urine. Novel urinary biomarkers have been proposed to localize the damage to the proximal and or distal tubules vs. the glomerulus as well as to serve as an earlier indicator of kidney damage than SCr and BUN. Renal urinary biomarkers specific to tubular damage include kidney injury molecule - 1 (Kim-1) and neutrophil gelatinase-associated lipocalin (NGAL). The purpose of this study was to use Kim-1 and NGAL urine measurements in rats and canines to screen compounds for clinical use and to identify compounds that were less nephrotoxic. Kim-1 and NGAL results were compared to the more classical markers of renal injury including N-acetyl-glucosaminidase, SCr, BUN and urine protein. Animals were dosed for 2, 7 or 15 days at low and high doses with different compounds. Urine was collected at pre and post dose time points and tested for Kim-1 (rats only), NGAL, creatinine, total protein and N-acetyl-glucosaminidase. The results of Kim-1 and NGAL measurements in urine were compared to the other parameters and significant differences in results were detected between the compounds. These results combined with histopathological examination provide more comprehensive and optimal assessment of nephrotoxicity effects for drugs being developed.

PS 1426 SERUM LEVELS OF THE PHOSPHOLIPID BIS(MONOACYLGLYCEROL) PHOSPHATE AS AN INDICATOR OF PHOSPHOLIPIDOSIS INDUCED IN THE RAT BY CORALGIL.

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Phospholipidosis (PLD) is a lipid storage disorder that can be induced in preclinical studies by more than 250 approved or investigational drugs and is characterized by the excessive accumulation of phospholipids in tissues. The incidence of PLD in patient populations is unknown due to the absence of a qualified accessible biomarker. A promising non-invasive biomarker of PLD is the rare phospholipid bis(monoacylglycerol) phosphate (BMP). The relationship between tissue incidence of PLD and BMP levels in biofluids was investigated using the drug Coralgil, which induces hepatic PLD in both patients and laboratory animals. Coralgil (4,4'-diethylaminoethoxyhexestrol dihydrochloride) was synthesized at the FDA and administered by gavage at 25, 50, or 100 mg/kg/day to male Fisher rats for 1 or 2 weeks. The drug induced a dose and time dependent increase in vacuolation in liver, lung, spleen, and mesenteric lymph node that was confirmed as PLD by electron microscopy and Lamp-2 immunohistochemistry. Liver PLD coincided with the accumulation of high levels of Coralgil and two of its metabolites in liver and with an increase in the hepatic expression of genes involved in lipid synthesis. Twelve species of phospholipid were measured in urine and serum samples. Dose related increases in serum levels of di-20:6-BMP and phosphatidylcholine species were observed after 1 week of treatment. In contrast, levels of di-20:6-BMP in urine were nonspecifically elevated in the high dose group at 2 weeks but not significantly changed at earlier times or lower doses. In this study, an increase in serum levels of BMP was an early indicator of the development of a generalized PLD induced in the rat by oral administration of Coralgil.

PS 1427 INVESTIGATION OF PREDICTIVE GASTROINTESTINAL TOXICITY BIOMARKERS FOR ONCOLOGY DRUG DEVELOPMENT IN RATS.

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A known side effect of anti-proliferative cancer drugs is gastrointestinal (GI) injury. Early detection of GI toxicity using sensitive, predictive and reliable biomarkers would be invaluable for drug candidate safety profiling and dose selection. In this study, plasma L-citrulline, diamine oxidase, fecal calprotectin and fecal bile acids were evaluated in animal GI toxicity models induced by anti-cancer drugs with well-known but different mechanism of action. Small molecules inhibiting protein kinase CHK1, PAK4 and heat shock chaperone protein HSP90 were given at different dose levels via oral route for five days to male Wistar Han rats. Clinical signs observed were soft and watery feces, decreased skin turgor and activity, and hunched posture. Sections of the GI were collected at termination for histological and morphological evaluation, with H&E and NBT staining, respectively, to confirm GI injury. Blood and fecal samples were collected at various time points for measuring potential GI toxicity biomarkers. There was a dose- and time-dependent decrease in plasma L-citrulline levels during the 5 day study for all the compounds tested. The animals dosed with the PAK4 inhibitor (15 mg/kg) showed the most significant decrease (90%) in L-citrulline, compared to a significant decrease (75%) by CHK1 inhibitor (200 mg/kg) and a moderate decrease (35%) by HSP90 inhibitor(30mg/kg). Pathologically, rats treated with CHK1 inhibitor exhibited dose-dependent gastric ulceration, hemorrhage, and necrosis affecting the nonglandular stomach. Treatment with HSP90 or PAK4 inhibitors resulted in dose-dependent gastrointestinal lesions, with more severe, extensive findings in animals administered the latter. In conclusion, blood L-citrulline correlates with clinical observations, morphological and histological changes in both incident and severity of GI injuries and appears to be a sensitive and early predictive biomarker of GI toxicity for a variety of anti-proliferative agents.

