involved in the biodegradation process. Computer modeling was used to structurally characterize possible nanotube interaction sites with EPO. Studies are underway to assess oxidative biodegradation of CNT by EPO-rich activated human eosinophils. We conclude, that EPO can participate in enzymatic biodegradation of CNT after respiratory exposures during their production and handling. Supported by NIOSH OH008282; NIH NIAID U19 AI068021, HL70755, HL094488, EC-FP7-NANOMMUNE-214281

PL 55 LONG, FIBROUS CARBON NANOTUBES ACTIVATE THE NLRP3 INFLAMMASOME IN HUMAN MACROPHAGES AND INDUCE NEUTROPHILIA IN MICE LUNGS AFTER INTRATRACHEAL ADMINISTRATION.

J. Palomäki, E. M. Rossi, K. Savolainen, S. Matikainen and H. Alenius. *Finnish Institute of Occupational Health, Helsinki, Finland.*

Carbon nanotubes (CNT) are of great interest because of their multiple applications in industry but also because of their unknown health effects. Recent studies suggest that the high aspect ratio, a feature common with asbestosis, is a key factor for reported toxicity of certain CNT. The mechanism behind this phenomenon is, however, not known. In the present study, we studied whether different carbon nanomaterials are able to induce differences in pro-inflammatory reactions in human macrophages *in vitro*. Carbon black (Evonik Industries AG); short CNT (Baytubes C150HP); long, tangled CNT (CheapTubes Inc©); long, fibrous CNT (Mitsui&Co, Ltd) and crocidolite asbestos (PRC, South-Africa) were used for *in vitro* studies. We also exposed C57BL6 mice intratracheally to fibrous and tangled CNT to investigate their effects *in vivo*. Our results showed that only long, fibrous CNT and asbestos were able to induce robust IL-1β secretion from LPS-primed macrophages. The western blot (WB) analysis confirmed that the secreted IL-1β was biologically active. Ribonucleic acid interference-mediated gene knockdown experiments demonstrated cytoplasmic NLRP3 inflammasome is essential for fibrous CNT- and asbestos-induced IL-1β secretion. Moreover, we showed that CNT-induced NLRP3 inflammasome activation is dependent on P2X7 receptor and cathepsin B activity. *in vivo* experiments demonstrated that in contrast to tangled CNT, fibrous CNT exposure elicited prominent neutrophilia accompanied by the expression of neutrophil attracting chemokines confirming our *in vitro* findings. Taken together, our results demostrate that long, fibrous CNT have asbestos-like effects being clearly more hazardous than other CNT. Fibrous CNT activated NLRP3 inflammasome causing high production of pro-inflammatory cytokine IL-1β in human macrophages. In addition, fibrous CNT exposure induced significant neutrophilia in the mouse lungs *in vivo*. Further studies are needed to make reliable risk assessment of carbon nanotubes.

PL 56 PULMONARY FIBROTIC RESPONSE TO SUB-CHRONIC MULTI-WALLED CARBON NANOTUBE EXPOSURE.

R. R. Mercer, A. F. Hubbs, J. F. Scabilloni, L. Wang, L. A. Battelli, V. Castranova and D. W. Porter. *PPRB, NIOSH, Morgantown, WV.*

