PS 1223 Quantification of Biomarkers of Cardiac Toxicity across Four Animal Species

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In order to validate the use of troponins I (cTnI) and T (cTnT), fatty acid binding protein 3 (FABP3), and myosin light chain 3 (Myl3) as biomarkers of acute myocardial injury, we have conducted experiments in four species of animals: mouse, rat, dog and non-human primates. Four Beagle dogs were outfitted for continuous ECG monitoring using the DSI JET system. Acute myocardial injury was induced by a bolus s.c. administration of 1 mg/kg of Isoproterenol HCl (ISO). Pulse oximetry parameters and blood samples for determination of plasma concentration of cTnI, cTnT, FABP3, MYL3 and sTnI were collected from each dog prior to and at 2, 4, 6, 8, and 10 hours after ISO administration. ISO produced tachycardia and tachypnea, which were sustained throughout the 10-hour monitoring period. All dogs demonstrated shortening of RR and PR intervals and prolongation of QTcf on ECG, as well as transient arrhythmias. Acute myocardial injury was evidenced by a significant increase in circulating biomarkers: cTnI (370-fold), cTnT (70-fold) and Myl3 (20-fold), FABP3 (8-fold) by the end of 10-hour observation. The level of skeletal TnI remained below detection threshold throughout the experiment. The chemiluminescence Mesoscale Discovery (MSD) platform was used to measure the biomarkers of cardiac injury described. ISO at equivalent doses have also been used to induce myocardial injury in mice, rats and non-human primates in our laboratory. These biomarkers were proven to be useful and were quantified in the ISO-induced cardiac injury model across all 4 tested species. In addition, we developed custom in-house MSD CK-MM and CK-BB MSD assays. The normal and abnormal historical levels of biomarkers, sensitivity, accuracy and precision, LLOQ and freeze-thaw stability in plasma and serum were also determined. Positive plasma and serum controls with high and low levels of cardiac toxicity biomarkers were prepared and frozen in multiple aliquots to verify the assay performance between MSD plates by running a positive biological matrix control on multiple plates.

PS 1224 Biomarkers of Pathologic Cardiac Hypertrophy: Investigation of NTproANP and NTproBNP in Rats

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Cardiovascular (CV) toxicity is a primary cause of attrition during drug development and following product launch. Better understanding and application of biomarkers of cardiac structure or function during development may aid in selection of molecules with improved CV safety. Natriuretic peptides (NP) are hormones secreted from the myocardium with a central role in maintaining CV tone, plasma volume, and cardiac growth. Their structure and function, and in the case of ANP, sequence, is well-conserved across veterinary species commonly used in drug development, making them important translational CV biomarkers. In humans with adaptive increases in left ventricular mass (LVMI), for instance as a result of exercise, plasma NP concentrations are normal, whereas patients with increased LVMI as a result of prolonged hypertension have increased NP concentrations. We hypothesized that NTproANP and/or NTproBNP could distinguish between adaptive (physiological) and maladaptive (pathological) increases in cardiac mass in rodents. Male Sprague Dawley rats were administered a PPARγ agonist daily or participated in a swimming protocol, working up to two 90 minute swim sessions each day. Heart weights and NP concentrations were compared to control and active control (2 minutes swimming twice daily) groups. Comparably increased heart weights (~15%) were observed in PPAR and swim group rats after 28 days. Increased NPs were observed in rats administered the PPARγ agonist, but not in swimming rats. These data support the use of NPs in rats as translational safety biomarkers for detection of pathological changes in cardiac mass during drug development.

PS 1225 Evaluation of Calcineurin Activity As a Biomarker of the State of Immunosuppression in Heart Transplantation

