PS 2313 THE SYNERGISTIC EFFECT OF SODIUM CHLORITE AND BROMOCHLOROACETIC ACID ON BROMATE-INDUCED RENAL CELL DEATH.

X. Zhang[1], R. J. Bull[2], J. A. Cotruvo[3], J. Fisher[4] and B. S. Cummings[1].
[1]Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA, [2]MoBull Consulting, Richland, WA, [3]Joseph Cotruvo & Associates, LLC, Washington, DC and [4]National Center for Toxicological Research, U.S. FDA, Jefferson, AR.

Our previous study demonstrated that bromate, a drinking water disinfection by-product, induced renal cell death via DNA-dependent and independent mechanisms. In addition to bromate, drinking water contains other disinfection by-products, which may work in tandem to enhance cytotoxicity. Therefore, we tested the combined effect of two of these by-products, sodium chlorite and bromochloroacetic acid (BCAA) on bromate cytotoxicity in normal rat kidney (NRK) cells. Sodium chlorite and BCAA alone induced cytotoxicity at concentrations of 20 and 50 ppm, respectively, while bromate was only moderately cytotoxic at concentrations of 200 ppm. Combining bromate with sodium chlorite or BCAA alone enhanced cytotoxicity 1.5-4 folds. Exposing cells to all three compounds simultaneously resulted in synergistic-like increases in cytotoxicity. This effect did not correlate to increases in reactive oxygen species (ROS), even though all three compounds induced ROS formation alone. Sodium chlorite, but not BCAA increased bromate-mediated DNA damaged as measured by 8-hydroxydeoxyguanosine staining. In addtion, sodium chlorite, other than BCAA, partially reversed bromate-induced G2/M cell cycle arrest. Both compounds increased apoptosis as assessed by annexin V, PI, and DAPI staining. This is in direct contrast to bromate treatment alone which induced necrosis. Western blot results showed that both sodium chlorite and BCAA increased bromate-induced histone H2AX phosphorylation, a marker of DNA damage, and p38 MAPK phosphorylation, but only BCAA increased bromate-induced phosphorylation of p53. Collectively, these data suggest that sodium chlorite potentiates bromate-induced renal toxicity by enhancing DNA damage; while BCAA may increase toxicity using pathways independent of DNA damage.

PS 2314 CELL SIGNAL TRANSDUCTION TO PREDICT MIXTURE INTERACTIONS.

J. Boyd, X. Gao, H. Williams, C. Kinzer and J. Vrana. *Bennett Department of Chemistry, West Virginia University, Morgantown, WV.*

While exposure to chemical mixtures is an everyday reality, an understanding of their combined effects, and any potential prediction thereof, is extremely limited. Realistic exposures potentially consist of hundreds to thousands of chemicals per day, but even relatively simple binary mixture interactions can be inherently difficult to predict based upon the lack of temporal and spatial mechanisms for the individual constituents. To this end my laboratory has been developing in vitro assays to enable a high-throughput means of defining general toxicodynamic response pathways in vitro, with a full dosing regimen, which has the potential to identify and predict possible interactions. A central aspect of our assays elucidates altered activity for cell signaling cascades following multiple time-course exposures to toxicants which allows for the identification of both their common and disparate response pathways. Preliminary results will be presented with data gathered from hepatocytes exposed to deguelin (0.001-100 uM), potassium cyanide (0.001-100 uM), staurosporine (10 uM) and SB202190 (350 nM), alone and in combination, for 24 hours. Oxygen consumption kinetic profiles suggested key changes in ATP production at 400 minutes post-dose which initiated an investigation into the activity of the signaling cascades via a bead-based multiplexed (8-plex) immunoassay at that time point. Dose-dependent cascade initiation indicates a clear identification of threshold response to low-level exposures, and crosstalk amongst selected proteins correctly predicts mixtures interactions. In this study, we demonstrate the potential of a new in vitro approach for the prediction of toxic mixtures interactions that is fundamentally driven by the interdependence of energy metabolism, signal transduction, and cell survival.

