and protein levels in the ear, but not in the lymph nodes or blood serum. *In vivo* administration of neutralizing anti-TSLP antibody impaired allergic responses augmented by dermal exposure to 3% triclosan during sensitization to ovalbumin. These effects include a significant decrease in skin pathology with a reduction in skin hyperplasia, redness and scabbing. There was also reduced cellularity of the skin draining lymph nodes, decreases in B cell frequencies, and reduced cytokine and GATA-3 transcription factor protein expression in Th2 CD4 T cells. These observations were further extended to human skin tissue cultures where we found that *in vitro* application of triclosan also induced TSLP expression. To our knowledge, this is the first report that triclosan can induce TSLP expression as a possible mechanism for augmenting allergic diseases.

PS 672 Interrelationship of TDI- and HDI-Induced Respiratory Tract Irritation and Allergy in Rats

J. Pauluhn. *Toxicology (retired), Bayer AG, Wuppertal, Germany.*

Brown Norway rats were skin-sensitized either with toluene diisocyanate (TDI) or 1,6-hexamethylene diisocyanate (HDI) by two topical administrations to pre-dispose them for respiratory allergy. This was followed by three subsequent inhalation priming exposures, each spaced by 2 weeks. Such protocol was shown to minimize any aggravation of irritation-related inflammation but to amplify the allergic-inflammation. A dose-escalation bronchoprovocation challenge followed at either the first or fourth inhalation encounter with TDI or HDI. The escalation protocol utilized a constant concentration (Cconst) x variable bronchoprovocation time (tvar) protocol to quantify the elicitation-threshold Cxt in the absence and presence of prior inhalation priming exposures. The inhalation elicitation threshold dose on elicitation was determined based on measurements of neutrophilic granulocytes (PMN) in bronchoalveolar lavage fluid (BAL). The most appropriate Cconst was selected based on multiples ancillary pre-studies to achieve an 'optimal' penetration of the reactive and water soluble vapor into the bronchial airways at stable breathing conditions. PMNs in BAL of naïve and sensitized and inhalation primed rats were essentially identical at 900-1000 mg diisocyanate/m3 x min. In summary, this study supports the conclusion that TDI- and HDI-induced respiratory allergy is likely to be caused by multiple, sequentially occurring mechanisms: first, dermal sensitizing encounters high enough to cause systemic sensitization (by circulating Th2-lymphocytes). Second, when followed by inhalation exposure(s) high enough to initiate and amplify an allergic airway inflammation (irritant threshold dose exceeded), then a progression into asthma may occur. The workplace human-equivalent threshold Cxt-product (dose), from 'asthmatic' rats, was estimated to be 0.003 ppmV when applying a time-dosimetry-susceptibility adjustment factor of 100. This threshold is in close agreement of the current ACGIH TLV® of TDI and HDI and published human evidence.

PS 673 Differential Analysis of Protein Expression in RNA Binding Protein-Transgenic and Parental Rice Seeds Cultivated under Salt Stress and Allergenicity Test of the Rice Extracts

R. Teshima[1], R. Adachi[1], T. Shindo[2], A. Yamada[3], M. Ohsawa[2] and Y. Ozeki[3]. *[1]National Institute of Health Sciences, Setagaya-ku, Tokyo, Japan, [2]Food and Drug Safety Center, Hatano Research Institute, Hatano, Kanagawa, Japan and [3]Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan.*

Transgenic plants tolerant to various environmental stresses are being developed to ensure a consistent food supply. We used a transgenic rice cultivar with high saline tolerance by introducing an RNA-binding protein (RBP) from the ice plant (Mesembryanthemum crystallinum); differences in salt-soluble protein expression between non-transgenic (NT) and RBP rice seeds were analyzed by two-dimensional difference gel electrophoresis (2D-DIGE), a gel-based proteomic method. To identify RBP-related changes in protein expression under salt stress, NT and RBP rice were cultured with or without 200 mM sodium chloride. Only 2 protein spots differed between NT and RBP rice seeds cultured under normal conditions, one of which was identified as a putative abscisic acid-induced protein. In NT rice seeds, 91 spots significantly differed between normal and salt-stress conditions. Two allergenic proteins of NT rice seeds, RAG1 and RAG2, were induced by high salt. In contrast, RBP rice seeds yielded 7 spots and no allergen spots with significant differences in protein expression between normal and salt-stress conditions. Therefore, expression of fewer proteins was altered in RBP rice seeds by high salt than those in NT rice seeds. We also assessed allergenicity of NT and RBP rice seeds cultured with or without 200 mM sodium chloride in mice. BALB/c mice were sensitized orally for three weeks with rice protein extracts in linoleic acid/lecithin emulsion, and after the sensitization, they were twice orally challenged with the protein extracts. Significant differences in systemic anaphylaxis score and antigen-specific IgG1 titer were not observed between NT and RBP rice fed mice groups.

