2033 Visceral Adiposity-Stimulated Genotoxicity and Malignant Transformation of Epithelial Cells

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Obesity is a global epidemic with a predicted rate of 42% in the USA by 2050. Epidemiological studies show that obesity is a risk factor for developing cancer, however; the molecular mechanism has not been fully elucidated. Our published data demonstrate that fibroblast growth factor-2 (FGF2) released from fat cells (adipocytes) in the visceral adipose tissue (VAT) induces transformation/tumorigenicity in the skin and mammary epithelial cells. Specifically, FGF2 released from VAT stimulates epithelial cell growth in soft agar by inducing the proto-oncogene c-Myc. Growth in soft agar is a measure of transformation/tumorigenicity; neither transformation nor c-Myc induction in epithelial cells was reversible. c-Myc overexpression can initiate a process of genetic instability linked to tumor initiation. Our discovery of this novel direct path of VAT-stimulated tumorigenesis adds mechanistic insight to our earlier discovery that VAT secretions promote UVR-induced non-melanoma skin cancer. The objective of our current study was to determine the mechanism by which FGF2 stimulates malignant transformation. We hypothesized that FGF2 from VAT induces c-Myc and subsequent genomic instability in epithelial cells leading to increased carcinogenesis. To test hypothesis we generated a filtered conditioned-medium from the human VAT, treated MCF-10A (mammary epithelial) and JB6 P+ (skin epithelial) cells and measured several downstream mediators of FGF2 and activation of FGFR-1 (FGF2 receptor). Following VAT treatment, epithelial cells demonstrated induced c-Myc protein expression along with ROS accumulation, elevated γ-H2AX foci, and increased micronucleus (MN) formation. We found that inhibition of c-Myc attenuated VAT-induced neoplastic transformation of MCF-10A and JB6 P+ cells, while constitutive activation of the c-Myc induced spontaneous neoplastic transformation of JB6 P+ cells. Collectively, our data suggested FGF2 released from VAT interacts with FGFR-1 and activates c-Myc. The role of c-Myc in the formation of MN and DNA damage is under investigation. Determining the impact of excess VAT on cancer will lead to strategies to help prevent adiposity-associated cancers and identify individuals at risk for disease or individuals that may be susceptible to compounded genotoxicity due to DNA damaging environmental exposures.

2034 Mechanistic Analysis of Benzo[*a*]pyrene on Carcinogenesis Using Mouse Lung Organoids

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The mechanisms of multistep carcinogenesis by long-time treatment of chemicals *in vivo* have not been comprehensively understood. We previously reported that subcutaneously injected mouse lung-derived organoids, to which genetic reconstruction, e.g., K*ras* activation, *p16* and/or *Pten* knockdown have been introduced *in vitro*, showed tumorigenicity. In the present study, to clarify chemical carcinogens could affect similarly to the genetic reconstruction, carcinogenic potentials and molecular changes by a short-time treatment of benzo[*a*]pyrene (B[*a*]P) were analyzed using organoids. Lung tissue was excised from B6 mice and its organoids were established by a 3D-culture method using Matrigel and growth factors. After introduction of sh*Luc* (control) or sh*Pten* followed by three times of B[*a*]P (0, 0.4-3.0 microM) treatment, the organoids were injected to nude mouse subcutis. At 59 days after the injection, subcutaneous tissues were histopathologically examined. While multi-layered/invasive regions with nuclear atypia were observed in organoids of sh*Luc*+B[*a*]P-High, sh*Pten*+B[*a*]P-Low and -High-treated groups, single-layered organoids only were observed in B[*a*]P-0 microM and sh*Luc*+B[*a*]P-Low groups. [Conclusion] A carcinogenic potential of B[*a*]P was histopathologically detected using mouse lung-derived organoids and an additive effect of *Pten* knockdown to the B[*a*]P-induced neoplastic changes appeared *in vitro*. We now conduct whole exome sequencing to clarify initial target genes of B[*a*]P on lung tumorigeneis.