Multi-walled carbon nanotubes (MWCNTs) are manufactured carbon compounds with many commercial applications. To address the hypothesis that MWCNTs cause persistent pulmonary pathology, C57BL/6J mice were exposed by pharyngeal aspiration to 10, 20, 40 or 80 μg MWCNTs (mean dimensions of 3.9 μm x 49 nm) or vehicle. Lungs were preserved at 1, 7, 28 and 56 days post exposure to analyze the distribution of lung burden. Morphometric measurement of Sirius Red staining was used to assess the connective tissue response. At day 1 post-exposure 62.0±2.5 and 9.9±2 percent of the lung burden (mean±SE, N=7) were in alveolar macrophages and alveolar tissue, respectively. The remainder of the lung burden (18.0±3.2) was in the airways. By 56 days post-exposure, 68.7±3.9, 7.5±1.9 and 22.0±5.1 percent of MWCNT were in alveolar macrophages, alveolar tissue and granulomatous lesions, respectively. No MWCNTs were found in the airways at 56 days. At 56 days post-exposure the average thickness of connective tissue in alveolar regions was 0.11±0.01, 0.12±.01, 0.12 ±0.01, 0.16±0.01 and 0.19±0.01 μm (mean±SE, N=6) for vehicle, 10, 20, 40 and 80 μg dose groups, respectively. The connective tissue in the alveolar region demonstrated a progressive increase in thickness over time in the 80 μg exposure group (0.11±0.01, 0.14 ±0.01, 0.16±0.01 and 0.19±0.01 μm for 1, 7, 28 and 56 day). The distribution of lung burden was predominately within alveolar macrophages with approximately 8% delivery to the alveolar tissue. Despite the relatively low fraction of the lung burden being delivered to the alveolar tissue (7.5% at day 56), the average thickness of connective tissue in the alveolar region was increased over vehicle control by 45% in the 40 μg and 72% in 80 μg exposure groups. These results demonstrate that MWCNT have the potential to produce a progressive, fibrotic response in the alveolar tissues of the lungs.

PL 57 THE ROLE OF IL-1β SIGNALING IN NICKEL ASSOCIATED MULTI-WALLED CARBON NANOTUBE-INDUCED PULMONARY INFLAMMATION.

T. A. Girtsman. *CEHS, University of Montana, Missoula, MT.*

Exposure to certain engineered nanomaterials (ENM) has been associated with pathological changes in animal models raising concern that human health effects will emerge with increasing use. Some, but not all, ENM have been shown to activate the NLRP3 inflammasome. We have shown that nickel containing multi-walled carbon nanotubes MWCNT (Ni-MWCNT) can activate the NLRP3 inflammasome (NLRP3) in vitro using primary alveolar macrophages (AM) or THP-1 cells. Furthermore, we have also demonstrated NLRP3 activation in vitro correlates strongly with lung inflammation and pathology. Activation of caspase-1 via assembly of the NLRP3 inflammasome results in the conversion of pro-IL-1β to the active form of this proinflammatory cytokine (mature IL-1β), which is released by AM and is an important mediator of inflammation during infection. In this study, we investigated the role of IL-1β signaling to induce a pulmonary neutrophilic response using C57Bl/6 wild type or IL-1 receptor null mice (IL-1R-/-) after exposure to Ni-MWCNT. We found that Ni-MWCNT was effective in inducing pulmonary inflammation as indicated by neutrophilic influx and IL-1β secretion into the airways of wild type mice. The inflammatory response however, was abolished in mice deficient in the type I IL-1R, as indicated by significantly lower neutrophils in the inflammatory infiltrate. These data suggest an important role for IL-1β signaling in Ni-MWCNT-induced pulmonary inflammatory responses. This work was supported by NIH grants RC2-ES018742 and P20-RR017670.

PL 58 PULMONARY INFLAMMATION, EPITHELIAL HYPERPLASIA, AND LYMPH NODE TRANSLOCATION AFTER MULTI-WALLED CARBON NANOTUBE INHALATION.

A. Hubbs, V. Castranova, B. T. Chen, D. G. Frazer, W. McKinney, R. R. Mercer, M. L. Kashon, L. A. Battelli, P. Willard and D. W. Porter. *HELD, NIOSH, Morgantown, WV.*