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Introduction: The standard for rejection prevention after heart transplantation (HTx) includes a calcineurin inhibitor (CNI), but the optimal balance is difficult to achieve. Indeed, inadequate immunosuppression (IS) may lead to rejection while excessive IS facilitates the development of cancer and infections. Therefore in order to circumvent possible adverse events related to their clinical use, we have investigated whether calcineurin activity (CN-a) could be a biomarker of IS. Methods: A Pilot monocenter study was first conducted in which 56 patients were enrolled. CN-a was determined in mononuclear cells. Endomyocardial biopsies were routinely performed to assess acute cellular rejection (ACR). Coronarography was yearly performed to assess chronic rejection/chronic allograft vasculopathy (CAV). The occurrence of infections was recorded to assess adverse events related to over-IS. Then in order to validate our results, a tricenter study was conducted in which 23 patients were enrolled. Results: In the Pilot study, we reported that the recipients who displayed extreme CN-a values were mainly those who developed ACR, and a therapeutic range for CN-a, associated with a significantly lower risk of developing ACR, was defined between 12 and 120 pmol/mg/min. Patients who displayed extremely high CN-a values were mainly those who developed CAV, and a therapeutic range for CN-a, associated with a significantly lower risk of developing CAV, was defined below 73 pmol/mg/min. The occurrence of bacterial and viral infections was significantly lower in patients who exhibited CN-a values higher than 16 and 7 pmol/mg/min, respectively. These results were confirmed in the Tricenter study. Conclusion: These results are similar to those we have recently reported in lung transplantation and they suggest that CN-a could be a biomarker of the IS state. CN-a could be useful to reduce both the occurrence of rejection related to an inadequate IS and the development of severe complications related to exceedingly IS.

PS 1226 Clinical Protein Array Screening to Discover Preclinical Biomarkers of Drug-Induced Vascular Injury

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Drug-induced vascular injury (DIVI) continues to be a major obstacle in drug development. Attempts to correlate this observation in preclinical toxicity studies with hemodynamic changes are not always successful. Additionally, no accessible and specific biomarkers of DIVI exist, making risk assessment and monitoring in humans a challenge during drug development and regulatory approval. Previous reports using antibody microarrays have shown increases in the circulating levels of several vascular-associated proteins in patients with various vasculitides compared to control populations. For example, circulating levels of matrix metalloproteinases, specifically matrix metalloproteinases-3 (MMP-3), were able to distinguish between patients with active antineutrophilic cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and remission. In order to determine whether a similar approach would uncover novel biomarkers of arterial pathology associated with DIVI observed in animal toxicology studies, we screened serum from DIVI positive non-human primate models (NHP) against biomarkers associated with vascular development and function, immune cell trafficking and inflammatory responses. The human arrays were tested for limits of detection, background, and cross reactivity against NHP serum, reducing our proposed 65 analyte antibody array to 29. In summary, MMP-3 was the only analyte identified using this approach as a potential translational accessible biomarker to also monitor for DIVI in animals. These findings are supportive of a strategy for discovering analytes that relies on a translational circulating biomarker for monitoring of vascular injury both pre-clinically and clinically, and on antibody reagents that will cross react with both human and NHP plasma samples.

PS 1227 Shotgun Proteomics of Human Sputum and Plasma Identifies Biomarkers of Acute Exposures to Diesel and Biodiesel Emissions

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Exposures to diesel particulate matter (DPM) are linked to a broad range of illnesses. In recent years, biodiesel has been used to reduce respirable DPM (rDPM) but there are few human studies that analyze the health effects of this fuel. Sputum and plasma are attractive sources of proteins and these bio-fluids have been used to

