

results along with our previous findings indicate that a murine RH model for low molecular weight chemicals may require additional IT exposures and/or exposures *via* a different route (dermal). Additionally, the increase in BALF total cell counts over the exposure time course and the slight changes in lung histopathology suggest that additional IT exposures may be helpful in producing a more robust murine model for respiratory hypersensitivity if exclusive respiratory exposure is desired. (This abstract does not reflect EPA policy.)

826 THE ROLE OF TUMOR NECROSIS FACTOR (TNF) α IN TOLUENE DIISOCYANATE (TDI) ASTHMA.

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Nearly 9 million workers are exposed to chemical agents associated with occupational asthma with isocyanates representing the chemical class most responsible. Isocyanate-induced asthma has been difficult to diagnose and control, in part because the biological mechanisms responsible for the disease and the determinants of exposure have not been well defined. Isocyanate-induced asthma is characterized by airway inflammation and we hypothesized that inflammation is a pre-requisite of isocyanate-induced asthma with tumor necrosis factor (TNF) α being critical to this process. To explore this hypothesis, TNF α receptor knockout (TNFR) and anti-TNF α antibody treated C57BL/6J mice were sensitized by subcutaneous injection (20 μ l on day 1; 5 μ l, days 4 and 11), and challenged 7 days later by inhalation (100ppb; days 20, 22 and 24) with toluene diisocyanate (TDI). Airway inflammation, goblet cell metaplasia, epithelial cell damage and non-specific airway reactivity to methacholine challenge, measured 24 hrs following the last challenge, were reduced to baseline levels in TNF α null mice. TNF α deficiency also markedly abrogated TDI-induced Th2 cytokines in airway tissues indicating a role in the development of Th2 responses. Intratracheal instillation studies (50 μ l, single dose, 0-58 mg/kg) with fluorescein-conjugated isothiocyanate (FITC) and intranasal studies (20 μ l, single dose, 0-10%) with TDI suggested that TNF α deficiency also resulted in a significant reduction in the migration of airway dendritic cells to the draining lymph nodes. Taken together, these results suggest that TNF α has multiple and central roles in TDI-induced asthma influencing both non-specific inflammatory processes as well as specific immune events.

827 TIME COURSE OF SPECIFIC ANTIBODY PRODUCTION FOLLOWING TOPICAL SKIN EXPOSURE TO DRY TRIMELLITIC ANHYDRIDE (TMA) POWDER.

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TMA is a low molecular weight chemical used in the solid form as a fine powder in industry. Workers have been reported to develop asthma after TMA exposure and specific anti-TMA IgE has been found in these workers. Although inhalation has been considered the major route of exposure leading to sensitization, the importance of dermal exposure in allergic sensitization is not known. The consequences of dermal exposure to dry TMA powder in a Brown Norway rat (BNR) model are explored in the present study. Dry TMA powder was applied to an area of the back which the hair had been carefully clipped, and occluded with surgical tape overnight. One group received a single application of TMA (20 mg) on day 0 and a second group received repeated exposure to 20 mg of TMA applied once per week for 4 weeks. Sera were taken on days 0, 7, 14, 21, 28 and 35, and analyzed for TMA specific IgE and IgG by ELISA. Skin from the application areas was taken for histopathological and immunohistochemical examination. Specific IgE and IgG could be detected in the sera of rats from both groups on day 14. Antibody levels increased and persisted through day 35 in both groups. Specific antibodies were not found in either unexposed or trimellitic acid exposed controls groups. Histopathology of TMA exposed skin was normal with no evidence of inflammation or tissue destruction. Immunohistochemical staining using autologous anti-TMA IgG demonstrated strong reaction of the TMA only at the stratum corneum. These data suggest that in BNR, TMA powder exposure to the skin produces persistent immunological sensitization, without adjuvants and at doses that do not produce inflammation at the application site. This persistent antibody response was demonstrated even after a single dermal exposure suggesting that the antigen(s) formed by TMA in the tissues is not readily cleared from the body.

