenylenediamine	+	0.13
Glyoxal	+	0.23
Cinnamaldehyde	+	0.33
IsoEugenol	+	0.56
Eugenol	+	0.75
Resorcinol	+	2.90
α-HCA	+	8.08
Cinnamyl Alcohol	-	na
Irritants		
Lactic Acid	-	na
Phenol	-	na
Sodium Dodecyl Sulfate	-	na
Methyl Salicylate	-	na
Chlorobenzene	+	na
Salicylic Acid	-	na
Tween® 20	-	na
Non-Toxics		
Glycerol	-	na
Isopropanol	-	na

^aChemical concentration (%) calculated to induce an SI-2 with IL-18 in epiCS® tissues.
na = not applicable

Table 2. Contingency Table for Chemicals (SI-2 of IL-18)

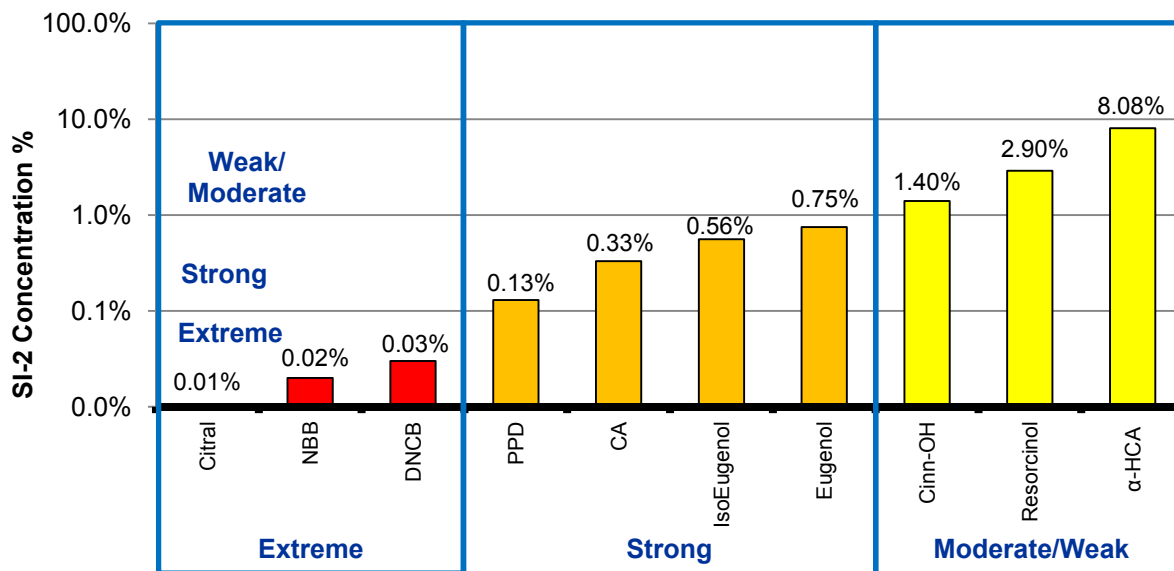
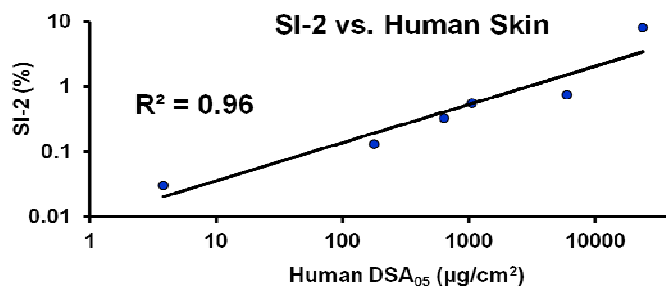
	Known +	Known -	Total
Tested +	10	1	11
Tested -	1	8	9
Total	11	9	20
Accuracy	90%	(18/20)	
Sensitivity	91%	(10/11)	
Specificity	89%	(8/9)	
Positive Predictivity	91%	(10/11)	
Negative Predictivity	89%	(8/9)	

RESULTS (continued)

Table 3. Mixtures Tested in EpiCS® tissues

Off-the-Shelf Mixtures Evaluated	IL-18 Result	IL-18 IVSA SI-2 TA%
Mixtures in which the SDS indicates sensitization potential		
Thread locker/Adhesive	+	0.83
Biocide Fuel Additive	+	0.87
Caulking Material	+	7.50
Dietary Supplement (Propolis Mixed in Honey)	+	26.17
Hair Dye	+	nd
Mixtures in which the SDS has no available data for sensitization		
Hypoallergic Shampoo	+	1.13
Fuel and Tank Cleaner	+	1.50
Hair Dye (PPD and Ammonia Free)	+	4.11

