methylacrylate. Using relative ACD-elicitation potencies, $F = \Lambda_{p^*}/L_{p^*}$, to compare dermal loads $L = L_{p^*}$ at which $R(F\ L) = p^*$ for the five OSs, a common LN ACDER model, $R_o(F\ L)$, fit the PT data for Ni and all five OSs. Using this modeling approach with far more limited ACDER data (one $\{L_p, p\}$ observation/chemical), additional F values were estimated for nine (meth)acrylates. The $R_o$ risk model described is proposed as a default method to assess ACDER quantitatively for a wide range of organic dermal sensitizers, until better methods become available.

## 2952 Differential Skin Inflammatory Responses in Irritant Contact Dermatitis

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Irritant contact dermatitis (ICD) is characterized as an inflammatory response caused by topical exposure to irritants and is regulated by cytokines. However, intrapersonal and interpersonal variability is observed with ICD, thus suggesting that the pathophysiology is more complex than originally thought. Differential cytokine profiles and dermal cellular phenotypes are thought to be responsible for this variability, and are modulated by the nature of the irritant. Immune phenotype, such as Th1- and Th2-dominance, and interleukin 6 (IL-6) deficiency are also postulated to contribute to this variability. To determine how these factors modulate the pathomechanism of ICD, C57BL/6 ("Th1 dominant"), Balb/c ("Th2 dominant"), and IL-6 deficient (IL-6KO) mice were exposed to the occupational irritants, benzalkonium chloride (BKC) and JP-8 jet fuel for 3 days to induce ICD. Lesions were obtained for histological examination, assessment of cytokine expression, and identification of dermal cells. Histopathology revealed that BKC induces greater cellular infiltration than JP-8, especially in C57 and IL-6KO mice. However, Balb/c mice did exhibit epidermal hyperplasia after JP-8 exposure. Following BKC exposure, C57 showed up-regulation of IL-1β, IL-18, TNF-α, CCL3, CCL4 and CXCL2 compared to Balb/c mice and higher expression of IL-27, CCL3, CCL5, CCL11, and CXCL2 compared to IL-6KO. After JP-8 exposure, C57 exhibited decreased expression of IL-28, IL-18, CCL2, and CCL5 compared to Balb/c mice and reduced expression of IL-10 compared to IL-6KO. C57 mice exposed to BKC had greater infiltration of neutrophils and granulocytes compared to Balb/c, but following JP-8 exposure Balb/c mice had more granulocyte infiltration. IL-6KO mice responded to irritant exposure by increased infiltration of dendritic cells compared to C57 mice. Overall, C57 mice develop a more severe form of ICD in response to BKC in comparison to Balb/c; however, Balb/c mice manifest a quicker inflammatory response to JP-8 exposure. In both Th1- and Th2- dominant phenotypes, the pathomechanism of ICD induced by BKC involved activated neutrophils, whereas JP-8 induced ICD did not. IL-6 deficiency appears to cause greater dendritic cell infiltration into the dermis in response to BKC and JP-8. The aforementioned findings may provide a better understanding of the pathology of ICD and insight into specific targets for therapeutic immunomodulation.

## 2953 Understanding Chemical Allergen Potency: Strength of Dendritic Cells Activation

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For complete replacement of animals in the assessment of skin sensitization, evaluation of relative potency is necessary. The mechanism that explain the strength of allergic contact dermatitis reactions to weak, moderate, strong, and extreme sensitizers is still a challenge. Development of allergic contact dermatitis requires activation of innate immune cells. Starting from three presuppositions: 1) the extent of chemical allergen-induced dendritic cells (DC) activation/maturation and lifespan drive the quality and magnitude of T cell activation; 2) PKC activation is necessary and sufficient to drive dendritic cells differentiation; 3) PKCβ activation is central in chemical allergen-induced CD86 expression and IL-8 production, two markers selectively upregulated by allergens in DC; this work was designed to characterize *in vitro* the extent of DC maturation/activation and longevity induced by chemical allergens of different potency (i.e. Bandrowski base, benzoquinone, diethylmaleate, hydroxycitronellal and imidazolidinyl urea) by assessing markers related to signals required for full T cell activation (upregulation of CD80, CD86, HLA-DR; and IL-1, IL-6, IL-8, IL-12 production), together with cell survival and PKCβ activation. The human promyelocytic cell line THP-1 was used as surrogate of DC. Cells were treated with increasing concentrations (using the CV75 as the highest concentration) of the selected chemicals for 5-60 min for PKCβ activation, and for 24-72 h for surface marker expression and cytokine release. Results indicate that the strong allergen benzoquinone induces a more rapid PKCβ activation (5 min) and CD86 upregulation (24 h) compared to the moderate and weak allergens. Furthermore, HLA-DR was upregulated at 72 h only by Bandrowski base and benzoquinone. Cell viability and CD80 expression was similar for all allergens. Regarding cytokine production, all three allergens induced a dose related IL-8 release, while IL-10 was induced only by benzoquinone and diethylmaleate, and IL-12 only by Bandrowski base and benzoquinone. The release of IL-6 was below the limit of detection. Overall, results suggest that allergens of different potency differently activate DC, with strong allergen inducing a higher degree of maturation compared to moderate and weak allergens, which may explain the different potency observed *in vivo*. Acknowledgments. *(This project was funded by the Johns Hopkins CAAT Grant 2016)*

## 2954 Predictive Possibility of the *Ex Vivo* Cytokine Assay and the Flow Cytometric Measurement of the B/T Cell Population in LNCs on Skin Sensitization Potency

