1201 Two Novel Models for Study of Particle-Induced Alveolar Macrophage Toxicity: ASC-Transfected Raw 264.7 Cells and Max Planck Institute Cells

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Both human epidemiological and mouse experimental studies reveal that lung exposure to crystalline silica triggers autoimmunity. Silica-induced pathogenesis has been linked to NLRP3 inflammasome activation in alveolar macrophages (AM), which drives caspase-1 activation, extracellular release of mature IL-1beta, and pyroptotic cell death, thereby activating and recruiting additional immune cells to the lung. *In vitro* elucidation of early silica-triggered molecular events is limited by low numbers of AM that can be obtained from a single mouse (≈10^5). To address this, we compared two new *in vitro* AM models relative to silica-induced inflammasome activation. In the first model, RAW 264.7 murine macrophage cells were stably transfected with the ASC protein (adaptor protein essential for inflammasome assembly). Following priming with LPS for 2 h, RAW 264.7 + ASC cells were highly sensitive to silica-induced cell death and showed over a ten-fold increase in the release of IL-1beta as compared to non-transfected RAW 264.7 cells. In the second model, Max Planck Institute (MPI) cells were used. MPI cells are self-renewing macrophages, derived by culturing fetal mouse livers in GM-CSF-supplemented medium and are phenotypically similar to murine AM. Both cytotoxicity and IL-1beta release were evident in LPS-primed silica-treated MPI cells. Taken together, the responses of silica treated RAW 264.7 + ASC cells and MPI cells were reflective of responses seen *in vivo* upon exposure to inhaled silica. Both types of cells might be suitable *in vitro* alternatives to primary AM for characterizing the up- and down-stream events associated with silica- triggered activation of the NLRP3 inflammasome in AM. *Supported by NIH grant ES027353, Lupus Foundation grant 362470, the Dr. Robert and Carol Diebel Family Endowment, and the Institute for Integrative Toxicology NIEHS Training Grant T32ES007255.*

1202 Long-Term Culture of Nasal, Tracheal, Bronchial, and Small-Airways Human Airway Epithelia at Interconnected and Dynamic Liquid Flow Conditions

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We herein report the first interconnection of four fully differentiated epithelia reconstituted from primary human cells from different anatomical origins, namely from the nose, the trachea, and the bronchi (three versions of the MucilAir™ system) and small airways (SmallAir™). The system is composed of a culture plate allowing 3D models grown in transwell to be (i) interconnected via the basal compartment through meso-fluidics (0.3 ml/min of a common culture medium) and (ii) maintained at the Air-Liquid Interface. Stability in terms of morphology and function of the four fully differentiated human airway epithelia was evaluated. Endpoint measurement included longitudinal tissue integrity assessment (TEER); cilia activity (Cilia Beating Frequency), and histological evaluation (H/E-alcian blue staining). Quantitative immune-histological analysis didn't reveal major differences in cell proliferation (Ki67) or ciliation (FoxJ1) between interconnected and static conditions. The study concluded that minor differences are observed for all tested endpoints after 6 weeks of culture at interconnected and dynamic liquid flow conditions; therefore, this model allows testing the toxicity of the chemical compounds simultaneously on several anatomical regions of the respiratory tract, as well as the interplay of different organs/tissues *in vitro*.

1203 Respiratory Irritants Cause Reversible Up-Regulation of Pro-Inflammatory Cytokines on Human Nasal Mucosa Reconstituted *In Vitro*

