spots and corresponded to the ARP-reactive protein spots were serotransferrin, serum albumin, transthyretin, prostaglandin D-synthase, α-1-antitrypsin, and adducin gamma.
The use of ARP coupled with mass spectrometry represents a novel approach for the identification of carbonylated CSF proteins. The identified proteins might potentially lead to the development of novel markers for AD and other neurodegenerative diseases.

239 *IN SITU* BREAST CANCER METASTASIS CHARACTERIZATION BY MALDI MASS SPECTROMETRY.

S. Schwartz[1], L. A. Greene[1], P. A. Veno[2], J. Zhao[2], L. F. Bonewald[2] and S. L. Dallas[2]. *[1]Midwest Research Institute, Kansas City, MO and [2]School of Dentistry, University of Missouri-Kansas City, Kansas City, MO.* Sponsor: B. Astroff.

Breast cancer frequently metastasizes to bone, causing bone destruction, hypercalcemia, fracture and pain. To examine the mechanisms responsible for breast cancer metastasis and growth in bone, and study the tumors in their metastatic microenvironment, tumorigenic bone sections from nude mice inoculated with MDA-231 human breast cancer cells were analyzed by direct-tissue protein profiling using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS). This technique allows "in situ" mass spectrometry analysis to be performed directly on tissue sections, and protein profiles are generated from discrete tissue subregions such as bone, marrow, tumor and muscle. The described approach is applicable to a variety of studies including monitoring small molecule localization within tissues and identifying key biomarkers indicative of disease state, tissue response to exposure or therapy, or patient outcome.
Tissue from five control mice and five mice containing breast cancer metastases were harvested, processed and analyzed, including tumor-bearing bone, tumor-bearing adrenal gland, muscle, liver and kidney. To optimize mineralized tissue preparation techniques, specimens were either fresh frozen or fixed in 70% ethanol and decalcified, sectioned, and analyzed by MALDI MS. The resulting protein profiles were evaluated. Comparable protein patterns were generated for the two tissue preparation methods, with the fixation/decalcification procedure giving better histology and the fresh frozen preparation yielding enhanced tumor protein patterns. The fresh frozen sample preparation method was then applied to characterizing breast cancer metastasis to bone and adrenal gland. Comparison of the protein patterns from tumor metastatic regions demonstrated greater than 20 protein signals with potential differential expression between the tumor and surrounding non-tumor regions as well as between tumor metastases in different tissues.

240 URINARY PROTEOMIC-BASED BIOMARKER DEVELOPMENT FOR EVALUATION OF OCCUPATIONAL EXPOSURE USING SELDI-TOF MASS SPECTROMETRY.

P. I. Mathias and K. L. Cheever. *DART BHAB MGM, CDC NIOSH, Cincinnati, OH.*

The utility of four types of Ciphergen ProteinChips were evaluated by comparing protein profiles in control, and in in vitro acrylamide or glycidamide exposed urine using Surface Enhanced Laser Desorption Ionization Time of Flight (SELDI-TOF) mass spectrometry. Acrylamide (CAS 79-06-1), a widely used industrial chemical, which also may be formed in thermally processed food, can produce peripheral neurotoxicity and reproductive effects. Acrylamide has been classified as a probable human carcinogen. Changes in the levels of potential protein biomarkers in serum and urine have been reported in exposure to toxic agents, and bioactivation of acrylamide to glycidamide (5694-00-8), a reactive epoxide, suggests that urinary protein levels would be affected in acrylamide exposure. This work was designed to identify molecular biomarkers in human urine for use to assess acrylamide exposure or detect early disease onset. Urinary proteins were processed on NP20, CM10, H50, and IMAC30 chips having normal phase, cationic exchange, hydrophobic, and ionic surface chemistries, respectively. The number of peaks and mean peak intensity varied significantly with IMAC30 > CM10 > NP20 > H50. Various factors were optimized during the development of binding protocols for each chip, including surface pretreatment, sample binding time and washing methods. The data from this work suggest that the detection and further characterization of urinary protein levels using SELDI-TOF mass spectrometry can provide specific proteomic-based biomarkers of exposure for acrylamide.
Disclaimers: Mention of company names and/or products does not constitute endorsement by the Centers for Disease Control and Prevention (CDC). The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.