2035 Formaldehyde-Induced Loss of Transcription Factors HIF-1α and HIF-2α

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Formaldehyde (FA) is the most abundant endogenous aldehyde with exogenous exposures being associated with a range of adverse health effects, including neurotoxicity, bone marrow damage and leukemia. Mouse models with diminished detoxification of aldehydes also exhibit a high vulnerability of hematopoietic stem cells to endogenous FA. It has recently been determined that FA is a potent proteotoxic agent, triggering activation of the heat shock transcriptional factor HSF1 and accumulation of large amounts of proteins with Lys48-linked polyubiquitination (proteasome-targeting mark). Although polyubiquitination and a subsequent proteasomal degradation of damaged proteins are generally viewed as cytoprotective, we considered a possibility that a particularly extensive depletion of some critical proteins can be detrimental to cells. We found that FA induces a rapid and selective loss of hypoxia-inducible transcriptional factors HIF-1α and HIF-2α in human cells. Protein expression of HIF-1α/2α in cells is repressed by oxygen and the stabilization and nuclear translocation of these transcription factors occur only in hypoxic conditions. We found that the hypoxic stabilization of HIFs was rapidly inhibited when cells were exposed to FA, however this was reversible with the removal of FA. We determined that FA-induced HIF degradation occurred via the proline hydroxylation/VHL- and proteasome-dependent pathway. Consistent with the diminished protein abundance of HIFs, extended FA exposures led to the downregulation of HIF-dependent gene expression. As HIF-regulated genes are important for normal functioning and maintenance of stemness in hematopoietic stem cells that reside in hypoxic bone marrow niches, vulnerability of HIFs to FA damage can help explain the bone marrow toxicity of this ubiquitous aldehyde.

2036 Post-Translational Modifications and Functional Study of Aryl Hydrocarbon Receptor upon Co-Exposure to Dioxin and Nickel

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Aryl Hydrocarbon Receptor (AhR) plays a crucial role in mediating metabolism of a wide range of environmental toxicants including polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and dioxins. Extensive research in the past strongly suggests that deregulated activities of AhR may be responsible for its carcinogenic effect after exposure to multiple environmental toxicants. Recently, we found that nickel chloride significantly blocked the induction of P450 genes by dioxin or benzo[alpha]pyrene. Given that Ni^{2+} strongly activates HIF-1alpha, which also shares a common beta-subunit (ARNT) with AhR, we postulate that the inhibitory effect of Ni^{2+} on expression of P450 genes is mediated through competition for ARNT between AhR and HIF-1alpha. To further investigate the mechanism of AhR activation and its interaction with components from other signaling pathways, we asked whether lysine mutations that would potentially alter several types of post-translational modifications affected AhR functions in cells. We demonstrated that AhR was modified by SUMO3 and that two lysines (K63/K510) were crucial for mediating the sumoylation. Moreover, substitutions of lysines with arginines within the bHLH domain suppressed the ability of AhR to transactivate P450 genes upon exposure to dioxins.

2037 Welding Fumes: A New Group 1 Carcinogen

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Welding fumes were recently classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) based on strong epidemiological evidence and limited evidence in animals. It is estimated that 11 million workers worldwide weld full-time, and an additional 110 million have had some type of welding-related exposure. Welding exposures are complex because of the diversity of welding modalities used in the workplace; these modalities include exposures to non-carcinogenic and/or carcinogenic metal containing fumes. The objective of this study was to determine which welding fumes and their component metals are the most toxic and have the greatest tumorigenic potential. Male A/J mice received intraperitoneal injections of corn oil or the initiator 3-methylcholanthrene (MCA;10 µg/g) and one week later were exposed by whole body inhalation to air or gas metal arc-stainless steel (GMA-SS) or GMA-mild steel (MS) welding

aerosols for 4 h/d x 4 d/w x 8-9 w at a target concentration of 40 mg/m^3. Lung nodules were enumerated at 30 weeks post-initiation. GMA-SS and GMA-MS fumes significantly promoted lung tumor multiplicity in A/J mice initiated with MCA (16.11 ± 1.18; 21.86 ± 1.50, respectively) compared to MCA/air-exposed mice (7.93 ± 0.82; 8.34 ± 0.59, respectively). Oropharyngeal aspiration of GMA-SS and its component metals showed that GMA-SS fume was more pneumotoxic than the individual components. Component Fe_2O_3 was the most toxic and also the only metal to promote lung tumors in A/J mice. In conclusion, this study demonstrates that inhalation of GMA-SS and GMA-MS welding fume as well as Fe_2O_3 promote lung tumor formation *in vivo* and provides support for the epidemiology that shows welders, using mild and/or stainless steel, are at an increased risk for lung cancer.

