teins (e.g. C3, 4, and 5, APOB, VDBP, alpha-1 ACT, and ITIH1) were highly effective in classifying plasma samples to phenotype. These proteins should provide insights into systemic chronic inflammation, serve as biomarkers, or new drug targets for intervention. Supported by NIEHS U54016105

PS 1284 EXTENSIVE OXIDATIVE PROTEIN MODIFICATIONS OBSERVED IN HUMAN PLASMA AND CANDIDATE BIOMARKERS OF SYSTEMIC CHRONIC INFLAMMATORY AND OXIDATIVE STRESS.

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Smoking and obesity are two of the most important, yet preventable risk factors for human morbidity and mortality. Chronic inflammation and oxidative stress appears to be the unifying mechanism underlying the interaction of these risk factors with the genome, resulting in a variety of chronic human diseases. We are identifying potential protein biomarkers of oxidative stress in human populations that accurately and quantitatively reflect an individual's exposure/response to environmental stressors. A subset of plasma samples were analyzed from a cohort of 500 exposed to main-stream tobacco smoke or never-smoked with BMI above 35 or below 25. Eight plasma samples per group representing high BMI smoker and non-smokers, and low BMI smoker and non-smokers were Individual human plasma were depleted, digested with trypsin and analyzed via an LTQ-Orbitrap mass spectrometer in triplicate. Peptides were identified by accurate mass and time (AMT) tags. 4616 peptides passed quality control and were analyzied by ANOVA and G-test for statistical significance to experimental groups. 133 proteins are significant for either smoking status or BMI. 94 of these proteins had at least one oxidatively modified proteins. Statistical and graphical analysis revealed that the abundance of unmodified peptides are typically similar across the four groups while the abundance of modified peptides is group specific. This trend is particularly noted for the proteins Complement factor D, Plasminogen-like B2, and Fibrinogen. The differential abundance of modified 0and unmodified peptides/proteins were highly effective in classifying plasma samples to phenotype. These proteins should provide insights into systemic chronic inflammation, serve as biomarkers, or new drug targets for intervention. Supported by NIEHS U54016105

PS 1285 DEVELOPMENT OF MALDI-TOF METHODOLOGY FOR SELECTIVE MS-ANALYSIS AND IMAGING OF CARDIOLIPINS IN LIPID EXTRACTS AND TISSUES.

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Cardiolipin (CL) is an anionic phospholipid that plays a key role in mitochondrial electron transport but is also highly susceptible to oxidative stress. The molecular diversity of endogenous cardiolipins in some tissues and the low abundance of its individual species, particularly oxidized derivatives, makes in situ analysis and imaging by MALDI-Mass Spectrometry (MALDI-MS) challenging. The goal of this study was to develop a method to selectively analyze CL using existing CL probes in a novel way, to decrease CL mass spectral complexity. Nonyl Acridine Orange (NAO) is a commonly used fluorescent stain for cardiolipin. We have used NAO as a novel MALDI matrix, and have found that it allowed detection of anionic phospholipids, including cardiolipin in the negative ion mode. With careful laser tuning, NAO induced prompt decay of cardiolipin selectively - but not other phospholipids - at very high efficiencies. From these selective prompt decay products, much information about the structure of cardiolipin can be determined. Pseudo-MS^3 is possible on a MALDI-TOF instrument by doing MS/MS on these products of cardiolipin. We have also extended these findings to include oxidized species of CL's and have determined that NAO also induced prompt decay of oxidized CL. Prompt fragments are seldom seen with dihydroxy-benzoic acid (DHB) which has been employed as a standard matrix for lipid analysis in the negative ion mode. Our results indicate that NAO can be utilized as a discerning matrix for the assessment of CL in lipid extracts from cells and the distribution of CL - particularly in tissues with a high diversity of CL molecular species and their oxidation products - by taking full advantage of the unique ability to induce prompt decay of this phospholipid with NAO.
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PS 1286 PROTEOMIC IDENTIFICATION OF NITRATED PROTEINS IN THE SPLEEN OF ANILINE EXPOSED RATS.