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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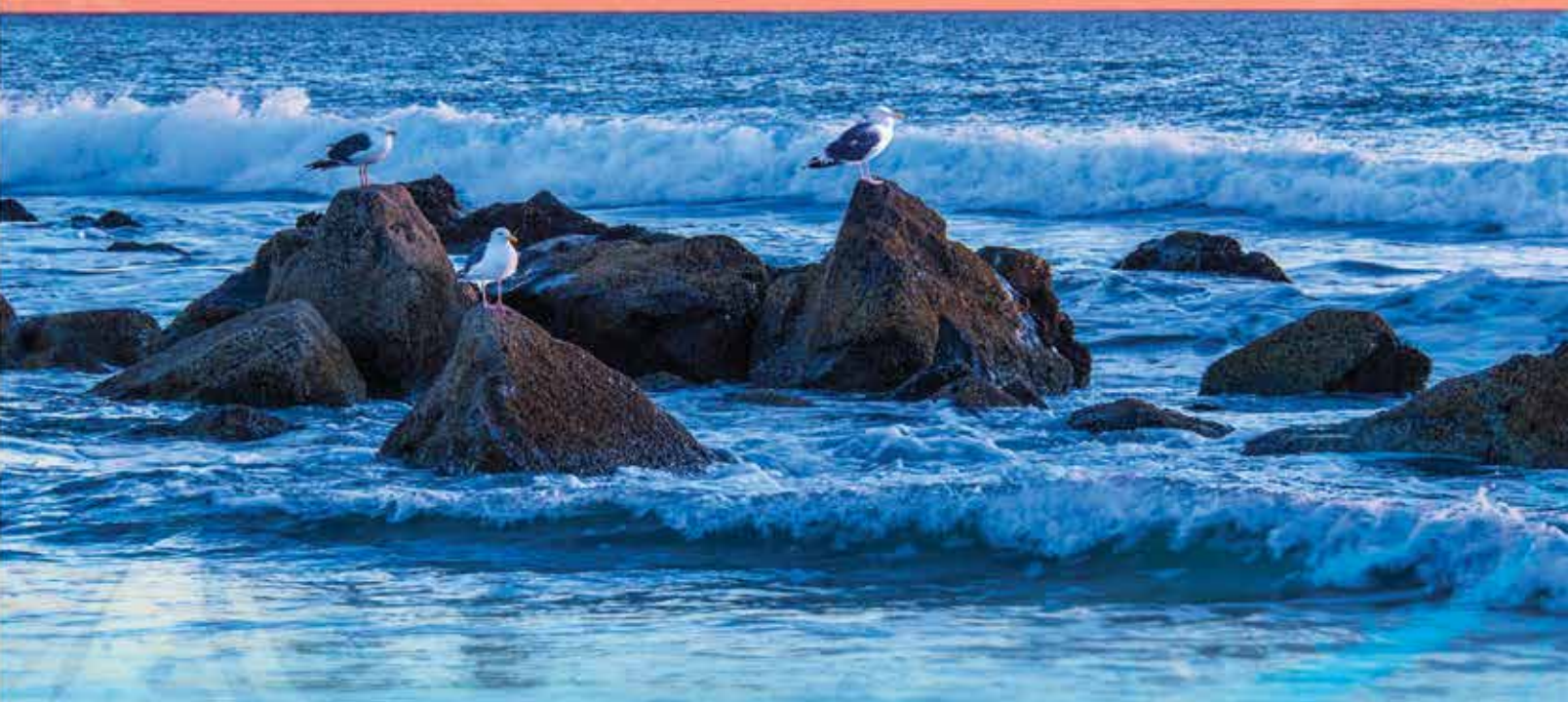

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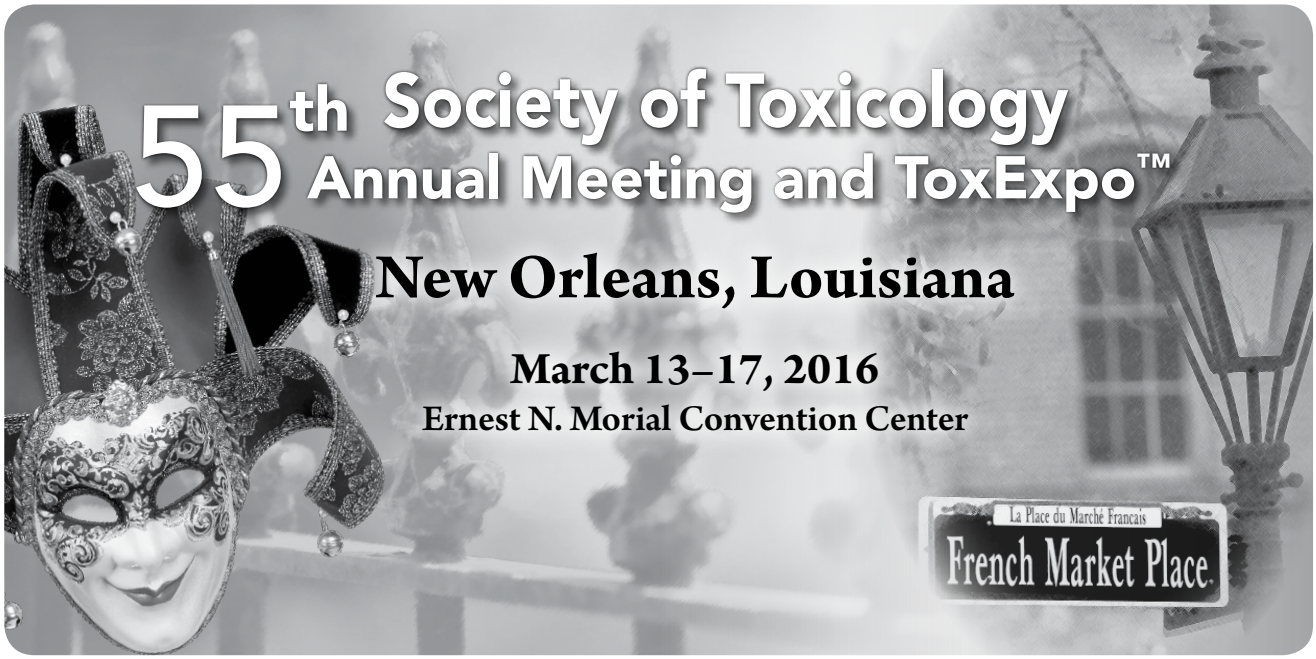