Multi-walled carbon nanotubes (MWCNTs) are engineered nanotubes with multiple fullerene carbon walls, a high aspect ratio, and rapidly increasing industrial uses. To investigate the toxicity of inhaled MWCNTs, mice were exposed 5 hours/day to 10 mg/m^3 MWCNTs (Mitsui, MWNT-7, count mode aerodynamic diameter 420 nm) for 4, 8 or 12 days and sacrificed 24 h post-exposures. Histopathologic sections of lung and tracheobronchial lymph nodes were examined at all time points and sections of nose (4 levels) were examined after the 12 day exposure. In lung, the principal changes were 1) inflammation centered around the bronchioloalveolar junction, 2) vasculitis, and 3) bronchiolar epithelial hypertrophy and hyperplasia. These were seen in all exposed mice (n=8, 6 and 6 at 4, 8 and 12 days, respectively). Peribronchiolar inflammation was principally histiocytic and neutrophilic with occasional giant cells. In many macrophages, cytopathologic changes included 1) MWCNT penetration of the cytoplasmic membrane, 2) MWCNT penetration of nuclei, and 3) karyolysis. Vascular changes were present in all exposed mice but manifestations varied and included medial hypertrophy and contraction, mural neutrophil infiltrates, and rare mural MWCNTs. Bronchiolar hypertrophy and hyperplasia were present after 4 days and persisted. After 12 days of exposure, all mice had foci of peribronchiolar fibrosis and bronchiolar epithelial mucous metaplasia. Pleural MWCNTs were seen in two mice. In lungs of air exposed controls (n=8, 6 and 6 at 4, 8 and 12 days, respectively), vasculitis and bronchiolar changes were absent; a single focus of inflammation was seen in one mouse. MWCNT translocation to the tracheobronchial lymph node progressed with time and localized to the deep paracortex, the normal location of T lymphocytes and dendritic cells. In the nose, neutrophilic rhinitis and hyaline droplet formation were consistent changes. These findings suggest that chronic inhalation toxicity studies are needed.

PL 59 UNDERSTANDING CARBON NANOTUBE GENOTOXICITY.

L. M. Sargent[1], S. H. Reynolds[1], A. F. Hubbs[1], S. A. Benkovic[1], D. T. Lowry[1], M. L. Kashon[1], K. J. Siegrist[1], J. Mastovich[2], J. L. Sturgeon[2], K. L. Bunker[2] and C. Z. Dinu[3]. *[1]Toxicology and Molecular Biology, CDC/NIOSH, Morgantown, WV, [2]RJLeeGroup, Pittburgh, PA and [3]Chemical Engineering, West Virginia University, Morgantown, WV.*

Carbon nanotubes have many applications in medicine, electronics, aerospace and computer circuits. However, in order to use nanotubes for such applications, their potential genotoxic and cytotoxic effects need to be understood. We are studying nanotube interaction with cells and isolated cellular components, to determine

mechanisms responsible for cell fate. Specifically, we exposed primary and immortalized human epithelial cells to single- and multi-walled carbon nanotubes and examined the potential of nanotubes to induce genetic damage. The microscopy results showed fragmented centrosomes, multipolar mitotic spindles and errors in chromosome number following exposure to single walled carbon nanotubes (SWCNT). The larger multi-walled carbon nanotubes (MWCNT) primarily induced mitotic spindles with one mitotic spindle pole. The nanotubes associated with microtubules and centrosomes and formed biohybrids localized at the nucleus. In order to explain this behavior, we polymerized microtubules in vitro and we used kinesin motors to show integration with nanotubes and manipulation of functional biohybrid assemblies. In eukaryotic cells, microtubules play roles in intracellular transport as well as cell division. Microtubules assemble into mitotic spindles, while the kinesin motors are responsible for microtubule-based transport and cellular division. Our results demonstrate disruption of the mitotic spindle by nanotubes and give further evidence of the mechanism responsible for the disruption of cell division. These results suggest caution should be used in the handling and processing of carbon nanotubes.

PL 60 IMPACTS OF STRUCTURE AND FUNCTIONALIZATION ON TOXICOLOGICAL RESPONSE OF THE MODEL ORGANISM *DAPHNIA MAGNA.*

D. A. Arndt and R. Klaper. *School of Freshwater Sciences, University of Wisconsin Milwaukee, Milwaukee, WI.* Sponsor: R. Hutz.

