

A Highly Differentiated 3D Epidermal Skin Model (epiCS®) to Characterize Skin Sensitizers in Mixtures

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ABSTRACT

Strong support exists in the consumer products, pharmaceutical and cosmetics industries to develop *in vitro* assays to identify and characterize skin sensitizers. We have adapted the keratinocyte interleukin-18 (IL-18) response assay developed by Corsini and colleagues in 2009 for use with epiCS®, a highly differentiated epidermal model. epiCS RHE comprises primary human keratinocytes, grown into a 3D skin model at the air-liquid interface. The IL-18 response assay measures release of this cytokine into the culture medium of test substance-treated tissues over 24 hours, by ELISA. Results are expressed as a Stimulation Index (SI) when compared to vehicle control treatment and an SI value of >1.6 was selected as indicative of a positive sensitizer response (based on the results here and previous experiments with other 3D skin models). Four vehicles – Ethanol, AOO 4:1, Ethanol:DMSO 4:1, and Petrolatum – were compared for their effects on three sensitizers (dinitrochlorobenzene, eugenol and citral). Ethanol:DMSO 4:1 yielded the highest SI values for the three sensitizers. The basal amount of IL-18 release for all vehicles ranged from 1.1 pg/ml to 17 pg/ml. DNCB at 0.15% consistently produced the highest SI values with the vehicles, and a clear dose-response at concentrations of 0.018%-0.3% was seen. In addition, a dose-response was caused by nitrobenzylbromide (0.025%-0.2%) and p-phenylenediamine (PPD, 0.1%-2.0%) in ethanol vehicle. However, resorcinol was clearly positive (SI = 2.7) in AOO 4:1 vehicle. Lastly, consumer product mixtures, specifically hair dyes containing PPD to yield light brown, dark brown and black colors were tested neat on the epiCS® tissues. A clear increase in IL-18 over sham-treated controls (13 pg/ml) was observed in hair dyes ranging from 71 pg/ml to 76 pg/ml to 85 pg/ml, respectively (SI values 5.7, 6.0 and 6.8). Further work is necessary to investigate different categories of chemicals and mixtures and their mechanisms of toxicity, to adapt and improve the prediction model accordingly.

INTRODUCTION

The 3D human epidermis equivalent epiCS® is reconstructed from normal human primary epidermal keratinocytes. epiCS® is validated for the classification of compounds according to OECD test guideline 431: "In Vitro Skin Corrosion: Human Skin Model Test." The European Centre for the Validation of Alternative Methods (ECVAM) has accepted this method to distinguish between corrosive and non-corrosive chemicals. Many final consumer products cannot be solubilized, a requirement for testing in cell lines. The use of a 3D human skin model overcomes this limitation by allowing direct topical application of products in many chemical forms and complex mixture formulations (e.g., liquids, solids, gels and lotions).

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 (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 pure chemicals as well as mixtures.

MATERIALS AND METHODS

Sensitizers and irritants/non-sensitizers were topically applied directly to the *stratum corneum* of epiCS® tissues. The primary vehicle used was ethanol and Acetone:Olive Oil (AOO). At 24 hr post-chemical application, media was sampled and analyzed by ELISA (MBL, Nagano, Japan) for secreted IL-18. IL-18 responses were measured as a fold increase above vehicle control. Tissues were washed and then tissue viability was measured by the MTT assay.

