pharmacokinetic uncertainties. Studies with the widely used herbicide 2,4-dichlorophenoxyacetic acid (2,4D) have demonstrated how internal dose measurements can be routinely and effectively quantified from animal dietary toxicity studies, a common mode of administration for many chemicals. In addition, units of internal dose common to human biomonitoring and toxicity studies can be further related to health protective regulatory reference standard doses, e.g., RfDs and RfCs, by development of "biomonitoring equivalent" (BE) values. Developed from pharmacokinetic modeling, BEs represent estimated blood/urine concentrations resulting from exposures to RfD/RfC exposure standards (Aylward et al., EHP 118: 177, 2010). These refinements to comparative internal dose quantitation offer important dose/exposure context to informing toxicologic plausibility and interpretation of observations reported in epidemiology studies.

W 50 THE ROLE OF EPIDEMIOLOGIC RESEARCH IN RISK ASSESSMENT: SOME CHALLENGES AND OPPORTUNITIES.

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Epidemiologic data obtained from well designed and executed human studies can inform the risk assessment process. Because of the nature of environmental and occupational exposures, most studies are observational in design and thus subject to numerous limitations; the primary limitation is typically the validity and reliability of exposure assessment. An overview of epidemiologic study design, with a focus on those studies most useful for risk assessment, will be presented. Traditional methods used for exposure assessment and absorbed dose measurement for registration and regulatory purposes will be reviewed, and contrasted with those typically used in epidemiologic research. Opportunities in exposure assessment in large occupational or environmental epidemiologic studies (such as the Ontario Health Study) and promising methods for incorporation of dose information into epidemiologic research will be discussed. Continued collaborative efforts amongst epidemiologists, exposure scientists, toxicologists, and risk assessors will be necessary to develop and apply the tools necessary to allow for the better integration of epidemiologic study results into the risk assessment process.

W 51 NEW TOOLS AND APPROACHES TO LINK TOXICOLOGICAL MODELS TO HUMAN HEALTH EFFECTS.

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Due to limitations on human testing, the use of surrogate models and endpoints are necessary when studying the effects of environmental chemicals on human health. However, translating the results obtained using the surrogate models and endpoints to human health effects poses many unique challenges. The development of new tools and approaches for linking toxicological models with human health effects will be highlighted using two examples. The first example focuses on the incorporation of human dosimetry and exposure into high-throughput in vitro toxicity screening. In this example, in vitro assays were used to estimate human metabolic clearance and plasma protein binding for ToxCast chemicals. Computational in vitro-to-in vivo extrapolation methods were then used to estimate the human oral equivalent doses that would be required to produce steady state in vivo concentrations equivalent to in vitro AC50 values from the high throughput screens. These oral equivalents were compared with human oral exposure estimates to assess whether significant in vitro bioactivity occurred within the range of expected human exposure. The second example focuses on the translation of biomarkers of effect from rodent models to humans. In this example, circulating mRNAs were evaluated as biomarkers of hepatotoxicity. In a rat model, increases in liver specific mRNAs were observed in the cell-free plasma of rats treated with two different hepatoxicants. Characterization of the circulating mRNAs showed an association with both necrotic debris and encapsulation within microvesicles. The increase in circulating liver mRNAs was confirmed in human subjects following drug-induced liver injury demonstrating translation of the biomarkers from animals models to humans.

W 52 THE ITERATIVE ROLE OF EXPOSURE SCIENCE IN RISK ASSESSMENT.