Over the past decade there has been increasing economic investment in carbon nanotechnology resulting in an increased potential for the release of these particles to the environment, particularly to freshwater and marine systems. Previous studies have demonstrated the toxicity of these particles; however comparative data on these nanoparticles and the mechanism of their toxicity remains largely unknown. To resolve this, we examined how alterations of core structure and surface chemistry affect the interaction of a particle with the aquatic ecological, toxicological and genomic model species, *Daphnia magna.* Daphnia were exposed to carbon nanoparticles with differing core structures and functionalizations, and acute toxicity and chronic toxicity and reproduction were measured. In addition, global gene expression responses were used to determine the physiological impact at sub-lethal concentrations. High throughput sequencing was used to create libraries of genes with altered expression patterns for each nanomaterial. Life cycle toxicity analysis indicates a particle's core structure and surface functionalization have an influence on its relative toxicity. Sequencing analysis indicates that different genomic pathways are activated in the presence of an unfunctionalized fullerene (nC60) compared to a hydroxylated fullerene (C60-OH). This indicates that Daphnia differ in their physiological response to alterations in particle chemistry and structure. There is a potential to use these expression patterns to not only determine the genetic mechanism of a particle's toxicity, but also to use these molecular clues as biomarkers to determine environmental exposures to nanomaterials in aquatic environments.

PL 61 TOXICOGENOMICS EFFECTS OF WATER-SOLUBLE CARBON NANOTUBES.

D. A. Sarigiannis[1, 2], G. Cimino Reale[1], A. Collotta[1], R. Brustio[1], B. Casati[1], E. Roda[3], A. Profumo[6], D. Merli[6], E. Quartarone[5], P. Mustarelli[5], M. Fagnoni[5], T. Coccini[4], L. Manzo[3, 4] and E. Marafante[1]. *[1]Institute for Health and Consumer Protection, European Commission - Joint Research Centre, Ispra, Varese, Italy, [2]Chemical Engineering Department Aristotle University of Thessaloniki, Thessaloniki, Greece, [3]Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Pavia, Italy, [4]Toxicology Division, Salvatore Maugeri Foundation IRCCS, Pavia, Pavia, Italy, [5]Department of Physical Chemistry, University of Pavia, Pavia, Pavia, Italy and [6]Department of General Chemistry, University of Pavia, Pavia, Pavia, Italy.* Sponsor: L. Birnbaum.

The widespread use of functionalized carbon nanotubes (CNTs) makes the understanding of potential harmful effects highly important. Two cell culture systems, human A549 pneumocytes and HaCaT keratinocytes, were used to assess the modulation of gene expression due to exposure to single and multi-walled CNTs. Moreover, CD-1 male mice were exposed to the CNTs tested by intra-tracheal instillation and lung samples were taken and analyzed after 1 day of exposure. Differentially functionalized CNTs (MW-COOH and MW-NH2) were tested in comparison with pristine MWCNTs and SWCNTs. Toxicogenomic analysis included whole genome micro-array analysis and quantitative PCR using micro-fluidic cards for inflammation genes.
Comparison of gene expression between in vitro and in vivo exposure to NTs revealed differences in the level of biological response induced towards oxidative stress, inflammation and apoptosis. Differential modulation in gene expression after in vivo exposure was observed as a function of single or multiple wall geometry and presence of specific functional groups. MW-COOH showed a very high degree of up-modulation of the genes coding for chemokine ligands clinically associated with the onset of lung fibrosis in humans. This effect was much less pronounced with MW-NH2 or SWCNT, whereas pristine MWVNT did not show any statistically significant modulation in gene expression. The main biological pathways induced by the tested CNTs were chemokine and cytokine induced inflammation and oxidative stress. This study indicates that CNT functionalization modulates the advent of early biological events affecting their health effects.

PL 62 DECIPHERING MECHANISMS UNDERLYING PROLONGED MALE INFERTILITY FOLLOWING A CLINICALLY-RELEVANT MULTI-CYCLE CISPLATIN TREATMENT.