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Respiratory irritants are considered as substances of higher risk, at the same level as carcinogens, mutagens, and toxic chemicals for reproduction. Respiratory irritation is defined as a non-corrosive effect inducing a reversible local inflammation on the mucosa. However, until now there is no validated *in vitro* cell model for identifying the respiratory chemical irritants. The aim of this study is to develop an *in vitro* cellular assay for identification of respiratory chemical irritants based on human 3D nasal airway epithelium (MucilAir™). Epithelia were reconstituted with primary human nasal cell pooled from 14 donors. MucilAir™ is not only morphologically and functionally differentiated; it can also remain at a homeostatic state for more than one year, allowing repeated-dose and long-term toxicity testing. 11 chemical compounds, belonging to 3 classes (irritants "H335", highly toxic "H330," and nontoxic chemicals through inhalation), were tested. As testing strategy, 30 µl of chemical solution were applied on the apical surface of the epithelium. In order to monitor recovery, endpoints were recorded 1, 2, 5, and 7 days post-exposure. Effect on tissue integrity was monitored by Trans-Epithelial Electrical Resistance (TEER), cell viability through LDH release quantification, effect on cilia motion by monitoring Cilia Beating Frequency, and morphological changes through visual inspection. Proinflammatory cytokines IL-8 and IL-6 were used as biomarkers for discriminating these molecules. Interestingly, at subtoxic doses, only the respiratory irritants upregulated reversibly the secretion of IL-8 and IL-6 upon acute challenge. As conclusion, this standardized human nasal epithelium model MucilAir™ is a promising *in vitro* platform for identifying the respiratory irritants, and IL-8 seems to be a reliable biomarker.

1204 Low-Molecular Weight PAH-Induced Early Signaling in Lung Epithelial Cells Involves Eicosanoids

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Low-molecular-weight (LMW) polycyclic aromatic hydrocarbons (PAHs) are more prevalent in the environment and occupational settings, as well as in secondhand smoke (SHS), when compared to their high-molecular-weight counterparts, such as benzo[a]pyrene (B[a]P). Previously, we demonstrated that SHS-prevalent LMW PAHs activate p38-MAPK-dependent dysregulation of gap junction intercellular communication (GJIC) and increased cytokines involved in inflammatory lung diseases. However, there is little known about the early mechanistic events leading to inflammation, specifically those mediated through lipid signaling and eicosanoids. Secondhand smoke is a complex mixture, and to model this feature *in vitro* we examined the effects of a binary mixture of 1-methylanthracene (1-MeA) and fluoranthene (Flthn) in C10 cells, a mouse, non-tumorigenic alveolar type II cell line for the ability to dysregulate GJIC (scalpel-loaded/dye transfer assay) via activated phospholipases (e.g., cytosolic phospholipase A_2 ($cPLA_2$)), and induce cyclooxygenase-2 (COX-2), downstream prostaglandins, and proinflammatory mediators via immunoblotting, focused metabolomics approaches, and quantitative RT-PCR from 30 min-8 hr. Specific inhibition of $cPLA_2$ led to significant reversal of dysregulated GJIC in response to the binary PAH mixture after only 30 min. COX-2 mRNA and protein was also significantly induced in response to these PAHs following activation of the lipases as early as 2 hr. The subsequent downstream effects were significant prostaglandin production (PGE_2 and PGF_2) and pro-inflammatory cytokine mRNA induction, including *Il1β*, *Il6*, *Ccl2*, and *Kc*. These effects were all prior to PAH metabolism, determined via GC/MS analysis. Our results represent a mechanistic pathway of inflammatory lung disease from exposure to a more relevant real-world binary mixture of LMW PAHs through the early induction of lipid-mediated GJIC dysregulation and downstream production of eicosanoids and proinflammatory cytokines. *Funded by R15 ES 024893-01 (AKB) and the Flight Attendant Medical Research Institute (FAMRI) CIA 130022 (AKB).*

1205 *In Vitro* Model of the Hepatic Contribution to Lung Epithelial Cell Toxicity Induced by Ethylbenzene, Styrene, and Naphthalene

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Ethylbenzene (EB), styrene (ST), and naphthalene (NA) cause toxicity in the lung, but this is thought to require metabolic activation by cytochrome P450 monooxygenases (P450s) in either the liver or the lung. We are developing an *in vitro* approach to determine whether hepatic bioactivation of these aromatic hydrocarbons can contribute to lung toxicity. We hypothesize that hepatic enzymes are capable of escalating lung epithelial cell damage caused by EB, ST, and NA. Airway epithelial cells from either female mouse trachea (MuEC) or the human bronchiolar epithelial cell line, HBE1, were grown and differentiated at the air-liquid interface in transwell inserts. Cells were exposed to 5µM or 10µM EB, 5µM or 10µM ST, or 5µM to 3mM NA, in media containing female mouse liver microsomes. Liver microsomes were boiled to inactivate P450s for negative control experiments. Lung epithelial cell toxicity was