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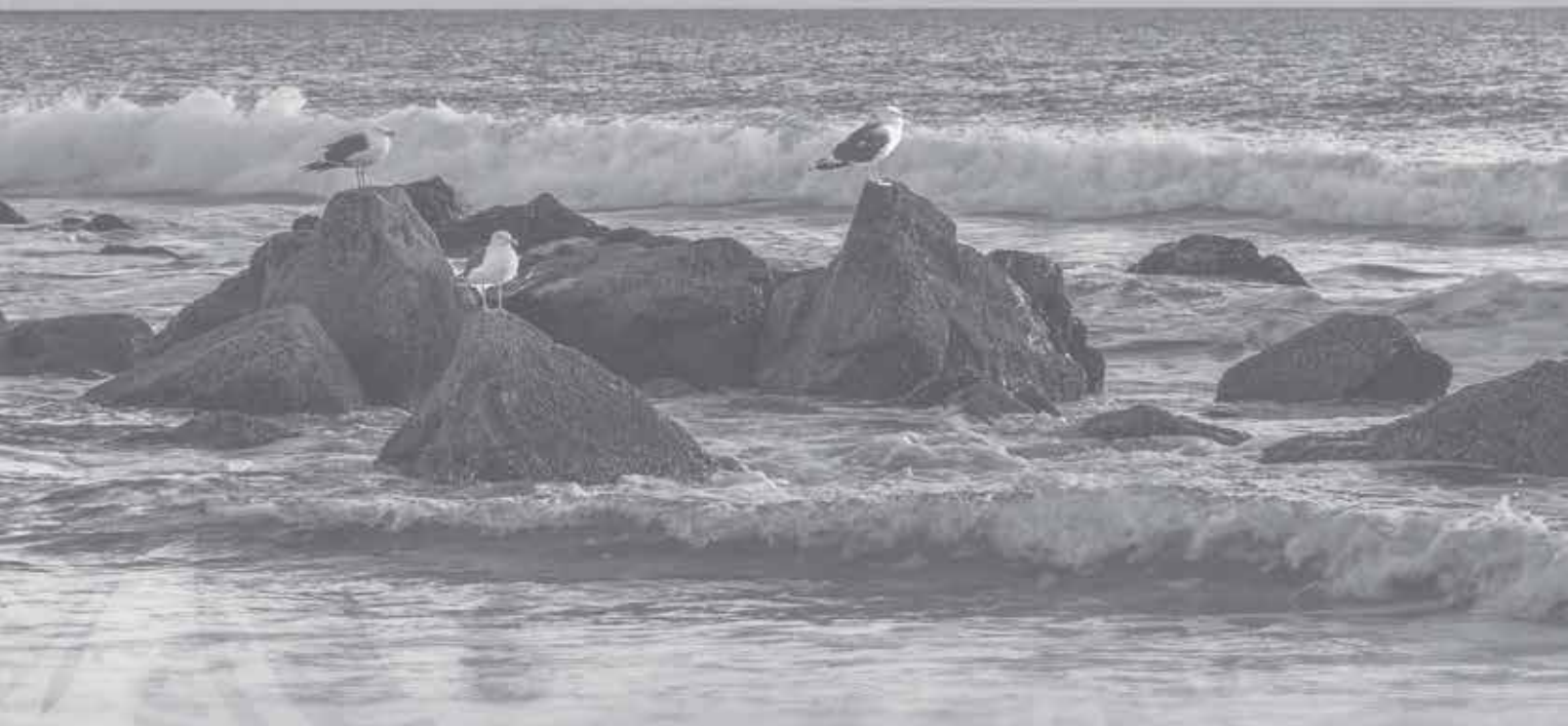

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 54th Annual Meeting of the Society of Toxicology, held at the San Diego Convention Center, March 22–26, 2015.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 529.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 553.

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

Scientific Session Types:

- EC Education-Career Development Sessions
- FS Featured Sessions
- IS Informational Sessions
- PL Platform Sessions
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