PS 2315 PIFITHRIN-α INCREASES IGF-1R INHIBITOR MEDIATED HEPG2 HEPATOCARCINOMA DEATH.

X. Gao, H. Williams, C. Kinzer, J. Vrana and J. Boyd. *Bennett Department of Chemistry, West Virginia University, Morgantown, WV.*

Insulin-like growth factor receptor I (IGF-1R) has been shown to be crucial for tumor transformation, and it expression has been related to p53 activity. Conversely, p53 expression in response to DNA damage may be regulated by IGF-1R. Additionally, p53 has been shown to have regulatory control over mitochondrial respiration which can lead to an increase in the reliance on glycolysis for energy metabolism in cancer cells. We have investigated the effect of a p53 inhibitor, Pifithrin-α, and an IGF-1R inhibitor, AG1024, on HepG2 hepatocarcinoma cells. Briefly, we measured oxygen consumption, glucose uptake, and viability of HepG2 cells treated with Pifithrin-α and AG1024 (individually or in combination) in the presence and absence of glucose. Our findings show that Pifithrin-induced HepG2 cytotoxicity is independent of the presence or absence of glucose. However we also found that Pifithrin decreases oxygen consumption in glucose-rich media, but increases oxygen consumption in its absence. Further, the viability of HepG2 cells in response to AG1024 is greatly decreased in the absence of glucose, while oxygen consumption remains relatively stable. Additionally, we have found that combinations of the inhibitors appear additive in presence of glucose, but result in greater than additive toxicity in the absence of glucose. Overall, our findings strengthen the case for the presence of a glycolytic modulator upstream of p53, but downstream of IGF-1R signaling in this type of cancer. Understanding the relationship between IGF-1R, p53, and energy metabolism may help elucidate their role in carcinogenesis, and further define additional mechanisms that may enhance chemotherapeutic discovery.

PS 2316 PBPK MODELING OF THE AGGREGATE AND CUMULATIVE EXPOSURES OF RATS TO TOLUENE, N-HEXANE, CYCLOHEXANE, AND ISOOCTANE.

N. Kaveh, M. Gagné, G. Charest-Tardif, R. Tardif and K. Krishnan. *Université de Montréal, Montreal, QC, Canada.*

Mixture risk assessments focusing on both the aggregate and cumulative exposures can be improved with an understanding of the change in internal dose. The modeling of the internal dose and pharmacokinetics of hydrocarbons following cumulative and aggregate exposures has not yet been undertaken. The objective of this study was to develop a PBPK model to simulate the mixed exposure of rats to four hydrocarbons (toluene (T), n-hexane (H), cyclohexane (C) and isooctane (I); chosen to represent chemical classes in large volume petroleum products (e.g., gasoline, kerosene, gas oils)) by inhalation and oral routes. The PBPK models of individual chemicals were initially developed, and then were interconnected using the metabolic interaction terms. The resulting model was first used to simulate the kinetics of each chemical following a single route of exposure, and then to simulate the data collected following multiroute exposure. The capacity of the PBPK model to adequately simulate the kinetics following aggregate and cumulative exposures was evaluated by comparing with the experimental data. These data were collected in groups of male Sprague-Dawley rats (n=5) exposed either to a single dose of each substance by inhalation (50 ppm of T and H; 300 ppm of C and I; 2-hr) or oral gavage (8.3, 5.5, 27.9, and 41.27 mg/kg, respectively, for T, H, C and I) or to multi-products given by both routes together at the same dose levels. The data-derived area under the blood concentration vs time curve (AUC; mg/L x hr) were 106.62, 12.97, 117.56 and 122.27 whereas the AUC values derived from the multiroute, multichemical PBPK model were: 114.04, 12.68, 58.99, and 123.42 mg/L x hr, respectively, for T, H, C and I. Overall, the PBPK model developed in this study is a useful tool for simulating kinetics of hydrocarbons, by accounting for not only the saturable metabolism but also for the interactive effects during aggregate and cumulative exposures.