assessed by measuring cell permeability and cell density. MuEC cells exposed to 10μM EB with liver microsomes present resulted in a 50% decrease in cell density. No significant MuEC cell death occurred with 10μM EB and boiled liver microsomes. The 5μM EB exposure did not result in significant cell death for MuEC cells. MuEC exposed to ST or NA at 5μM and 10μM resulted in no significant cell death with or without active liver microsomes. For a higher dose of NA, 20μM, MuEC cell death was observed, but the extents were similar in the active and boiled microsome groups (~50% decrease in cell density). No significant cell death was detected in HBE1 cells exposed to 5μM to 3mM NA without active liver microsomes present. With the active microsomes present during exposure to 3 and 0.3mM of NA, a significant (20%) drop in HBE1 cell density was detected. Liver microsome-generated EB metabolites are more potent than liver microsome-generated NA or ST metabolites in causing toxicity in the mouse trachea epithelial cells. MuEC, which are primary mouse trachea cells, had apparently greater intrinsic ability to generate toxic NA metabolites, and were more susceptible to NA toxicity, compared to the HBE1 cells. *Supported by R01 ES020867, P30 ES023513, NIOSH 2U54OH007550, and T32 HL007013.*

1206 Resveratrol Inhibits Metabolism of SEB-Activated T Lymphocytes by Epigenetic Dysregulation

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Staphylococcal enterotoxin B (SEB) is a Centers for Disease Control and Prevention-designated select agent of bioterrorism. It is also a superantigen activating Vβ8-specific T cells, inducing massive T cell proliferation and cytokine production as well as changes in the metabolic status of naïve T cells from normal quiescent state to metabolic energetic, glycolytic cells. Resveratrol, RES, a phytoalexin produced by various plants, mainly grapes and berries, has a well-known role as antioxidant and anti-inflammatory agent. In addition, it has been shown to be an inhibitor of cancer cell metabolism through suppression of pyruvate kinase. In our previous studies, we found that RES has an ameliorative effect in mice with SEB-induced acute lung injury, which led to the survival of the mice while vehicle-treated mice succumbed. In the current study, we examined the effects of resveratrol treatment on cell metabolism of the SEB-activated T cells, specifically following activation of isolated splenocytes with SEB along with treatment with vehicle or RES. Seahorse Realtime metabolic analyzer was used to study the metabolic changes of RES- and vehicle-treated cells. The data generated showed that RES-treated T cells reverted toward quiescent phase and underwent cell cycle arrest when compared with vehicle-treated cells, which were more energetic as demonstrated by increase in the glycolytic activity represented by extracellular acidification rate, which started to increase after 3 hours of SEB exposure. Moreover, RES treatment shifted the cell metabolism of SEB-activated T cells from glycolysis to totally mitochondrial phosphorylation, which was due to probably upregulation of up to a 3-fold change of microRNA-100 that targets a key enzyme, pyruvate kinase, and prevents transformation of phosphoenol pyruvate into pyruvate, the main precursor of acetyl CoA, before entering the mitochondria for oxidation to ATP molecules. Using RT-PCR, we found that PKM gene was significantly suppressed, while expression of TOB, a T cell anti-proliferative gene and DEPTOR, an mToR suppressor, were statistically elevated in RES-treated cells. mToR quantity was found in lower amounts in RES-treated cells when compared to vehicle-treated cells. Together, RES treatment has a significant impact on SEB-activated T cells through effects on the metabolic pathways.

1207 Modeling Toll-Like Receptor 4-Mediated Apoptosis Resistance in Lung Fibroblasts to Investigate Pulmonary Fibrosis Pathogenesis