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Aniline exposure is associated with toxicity to the spleen. However, mechanisms by which aniline elicits splenotoxic response are not well understood. Earlier we have shown that aniline exposure leads to increased nitration of proteins in the spleen. However, nitrated proteins remain to be identified and characterized. Identification of nitrated proteins is important because nitration of proteins could result in their inactivation, increased degradation and altered tyrosine phosphorylation, and thus, could contribute to splenic toxicity. Therefore, the current study, using proteomic approaches (2D Western blot, MALDI TOF/TOF MS/MS and Progenesis SameSpots, etc.), was focused on identifying and characterizing nitrated proteins in the spleen of aniline exposed rats (0.5 mmol/kg/day for 30 days via drinking water). Aniline exposure led to increased tyrosine nitration of proteins, as determined by 2D Western blotting with specific anti-3-nitrotyrosine antibody, compared to the controls. The nitrated proteins were found in the molecular weight range from 27.7 to 123.6 kDa. A total of 37 nitrated proteins were identified in the protein extracts of aniline-treated and control spleens. Among them, 25 were found only in aniline-treated rats, 11 were present in both aniline-treated and control rats, while one nitrated protein was found in controls only. The nitrated proteins identified mainly represent skeleton proteins, chaperones, ferric iron transporter, enzymes, nucleic acids binding protein, and signaling and protein synthesis pathways. The increased nitration of proteins not only supports our previous findings, but more importantly, provides a global map to further investigate alterations in their structural and functional properties, and thus, a better understanding of the role of the protein nitration in splenic toxicity. Supported by NIH ES006476.

PS 1287 CIRCULATING LEVELS OF NITRATED PROTEINS ARE SUPPRESSED BY CIGARETTE SMOKING.

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Nitric oxide (NO) is a physiological regulator of endothelial function and hemodynamics. Oxidized products of NO can form nitrotyrosine in proteins, a general marker of nitrative stress. Cigarette smoking decreases exhaled NO, and the underlying mechanism may be important in cardiovascular toxicity. Even so, it is unclear if this decrease in exhaled NO is due to lower NO production or increased NO degradation to nitrating species. Since these two processes should have opposing effects on nitrotyrosine levels, our goal was to examine nitration levels in circulating proteins in order to gain insight into which of these processes predominates in smokers. A custom antibody microarray platform was developed to analyze the levels of 3-nitrotyrosine modifications on 24 proteins in plasma. Plasma samples from 458 individuals including non-smokers, former smokers, and active smokers with or without chronic obstructive pulmonary disease (COPD) were analyzed. Levels of nitrated plasma proteins were consistently lower in current and former smokers than in plasma from non-smokers; protein nitrotyrosine levels in individuals at risk for environmental cigarette smoke exposure was intermediate between smokers and non-smokers. These results are consistent with evidence that soluble cigarette-smoke components suppress endothelial NO synthase activity, leading to endothelial dysfunction and cardiovascular disease. In contrast, we observe an increase in nitrotyrosine levels in individuals with COPD that likely reflects an increase in inflammation. This study provides the first evidence that smoking has irreversible effects on endothelial production of nitric oxide in humans, and provides insight into how smoking could induce endothelial dysfunction and a long-term increase in the risk of cardiovascular disease. Supported by NIEHS Exposure Biology Program, U54 ES106015.

PS 1288 URINARY 8-OXO-DEOXYGUANOSINE IN SMOKERS: ASSOCIATIONS WITH RACE, SEX, AND MENTHOL SMOKING.