PS 2317 NAIL MANGANESE AS A BIOMARKER OF WELDING FUME EXPOSURE.

J. M. Antonini, J. R. Roberts and K. Sriram. *NIOSH, Morgantown, WV.*

Manganese (Mn) is a common metal in welding fume aerosols and suspected of inducing adverse neurological effects in exposed workers. Measurements of Mn in blood and urine have proven to be unreliable indicators of exposure. The goal of the current study was to determine if Mn accumulation in the nail (claw) can be used as a potential biomarker. To model this, male Sprague-Dawley rats were treated by intratracheal instillation (IT) with 2 mg/rat of shielded manual metal arc-hardsurfacing (MMA-HS; high Mn) or gas metal arc -mild steel (GMA-MS; low Mn) welding fumes once a week for 28 weeks. Vehicle controls received saline by IT. The percentage of Mn, relative to the other metals in the collected fume, was more than double (50.9 %) in the MMA-HS fume compared to GMA-MS (21.7 %). At 7 days after the last exposure, right lung lobes, specific brain regions, blood, and nails were harvested from each group. Mn levels were determined by inductively coupled plasma atomic emission spectroscopy. Significant elevations in lung Mn were observed following repeated exposure to MMA-HS and GMA-MS fumes for 28 weeks. Despite the difference in Mn composition of the two fumes, repeated exposure to equal mass doses of the fumes produced comparable Mn lung burden, suggesting a steady-state may have been achieved. Measurements of blood Mn were near or at the limit of detection and, as a consequence, variable. In contrast, Mn

levels significantly increased in the nails, as well as in striatum and midbrain, potential targets of Mn neurotoxicity, for the MMA-HS group compared to the GMA-MS group and controls. Our results demonstrate nail Mn to be a potentially viable and sensitive biomarker for welding fume exposure. The ease with which nails can be harvested, transported, and stored makes them an attractive surrogate for monitoring exposures in occupational settings.

PS 2318 PERIPHERAL BLOOD GENE EXPRESSION PROFILING REVEALS SILICA-INDUCED PULMONARY TOXICITY.

R. Sellamuthu, C. Umbright, J. Roberts, R. Chapman, S. Young, D. Richardson, D. Leonard, W. McKinney, B. Chen, D. Frazer, S. Li, M. Kashon and P. Joseph. *National Institute for Occupational Safety and Health (NIOSH), Morgantown, WV.*

The present research aimed to investigate peripheral blood gene expression profiling as a minimally invasive surrogate approach to study silica-induced pulmonary toxicity. Rats were exposed to crystalline silica by inhalation (15 mg/m3, 6 hours/day, 5 days). Pulmonary damage and blood gene expression profiles were determined at various latency periods (0 - 16 weeks). Silica exposure resulted in pulmonary toxicity in the rats as evidenced by histological changes in the lungs and elevation of LDH activity in the bronchoalveolar lavage fluid (BALF). Analysis of global gene expression profiles in the blood of the rats identified genes that were differentially expressed in response to silica exposure. The differential blood gene expression profiles correlated with the pulmonary toxicity parameters in the silica exposed rats. Genes involved in biological functions such as inflammatory response, cancer, pulmonary damage, oxidative stress, energy metabolism, fibrosis, etc, were found differentially expressed in the blood of the silica exposed rats compared with the controls. Induction of pulmonary inflammation in the silica exposed rats, as suggested by differential expression of inflammatory response genes in the blood, was supported by significant increases in the number of neutrophils and macrophages as well as the activity of pro-inflammatory chemokines – MCP1 and MIP2, observed in the BALF of the silica exposed rats. A silica-responsive blood gene expression signature developed using the gene expression data predicted with significant accuracy the exposure of rats to lower concentrations (1 and 2 mg/m3) of silica. Taken together our findings suggest the potential application of peripheral blood gene expression profiling as an efficient surrogate approach to study silica-induced pulmonary toxicity.
Disclaimer: The findings and conclusions in this abstract have not been formally disseminated by NIOSH and should not be constructed to represent any agency determination or policy.