PS 674 Lung Toxicity and Allergy Responses in Mice Exposed to Nanoparticle Silver

C. E. McLoughlin, S. Anderson, J. R. Roberts, B. T. Chen, D. Schwegler-Berry, K. L. Anderson and K. Roach. *NIOSH, Morgantown, WV.*

With expansive use of silver nanoparticles (AgNP) in medical applications and consumer products, potential for worker exposure during manufacturing has become a concern. The goal of the current study was to characterize the potential effects of AgNP in an ovalbumin (OVA)-induced allergy model in BALB/c mice. To characterize the effects of AgNP alone, mice were exposed via pharyngeal aspiration (PA) to physiological dispersion medium (DM), 6.1 μg, 18.2 μg, or 73 μg AgNP. Twenty nm diameter AgNP with 0.3% wt polyvinylpovidone coating (NanoAmor, Inc.) were suspended in DM and sonicated before exposures. For all studies lung function was assessed using enhanced pause (Penh); bronchoalveolar lavage (BAL) was performed on the whole lung; BAL cells and fluid were retained for analysis of lung-associated injury, inflammation, phenotyping; and lymph nodes (LN) were harvested for enumeration and immune cell phenotyping. AgNP alone did not result in changes in Penh, while cellular responses in the lung indicated a dose-dependent injury and inflammation by post-exposure day 10, which began to resolve by day 29. Our previous studies have shown that exposure to AgNP prior to OVA-sensitization results in a trend for the development of airway reactivity. In this study, effects of AgNP on the elicitation phase were examined. Animals were sensitized with i.p. injections of OVA (dose) + aluminum hydroxide gel on days 1 and 10. To elicit an OVA-specific response, two PA challenges with OVA were given on days 19 and 28. AgNP were administered by PA on day 27. AgNP did not appear to significantly enhance Penh, and lung-associated LN total cell numbers, BAL cell numbers and IgE levels in serum were not increased above those of the allergy model control (OVA). The results indicate that although AgNP may have a moderate effect on airway resistance in the lung when administered before sensitization, they do not significantly alter the course of allergy development when given either prior to sensitization or during the elicitation phase.

PS 675 Gene Expression Changes Induced by Skin Sensitizers in THP-1 Cells: Possible Relationship to Protein Binding Domains

C. Ryan[1], Y. Shan[1], X. Wang[1], R. J. Dearman[2], I. Kimber[2] and G. Gerberick[1]. *[1]The Procter & Gamble Company, Mason, OH and [2]University of Manchester, Manchester, United Kingdom.*

The objective of our study is to generate a global view of transcriptional changes induced in target cells exposed to sensitizing chemicals with the goal of identifying common functional and regulatory pathways/molecules. Six replicate wells of THP-1 cells (human monocytic cell line) were exposed for 24h to single concentrations of 9 skin sensitizers (SS), 8 low molecular weight respiratory sensitizers (RS), and 9 non-sensitizers (NS) along with 11 vehicle control replicates. Cells were harvested for transcript profiling using the Affymetrix GeneTitan® U219 array plates. Statistical analyses at the individual gene level using cut-off values of a 1.5 fold-change with a false discovery rate <0.05 identified 181, 72, and 273 unique gene expression changes for the SS, RS and NS, respectively. The 181 unique genes for the SS were analyzed by hierarchical clustering. Examination of the SS heat map revealed possible clustering by protein reactivity domains. Enrichment analysis using MetaCore ™ software (Thomson Reuters) was conducted on all of the SS and individual SS grouped by domain using genes common to all in the group. The most significant pathways for all SS grouped together and the SN2 electrophiles were similar and included cholesterol biosynthesis, TREM1 and GM-CSF signalling. The top pathways for the MA electrophiles were related to oxidative stress and the activation of antioxidant defense system. Pathways activated by the one SS classified as an acylating agent included immune response pathways associated with antigen presentation, complement, and histamine signalling. These results suggest that the nature of the hapten-protein binding chemistry may influence activation of specific cellular pathways in immune cells.

PS 676 Increased Expression and Immunoregulatory Potential of microRNA 210 in a Murine Model of TDI Sensitization

C. M. Long[1, 2], N. B. Marshall[2], E. Lukomska[2], A. P. Nayak[2], P. D. Siegel[2], B. J. Meade[2] and S. E. Anderson[2]. *[1]Immunology and Microbial Pathogenesis Graduate Program, WVU, Morgantown, WV and [2]CDC-NIOSH, Morgantown, WV.*