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Several recent studies have found no significant differences in either biomarkers of exposure or biomarkers of potential harm in the blood and urine of menthol smokers compared to non-menthol smokers. Differential uptake or metabolism of cigarette smoke constituents can induce oxidative stress and lead to the oxidative modification of the guanine base of DNA. This modification results in the formation of

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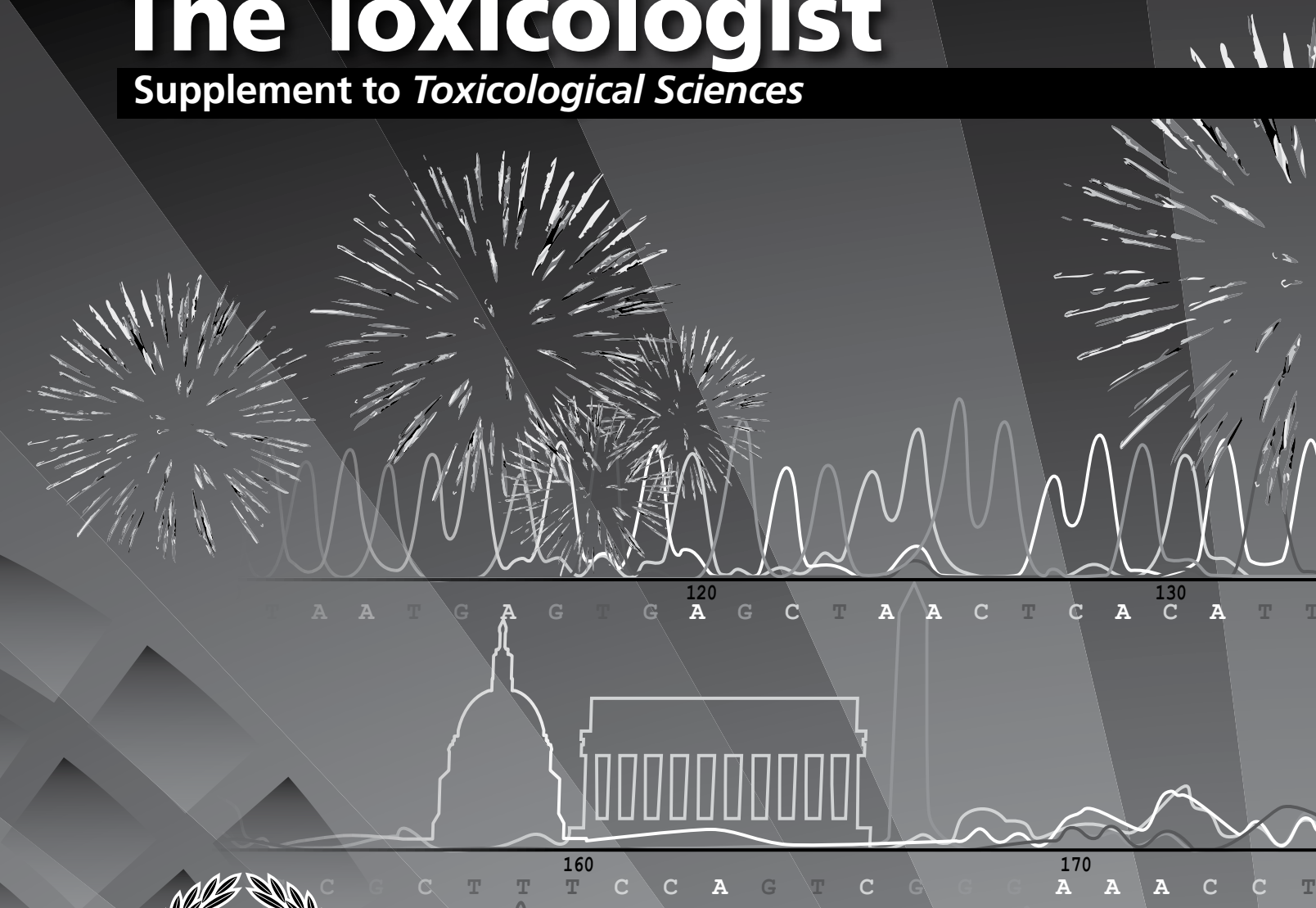

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 50th Annual Meeting of the Society of Toxicology, held at the Walter E. Washington Convention Center, March 6–10, 2011.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 578.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 606.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

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