J. Harman[1, 2] and J. Richburg[1, 2]. *[1]College of Pharmacy, Division of Pharmacology/Toxicology, University of Texas at Austin, Austin, TX and [2]Center for Molecular and Cellular Toxicology, University of Texas at Austin, Austin, TX.*

Cisplatin is a chemotherapeutic compound that initiates apoptosis. A typical clinical regimen consists of repeated cycles of 5-7 daily injections of low dose cisplatin with a 1-2 week recovery period. An unfortunate side effect of cisplatin exposure in males is a prolonged, sometimes permanent, infertility. Previously, we developed a clinically-relevant treatment paradigm in adult C57/Bl/6J mice (repeated cycles of 2.5mg/kg/day for 5 days followed by a 7 day recovery period) and discovered that the severity of testicular damage is more dependent on the number of cycles of treatment than the cumulative dose. Theoretically, spermatogonial stem cells (SSCs) should be able to repopulate the testis after cisplatin exposure has ended. We hypothesize that an increase in the mitotic activity of SSCs during the initial exposure to cisplatin renders them increasingly susceptible to induced injury during the next treatment cycle and underlies the mechanism of treatment-induced infertility. Here we investigate Sertoli cell (SC) factor(s) that stimulate SSCs to exit quiescence and enter the cell cycle after cisplatin exposure. IHC analysis of BrdU incorporation after one cycle of cisplatin exposure showed a 13-fold increase in the proliferative rate of early germ cells over controls. Glial cell line-derived neurotrophic factor (GDNF) is secreted by SCs, and has been implicated in SSC regulation. Analysis of testicular cross sections revealed an increase in GDNF protein immunostaining in treated mice that was particularly prominent along the basal membrane, the region where SSCs reside. Taken together, these preliminary data provide evidence that an increased secretion of GDNF from SCs occurs at a time that correlates with an increased proliferative rate of SSCs after cisplatin-induced testicular injury. Future experiments are targeted to test the direct role of GDNF in increasing the sensitivity of SSCs to cisplatin-induced injury.