PS 1428 EVALUATION OF A TEST METHOD FOR THE MEASUREMENT OF THE URINARY BIOMARKERS S-BENZYLMERCAPTURIC ACID AND S-PHENYLMERCAPTURIC ACID.

C. B'Hymer and J. E. Snawder. *BHAB, NIOSH, Cincinnati, OH.*

High-Performance Liquid Chromatography-Mass Spectrometric (HPLC-MS) conditions were developed for use to detect and quantitate urinary levels of S-benzylmercapturic acid (S-BMA) and S-phenylmercapturic acid (S-PMA). S-BMA and S-PMA are the metabolites from toluene and benzene, respectively, and these compounds are important for occupational health studies owing to their use as biomarkers of exposure. Toluene is used extensively as a solvent, and the health hazards of benzene exposure have been well established. The developed test method included the use of the deuterated analog of both analytes for use as internal standards. Spiked urine specimens were passed through solid-phase extraction cartridges; then the analytes were removed by acetone washes. The liquid volume was reduced by evaporation, and the dry extracts were reconstituted in mobile phase solvent for introduction into the liquid chromatograph. A triple quadrupole mass spectrometer (MS/MS) was used as detector with gradient reversed-phase HPLC conditions to measure the target analytes. Recovery experiments using 1, 2, 6, 8 and 30 ng/ml S-BMA and S-PMA fortified urine samples demonstrated good accuracy and precision. Recovery of both target analytes varied between average values of 99 to 110% of theory for the various spiked levels. Linearity of response was verified for concentrations of 0.5 to 50 ng/ml, and correlation coefficients of 0.98 or greater were obtained for all standard curves. This method offers a valid test for the determination of the urinary levels of both of these biomarkers of exposure as demonstrated by the accuracy and precision of the recovery studies.
Disclaimers: Mention of company names and/or products does not constitute endorsement by the National Institute for Occupational Safety and Health (NIOSH). The findings and conclusions in this poster have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

PS 1429 MIR-34A EXPRESSION WAS UP-REGULATED BY SEVEN DIFFERENT GENOTOXIC AGENTS IN A DOSE-DEPENDENT MANNER IN TK6 CELLS.

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MicroRNAs (miRNAs) are a class of small RNAs and play an important role in carcinogenesis. miR-34a is a tumor suppressor miRNA and its expression is directly controlled by the p53 gene to responds to DNA damage. In this study, alternation of miR-34a expression by genotoxins was determined with TaqMan real-time PCR methods to evaluate whether this miRNA could be used as an indicator for genotoxic damage. To determine the sampling time for the miRNA expression, a preliminary study was conducted. TK6 cells were treated with N-ethyl-N-nitrosourea (ENU) for 1, 2, 4, 8, 24 and 48 hours and miR-34a expression at the different time points was determined. miR-34a expression increased steadily over time and reached the highest at the 24 hour point. For the main experiment, TK6 cells were treated with 7 genotoxins: ENU, cisplatin, etoposide, mitomycin C (MMC), methyl methane sulfonate (MMS), toxal and NaCl at low, medium and high doses for 4 hours. Each treatment was in triplicate and the high doses were based on 50% cytotoxicity (relative growth rate). After the treatment, the cells were washed and cultured for additional 20 hours, and then their miR-34a expression was determined. The miR-34a expression was significantly up-regulated by each of the genotoxins in a dose dependent manner. The expression changes for the low, medium and high doses were 1.5-, 4.5-, and 5.9- fold for ENU; 1.6-, 2.8 and 7.1-fold for