PS 2319 BIOACTIVATION OF THE NASAL TOXICANT 2, 6-DICHLOROBENZONITRILE: AN ASSESSMENT OF METABOLIC ACTIVITY IN HUMAN NASAL MUCOSA AND IDENTIFICATION OF BIOMARKERS OF EXPOSURE AND POTENTIAL TOXICITY.

F. Xie[1,2], X. Zhou[1], J. D'Agostino[1], D. Spink[1] and X. Ding[1]. *[1]Wadsworth Center, New York State Department of Health, Albany, NY and [2]School of Public Health, State University of New York at Albany, Albany, NY.*

The herbicide 2,6-dichlorobenzonitrile (DCBN) is a potent nasal toxicant in rodents. However, it remains unknown whether DCBN causes nasal toxicity in humans. CYP2A5, a P450 enzyme predominantly expressed in mouse nasal mucosa, is largely responsible for converting DCBN into electrophilic intermediates, which, through formation of glutathione conjugates (DCBN-GS) and protein adducts, cause tissue damage. The human orthologs of CYP2A5, CYP2A6 and CYP2A13, are both expressed in the nasal mucosa, and are capable of activating DCBN. Therefore, we hypothesized that 1) human nasal tissues can activate DCBN; and 2) DCBN-GS or its derivatives can serve as biomarkers of potential DCBN exposure and toxicity. To test these hypotheses, we first established a sensitive LC-MS/MS method for detection and quantification of DCBN-GS in biological matrices. We then demonstrated that human fetal nasal mucosa microsomes catalyzed the formation of DCBN-GS *in vitro*, with a Km value comparable to that for DCBN-GS formation in adult mouse nasal microsomes. The involvement of CYP2A enzymes in this bioactivation in human nasal microsomes was confirmed by the finding of an inhibition of the activity by 8-methoxyporsalen, a known CYP2A inhibitor. Furthermore, DCBN-GS was detected in the nasal mucosa and nasal-wash fluid obtained from DCBN-exposed mice; the detected amounts of DCBN-GS increased, with increases in DCBN dose, and the conjugate was detectable at as early as 30 min after DCBN exposure. Moreover, metabolites of DCBN-GS, including DCBN-Cys, which may serve as alternative biomarkers, were also detected in the nasal mucosa and nasal-wash fluid. Thus, our data indicate that DCBN is potentially toxic to human nasal tissues, and that DCBN-GS (or its derivatives) in nasal-wash fluid may serve as biomarkers of DCBN exposure and potential nasal toxicity in humans. (Supported in part by NIH grant ES007462)

PS 2320 SYSTEMIC UPTAKE OF ^{14}C 2, 3-BUTANEDIONE ADMINISTERED BY INTRATRACHEAL INSTILLATION IN MALE SPRAGUE-DAWLEY RATS AND OROPHARYNGEAL ASPIRATION IN MALE B6C3F1 MICE.

T. Fennell[1], D. Morgan[2], S. Watson[1] and S. Waidyanatha[2]. *[1]RTI International, Research Triangle Park, NC and [2]National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