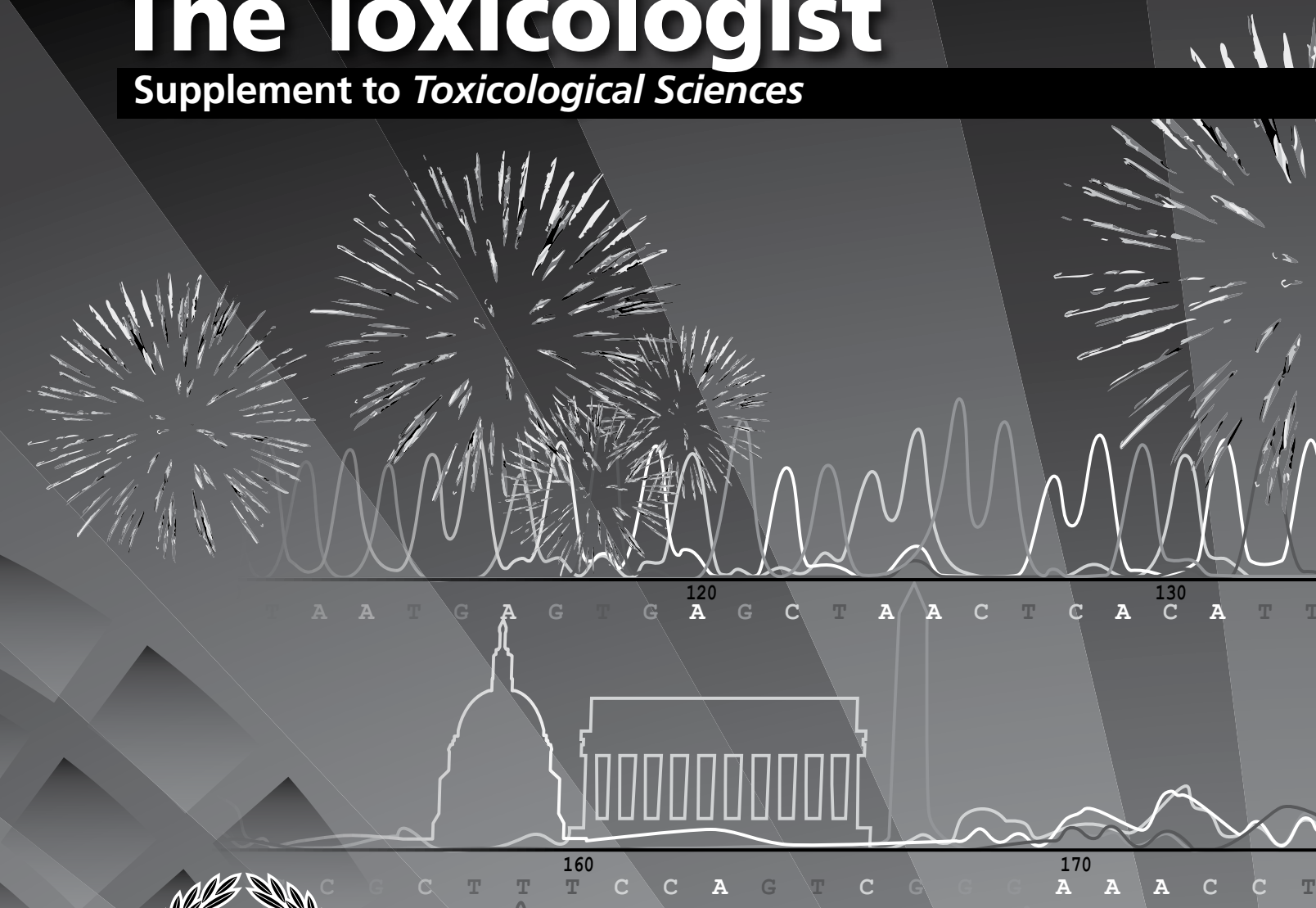
PL 63 DRAMATIC STRAIN DIFFERENCES IN SENSITIVITY OF RAT SPERMATOGENESIS TO IRRADIATION AND OTHER GONADAL TOXICANTS.

M. Meistrich, C. Weng and M. AbuElhija. *Experimental Radiation Oncology, MD Anderson Cancer Center, Houston, TX.*

We previously reported (J Androl 14:257,1993) dramatic differences in the recovery of spermatogenesis in rat testes from the chemotherapy drug procarbazine; Lewis and Lewis-Brown Norway (BN) F1 hybrids were uniformly sensitive and outbred Sprague-Dawley (SD) rats were resistant but with wide variations, suggesting genetic differences. To systematically investigate these differences, we measured the recovery of spermatogenesis after irradiation in 5 inbred strains (BN, Lewis, Fischer 344, Wistar Kyoto, and SHR, which is derived from Wistar) and 2 outbred stocks (Long-Evans and Sprague-Dawley [SD]). Rat testes were irradiated with 5 Gy and tissue harvested 10 weeks later for testicular sperm counts and histological analysis, particularly the tubule differentiation index, TDI, which is the percentage of tubules containing differentiated cells. Lewis and BN rats were by far the most sensitive, Long-Evans, Wistar, Fischer, and SHR (listed in order of increasing recovery) were more resistant, and SD had the highest spermatogenic recovery. For example, testis weights were decreased to 23%, 29%, and 35% of control in BN, Wistar, and SD rats respectively. Sperm counts were decreased to $0.005x10^6$, $4x10^6$, and $19x10^6$ for BN, Wistar, and SD, respectively, compared to about $200x10^6$ for controls. By histology, the BN testes showed atrophy with almost complete germ cell loss while the SD appeared relatively normal, with only a reduction in the fractions of tubules with spermatids; TDIs were 1%, 57% and 98% in BN, Wistar, and SD, respectively. The atrophic tubules in all rats showed the presence of type A stem spermatogonia, indicating that the failure of recovery was not due to the loss of

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