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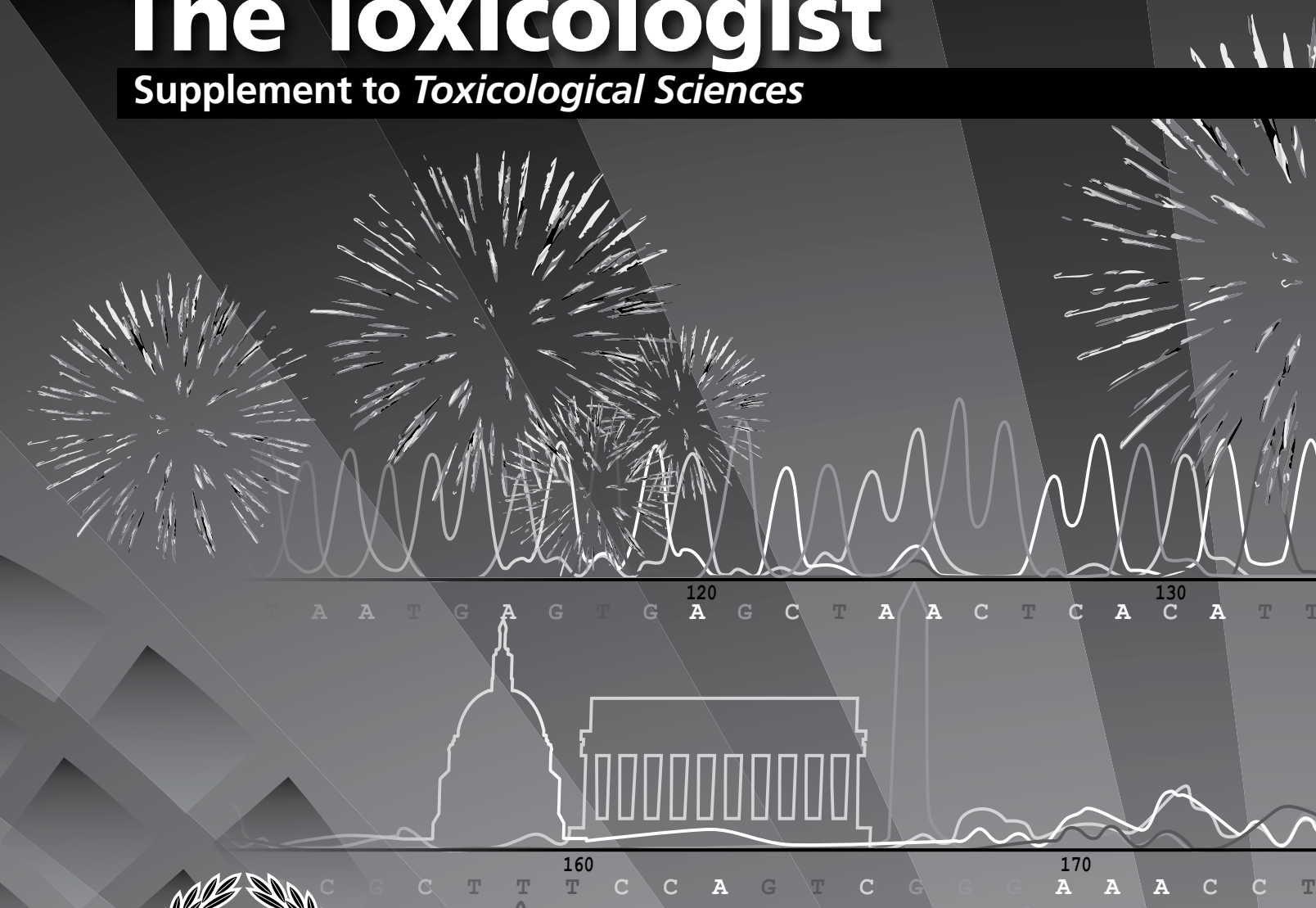

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 50th Annual Meeting of the Society of Toxicology, held at the Walter E. Washington Convention Center, March 6–10, 2011.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 578.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 606.

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

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