2038 Direct Formalin Fixation Induces Widespread Genomic Effects in Archival Tissues

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Recent advances in next generation sequencing have dramatically improved transcriptional analysis of degraded RNA from formalin-fixed paraffin-embedded (FFPE) samples. However, little is known about potential genomic artifacts induced by formalin fixation, which could affect toxicological and clinical studies being conducted in FFPE samples. Previously, we identified a consistent shift in RNA-sequencing profiles between matching frozen and FFPE samples. We hypothesized that this shift was due to a core set of transcriptional changes induced when fresh tissue is fixed in formalin. To test this idea, liver samples were collected from male B6C3F1 mice treated with 600 ppm of phenobarbital (PB) or vehicle control (Con) for 7 days. Samples were divided into the following conditions:1) fresh-frozen (FR); 2) directly fixed in 10% buffered formalin for 18 hours and processed to FFPE (FIX); and 3) processed as for FIX but initially frozen (FR>FIX) (n=6/group/condition). The FR>FIX group served as a control for tissue processing and sequencing after collection. Total RNA libraries were prepared and ribo-depleted prior to sequencing on an Illumina Hi-seq 2500. Reads were aligned using Star (2.4) and analyzed in Partek Flow (6.0). Direct fixation (FIX *vs*. FR) resulted in 2946 differentially expressed genes (DEGs), 98% of which were down regulated. Freezing prior to fixation (FR>FIX *vs*. FR) resulted in 95% fewer DEGs, indicating that the majority of formalin effect occurred at the time of fixation (i.e., as a transcriptional response) rather than during tissue processing or sequencing. Comparative analysis of this formalin-induced gene set with two independent studies in Ingenuity Pathway Analysis identified consistent enrichment in oxidative stress, mitochondrial dysfunction, and transcription elongation pathways. However, direct fixation did not have a clear impact on chemical response. PB treatment induced 180 DEGs within the FIX group and 159 in the FR>FIX group of which 120 were shared. The DEGs in each list were consistent with CAR/PXR activation and PB exposure, suggesting the formalin signature did not confound the chemical response. Our results highlight distinct transcriptional effects of formalin fixation that could impact RNA-sequencing studies using FFPE samples. *This abstract does not reflect US EPA policy.*

2039 Effect of Resveratrol on Gut Microbiome in Colorectal Cancer

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Colorectal cancer (CRC) is a heterogeneous disease with recognizable clinical and molecular features, with wide range of prognostic and treatment responses observed through CRC patients worldwide. Currently, there is no cure for patients suffering from CRC, and most treatments involve the surgical removal of the cancer and chemotherapy that are not effective in most cases and have adverse side effects or increase the toxicity. In current study, we investigated the effects of resveratrol (RES), a natural component found in grapes, strawberries and raspberries on murine azoxymethane-dextran sodium sulfate (AOM-DSS) induced CRC model. Our data shows that administration of RES alleviates symptoms associated with CRC in this model, which includes reversal of weight loss and decreased colon polyps. Flow cytometry data showed significant increase in both blood and splenic MDCSs in AOM-DSS group while significant decreases in blood, spleen and mesenteric lymph node (MLN) CD3+, CD4+ and CD8+ T cells population Also, flow cytometry data showed significant increase in spleen and MLN Foxp3+ T cells and IL10 in AOM-DSS fallow by RES treatement while significant decreases in IFN-γ and Th17 cells in spleen and MLN in AOM-DSS group. Endoscopy and histopathology also showed decreased colonic tissue damage, tumor growth and cellular infiltration in the colon after RES treatment. To better understand the beneficial effects of RES in CRC, we performed 16S rRNA metagenomic sequencing to investigate alterations in the gut microbiome in vehicle- or RES-treated AOM-DSS mice. Analysis of cecal flushes revealed that AOM-DSS administration led to significant decrease in Ruminococcus gnavus, Akkermansia muciniphila, Bacteroides acidifaciens and Mucispirillum schaedleri. However, mice that were treated with RES showed a remarkable reversal in these gut microbial alterations caused by AOM-DSS CRC induction, which had gut microbiome similar to that of naive mice. Collectively, these data suggest that RES can ameliorate CRC by preventing gut microbial dysbiosis and restore gut microbiome composition to a more homeostatic state. *Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20RR032684 and VA Merit Award BX001357.*