MicroRNAs are single-stranded RNAs that exhibit functional significance through the regulation of gene expression; however, their roles in chemical sensitization have not been elucidated. Toluene 2,4-diisocyanate (TDI) is a low molecular weight chemical sensitizer that causes occupational asthma. In order to investigate

the functional role of miR-210 during TDI sensitization, BALBc mice were dermally exposed to TDI (0.5-4% v/v) and gene and protein expression were evaluated in the draining lymph nodes (dLN) and ears using RT-PCR and Western blot/flow cytometry, respectively. Increased total serum IgE levels confirmed sensitization in these mice. Augmented miR-210 expression was observed in the dLN and ears following exposure to both irritating and non-irritating TDI concentrations. Increased expression of a potential miR-210 inducer, hif1α, was observed in the ears following TDI exposure. Alterations in expression of confirmed miR-210 transcription factor target foxp3 were observed in the dLN and ears and decreases in predicted targets' (foxp3, runx1, runx3, and smad4) mRNA expression were observed in the dLN of TDI-exposed mice. These transcription factors are involved in regulatory T cell (T-reg) expansion and function; therefore, miR-210 may play a functional role in TDI sensitization by potentially inhibiting T-reg differentiation and function. This hypothesis is supported by the T-reg population's kinetics and the presence of miR-210 in CD4 T cells during TDI sensitization. Because the roles of T-regs and miR-210 in chemical sensitization have not been elucidated these data contribute to the understanding of the immunologic mechanisms of chemical induced allergic disease and are critical for the development of preventative and therapeutic strategies. This work was supported by internal funds from NIOSH/HELD.

PS 677 A Weight-of-Evidence Investigation of Novel Amino Alcohols for Skin Sensitization Potential Using *In Silico*, *In Vitro*, *In Vivo*, and Genomic Approaches

R. S. Settivari[1], N. N. Ball[1], N. Visconti[1], B. J. Hughes[1], D. Wilson[1], H. M. Hollnagel[1], R. Hunziker[1], R. M. Golden[1], M. B. Black[2] and D. R. Boverhof[1]. [1]*Predictive Toxicology, The Dow Chemical Company, Midland, MI and* [2]*The Hamner Institute for Health Sciences, Research Triangle Park, NC.*

Assessment of the skin sensitization potential of chemicals is an important component of the safety evaluation process. Key chemical and biological events underlying the skin sensitization process have been outlined, facilitating a weight of evidence (WoE) approach which aligns with an adverse outcome pathway. In a previous study, we evaluated the sensitization potential of four amino alcohols (aminocyclohexane (ACyHM), 2-aminopropanol (2-AP), aminomethylcyclohexanol (AMCyHOL) and aminododecane (ADD)) using *in silico* (DEREK, TIMES SS), *in vitro* (peptide reactivity assay; DPRA; KeratinoSens assay) and *in vivo* (Local Lymph Node Assay (LLNA) and guinea-pig maximization test (GPMT)) test procedures. The test procedures did not provide consistent results to firmly characterize the tested compounds as sensitizers or non-sensitizers (Hughes et al., *Toxicologist*, 126, 763, 2012). Therefore in the present study, a modified LLNA was conducted in which targeted gene expression changes were analyzed to identify LLNA sensitizers and false-positives. Gene expression changes were analyzed using 384-well TaqMan Array micro fluidic cards pre-plated with primers and probes for 38 prior generated gene signatures (Adenuga et al., *Toxicol. Sci.* 2012). The gene expression data was analyzed in a SAS-based JMP Genomics software program. The LLNA-gene expression data predicted AMCyHOL and 2-AP as sensitizers, ACyHM as border-line positive and ADD as a false-positive in LLNA. Taken together, AMCyHOL and 2-AP were concluded as sensitizers, ACyHM as a borderline sensitizer, while ADD as a non-sensitizer. When considering the present study results in the context of overall data generated, the lack of sufficient metabolic capability of *in vitro* studies posed challenges to provide conclusive evidence, while genomic approach provided added insight for WoE.

PS 678 An Integrated *In Vivo* Approach to Identify and Characterize Respiratory Sensitizers

K. K. Greenwood, N. Quay, K. M. Mahoney, R. Sura, M. R. Woolhiser and J. A. Hotchkiss. *TERC, Dow Chemical Company, Midland, MI.*

