THE UNIVERSITY OF IOWA

March 19, 1996

Brenda Boutin
NIOSH, EID-C31
4676 Columbia Prkwy.
Cincinnati, OH 45226

Dear Ms. Boutin:

Please find enclosed the manuscripts you requested from Peter Thorne. Other manuscripts regarding metal-working fluids are in various stages of progress and will be forwarded to you upon acceptance. Please let us know if we can be of any further assistance to you.

Sincerely,

Jeannine DeKoster

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Attachment A

The Toxicologist

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to proceed with or halt cell cycle progression. Mutations in any part of these cellular pathways can have the ultimate effect of disrupting chromosomal integrity. We have used viral proteins involved in malignant transformation to investigate cellular pathways that may be perturbed during loss of genomic instability. Recent studies have identified cellular proteins which are targets for the viral oncoproteins, stressing the importance of these cellular proteins in controlling neoplasia. Among the targets of the viral oncoproteins are the products of p53 and retinoblastoma (Rb) tumor suppressor genes. We demonstrate that the expression of human papillomavirus type 16 E6 and E7 oncoproteins in normal, mortal cells disrupts the integration of the network of signals that maintain genomic integrity. E6-expressing cells, in which cellular p53 protein is bound and degraded exhibited alterations in cell cycle control and displayed the ability to amplify the endogenous CAD gene when placed in the drug PALA. Expression of E7, which complexes with a variety of cellular proteins, including Rb, resulted in a p53-independent alteration in cell cycle control, massive cell death and polyploidy upon PALA treatment. These results demonstrate that the viral proteins disrupt cellular processes that safeguard the genome and growth of normal cells.

698 HUMAN SOMATIC MUTATION: INFLUENCES OF GENOTYPE AND CELL LINEAGE ON MUTATION ACCUMULATION *IN VIVO*

Raymond J. Monnat, Jr. *Department of Pathology, University of Washington, Seattle, WA*

Human somatic mutation accumulation may play an important role in the pathogenesis of human neoplastic and degenerative diseases. This presentation will review what we know about quantitative and molecular aspects of human somatic mutation; will discuss recent advances in our understanding of the pathways that control the generation of DNA structural rearrangements in human cells; and will delineate several of the most important conceptual issues and unanswered mechanistic questions concerning the generation and the persistence of mutations in the human body. For this discussion I will draw on work we have done to understand Werner syndrome (WS), an uncommon human autosomal recessive disease that is a caricature of premature aging, is associated with an elevated risk of developing cancer, and displays several different types of genetic instability including a spontaneous deletion mutator phenotype. I will also discuss recent results with a new system we have developed to study quantitative and molecular aspects of somatic mutation *in vivo* in a human epithelial cell lineage.

Work supported by the NCI, NIA and NIEHS.

699 SHORT-TERM TESTS FOR THE DETECTION OF CHEMICAL INDUCED MITOTIC RECOMBINATION

F K Zimmermann. *Institute for Microbiology und Genetics, Darmstadt Institute of Technology, Darmstadt, Germany.* Sponsor: R H Schiestl

Mitotic or somatic recombination can lead to a loss of heterozygosity, LOH, which causes the expression of recessive tumor alleles and thus can lead to cancer. A considerable part of the mammalian genome is subject to genomic imprinting, a situation where some segments of a chromosome are inactive when derived from the maternal parent while other segments are inactive when inherited from the paternal parent. Consequently, in certain segments, retention of both parental genomes is required for proper function and LOH can cause characteristic defects. Mitotic recombination has been induced with a wide spectrum of chemicals without any indication of mutagen specificity. This is shown by ionizing radiations which are poor inducers of base substitutions, but extremely efficient inducers of mitotic recombination. Mitotic recombination test systems have been well validated in *Saccharomyces cerevisiae*, *Aspergillus nidulans* and *Drosophila melanogaster*. Well over 50 chemicals from all categories have been tested in these organisms and shown that assays for the induction of mitotic recombination indeed uncover a wide spectrum of genotoxins. A major problem in genotoxicity testing is caused by the fact that many chemicals are indirect mutagens i.e. the genotoxic effects are only exerted by metabolic derivatives. Predicting human genotoxicity and carcinogenicity requires not only appropriate genetic assays, but also metabolic activation systems which mimick the human conditions. Genes coding for human activating enzymes were expressed in *Saccharomyces cervisiae* to the effect that metabolizing activity could be demonstrated at the biochemical level and mitotic recombination induced.