2040 Cells That Escape Cr(vi)-Induced Cell Death Exhibit Chromosome Instability

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Hexavalent chromium (Cr(VI)) compounds are established human lung carcinogens; however, the carcinogenic mechanism remains poorly understood. Cr(VI) induces DNA damage which under normal circumstances triggers the protective apoptotic machinery to avoid transformation and carcinogenesis. Evasion of apoptosis is a hallmark of carcinogenesis, however it is unknown how Cr(VI)-damaged cells are able to escape cell death and become tumorigenic. We exposed human lung cells to low concentrations of zinc chromate continuously for 6 months. At various intervals during treatment we assessed growth parameters using a colony forming assay. We also monitored changes in chromosome number and structure of surviving cells through traditional karyotyping methods. We found that Cr(VI) induced a measure of cell death in the first 25 days of exposure. The lower concentrations (0.0125 and 0.025 ug/cm^2) showed decreased plating efficiency relative to the control, indicative of cell death, for the entire length of treatment. Interestingly, the highest concentration, 0.05 ug/cm^2, initially caused significant cell death, but was followed by a period of enhanced survival at day 70 of treatment, and then decreased plating efficiency after day 120 which remained throughout exposure. Although plating efficiency decreased, cell growth was accelerated in the high treatment group. Additionally, cells that escaped particulate Cr(VI)-induced cell death exhibited significant amounts of both structural and numerical changes. Control cells at all time points showed normal chromosomes; at day 5 there were 24, 26, and 30 percent of cells with abnormal karyotypes at 0.0125, 0.025 and 0.05 ug/cm^2 zinc chromate, respectively; at day 70 there were 46, 52, and 48 percent of cells with abnormal karyotypes; at day 180 there were 18, 72, and 84 percent of cells with abnormal karyotypes. Increases in aneuploidy were observed at earlier time points compared to structural alterations. These data support a hypothesis that Cr(VI)-treated cells can evade apoptosis and transform into chromosomally unstable cells yet continue to survive. These cells have the potential to become carcinogenic. Further work will elucidate the mechanism behind the evasion of apoptosis by considering genetic and epigenetic changes in the apoptotic pathway. *This work was supported by NIEHS grant ES016893 (J.P.W.) and the Jewish Heritage Fund for Excellence in Research Enhancement Program at the University of Louisville School of Medicine.*

2041 *In Vitro* Investigations of Adjuvant Chemotherapy for Prevention of Breast Cancer Tumor Recurrence

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Breast cancer is the most common cancer among women. Once identified and tumor excised, the risk of local breast cancer recurrence (or return of cancer within the breast) depends on tumor characteristics. Adjuvant therapies are used to ensure that remaining microscopic disease will be eradicated, and to help extend survival time of the patients. A standard clinical regimens is a combination of cyclophosphamide, adriamycin (doxorubicin) and 5-fluorouracil (CAF) that is administered for four months. Despite the initially successful multimodal therapy, tumor recurrence remains a major cause of mortality in breast cancer patients [1]. Hence, better treatment options are necessary. In this study, responses from murine H8N8 and H8N8 T3.2 cells were investigated via two different assays: via image-based live-cell analysis and an impedance-based cell monitoring system. The H8N8 cells are an immortal mammary carcinoma cell line with tumor stem cell properties, and the H8N8 T3.2 cells are a recurrent tumor variant. H8N8 T3.2 cells were established from a solid breast tumor that received the CAF clinical regimen *in vivo*. For further *in vitro* tumor recurrence investigation,