828 QUANTITATIVE ANALYSIS OF ALLERGEN-PROTEIN REACTIVITY.

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Skin sensitization is an immunological response caused by low-molecular weight chemicals that come in contact with the skin. Currently, it is believed that four major factors are important for skin sensitization by a chemical: 1) ability to penetrate the skin, 2) possible biotransformation, 3) reactivity to proteins, and 4) recognition by the immune system to trigger a response. Currently, we rely on the Local Lymph Node Assay (LLNA) to identify potential skin sensitizers. In an effort to develop *in vitro* method(s) to predict skin sensitization, we are evaluating protein and peptide reactivity to skin allergens that have been tested by LLNA. Ten compounds, allergens and non-allergens, were selected: oxazolone, isocugenol, diethylenetriamine, sodium lauryl sulfate, lauryl gallate, hexane, hydroxycitronellal, phthalic anhydride (PA), 2,4-dinitrofluorobenzene (DNFB), 2,4-dinitrochlorobenzene (DNFB). To quantify protein reactivity, human serum albumin was reacted with the chemicals. The protein-ligand conjugates were determined by MALDI MS. The reactivity was rated based on the Michaelis-Menten kinetics data. Of the 10 compound tested, PA showed the greatest reactivity ($V_i = 7.7 \times 10^{-2} \text{ s}^{-1}$), followed by DNFB ($V_i = 7 \times 10^{-4} \text{ s}^{-1}$) and DNFB ($V_i = 4.2 \times 10^{-6} \text{ s}^{-1}$). In addition, we used the peptide Ac-RFAAKAA to test the reactivity of the chemicals to lysine. Of the 10 compounds, only PA, DNFB, and DNFB bound to lysine. Comparison of the protein reactivity data with LLNA data shows good, but not identical correlation, suggesting that other mechanisms such as skin penetration and immune response need to be considered for development of *in vitro* model. In summary, the results demonstrate a good correlation between the quantitative protein reactivity and LLNA data. Generation of quantitative protein reactivity data with allergens shows potential for use in the development of *in vitro* skin sensitization models.

829 EXPERIENCE WITH A RAT BASOPHILIC LEUKEMIA CELL LINE ASSAY FOR MEASUREMENT OF RAT OR MOUSE SPECIFIC IGE AS AN ALTERNATIVE TO PASSIVE CUTANEOUS ANAPHYLAXIS.

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Measurement of antigen specific IgE is a key endpoint in the evaluation of the allergenic potential of proteins. Currently, the most robust means of assessing this endpoint in rodents is passive cutaneous anaphylaxis (PCA). In this test, sera to be analyzed are injected intradermally in naïve recipients which are then challenged with the potential allergen. Although this approach is sensitive and specific, it does use animals. We report an *in vitro* alternative to the rat/mouse PCA test which involves passive sensitization of the cultured rat basophilic leukemia cell line (RBL-2H3) with serum from rats or mice sensitized to the protein of interest. The protein itself is then added to the culture, provoking the cross-linking of any specific IgE bound to the RBL cells, inducing mediator release in direct proportion to the amount of specific IgE present in the sera. The quantity of IgE is determined using a colorimetric estimate of one of those mediators, b-hexosaminidase, in the cell supernatant. The IgE dependency of the RBL-2H3 mediator release assay was verified by sensitizing RBL-2H3 cells using a panel of immunoglobulin (Ig) isotypes followed by an anti-mouse antibody. Heat treatment at 56°C for 30 minutes ablated both PCA and RBL-2H3 responses, confirming the specificity for IgE. Comparison of the mediator release from the RBL-2H3 with the reactions from PCA tests on the same individual sera ($n=19$) supported the utility of the RBL cell data. The coefficient of variation was typically <10% for sample replicates (normally triplicates), and <15% between assays. We have demonstrated, for the allergens examined to date (ovalbumin, peanut and soya), that the RBL-2H3 mediator release assay may be a potential replacement for PCA analysis.

830 IMMUNOMODULATION OF THE IGE RESPONSE TO NATURAL RUBBER LATEX (NRL) PROTEINS BY ENDOTOXIN AND GLUTARALDEHYDE.

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Reduction of the protein and powder content of NRL gloves has been recommended to reduce the incidence of latex sensitization and symptoms associated with NRL exposure. A better understanding of the effects of co-exposure with other



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An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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