241 IDENTIFICATION OF PROTEIN EXPRESSION PROFILES AFTER TREATMENT WITH THE HEAT SHOCK PROTEIN (HSP)90 INHIBITOR, 17-DIMETHYLAMINOETHYLAMINO-17-DEMETHOXYGELDANAMYCIN (17-DMAG) IN HUMAN AND RAT PBMCS USING THE SELDI PROTEIN CHIP SYSTEM.

S. Qin, D. Zhou, X. Zhang, L. Li, J. Ye, X. Zhang and J. Barsoum. *Synta Pharmaceuticals Corp., Lexington, MA.* Sponsor: E. Kitayama.

Proteomic techniques hold the promise to improve efficiency of protein profiling and biomarker identification for treatment-responsive proteins. In this study, we use a surface-enhanced laser desorption/ionization time-of-flight mass spectroscopy (SELDI TOF-MS) system to identify protein profiles in normal PBMCs treated with Hsp90 inhibitor in vitro. The fresh human and rat PBMCs were isolated and treated with 17-DMAG overnight at different concentrations from 1 to100 nM. The cells were harvested and proteins were extracted, quantified, and then applied to weak cation exchange (CM10) and strong anion exchange (Q10) ProteinChip® arrays for protein profiling by SELDI-TOF MS. Ciphergen Express and Biomarker Patterns Software package was used to compare the protein profiles from different samples acquired from different protein chips. When the peak intensity was calculated as a fold increase or decrease vs. control in a range from 1-13 kDa, 49 protein picks were identified in rat and human PBMCs, which all relevant to the treatment of 17-DMAG. Almond those, 29 (16 from Q10 and 13 from CM 10 chips) proteins identified in the treatment groups were differentially by 3 folds or greater relative to the vehicle control with a trend of dose-dependency. Overall, the number of protein picks identified in the rat was higher than that in the human PBMCs based on the peak intensity. Our results suggest that SELDI-TOF-MS has the potential to identify a protein profile that distinguishes action of the testing compounds vs. vehicle controls in both rat and human PBMCs. These protein expression profiles determined by SELDI TOF-MS can provide a new resolution to identify drug-responsive biomarkers and potentially lead to a better understanding of mechanisms related to the activity/toxicity of Hsp90 inhibitors.

242 DEVELOPMENT OF A PROTEOME DATABASE FOR QUANTITATIVE ANALYSIS OF MACROPHAGE RESPONSES TO NANOMATERIALS AND ENVIRONMENTAL PARTICULATES.

L. M. Masiello, J. M. Jacobs, K. M. Waters, R. C. Zangar, D. Smith, C. Sacksteder and B. Thrall. *Pacific Northwest National Laboratory, Richland, WA.*

The increase in industrial use of engineered nanoparticles and the link between particulate matter exposure and cardiovascular and respiratory effects in humans has created a need to understand the biological pathways stimulated by these agents. Identification of protein biomarkers of exposure to particulates and nanomaterials may aid in early detection of toxicity and highlight differential mechanisms of action. Toward this goal, we have established a reference macrophage proteome database for comparison of responses to different activating stimuli, which can be applied for future quantitative comparisons. We used a global proteomic approach to identify proteins in the extracellular compartment following treatment with particulate, biological, and biochemical stimuli. Conditioned medium from RAW 264.7 cells treated with urban particulate matter (UPM), crystalline silica, lipopolysaccharide (LPS), phorbol ester (PMA), or serum-free media (control) was analyzed by LC-MS/MS following tryptic digestion. We confidently identified 623 proteins, which is a significant increase in coverage compared to previous 2-D gel based studies of the extracellular proteome. Based on spectral counts, we found proteins that were differentially detected compared to control for UPM (80), silica, (84) LPS (87), and PMA (117), as well as several proteins that were unique to each stimulus. We identified proteins associated with inflammatory (74), proteolytic (55), and apoptotic (15) processes among other biological pathways. Several of the proteins responses identified by MS/MS have been validated by orthogonal protein microarray analysis. This study established a reference database that is being applied for high-throughput investigations of macrophage responses to an array of nanomaterials using quantitative mass spectrometry-based approaches.

243 METABOLOMIC DETERMINATION OF BIOMARKER CANDIDATES OF PHOSPHOLIPIDOSIS USING CAPILLARY ELECTROPHORESIS TIME-OF-FLIGHT MASS SPECTROMETRY.

N. Masutomi[1], T. Ishikawa[2], J. Abe[2], A. Hirayama[2], N. Tsutsui[2] and T. Soga[2,3]. *[1]Toxicology Laboratory, Mitsubishi Pharma Corporation, Chiba, Japan, [2]Human Metabolome Technologies, Inc., Yamagata, Japan and [3]Institute for Advanced Biosciences, Keio University, Yamagata, Japan.* Sponsor: J. Sugimoto.

