monitor airway and systemic responses to a variety of toxicants. The goal of this study was to analyze these bio-fluids for novel protein biomarkers in response to acute diesel and biodiesel emission exposures. Using a cross-over experimental design, 48 subjects operating a load-haul-dump vehicle in an underground mine were exposed on separate days for 200 minutes each alternating use of diesel and 75% biodiesel/ 25% diesel (B75) blend fuels. Switching to B75 reduced rDPM exposure by 20%. Sputum and plasma were then collected before and after diesel and B75 exposures. Proteins from the sputum and plasma were extracted from 6 subjects and were enzymatically digested into peptides that were then run in triplicates using LC-MS/MS strategies. 848 sputum and 407 plasma proteins were identified. Label-free quantitation was also employed and based on criteria of identification in four or more of the subjects and at least two-fold increase or decrease, 42 and 32 novel candidate biomarkers were selected in the sputum and plasma respectively. Two sputum (matrix metalloproteinase-8 (MMP-8) and growth-regulated alpha protein (GRO-α)) and one plasma (tenascin-C (TN-C)) were further validated in all samples using enzyme-linked immunosorbent assay (ELISA). MMP-8 significantly increased following exposures to emissions from both fuel-types. GRO-α was only significantly elevated in post-B75 exposures. Plasma TN-C was significantly increased following diesel exposure, and, not quite significantly, in the post-B75 exposures. This study gives us a better understanding in evaluating the comparative toxicity of the emissions from diesel and B75. Supported by NIEHS Training Grant T32 ES007091 and NIOSH RO1 OH009878

PS 1228 Comparative Analyses of Methods Used to Prepare Diisocyanate Conjugates; Implications in Clinical Assay Development

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Exposure to diisocyanates (dNCOs), such as methylene diphenyldiisocyanate (MDI) can cause occupational asthma. Recently we observed differences in MDI-specific mAb reactivity with MDI-protein conjugates prepared using different methods. The consistent preparation of dNCO-protein adducts is crucial for dNCO-specific antibody screening. Therefore, the aim of this study was to identify the dNCO-protein conjugation method that resulted in the most extensive and consistent conjugation. Four methods were used: 1) MDI was slowly dripped into human serum albumin (HSA) while vortexing (drip), 2) MDI was quickly dispensed into HSA and vortexed (fast dispense), 3) MDI was pumped into HSA while vortexing (infusion), and 4) MDI was dissolved in an non-water miscible solvent forming 2-phases and was stirred overnight (2-phase). On average the infusion method resulted in approximately 1.5, 3.7, and 3.9 times more reactivity in a MDI specific sandwich ELISA than the drip, fast dispense, or 2-phase methods, respectively. Interestingly, the amount of crosslinking, as measured by the binding of free amines by trinitrobenzene sulfonic acid, was similar amongst samples prepared using all of the described methods. Intrapersonal variability was examined by instructing 3 individuals to prepare conjugates using either the drip or fast dispense methods. A high variability in protein conjugation was observed between users, particularly in the drip method. This may correlate to the drip speed of the MDI and vortex speed of the HSA. This work demonstrates intra- and interpersonnel variability in non-automated methods for conjugate preparation and suggests utility of infusion pump for removal of that variability.

PS 1229 Variability of Cytokine Response following *Ex Vivo* Stimulation of Blood from Cynomolgus Monkeys