2,3-Butanedione (BD) is a reactive diketone that is found in butter and has been used in artificial butter flavor, and is thought to cause bronchiolitis obliterans in popcorn workers. The extent of uptake of BD from inhalation exposure is a concern. The objective of this study was to evaluate the ability of BD to be taken up in the lung, enter the systemic circulation, and bind to hemoglobin and albumin. [1-^{14}C] BD was administered to male Sprague Dawley rats (100 mg/kg) by intratracheal instillation (ITI), and to male B6C3F1 mice (200 mg/kg) by oropharyngeal aspiration (OPA). After 24 h, animals were euthanized and blood collected. Blood and plasma were analyzed for ^{14}C to estimate the systemic dose at 24h. Binding to plasma albumin was assessed following isolation by trichloroacetic acid precipitation, and ultrafiltration, or by ammonium sulfate precipitation. Binding to hemoglobin was assessed by dialysis of hemolysate followed by size exclusion HPLC, or by precipitation of globin from hemolysate with acidic acetone. At 24 h, 1.2 ± 0.1 % of the dose was found in rat blood, 0.66 ± 0.06 % in rat plasma, 0.35 ± 0.12 % in mouse blood and 0.17 ± 0.05 % in mouse plasma. Albumin binding in rats was 3.12 ± 0.28 nmol equiv/mg albumin, with 38% of the radioactivity in plasma bound to albumin. In mice, binding was 0.99 ± 0.26 nmol equiv./mg albumin, with 45% of the radioactivity in mouse plasma bound to albumin. The extent of binding to hemoglobin in the rat was 0.44 ± 0.20 nmol equiv/mg hemoglobin, and 0.31 ± 0.05 nmol/mg globin. In mice, the extent of binding to hemoglobin was 0.19 ± 0.10 nmol/mg. This study demonstrated that BD, following administration by ITI in rats or by OPA in mice, can enter the systemic circulation and react with hemoglobin and albumin. This shows the promise for evaluating hemoglobin and albumin adducts as biomarkers of exposure in humans.

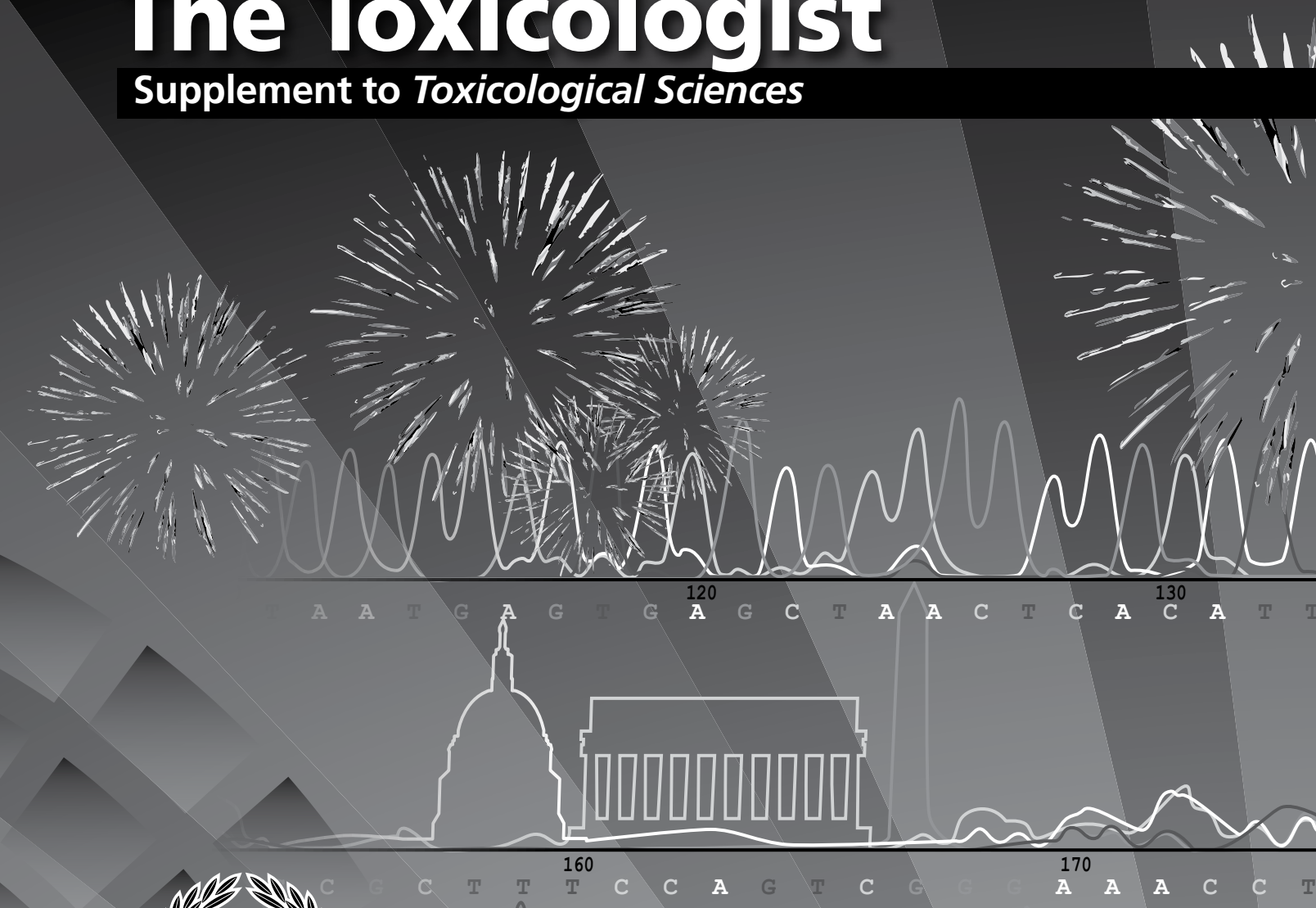
PS 2321 RESVERATROL, AN AHR LIGAND, ALLEVIATES BACTERIAL ENTEROTOXIN-INDUCED ACUTE LUNG INJURY *VIA* UPREGULATION OF MIRNA 155 AND SUBSEQUENT INCREASE IN MYELOID-DERIVED SUPPRESSOR CELLS.