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The mapping of the human genome and the emergence of tools in computational, informational, and molecular sciences has led to new possibilities for improving chemical risk assessment. For the first time, these advances allow for a high throughput and broad scale examination of the molecular and cellular targets of chemicals. In 2007, EPA launched ToxCast™ in order to rapidly and cost-effectively prioritize the toxicity testing of a large number of chemicals. Because hazard and exposure mutually determine risk, the implementation of toxicity screening has led to awareness of the need to develop exposure screening approaches. Furthermore, there is the need to integrate the two approaches to focus toxicity testing on those chemicals and endpoints, at those life-stages and via those exposure routes, most likely to present a risk of concern (Dellarco et al., 2010). Thus, exposure assessment should not be relegated to the fourth step in risk assessment (following dose-response, as specified by the 1983 NRC "Red book") but rather should be considered iteratively with allocation of resources as guided by a risk screening approach. Herein, I describe approaches that make use of two types of exposure survey data, the National Health and Nutrition Examination Survey (NHANES), a cross-sectional survey of population biomarkers, and national surveys of pesticide residues in homes and daycare facilities to inform development of risk assessment models for a group of pesticides. *EPA reviewed this work but it does not necessarily reflect official Agency policy.*

PL 53 ACUTE PULMONARY RESPONSES TO MWCNT INHALATION.

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The large scale manufacture of multi-walled carbon nanotubes (MWCNT) suggests occupational exposures may occur. In order to investigate the pulmonary toxicity of MWCNT, male C57BL6/J mice (6 weeks old) were exposed to aerosolized MWCNT (10 mg/m^3, 5 hours per day; mass mode aerodynamic diameter 1.3 μm, count mode aerodynamic diameter 0.4 μm) for 2, 4, 8 and 12 days. All mice were sacrificed at one day post-exposure. Whole lung lavage (WLL) was conducted and polymorphonuclear leukocytes (PMNs) were assessed to index pulmonary inflammation. WLL fluid assays included lactate dehydrogenase (LDH) activity as a measure of cytotoxicity, albumin as a marker of the lung air-blood barrier integrity, as well as various cytokines. MWCNT lung burden ranged from 6.6 ± 0.5 μg/lung (2 days exposure) to 30.6 ± 1.2 μg/lung (12 days exposure). MWCNT-exposure induced increases in PMN levels from 152 ± 27 (x 10^3) PMNs/mouse (2 days exposure) to 1,893 ± 462 (x 10^3) PMNs/mouse (12 days exposure). MWCNT-exposure increased WLL fluid albumin from 0.18 ± 0.01 mg/ml (2 days exposure) to 0.41 ± 0.04 mg/ml (12 days exposure), while WLL fluid LDH increased from 131 ± 6 units/L (2 days exposure) to 286 ± 35 units/L (12 days exposure). MWCNT exposure caused increased interleukin-6 (IL-6), chemokine ligands 1 and 2 (CXCL1, CCL2), and granulocyte macrophage colony stimulating factor (GM-CSF) levels relative to air-exposed controls. In summary, these data indicate that exposure to aerosolized MWCNTs results in dose-dependent increases in pulmonary inflammation and damage, suggesting that aerosolized MWCNT may pose an occupational health hazard.

PL 54 BIODEGRADATION OF CARBON NANOTUBES BY EOSINOPHIL PEROXIDASE.

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Single-walled carbon nanotubes (SWCNT) having high potential for applications in nanotechnology, electronics and medicine can have adverse effects on human health and the environment. In vitro data indicate that SWCNT may be cytotoxic, largely by inducing oxidative stress. It was shown previously, that horseradish peroxidase and human myeloperoxidase (MPO) catalyse the biodegradation of SWCNT. Biodegradation of nanotubes by another mammalian peroxidase - eosinophil peroxidase (EPO) has not been studied. EPO exocytosed by eosinophils is actively involved in increased oxidant production during lung inflammation after treatment with pollutants and cigarette smoke. We found that incubation with EPO and H2O2 caused degradation of CNT over time, and the CNT suspension turned translucent. Neither EPO alone nor H2O2 alone caused nanotube degradation. Degragation of CNT was confirmed by: 1) visible-near-infrared spectroscopy showing decrease of characteristic metallic band (M1) and semiconducting (S2) transition band and 2) Raman spectroscopy demonstrating increase of disorder-induced D-band and decrease of tangential-mode G-band. Drastic changes in nanotube morphology were demonstrated by transmission electron microscopy. Biodegradation of CNT was higher in the presence of NaBr suggesting that not only reactive radical intermediates of EPO but also generated HOBr was

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.