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In order to evaluate endogenous cynomolgus cytokines, whole blood or peripheral blood mononuclear cells (PBMCs) were stimulated with 15 ug/mL of either pokeweed mitogen (PWM), lipopolysaccharide (LPS), or concanavalin A (ConA) for 2-48 hours at 37°C. Sixteen cytokines were evaluated on two multiplex plates (IFN-γ, IL1β, IL-2, IL-6, IL-8, IL-10, MIP-1α, MIP-1β, Eotaxin-3, TARC, IP-10, MCP-1, MDC & MCP-4). For each cytokine, the lower limit of quantification (LLOQ, 0.8 to 17 pg/mL) and upper limit of quantification (ULOQ, 201 to 6,520 pg/mL) were established. All of the cytokines were below the LLOQ prior to stimulation. MIP-1α and MIP-1β had the largest increase following 18 hour stimulation,, rising to over 70 ng/mL, and IL-6 levels increased significantly following stimulation of whole blood or PBMCs with PWM or LPS (11,300 to 13,450 pg/mL) but not ConA. Eight out of 13 of the other cytokines had detectable increases following stimulation. A nominal concentration for NHP derived material was spiked into individual or pooled serum and assessed for precision, accuracy, selectivity/spike recovery, specificity, dilutional linearity/parallelism, and biomarker stability. Additional validation tests such as suitability of calibration standards, determination of endogenous biomarker levels in matrix were conducted, but did not use the NHP stimulated product. Inter-Assay Precision and Accuracy was demonstrated in both the Kit controls and NHP derived QCs for 12 out of the 14 cytokines at <19.9% CV and <15.6% RE. Eotaxin-3 and TARC did not meet acceptance criteria with precision <72% CV. These results define the limits for measurement of endogenous cynomolgus cytokines in peripheral blood.

PS 1230 Impairment of Skin Function—Defining Biomarkers from Gene Expression Datasets

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Exposure of skin to chemicals can induce changes on gene/protein levels and affect range of biological pathways responsible for its normal function. Similarly, certain systemic diseases can in addition modify skin molecular composition, leading to changes in its structural proteins, inflammatory mediators, nucleic acids and small molecules. Those affected molecules represent a collection of potential biomarkers valuable for toxicity assessment, diagnosis, or therapeutic outcome. Microarrays are a powerful method to deduce potential sets of genes indicating a particular response, but require additional interpretation and processing to identify true biomarker candidates. We created a hand curated database of over 2,000 of gene expression signatures from *in vitro* and *in vivo* experiments, manually interpreted from 400 studies in the literature, and an integrated pipeline of computational tools for defining biomarkers from gene expression datasets. We benchmarked the tool, using two types of data: 1) data arisen from exposure of dendritic cells to few skin sensitizers and 2) data from few systemic diseases (such as cancer, diabetes and psoriasis), and arrived at sets of high-confidence and mechanistically plausible biomarkers for use in predictive models. Those biomarkers are shown to have capacity in assessing sensitizing potential of chemicals or as endpoints for therapeutic interventions in diseases.

PS 1231 Arsenic (+3) Methyltransferase (AS3MT) and Glutathione S-Transferase Omega (GSTO1) Genetic Variants Associated with Arsenic Susceptibility: Influences on As Metabolism and Skin Lesions

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Geological studies have recently shown that arsenic (As) levels in drinking water ranged from 11 to 500 ugL-1 in Nevsehir province, Turkey. This study is a part of molecular epidemiology research carried out to collect human data on iAs exposure in this area. For this purpose,peripheral blood samples were collected from the residents of villages with levels of As > 50 ug L-1 (n=230) and 10-50 ug L (n=151) and from four villages with levels of As <10 ug L-1 (n=182) in drinking water. The polymorphisms in AS3MT and GSTO1 genes were studied by PCR-RFLP method. The influences of these genotypes on As methylation index (as DMA/MMA) and frequency of skin lesions were also investigated. The genotypic distributions of AS3MT in As exposed group were not significantly different from that of controls. The frequencies of GSTO1 genotypes were also similar in As exposed and control groups. The methylation index was significantly decreased (p=0.005) in individuals having the variant genotype of AS3MT. The frequency of skin lesions were associated with neither AS3MT nor GSTO1 genotypes. Our results indicate that variations in AS3MT or GSTO1 do not play a major role in individual susceptibility to As-induced health effects. However As metabolism (DMA/MMA) is slightly influenced by AS3MT polymorphism which may result in increased levels of toxic metabolites of As. This study was funded by The Scientific and Technological Research Council of Turkey (TUBITAK) project no:109S419.

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