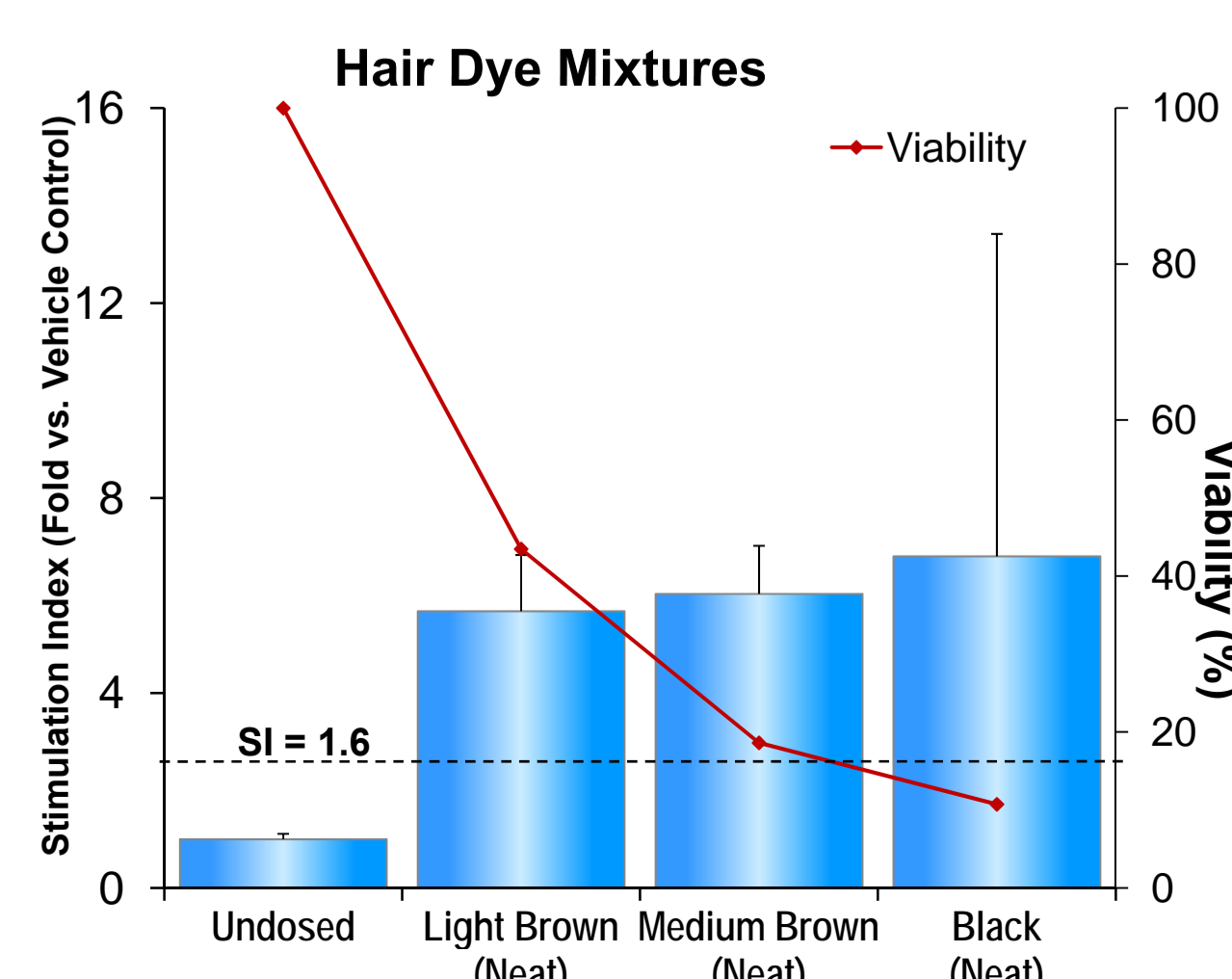
RESULTS

Table 1. Chemicals Tested in epiCS®

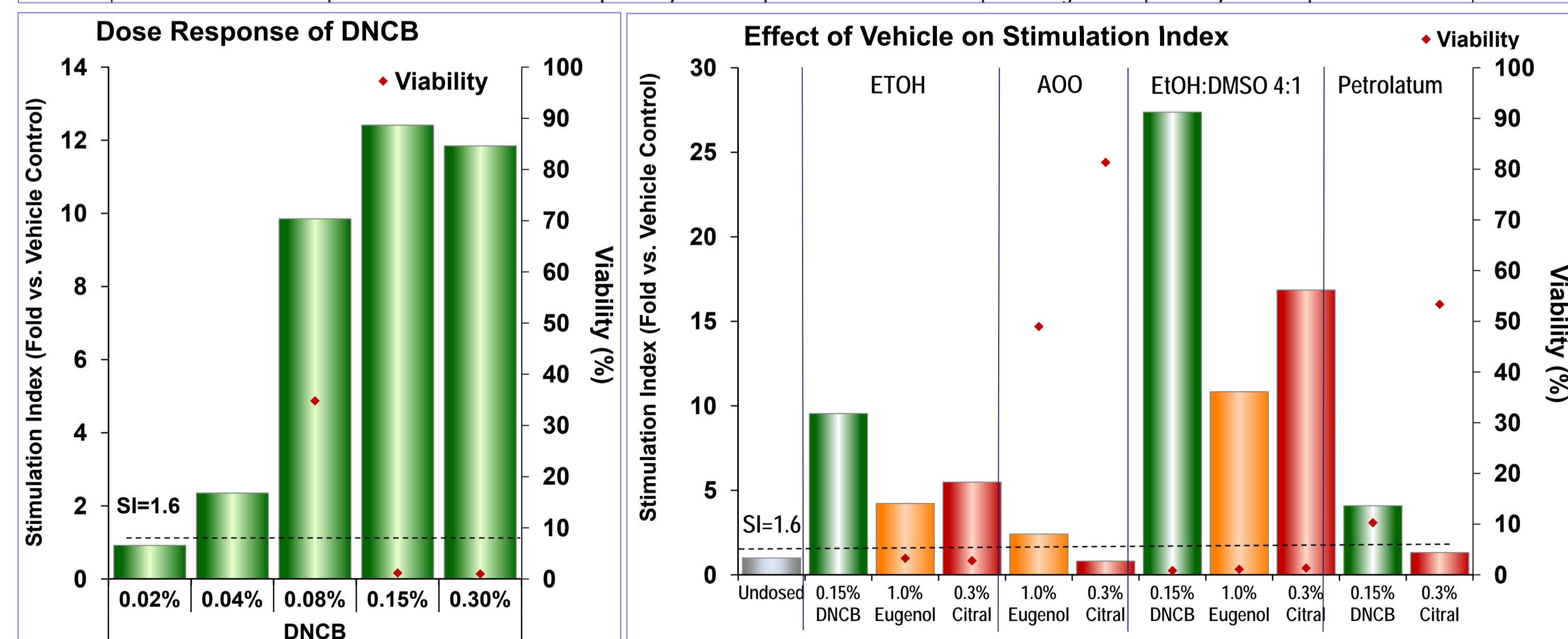
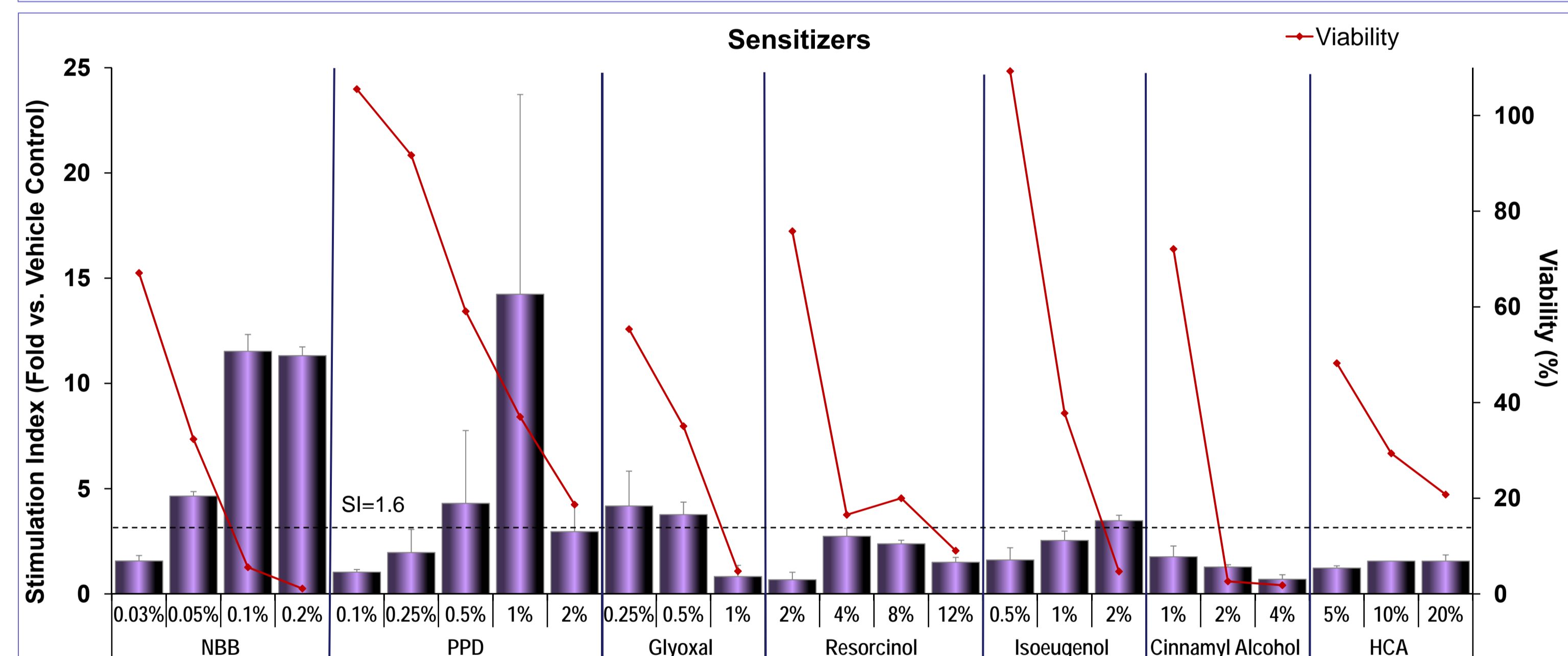
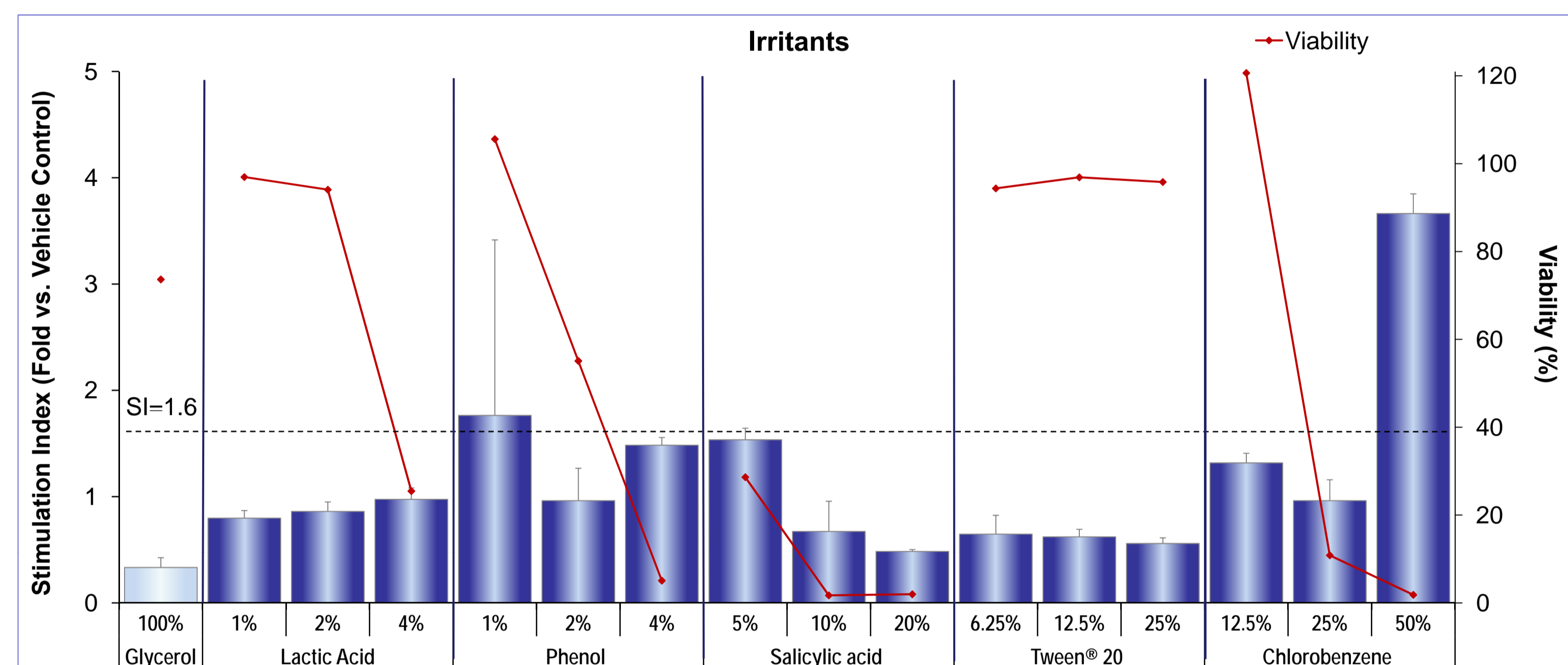
Chemicals	LLNA Classification	IL-18 Result
Sensitizer		
4-Nitrobenzylbromide (NBB)	Extreme	+
1-Chloro-2,4-Dinitrobenzene (DNCB)	Extreme	+
p-Phenylenediamine (PPD)	Strong	+
Glyoxal	Moderate	+
Resorcinol	Moderate	+
α-HCA	Moderate	+
Isoeugenol	Moderate	+
Eugenol	Weak	+
Cinnamyl Alcohol (Cinn-OH)	Weak	+
Citral	Weak	+
Irritants		
Lactic Acid	Non-sensitizer	-
Phenol	Non-sensitizer	+
Chlorobenzene	Non-sensitizer	+
Salicylic Acid	Non-sensitizer	-
Tween® 20	Non-sensitizer	-
Non-Toxins		
Glycerol	Non-sensitizer	-

Table 2. Contingency

	Known +	Known -	
Tested +	10	2	12
Tested -	0	4	4
	10	6	16
Accuracy	88%	(14/16)	
Sensitivity	100%	(10/10)	
Specificity	67%	(4/6)	
Positive Predictivity	83%	(10/12)	
Negative Predictivity	100%	(4/4)	



RESULTS



CONCLUSIONS

- An SI of 1.6 was calculated to be the best fit cutoff to distinguish sensitizers from irritants and non-toxic chemicals.
- epiCS® tissues generated IL-18 responses with 88% Accuracy and 100% Sensitivity using a panel of 16 test chemicals.
- Commercially available hair dyes of different colors generated significant increases in IL-18 responses. Demonstrating epiCS® tissues are capable of identifying and measuring sensitization responses from complex mixtures.