1312 LOCAL ANTIBODY CLASS SWITCHING IN THE NASAL MUCOSA OF MICE WITH TDI RHINITIS.

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The role of antibodies in the etiology of occupational rhinitis and asthma caused by diisocyanate exposure is controversial. Serum levels of diisocyanate-specific IgE and IgG antibodies are detectable in approximately 30% and 80% of exposed workers, respectively. Importantly, specific IgE antibodies have high specificity for disease whereas specific IgG is present in the serum of exposed workers without disease indicating that these antibodies may be markers of exposure. The low detection prevalence of diisocyanate specific antibodies may be due to methodological limitations or low production and utilization of these antibodies at sites of active inflammation. Therefore, we used a previously developed murine model of TDI rhinitis to test the hypothesis that inhalation of diisocyanate vapor results in antibody class switching at the site of exposure, the nasal mucosa. Antibody class switching involves expression of germline transcripts and activation-induced cytidine deaminase (AICD) leading to recombination and production of a mature immunoglobulin transcripts. We employed PCR-based assays to detect germline and mature immunoglobulin transcripts, AICD and IL-4, a cytokine known to drive the process. Mice with TDI rhinitis showed increased expression of IL-4 and AICD. Strong upregulation of mature transcripts for IgG1 and its corresponding germline transcript were observed in mice with TDI rhinitis. In addition, germline and mature transcripts for IgE were detected in some of the TDI treated mice but not in the control mice. Mature and germline transcripts for IgA were detected in approximately 50% of the control mice and were upregulated in all of mice exposed to TDI. Overall, these results provide evidence that antibody class switching occurs in the nasal mucosa following inhalation of TDI. Determining the specificity of local antibodies and their role in respiratory allergies caused by diisocyanates will be important in protecting the health of potentially exposed workers.

1313 EVALUATION OF IRRITANCY AND THE SENSITIZATION POTENTIAL OF METAL WORKING FLUIDS AND METAL WORKING FLUID COMPONENTS.

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Metalworking fluids (MWFs) are used to reduce the heat and friction associated with industrial machining and grinding operations. There are approximately 1.2 million workers potentially exposed to MWFs. Irritancy and sensitization potential of 9 National Toxicology Program nominated MWFs (TRIM 229, TRIM VX, TRIM SC210, CIMTECH 310, CIMPERIAL 1070, CIMSTAR 3800, SYNTILO 1023, SUPEREDGE 6768, and CLEAREDGE 6584) were examined in a combined local lymph node assay (LLNA). BALB/c mice were dermally exposed to each MWF at concentrations up to 50%. Significant irritation was observed after dermal exposure to all MWFs except CIMTECH 310 and SYNTILO 1023. Of the 9 MWF tested, TRIM VX yielded the highest sensitization potential with an EC3 value of 6.9%. Chemical components of TRIM VX identified using HPLC, were screened for sensitization potential using QSAR modeling and the LLNA. TOPKAT predicted Triethanolamine (TEA) and Triton-X as sensitizers while DEREK for Windows only predicted 4-Chloro-3-methylphenol (CMP). When tested in the LLNA only CMP (EC3= 28%) and OA (EC3=30%) were identified as sensitizers. All TRIM VX components tested resulted in a significant irritation response. In a further attempt to explain the low EC3 value for TRIM VX, combinations of the components were analyzed in the LLNA. Dermal application of mixtures of CMP, OA, TEA, and alpha-terpineol (TMP) resulted in an EC3 value for the mixture of 3.9%. Mixtures of CMP and OA resulted in an EC3 value of 5.9% while mixtures of CMP, TMP, and OA resulted in an EC3 value of 5.8%. These results suggest that the mixture may be responsible for the magnitude of sensitization potential of TRIM VX as compared to the sensitization potential of the individual components. This raises the question if testing individual components of a mixture or manufactured product will accurately predict the sensitization potential of final products.

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1314 EVALUATION OF A GENOMIC APPROACH TO THE LOCAL LYMPH NODE ASSAY (LLNA).

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Genomic technologies have the potential to enhance and complement existing toxicology endpoints. The present study was conducted to compare the sensitivity of genomic responses to the traditional LLNA endpoint of lymph node cell proliferation and to evaluate the responses for their ability to provide insights into mechanisms of action. Female BALB/c mice (9/group) were dosed on the dorsum of each ear on study days 1, 2 and 3 with vehicle, 0.1, 1 or 10% trimellitic anhydride (TMA), a strong sensitizer. On day 6, four animals from each dose group were injected with tritiated thymidine (3HTdR) and 5 hours later the auricular lymph nodes (ALN) were removed and processed for 3HTdR incorporation. The remaining 5 mice/group were sacrificed on day 6 and the ALN were harvested for gene expression analysis. Additional mice dosed with either vehicle or 10% TMA and sacrificed on day 3 or 10, were also included to examine temporal effects on gene expression. Analysis of 3HTdR incorporation revealed TMA induced stimulation indices of 2.8, 22.9 and 61.0 relative to vehicle with an EC3 of 0.11%. Examination of gene expression responses identified 9, 833, and 2122 differentially expressed genes relative to vehicle for the 0.1, 1 and 10% TMA dose groups, respectively, while no changes were identified in an untreated control group. Calculation of EC3 values for differentially expressed genes did not identify a response that was more sensitive than the 3HTdR value. However, a number of genes displayed comparable sensitivity including IL4, Akr1c18, Gzmb and IL21 which exhibited EC3 values of 0.12, 0.15, 0.27 and 0.39%, respectively. Furthermore, these and other responses could be mechanistically linked to sensitization/allergy and Th2 cellular responses. Collectively these results suggest gene expression responses may provide mechanistic surrogates for the detection of sensitizers. Furthermore, a comprehensive examination of this approach using a diverse spectrum of chemicals could aid in differentiating contact and respiratory sensitizers.

