Appendix E

Calculation of Weighted LLNA EC3 for CMIT/MIT

Composition of CMIT/MIT mixture = 10.8% CMIT + 3.4% MIT = 14.2% active ingredient

Calculation of a weighted average CMIT/MIT EC3 uses the CMIT EC3 and the MIT EC3 and assumes that the sensitization effects of these ingredients are additive and that no other ingredients in the mixture impact the sensitization potential. The EC3 of each component is multiplied by its fraction (%) in the mixture, the two products are added, and then the sum is divided by the fraction of active ingredients (14.2%) in the CMIT/MIT mixture.

The NICEATM LLNA database (https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/immunotoxicity/llna/index.html) has two EC3 values for CMIT: 0.009% (vehicle = dimethyl formamide) and 0.01% (vehicle = acetone:olive oil). The NICEATM approach averages these for EC3 = 0.0095% for CMIT. The Dow approach would use EC3 = 0.01% for CMIT because the vehicle for this test was acetone:olive oil.

Calculation of the weighted LLNA EC3 for CMIT/MIT using the NICEATM approach and comparison to NICEATM in vivo data:

- Weighted EC3 = [(0.0095 * 10.8) + (1.154 * 3.4)]/14.2 = **0.28%** EC3 for CMIT/MIT
- Measured LLNA EC3 for CMIT/MIT = **0.018%**

Calculation of the weighted LLNA EC3 for CMIT/MIT using the Dow approach, and comparison to Dow in vivo data:

- Weighted EC3 = [(0.01 * 10.8) + (0.863 * 3.4)]/14.2 = **0.21%** EC3 for CMIT/MIT
- Measured LLNA EC3 = **0.002%**

Chemical	Dow LLNA EC3 (%)	NICEATM LLNA EC3 (%)	Weighted EC3 (%)	DA: ANN D_hC[b] EC3 (%)[a]	DA: ANN D_hC_KS[c] EC3 (%)[a]
CMIT		0.0095			
CMIT/MIT	0.002	0.018 (0.0011-0.034)	0.21 (Dow) 0.28 (NICEATM)	0.121 (0.119 – 0.123)	0.492 (0.4 – 0.605)
MIT	0.863	1.154 (0-3.476)		1.775 (1.732 – 1.818)	0.826 (0.759 – 0.9)

[a] Numbers in parentheses are the 95% confidence intervals
[b] Model 1 from Hirota et al. 2015: DPRA + h-CLAT
[c] Model 4 from Hirota et al. 2015: DPRA + h-CLAT + KeratinoSens