Currently, there is no fully accepted approach to identify chemicals as respiratory sensitizers. This study was designed to provide an integrated exposure-response profile to identify low molecular weight respiratory sensitizers and differentiate them from dermal sensitizers. Brown Norway rats received 2 equipotent dermal applications of 2,4-dinitrochlorobenzene (DNCB), hexyl cinnamaldehyde (HCA), trimellitic anhydride (TMA) or orthopthalaldehyde (OPA) in methylethyl ketone (MEK) to induce systemic sensitization. Two weeks later sensitized and control (MEK only) rats were exposed via inhalation to equipotent concentrations for 15, 30 or 60 minutes (n=4/dose) to characterize the dose-response of the dermal (DNCB, HCA) and respiratory (TMA, OPA) sensitizers. Functional respiratory parameters were measured during and after exposure. Serum IgE, pulmonary inflammation, airway hyperreactivity and histopathology were evaluated 1 day post exposure. Serum IgE levels increased ~ 2-fold, relative to baseline, in both TMA and OPA sensitized rats with no change detected in DNCB or HCA sensitized rats. Bronchoalveolar lavage (BAL) and histopathology indicated only inhalation of TMA induced pulmonary inflammation in sensitized, compared to control rats. At peak response, rats exposed to TMA had increased total BAL protein (4-fold) and LDH (2.5-fold) and total BAL cells were increased 2.5-fold (2.5- and 17-fold increases in eosinophils and neutrophils, respectively). Inhalation of TMA, DNCB or HCA had no effect on respiratory parameters during or after exposure in sensitized or control rats. Inhalation of OPA, however induced a rapid 40% decrease in breathing rate in both sensitized and control rats during exposure, indicative of a sensory irritant. The difference in the magnitude and character of the responses to the respiratory sensitizers TMA and OPA underscore the need to examine multiple phenotypic endpoints in any *in vivo* sensitization model as the effects of epithelial irritation and chemical reactivity can impact regional dosimetry and confound interpretation of results.

PS 679 Asbestos-Induced Pleural Fibrosis Involves Autoantibody-Mediated Protein Tyrosine Phosphorylation Pathway

R. L. Hanson, J. Gilmer, K. Serve and J. C. Pfau. *Department of Biological Sciences, Idaho State University, Pocatello, ID.*

Asbestos induced Lamellar Pleural Fibrosis (LPF) is an emerging disease with the potential to affect thousands of lives through environmental exposures. Unlike typical pleural plaques, LPF is progressive and results in severe limitations in pulmonary function. Many details of the mechanism of pathology remain unknown, but a key factor in the pathology is the presence of mesothelial cell autoantibodies (MCAA) that have been generated in response to asbestos exposure. Excessive collagen deposition, a key event in the development of fibrosis, has been observed in cell cultures exposed to these antibodies, along with activation of matrix metalloproteinases (MMP). The focus of this study is to identify the cellular signaling pathways affected by these antibodies. Using a variety of methods including flow cytometry and ELISA, we have demonstrated that increased tyrosine phosphorylation occurs in mesothelial cells exposed to amphibole asbestos induced MCAA. This is consistent with other studies demonstrating a role for tyrosine phosphorylation in fibrotic pathways and MMP activation. Serine and threonine phosphorylation were also examined, but statistically significant changes were not observed. This data will influence future efforts in characterizing the signaling pathways and identifying the target receptor for MCAA.

PS 680 Anti-Mesothelial Cell Autoantibodies Upregulate Transcription Factors Involved in Collagen Pathways

J. Gilmer and J. C. Pfau. *Biological Sciences, Idaho State University, Pocatello, ID.*

Amphibole asbestos exposure leads to autoantibody production in both mice and humans. These autoantibodies have been linked with pleural disease in the asbestos contaminated vermiculite mining community of Libby, Montana. However, the exact intracellular mechanism of how these autoantibodies cause an increase in collagen deposition remains unknown. This study sought to gain insight into the signal transduction linking autoantibody binding to collagen production in human mesothelial cells. In this study, transcription factor activation profiles were generated from human mesothelial cells treated with sera from the patients of Libby. Analysis of these profiles indicated differential expression in 31 of the 48 transcription factors analyzed compared to the untreated control. Thirteen of these transcription factors are associated with type 1 collagen deposition. These data suggest autoantibodies are directly involved in type 1 collagen deposition and may elucidate potential therapeutic targets for auto antibody mediated fibrosis.

PS 681 A Retrospective on Drug Allergy in Dogs at a US Veterinary Teaching Hospital

F. T. Fosset, J. Schimansky and S. N. Lavergne. *Comparative Biosciences, University of Illinois, Urbana, IL.*

Adverse drug reactions affect approximately 7% of the general population, represent the 4th-6th cause of death. A 1/3 of these reactions are immune-mediated (drug hypersensitivity [HS] or allergy). These events can be immediate (IgE-mediated; e.g. anaphylaxis) or delayed (IgG- or T cell-mediated; e.g. toxic epidermal necrolysis). The dog has been proposed as a potential animal model to study drug HS. Indeed, canine patients experience similar drug HS as observed in humans. However, the incidence of drug HS in dogs remains unknown. This retrospective study aimed to estimate this incidence and further characterize drug HS reactions in dogs seen between 01/01/2003 and 04/28/2014 at the veterinary teaching hospital (University of Illinois, USA). We identified 99 cases (12 immediate and 87 delayed reactions). Our primary results suggest an overall incidence of 0.33%. The suspected drug was an antibiotic for 63 cases (72.4%; 22 penicillins; 25 cephalosporins; 11 fluo-

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