1315 HEME-OXYGENASE 1 AND NADPH QUINONE OXIDOREDUCTASE 1: NEW TARGET GENES TO PREDICT THE SENSITIZING POTENTIAL OF CHEMICALS ?

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Contact sensitizers can mimick danger signals such as LPS or cytokines by inducing the maturation of dendritic cells. In vitro studies has previously shown that nickel and dinitrochlorobenzene (DNCB) induced phenotypical changes in human dendritic cells generated from CD34+ hematopoïetic progenitors (CD34-DC). The purpose of this study was to identify new activation markers in response to contact sensitizers for developping in vitro methods to predict the sensitizing potential of chemicals. Recent in vitro studies have demonstrated that contact sensitizers could induce an oxidative stress in dendritic cells (DC). In this work, we focused our investigations on the Nrf2/Keap1 pathway, a highly sensitive pathway in response to oxidative stress. Two models known to respond to contact senzitizers have been used: the U937 monocytic cell line and CD34-DC. Our results showed that the expression of target genes of Nrf2 such as HMOX1 (heme-oxygenase 1) and NQO1 (NADPH quinone oxydoreductase 1) are up-regulated in a dose-dependent manner in response to NiSO4 and DNCB both in U937 cells and CD34-DC suggesting that these molecules induced an oxidative stress. In U937, both HMOX1 and NQO1 expression are induced by nickel and DNCB 8 hours after treatment. In CD34-DC, 14 hours are necessary to induce these two genes. We are currently investigating the potential of other contact sensitizers belonging to different classes of chemical such as formaldehyde, para-phenylenediamine and hexylcinnamaldehyde to induce HMOX1 and NQO1 gene expressions.

1316 ZINC DIETHYLDITHIOCARBAMATE ALLERGENICITY: POTENTIAL HAPTENATION MECHANISMS.

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Zinc diethyldithiocarbamate (ZDEC) and its disulfide, tetraethylthiuram disulfide (TETD) are rubber accelerant contact allergens that cross-react in some individuals. Rubber gloves are often worn while using oxidizing disinfectants. The present study explored potential protein haptenation mechanisms of ZDEC and its oxidation products. Oxidation of ZDEC by bleach, iodine or hydrogen peroxide resulted in production of TETD, tetraethylthiocarbamoyl disulfide (TETCD) and tetraethyldicarbamoyl disulfide (TEDCD). Cysteine, glutathione and albumin thiols reduced TETD with subsequent mixed disulfide formation suggesting a potential route of protein haptenation. Chelation of the copper ion bound on the active site of the monomeric Cu/Zn superoxide dismutase (SOD) by diethyldithiocarbamate was observed when ZDEC was reacted with SOD. The specificity of the ZDEC-Cu(II) reaction and lack of ZDEC reactivity with reduced Cys6 and Cys111 on SOD suggested formation of the DEC-Cu(II) co-ordinate bond as another haptenation mechanism. The murine local lymph node assay (LLNA) was used to evaluate sensitization potential of ZDEC, Cobalt-DEC (CoDEC), TETD and TEDCD. The ranking of the sensitization potency was ZDEC ≥ TEDCD >

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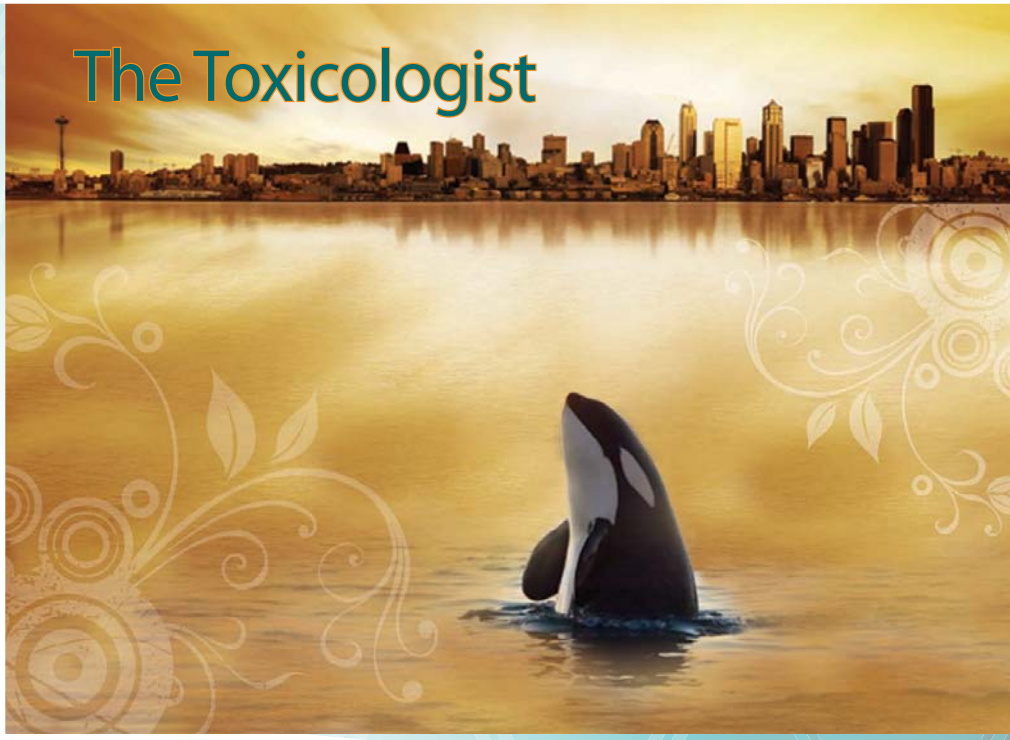

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