We conducted a metabolomic analysis based on capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS) to find biomarker candidates of phospholipidosis (PLD). SD rats received oral doses of a vehicle, amiodarone or MPC1 (a

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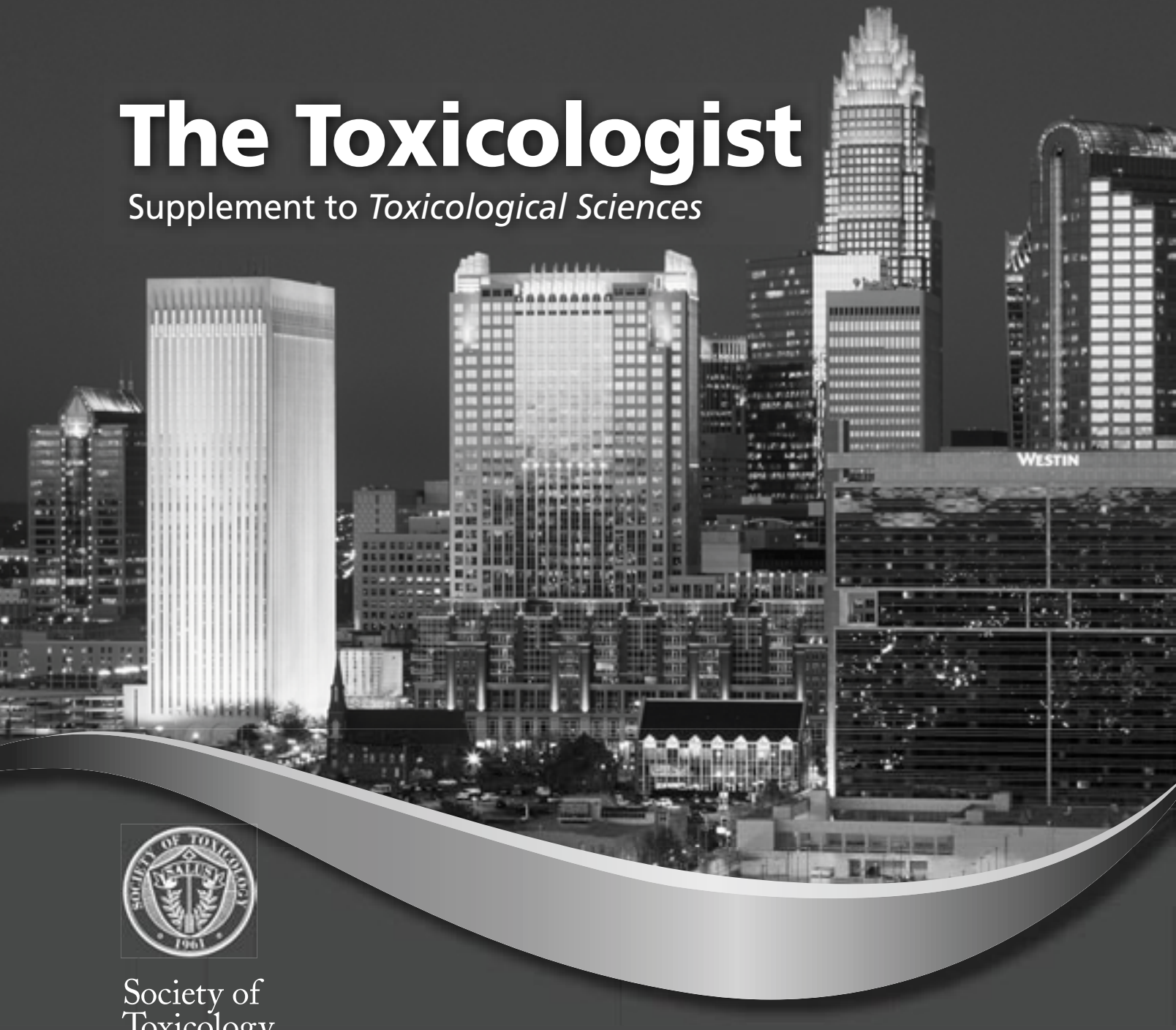

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 46th Annual Meeting of the Society of Toxicology, held at the Charlotte Convention Center, Charlotte, March 25–29, 2007.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 449.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 480.

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

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