outperformed by selected novel urinary novel biomarkers, indicating that the innovative multiplex IPLC/MS assay is a valueadded platform for simultaneous detection of urine-based proximal cortical tubule injury biomarker changes in dogs and potentially in other species. 1

1253 Sex Differences in Excretion Levels of Urinary Biomarkers of Nephrotoxicity in Rats

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Urinary biomarkers have been used widely in preclinical toxicity studies to detect dysfunctions and injuries of the kidney caused by drugs under development. While they have been well-studied for evaluating nephrotoxicity, knowledge of sex differences in excretion levels of urinary biomarkers remains inadequate. We conducted experiments focused on effects of endogenous sex hormones on urinary biomarkers using intact and castrated male and female rats. Comparisons of the urinary biomarker excretion levels between intact male and female rats at 5, 7, 9 and 12 weeks of age revealed higher excretion levels of leucine aminopeptidase (LAP), γ-glutamyl transpeptidase (γGTP), total protein, liver-type fatty acid-binding protein (L-FABP), cystatin C (Cys-C) and β2-microglobulin (β2-MG), and lower excretion level of kidney injury molecule 1 (Kim-1), in male rats as compared to female rats. Orchidectomized male rats showed lower urinary excretion levels of alkaline phosphatase (ALP), LAP, γGTP, N-acetyl-β-D-glucosaminidase, glucose, total protein, L-FABP, Cys-C, β2-MG and neutrophil gelatinase-associated lipocalin, and higher urinary excretion levels of clusterin and Kim-1, than sham-operated male rats. On the other hand, no significant differences in the urinary biomarker excretion levels excluding ALP were observed between ovariectomized and sham-operated female rats. In the present study, we demonstrated the existence of sex differences in excretion levels of urinary biomarkers that are universally used in preclinical toxicity studies, and also that these differences, especially in relation to the urinary excretions of ALP, LAP, γGTP, total protein, L-FABP, Cys-C, and β2-MG, may closely relate to the endogenous testosterone.

1254 Evaluation of Urinary Renal Safety Biomarkers in Non-Human Primates with Six Model Kidney Toxicants

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Certain classes of drugs (e.g. antibiotics like aminoglycosides or cephalosporins, chemotherapeutic agents, and antivirals) are known to cause drug-induced kidney injury (DIKI) in patient populations. Due to the close evolutionary relationship with humans, non-human primates (NHP) are an important nonclinical model for assessing drug-induced injuries. NHP models afford an opportunity not only to study the type of injury induced by such drugs but also to evaluate the performance of translational safety biomarkers that may be useful for monitoring similar injuries in humans. Novel urinary kidney safety biomarkers have been shown to be more sensitive than the conventional renal function biomarkers, blood urea nitrogen (BUN) and serum creatinine (SCr). To characterize the types of kidney injury caused by common drugs and to assess the relative performance of a growing list of novel kidney safety biomarkers, studies were run in NHP using six compounds that are clinically relevant - cefpirome, gentamicin, everninomicin, cisplatin, naproxen and cyclosporine. A comprehensive evaluation of nine urinary biomarkers (albumin, clusterin, cystatin C, Kim-1, lipocalin-2 or NGAL, N-acetyl-β-D-glucosaminidase (NAG), osteopontin (OPN or SPP1), retinol binding protein 4 (RBP4) and total protein) was performed on urine collected from these studies. Treatment with each of the six compounds resulted in kidney proximal tubule injury of various severities, and the performance of the urinary biomarkers was determined relative to the microscopic histomorphologic changes observed. Among the kidney injury biomarkers analyzed, Kim-1, clusterin, and albumin showed the highest overall performance for detecting drug-induced renal tubular injury in the NHP, and the majority of the biomarkers were able to detect the injury earlier than BUN or SCr. This comprehensive evaluation of six common nephrotoxic drugs characterized the histopathological changes in the kidney and demonstrated the monitorability of such changes using novel safety biomarkers, and provided additional supporting evidence for translating these biomarkers for use in clinical settings to further ensure patient safety.