S. A. Rieder, P. Nagarkatti and M. Nagarkatti. *Pathology Microbiology and Immunology, University of South Carolina, Columbia, SC.*

Acute lung injury (ALI) has many etiologies such as inhalation of a toxic substance, pneumonia, surgery and sepsis. The treatment options for ALI are very limited, and better therapeutic options are urgently needed. We administered Staphylococcal enterotoxin B (SEB, 50μg/mouse) through the intranasal route in order to induce ALI, and treated mice with resveratrol (RES), a natural plant product and an AhR ligand. We demonstrated that pre-treatment with 100 mg/kg bodyweight oral RES alleviated vascular leak and edema induced by SEB. SEB inhalation resulted in a significant increase in the percentage and absolute cell numbers of Gr-1+CD11b+ Myeloid Derived Suppressor Cells (MDSCs) in the lungs. Moreover, resveratrol pre-treatment further increased the percentage and absolute number of MDSCs. Arginase expression, a hallmark of MDSCs, was significantly increased in RES-treated mice when compared to vehicle controls. While SEB activation in vitro resulted in extensive T cell proliferation, addition of sorted MDSCs, particularly those from RES-treated groups, caused significant suppression of T cell expansion. Next, we carried out an affymetrix survey of 600 different miRNAs in lung infiltrating lymphocytes. Ingenuity pathway software analysis revealed 11 important miRNAs that were differentially regulated in SEB-administered mice versus RES-treated groups. Interestingly, miRNA 155 exhibited 18.764 fold change in expression in SEB+vehicle-administered group when compared to 46.474 fold change in SEB+RES-treated group. We also studied the target genes of miRNA 155, and found that IL-1β and NOS-2 were highly upregulated in SEB-administered group compared to the control. RES treatment further increased the expression of these

The Toxicologist

Supplement to *Toxicological Sciences*



SOCIETY OF TOXICOLOGY
50th Anniversary
1961–2011

Celebrating 50 Years of Service in Science

50th Anniversary Annual Meeting and ToxExpo™ Washington, D.C.

An Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 120, Supplement 2
March 2011

www.toxsci.oxfordjournals.org

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science of Toxicology

www.toxicology.org

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.

Copies of *The Toxicologist* are available at $45 each plus $5 postage and handling (U.S. funds) from:

Society of Toxicology
1821 Michael Faraday Drive, Suite 300
Reston, VA 20190

www.toxicology.org