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Radiation treatment (rt) of thoracic tumors frequently results in pulmonary fibrosis (PF), which is characterized by progressive fibroblast accumulation. This lung scarring adversely affects hundreds of thousands of patients globally, who have a median survival time of only 3 years after diagnosis. Although fibrosis is not uncommon after lung rt, treatment strategies are currently lacking. Recent studies suggest that Toll-like receptor 4 (TLR4) is a key element in PF. Lung rt often results in inflammation, causing release of TLR4 agonists such as HMGB1. TLR4 has been previously linked to pro-survival signaling, which may help cells evade apoptosis. Fibroblast apoptosis is critical for wound resolution, but in PF, fibroblasts become apoptosis resistant and proliferate excessively. We propose that TLR4 agonists released during rt pneumonitis cause pro-survival signaling and subsequent apoptosis resistance, thereby contributing to the onset of PF. Here, we present supporting evidence for this idea, as well as an *in vitro* model of TLR4-mediated apoptosis resistance in primary human lung fibroblasts (1° HLFs). To establish a model of TLR4-induced apoptosis resistance, 1° HLFs were treated with 6 doses of lipopolysaccharide (LPS), a potent TLR4 agonist, ranging from 0.0003 to 30 $^{EU}/_{mL}$. After 7 d LPS, 1° HLFs were treated with 6 hr 0.5 μM staurosporine (STS) to induce apoptosis. Western blotting for cleaved caspase 3 showed that low doses of LPS pre-treatment inhibited apoptosis. This paradigm was validated in 3 strains of 1° HLFs. We utilized this model to study the mechanism linking TLR4 to pro-survival signaling. 1° HLFs were treated with 7 d 0.3 $^{EU}/_{mL}$ LPS to induce apoptosis resistance. Apoptotic mediators downstream of TLR4, such as PI3K, IκBα, XIAP, TRAF1/2, and Bcl-x_L, were analyzed, revealing that LPS-treated cells exhibited increased pro-survival proteins and decreased pro-apoptotic proteins in comparison to untreated cells. These results give context to findings that individuals deficient in TLR4 signaling are less susceptible to PF. The current study suggests that TLR4 plays a pathogenic role during the lung rt response by causing apoptosis resistance in fibroblasts and thus unchecked proliferation of scar tissue. Further elucidation of this pathway is crucial to developing strategies to prevent and treat PF.

1208 Vascular Cell Dysfunction from Exposure to Polychlorinated Biphenyls Contributes to Lung Toxicity

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Polychlorinated biphenyls (PCBs) are ubiquitous environmental toxicants considered to be vascular disrupting compounds. PCB153 is a non-dioxin like congener most often found in human foods and accounts for approximately 25% of the total PCB content found in human tissues. PCBs produce pathological vascular remodeling in the experimental model, and PCB153 in the experimental model has been shown to selectively accumulate in the lung. High levels of PCBs are found in human lung tissue, and epidemiological studies have shown the association of lung toxicity by chronic exposure to PCBs. Our novel discovery of new roles for the transcription regulator ID3 in the acquisition of induced pluripotent vascular stem cells (iPVSCs), endothelial-mesenchymal transition, and 3D vascular sphere formation led us to test whether ID3 drives pulmonary vascular cell dysfunction upon PCB153 exposure. Our lab has tested the effects of environmentally relevant concentrations of PCB153 on human lung vascular endothelial and smooth muscle cells. We observed PCB153-exposed human pulmonary endothelial and smooth muscle cells overexpressing ID3 to show a significant two-fold increase in cell proliferation as determined by MTT, SRB, and BrdU assays. Similarly, a 3D HuBiogel vascular sphere model, which mimics *in vivo* conditions, showed a significant increase in size and number of vascular sphere formation upon PCB153 treatment. Pluripotent vascular stem cells showed the loss of VE-cadherin and gain of MMP9 and vimentin, which are markers of endothelial to mesenchymal transition (EndMT). Based on these findings, we evaluated accumulated PCBs in a large NHANES population cohort and showed a significantly higher accumulation of dioxin and non-dioxin-like PCBs in subjects at risk of pulmonary arterial hypertension. We applied a machine learning approach and identified a significant interaction between ID3 genome targets and PCBs. Based on these findings we propose that transcription regulator ID3 may serve as a novel diagnostic/prognostic indicator for evaluating vascular stem/progenitor cell toxicity.