1255 Alterations in the Expression of Shelterin Complex Genes in Crystalline Silica Exposed Rat Lungs

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Occupational exposure to silica can result in advanced pulmonary fibrosis and lung carcinoma through several complex mechanisms. Therefore, it is imperative to identify the key biomarkers of silica-induced pulmonary toxicity for the intervention of lung pathologies. Telomeres (the nucleoprotein structures with repetitive (TTAGGG) sequences at the end of chromosomes) are a molecular "clock of life" and alterations are associated with several chronic diseases. Shelterin complex: protection of telomerase1 (POT1), telomeric repeat-binding factor1 (TRF1), telomeric repeat-binding factor2 (TRF2), TRF1-interacting nuclear factor2 (Tin2), TRF2-interacting telomeric protein (Rap1), and POT1 and Tin2-organizing protein (TPP1) play an important role in maintaining telomere length and integrity and any alteration in telomeres activate DNA damage machinery resulting in telomere attrition. The goal of this study was to assess the effect of crystalline silica exposure on the regulation of shelterin complex genes in an animal model. Male Fisher 344 rats were exposed by inhalation to Min-U-Sil 5 silica for 3, 6, and 12 weeks at a concentration of 15 mg/m^3 for 6 hours/day for 5 consecutive days/week. After the final day of exposure the right lung was homogenized, total RNA was isolated and reverse transcribed to obtain cDNA, and expression of shelterin complex genes was assessed. At all-time points after exposure, mRNA expression of POT1, TRF1, TRF2, Tin2, Rap1, and TPP1 were significantly decreased ($p<0.05$) in the silica-exposed animals compared to air controls, and the decrease observed were exposure time dependent. POT1 and TPP1 which mediates telomerase-dependent telomere extension were significantly decreased in exposed animals. In conclusion, our results suggested that silica inhalation promoted shelterin complex instability. This study indicated that measurement of expressions of shelterin genes involved in telomere regulation may serve as a potential biomarker for silica-induced pathology including carcinogenesis. In addition, changes in shelterin complex could potentially promote telomere end-to-end fusions and cancer formation.

1256 Evaluation of Di-Docosahexaenoyl (C22:6)-Bis(monoacylglycerol) Phosphate(22:6 BMP) as a Biomarker of Drug-Induced Phospholipidosis in Rodents

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Drug induced phospholipidosis (DIPL), a lysosomal phospholipid storage disorder, has been known as a side effect of cationic amphiphilic drugs. Recently di-docosahexaenoyl (C22:6)-bis(monoacylglycerol)phosphate (22:6 BMP) has been proposed as a noninvasive and specific biomarker to monitor DIPL in animals and human. In this study, amiodarone (AMD), a well-known phospholipidosis-inducer, was administrated to rats to evaluate onset, dose-response and reversibility of 22:6 BMP in serum and urine. We also evaluated the specificity of 22:6 BMP by the analysis of rats treated with tetracycline (TC), a negative compound for DIPL. In the 1st study, AMD was orally administered to male Crl:CD(SD) rats at dose of 150 mg/kg/day for 3, 7 or 10 days. Additional animals were dosed 150 mg/kg/day of AMD for 10 days followed by 13 days recovery period. In the 2nd study, AMD and TC were orally administrated for 7 days at doses of 16, 50 and 150 mg/kg/day of AMD or 2000 mg/kg/day of TC to male rats. In both rat studies, urine was collected overnight via metabolic cages after last dosing or at the end of recovery period. Blood and tissues were collected at the necropsy, and the tissues were used for microscopic examinations. Serum and urine 22:6 BMP were measured by LC/MS/MS. DIPL-related histopathological changes were observed after administration of 150 mg/kg/day AMD for 3, 7 or 10 days, which caused the increase of 22:6 BMP in both serum and urine. AMD-induced histopathological changes and the increase of 22:6 BMP were returned to normal after the recovery period. There were no histopathological changes at doses of 16 and 50 mg/kg/day AMD for 7 days, and serum and urine 22:6 BMP were not increased significantly. Regarding the TC dosing group, slight to moderate fatty changes of hepatocyte were observed, but serum and urine 22:6 BMP were not increased. Serum and urine 22:6 BMP are considered to be enough sensitive, reversible and specific markers for DIPL. They only increase significantly when the histopathological changes related to DIPL are observed.