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The traditional LLNA (tLLNA) requires radioisotopes to evaluate skin sensitization response, but many countries have strict regulations on the use of radioisotopes on animals *in vivo*. For that reason, the Japanese Center for the Validation of Alternative Methods (JaCVAM) developed and validated the LLNA: DA (OECD TG 442A) and the LLNA: BrdU-ELISA (OECD TG 442B), in which radioisotopes are not needed. The Korean Center for the Validation of Alternative Methods (KoCVAM) also developed the non-radioisotopic LLNA: BrdU-FCM. This assay can be used to identify skin sensitizing chemicals and evaluate skin sensitization potency in the same way as the LLNA, the LLNA: BrdU-ELISA and the LLNA: DA. Activation of T lymphocytes is the fourth key event of the adverse outcome pathway for skin sensitization. The cytokines secreted by TH1/TH2 cells play a major role in immune response. Allergen treatment induces an increase in the lymphocyte population (B/T cells). Notably, the number of the B cell population significantly increases when skin sensitization response occurs. The B/T cell ratio can be calculated by cytometric analysis of B cells (B220 positive cells) and T cells (CD4ee positive cells). The LLNA: BrdU FCM allows for the conduct of the *ex vivo* cytokine assay and the flow cytometric measurement of the B/T cell population in LNCs without sacrificing extra animals. We performed the *ex vivo* cytokine assay and the flow cytometric measurement of the B/T cell population in LNCs in order to evaluate skin sensitization potency. We compared the test results of the LLNA: BrdU-FCM with those of the *ex vivo* cytokine assay and the flow cytometric measurement of the B/T cell population in LNCs using the reference chemicals listed in OECD TG 429. *(Funding Source: This research was supported by grants (16181MFDS386) from the Korea Ministry of Food and Drug Safety in 2016).*

## 2955 COCAT-Advanced *In Vitro* Assessment of Skin Sensitization Potency of Chemicals Using THP-1 Cells in Coculture With HaCaT Keratinocytes

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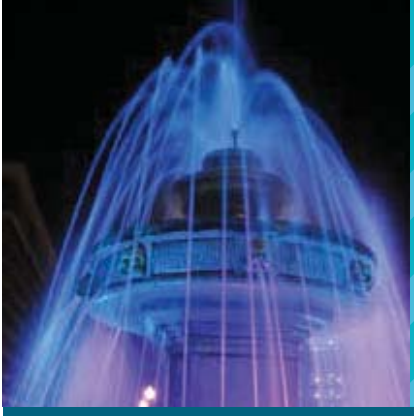
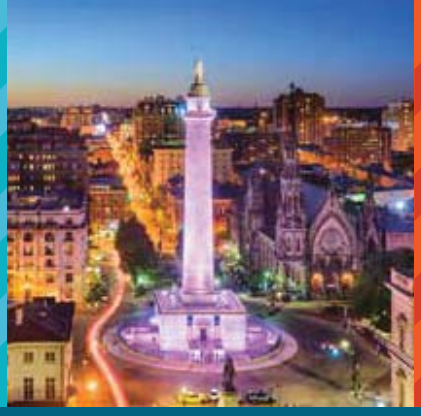
The identification of skin sensitization hazard as well as potency assessment is crucial for quantitative risk assessment of chemicals. While some *in chemico* and *in vitro* methods have already been validated and regulatory accepted for hazard identification, none of these methods have been validated for the estimation of skin sensitization potency. We evaluated the capacity of our coculture model (THP-1 cells in coculture with HaCaT keratinocytes, COCAT) for the prediction of sensitization potency. And further, we tested whether the COCAT can separate structurally related molecules with different potencies. We investigated the upregulation of CD86 and CD54 on THP-1 cells after exposure to 33 chemicals in the optimized 96 well plate format. The test set contained 21 sensitizers (8 haptens, 8 prohaptens and 5 prehaptens) and 12 non-sensitizers. As measure for potency we used the lowest concentration needed to exceed one of the empirically determined thresholds for positivity (ΔMFI ≥ 1.8 for CD86, ΔMFI ≥ 50 for CD54). Prediction of GHS (Globally harmonized system implemented in the Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures) potency sub-categories 1A or 1B applying a threshold of 300 µM resulted in an accuracy of 85%. Comparison of results obtained for all test chemicals with continuous ranking of *in vivo* skin sensitization potency (EC3, local lymph node assay) revealed a very good correlation (Spearman $r = 0.69$, $p = 0.006$). More in-depth analysis of the results obtained in COCAT for structurally related sensitizers (Bandrowski's base, *para*-phenylenediamine, *para*-toluylenediamine, 2-methoxymethyl-*para*-phenylenediamine; cinnamic aldehyde, cinnamic alcohol; 4-aminophenol, 3-aminophenol) revealed distinct and reasonable responses reflecting *in vivo* differences. These promising data demonstrate that the COCAT, which integrates keratinocyte responses with activation of THP-1, allows the simultaneous evaluation of sensitization potential and potency.

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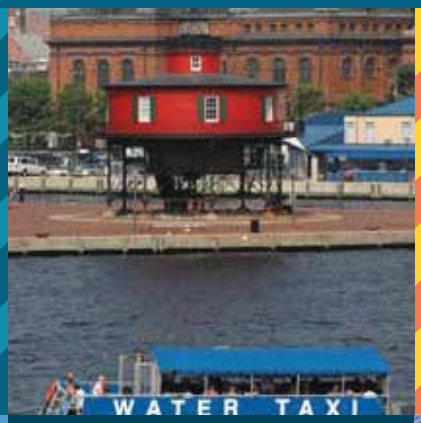


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