Figure 1. Concentration of Chemical Inducing an IL-18 SI-2 is Proportional to the Potency of the Sensitizer

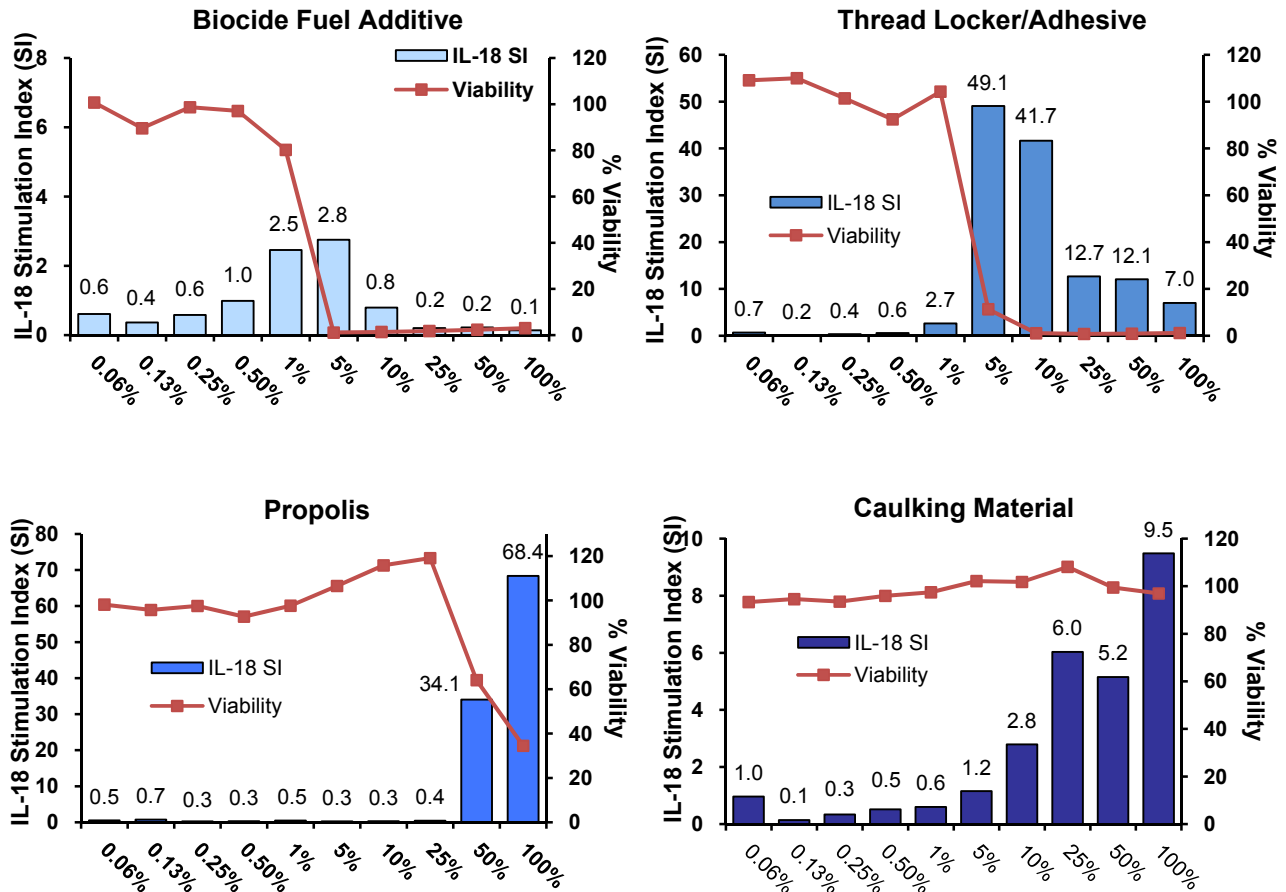

 Figure 2. Correlation of IVSA Results and Human DSA₀₅


SI-2 (%) = Test Article Concentration (%)
Inducing IL-18 SI-2

Human DSA₀₅ = Induction dose per skin
area, in µg/cm²

RESULTS (continued)

Figure 3. Examples of IL-18 SI and Viability Responses to Mixtures in IVSA



= SI \geq 2.0 (Positive IL-18 Response)

CONCLUSIONS

- The IVSA (using epiCS[®] tissues) correctly predicted sensitization potential with 90% Accuracy and 91% Sensitivity (20 chemicals).
- The IL-18 SI-2 can often predict the relative sensitization potency of a chemical.
- The presence of the stratum corneum on the tissue allows for direct application of a variety of materials, obviating the need for full solubilization of a test material, which is required for many other assays.
- Commercially available mixtures are able to generate significant and different types of IL-18 responses in epiCS[®] tissues.