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, TNFα receptor knockout (TNFR) and anti-TNFα antibody treated C57BL/6J mice were sensitized by subcutaneous injection (20 µl on day 1; 5 µl, 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 TNFα null mice. TNFα deficiency also markedly abrogated TDI-induced Th2 cytokines in airway tissues indicating a role in the development of Th2 responses. Intratracheal instillation studies (50 µl, single dose, 0-58 mg/kg) with fluorescein-conjugated isothiocyanate (FITC) and intranasal studies (20 µl, single dose, 0-10%) with TDI suggested that TNFα 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 TNFα 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 cornea. 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, isoeugenol, diethlenetriamine, sodium lauryl sulfate, lauryl gallate, hexane, hydroxycitronellal, phthalic anhydride (PA), 2,4-dinitrofluorobenzene (DNFB), 2,4-dinitrochlorobenzene (DNCB). 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 (Vi=7.7 x 10^-2 s^-1), followed by DNFB (Vi=7 x 10^-4 s^-1) and DNCB (Vi=4.2 x 10^-6 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, DNCB, 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 sensitising 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

irritant/sensitizers on the development of NRL allergy will be helpful in developing effective intervention strategies. These studies were conducted to investigate the effect of co-exposure to the common health care contaminants endotoxin and glutaraldehyde on the development of an IgE response to NRL. Endotoxin was tested at 50 –25,000 EU while concentrations of glutaraldehyde tested were representative of the concentrations found in cold sterilant solutions (0.1 - 1%) and aerosol levels surrounding the permissible exposure limits (PEL, 0.05 - 1ppm). Female BALB/c mice were concurrently exposed to endotoxin (intranasally, subcutaneously) or glutaraldehyde (dermally) and 25 µg of NRL proteins 5 days a week for up to 17 weeks. Mice were tail bled prior to exposure and bi-weekly thereafter to obtain total and specific serum immunoglobulin levels. Animals exposed concurrently to endotoxin and NRL exhibited a dose dependent decrease in total and NRL specific serum IgE levels reaching >50% suppression and increase in serum IgG1 and IgG2a levels. Mice exposed to glutaraldehyde in concentrations consistent with cold sterilant solutions exhibited decreases in total (>50%) or NRL specific IgE levels when compared to mice exposed to NRL alone. However, upon co-exposure to levels of glutaraldehyde in the range of the PEL a dose responsive increase in both total (3 fold) and NRL specific (>40 fold) IgE was observed. These studies demonstrated that endotoxin induced an immunoglobulin class switch whereas glutaraldehyde augmented the IgE response to NRL proteins.

831 IMMUNOGENICITY AND ALLERGENICITY OF SUBTILISIN PROTEASES ARE NOT DEPENDENT UPON ENZYME ACTIVITY.

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The subtilisin serine proteases derived from Bacillus spp. are occupational allergens in the detergent industry. These proteases will also induce robust immune and allergic responses in guinea pig and mouse models of allergy. Recent reviews of the characteristics of allergens suggest that for some, enzyme activity is important to inherent immunogencitiy and allergenicity (Stewart and Thompson, Clin Ex All, 1996, 26:1020; Aalberese, JACI, 2000, 106:228; Huby, et.al., ToxSci 2000, 55:235). In previous work, we showed that the proteolytic activity of subtilisins had adjuvant affects on the allergic response to non-protease proteins (Sarlo, et.al., JACI, 1997, 100:480). Here we show that proteolytic activity is not important to the inherent immunogenicity and allergenicity of the subtilisins. Proteolytic activity of the subtilisins Alcalase® and Savinase® was blocked by di-isopropyl flourophosphate (DFP). Weekly intratracheal (IT) instillation of 10ug Alcalase or DFP-Alcalase protein into Hartley female guinea pigs led to the production of equivalent allergic antibody titers to the active and inactive enzyme. IT instillation of 10ug Savinase or DFP-Savinase led to equal allergic antibody titers to active and inactive enzyme. Intranasal (IN) exposure of BDF1 mice on days 1, 3, 10, 17 and 24 to active subtilisin (MW = 26kD) or to inactive subtilisin fragments (MW </= 20kD) led to the production of IgG1 and IgE antibodies to the subtilisin. The IgE antibodies from the fragment exposed mice recognized the intact subtilisin and the fragmented protein in the rat PCA test. There was no difference between IgE titers of mice exposed to 1ug or 0.66ug subtilisin or fragmented subtilisin. Comparison of ED50 values from the IgG1 dose-response curves showed that the active protein was only 4.7x more immunogenic than the inactive fragmented subtilisin. These data show that de novo synthesis of subtilisin specific IgG and IgE antibody is not dependent upon the proteolytic activity of the enzymes.