The Toxicologist

Supplement to
Toxicological Sciences

57th Annual Meeting and ToxExpo™

San Antonio, Texas
March 11–15, 2018

OXFORD
UNIVERSITY PRESS

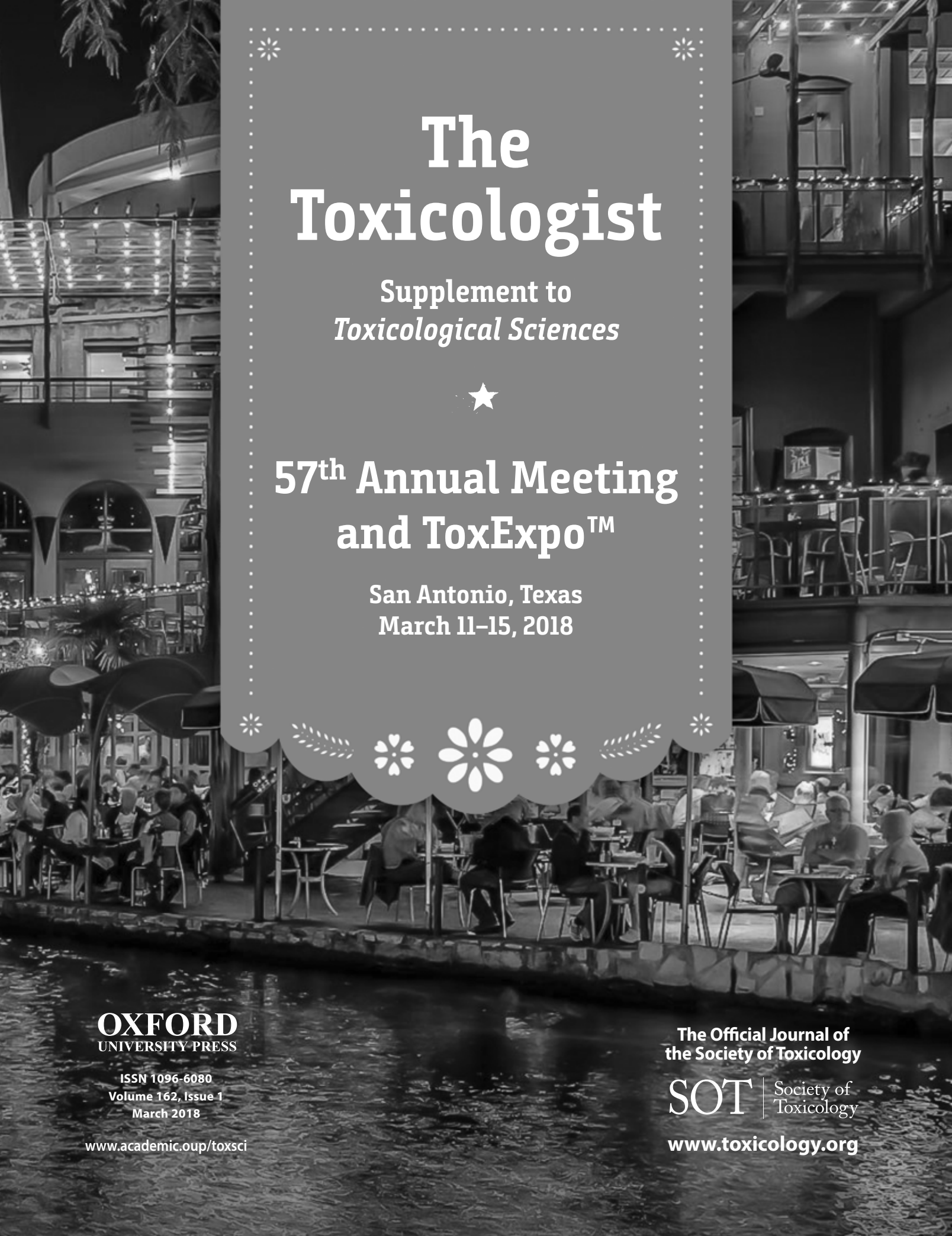
ISSN 1096-6080
Volume 162, Issue 1
March 2018

www.academic.oup/toxsci

The Official Journal of
the Society of Toxicology

SOT | Society of Toxicology

www.toxicology.org



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OXFORD
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ISSN 1096-6080
Volume 162, Issue 1
March 2018

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