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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

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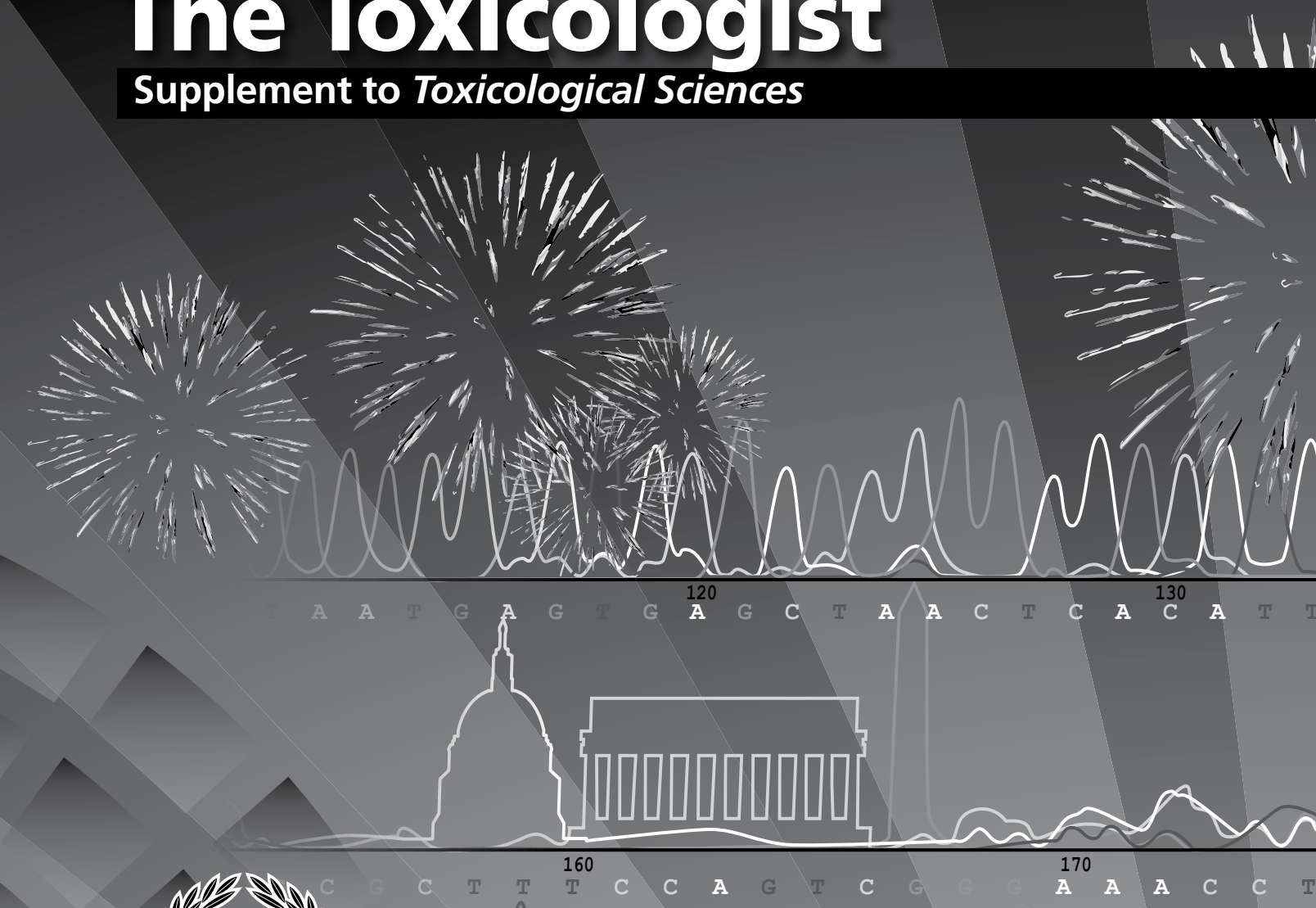

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Preface

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.

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