832 ASSOCIATION OF IGG1 AND IGE ANTIBODY RESPONSES TO BOVINE ALBUMIN AND OVALBUMIN IN BDF1 AND BALB/C MICE.

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The BDF1 mouse has been used in the mouse intranasal test (MINT) for the assessment of the immunogenic/allergenic potential of enzymes. Previous work showed a correlation between IgG1 and IgE antibody responses to enzymes (Horn, et.al., Toxicologist, 1999: 1967(a)) and a strain dependency for rank order of potency (Sarlo, et.al., ToxSci, in press). To see if these findings extend beyond enzymes, we tested bovine albumin (BSA) and ovalbumin (OVA) in BDF1 and Balb/c mice. Animals received intranasal doses of BSA, OVA or the reference enzyme Alcalase in detergent matrix adjuvant on days 1, 3, 10, 17, and 24. Sera were collected on day 29. The protein specific IgG1 titers were measured by ELISA and specific IgE titers measured by rat passive cutaneous anaphylaxis (PCA) test. The dose of protein needed to give ½ maximal IgG1 antibody response (ED50) was used to rank the proteins more potent > less potent. In BDF1 mice, BSA was 17x less potent than Alcalase and OVA was 9x less potent than Alcalase. The IgE data support the IgG1 data: Alcalase > OVA > BSA. In contrast, the IgG1 response in Balb/c mice did not distinguish Alcalase from OVA but ranked BSA as 3x more potent than Alcalase. Balb/c mice did not make IgE antibody to BSA. The IgE response showed OVA as slightly more potent than Alcalase. The IgE response follows the IgG1 response in BDF1 but not in Balb/c mice. The BDF1 strain can distinguish "potency" differences among the 3 proteins using both IgG1 and IgE endpoints; this cannot be done in Balb/c mice. These data are similar to data generated with different enzymes and support the continued testing of BDF1 mice in the MINT as a predictive model of protein allergy.

833 USE OF THE MOUSE INTRANASAL TEST TO EVALUATE THE POTENTIAL OF A QUATERNIZED WHEAT PROTEIN TO INDUCE ALLERGIC ANTIBODY.

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Modified Protein W is a quaternized wheat protein with applications in personal care products. As a part of the safety assessment, it was necessary to evaluate the potential for this material to cause respiratory allergies in consumers who will come into contact with products containing this ingredient. The mouse intranasal test (MINT) was chosen as the model for allergenicity resulting from exposure to the material *via* the respiratory tract. The presence of allergic antibody was evaluated using a Passive Cutaneous Anaphylaxis (PCA) assay in the rat. Savinase, a proteolytic enzyme known to produce allergic antibody *via* the respiratory route, was used as a positive control. Groups of young adult BDF1 female mice (10 per group) were dosed with 1, 3 or 10 µg/dose of either modified Protein W or Savinase on days 1, 3 and 10, and weekly thereafter for 6 weeks. Dosing was done on anesthetized mice by placing a 50 µl drop containing the appropriate amount of test material on the nostrils. An additional group of animals was treated with diluent (distilled water). Half of each group of animals was sacrificed on day 29 of the study (after the fifth dose), and the remaining animals were sacrificed on day 57 (after nine doses). For the PCA, sera from the mice were diluted and injected intradermally (100 µl/injection) on the shaved backs of CD rats. Twenty-four hours later, each rat was challenged intravenously with a mixture of Evans blue dye and either the Protein W or Savinase, as appropriate. The PCA data on the sera derived from both the interim and terminal sacrifice animals indicated no allergic antibody activity in the sera from animals dosed with Modified Protein W or distilled water. Conversely, there was positive PCA activity in sera from mice treated with the positive control (Savinase). Based on these data we concluded that Modified Protein W does not pose a risk of inducing respiratory allergy in consumers when used in personal care products. This work was conducted for The McIntyre Group, University Park, IL.

834 ALLERGIC RESPONSES TO AN EXTRACT OF THE TOXIGENIC FUNGUS *STACHYBOTRYS CHARTARUM* BY BALB/C MICE.

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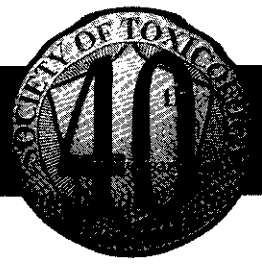
Exposure to *Stachybotrys chartarum* has been associated with the development of acute pulmonary hemorrhage in infants, allergic asthma in adolescents, and sick building syndrome in adult humans. The goal of this study was to assess soluble components of this fungus for allergenic potential. Five isolates of *S. chartarum* found in control houses (ie: no infant pulmonary hemorrhage) from the Cleveland, OH study [Etzel *et al., Arch. Pediatr. Adolesc. Med.* (1998) **152:** 757] were grown and combined in approximately equal weight amounts of mycelium and conidia. The resulting mass was extracted using Hanks' Balanced Salt Solution (HBSS) + 0.05% Tween-80, and sterile-filtered to form a crude antigen preparation (SCE-1). Female BALB/C mice were anesthetized and sensitized by 4 aspirations of 5, 10, or 20 µg of SCE-1 or HBSS (controls) over a 4-week period. Serum and bronchioalveolar lavage fluid (BALF) were collected before the fourth aspiration (day 0), and at 1, 3, and 7 days after the fourth aspiration. Lungs and kidneys also were fixed for histopathological examination. SCE-1-exposed mice displayed increased BALF total protein on days 0, 1, and 3, and increased LDH at days 1 and 3 at all doses compared to HBSS controls. BALF total cell numbers were elevated on each day for all SCE-1 doses. Differential counts of BALF cells showed neutrophilia on day 1 resolving by day 3, marked eosinophilia on all days, and increased numbers of lymphocytes at days 1, 3, and 7 for all doses. Serum and BALF total IgE levels were elevated at all days and doses. BALF IL-5 levels were greatly increased (7-fold) on day 1 for all doses, returning to control levels by day 3. No changes were observed



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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, roundtable, and poster sessions of the 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

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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