1209 Context Matters: Using an Organotypic Airway Model to Assess the Response to Inhaled Toxicants

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Exposure to air pollution is associated with increased morbidity and mortality from pulmonary and cardiovascular diseases worldwide. As a significant contributor to air pollution, diesel exhaust particles (DEP) are known to induce pulmonary oxidative stress and pro-inflammatory responses in both *in vivo* studies and *in vitro* models. Many *in vitro* studies of DEP exposure have focused only on bronchial epithelial cells grown in two-dimensional cultures on plastic substrata; however, the cellular microenvironment *in vivo* is more complex involving additional cell types such as fibroblasts in the stroma. Intercellular communication is an important aspect of response to environmental stimulants, and it has been shown that fibroblasts in the lung influence epithelial cell growth, differentiation, and gene expression. We hypothesized that incorporating fibroblasts into an organotypic model of the airway epithelium would alter epithelial cell response to DEP and provide an opportunity

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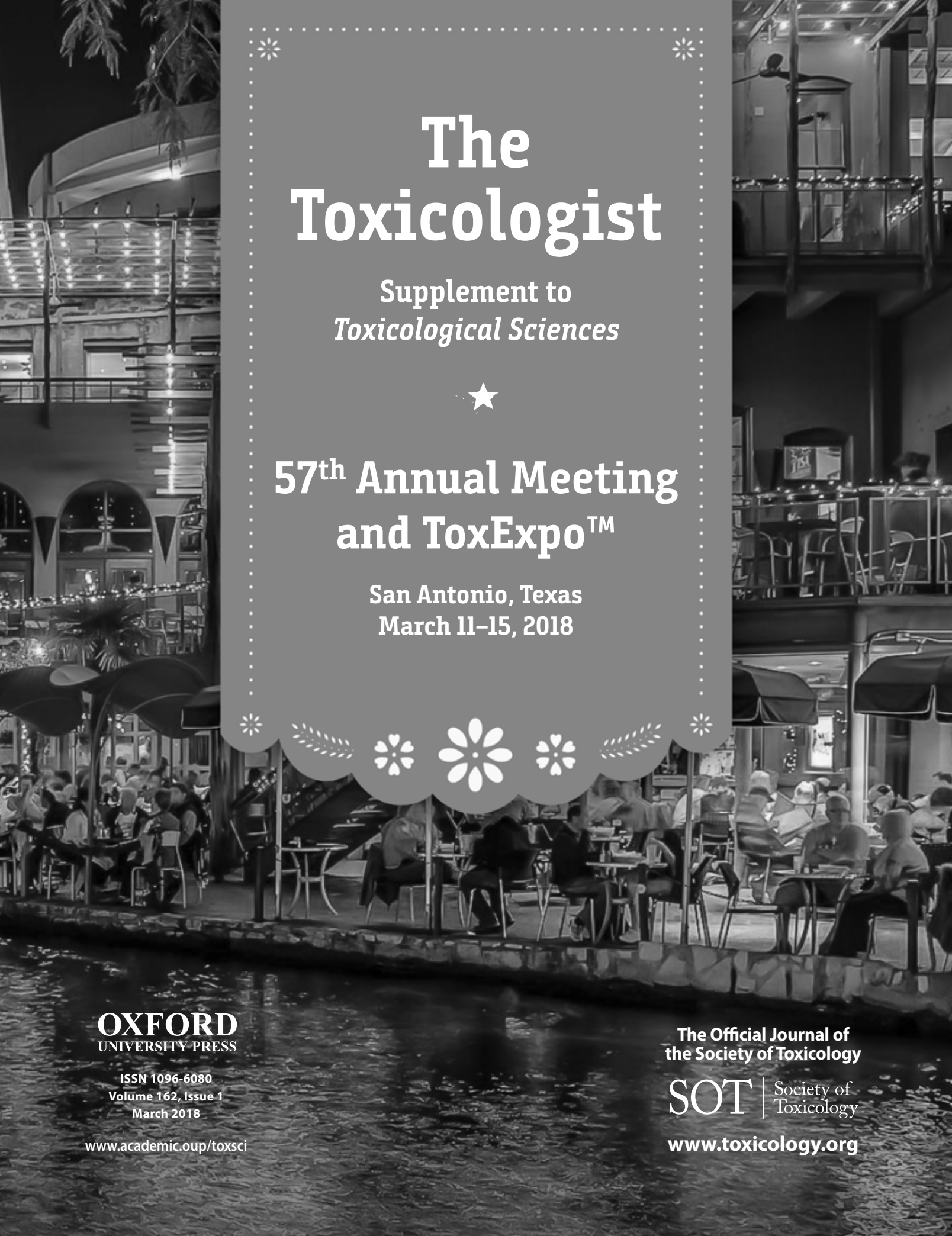

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