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

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the Society of Toxicology

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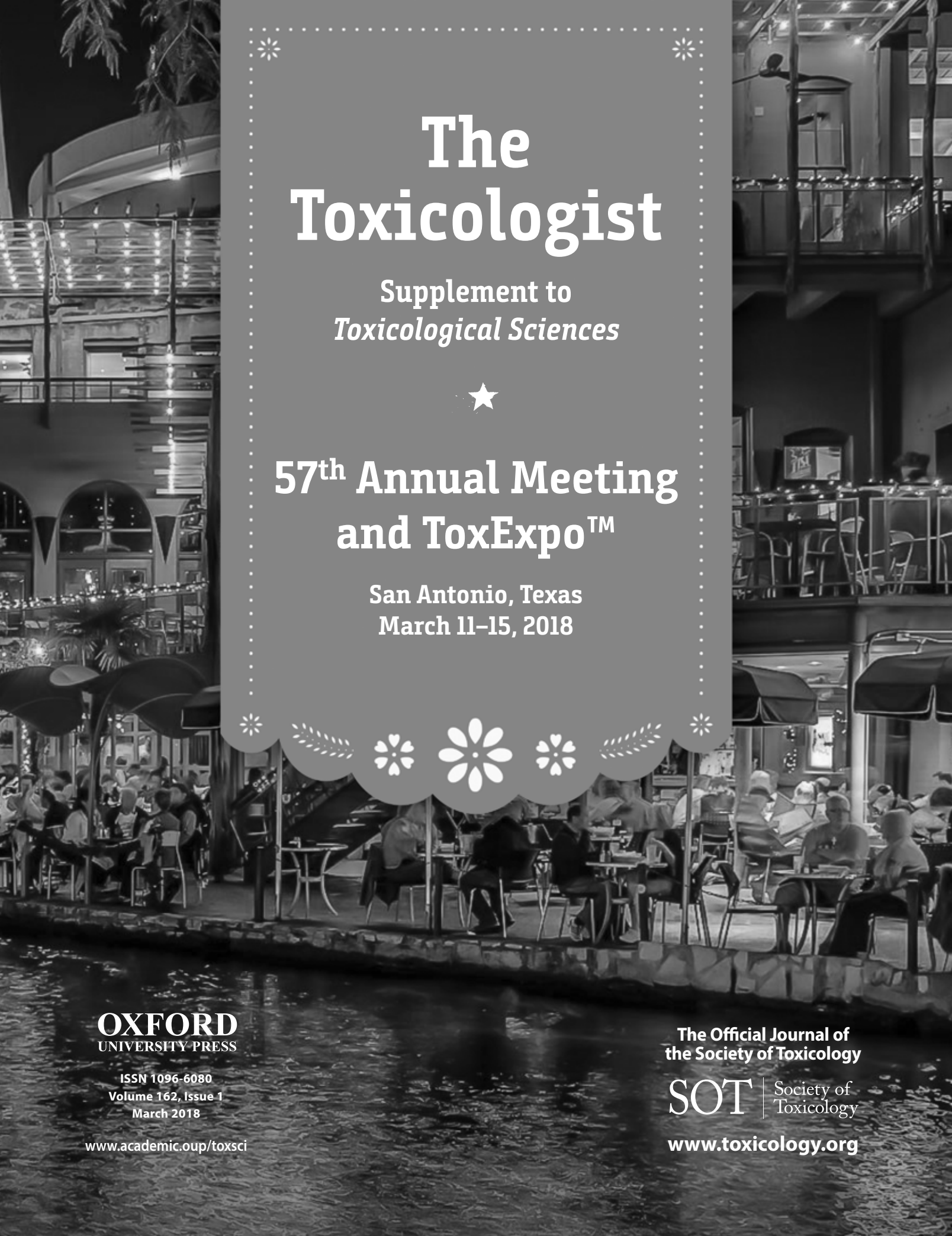
www.toxicology.org



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