700 ASSAYS FOR DELETIONS DETECT CARCINOGENS

R H Schiestl. *Department of Molecular and Cellular Toxicology, Harvard School of Public Health, Boston, MA*

DNA deletions are involved in carcinogenesis and in most genetic diseases. Assays scoring for deletions have been constructed and/or used in yeast, in human cells and *in vivo* in mice. In all three organisms deletion events are inducible by a variety of carcinogens including carcinogens that are negative in the Salmonella (Ames) assay and in most other short term tests. Of 47 chemicals of known carcinogenicity status the yeast DEL assay detects 85% correctly. In comparison, the Salmonella assay that detects only 34% correctly. Salmonella positive carcinogens induce DEL recombination with a linear dose response starting at several orders of magnitude below the lowest cytotoxic dose. On the other hand, Salmonella negative carcinogens show a threshold and induce DEL recombination only at high, already cytotoxic doses. Salmonella negative carcinogens induced oxidative stress whereas the Salmonella negative carcinogens did not. These results suggest that many Salmonella negative carcinogens may be active only at high, cytotoxic doses and may be indirect acting. DNA double strand breaks initiated DEL recombination. Alkylating agents like 4-NQO required DNA replication to induce deletions whereas agents directly causing double-strand breaks like X-rays induced deletions in the absence of DNA replication. The DEL assay in human cells is much more sensitive to some chemicals than the yeast DEL assay. Mouse DEL assays may detect tissue specific effects and allow *in vitro - in vivo* comparisons. More widespread use of the DEL assays may reduce the number of experimental animals required, may lead to a higher accuracy in predicting the carcinogenic potential of chemicals and may give more mechanistic information about the biological activity of carcinogens than current assays do.

701 MECHANISM OF PARTICLE-INDUCED INFLAMMATORY CYTOKINE GENE EXPRESSION AND PRODUCTION IN RAT ALVEOLAR MACROPHAGES

M I Luster and W Dong. *Environmental Immunology and Neurobiology Section, NIEHS, Research Triangle Park, NC*

Inflammatory cytokine gene expression and production induced by urban air (A1648) and diesel (D1651) particles were studied in rat alveolar macrophages. Tumor necrosis factor alpha (TNFα) and interleukin-6 gene expression and TNFα secretion were increased in cells treated with 50 to 200 μg/ml Particle A1648 in a dose-response manner. There was no increase in cytokine messenger or production by Particle D1651. A1648-induced increased in TNFα production was not significantly reduced by antioxidants such as TMTU or DMSO. However, the above effect was completely reduced by polymyxin B, an agent that is capable of neutralizing bacterial LPS activities. Endotoxin was found in A1648-treated culture supernatant using *Limulus* amebocyte lysate test, but not in D1651 treated one. We conclude that the mechanism of A1648-induced elevation of cytokine gene expression and production in rat alveolar macrophages was due to the presence of endotoxin incorporated on the particle.

702 DOSE-DEPENDENT PULMONARY FUNCTION CHANGES AND INFLAMMATION FROM INHALATION OF NEAT AND IN-USE MACHINING FLUIDS

J A DeKoster and P S Thorne. *Dept. of Preventive Medicine & Environmental Health, the University of Iowa, Iowa City, IA*

Acute inflammation and pulmonary function responses following inhalation exposure to neat and in-use metal working fluids (MWF) were investigated in guinea pigs and mice. Aims of the study were to characterize physiologic and biomarker responses to inhaled MWF, identify potential causative agents, and evaluate the potential for development of pulmonary hypersensitivity from constituents of in-use MWF. Inhalation exposures of guinea pigs to MWF demonstrated dose-dependent increases in respiratory rate and decreases in breathing volume that served to quantify MWF potency. In-use MWF were more toxic than their corresponding neat MWF. Lowering the pH of neat MWF or spiking neat MWF with active cultures of *Pseudomonas pseudoalcaligenes* did not increase potency to the level of the in-use MWF. These studies showed that significant predictors of respiratory responses were in-use over neat MWF ($p=0.0001$), MWF dose ($p=0.022$), MWF type ($p=0.031$), and the particular MWF sample tested ($p=0.032$). Inhalation exposures to biocides used in the MWF resulted in dose-dependent decreases in respiratory rate. The in-use MWF had endotoxin concentrations from 280

to 1.7 x 10^5 EU/ml. To investigate the role of this endotoxin, inhalation experiments were performed in normal mice (SEN) and endotoxin resistant mice (C3H/HeJ) (RES). These revealed a dose dependent 10,000-fold increase in neutrophils in BAL fluid and a 100-fold increased concentration of IL-6 and TNFα in SEN but not RES mice with exposure to in-use MWF. Smaller increases were seen for IL-1α. Inflammatory responses were not observed with exposure to neat MWF or with sham exposure. Removal of microorganisms from the MWF by filtration of the in-use MWF did not change the responses observed in either strain of mouse. These studies demonstrated that lung inflammation was an important outcome from exposure to in-use MWF and that the endotoxin in these fluids was an important toxicant.

703 EXPOSURE OF HUMAN BRONCHIAL EPITHELIAL CELLS (BEAS-2B) TO OCCUPATIONAL ALLERGENS INDUCES IL-6 PROTEIN SECRETION

P Stetkiewicz and M Wills-Karp. *Department of Environmental Health Sciences, Johns Hopkins University, Baltimore, MD.* Sponsor: T Sutter

Occupational asthma is defined as reversible airways obstruction induced by fumes, vapors or gases encountered in the workplace. The mechanism by which the occupational allergens, toluene 2,4-diisocyanate (TDI) and nickel sulfate ($NiSO_4$) cause occupational asthma are unknown, although it is thought that there is an immunologic and nonimmunologic component to the disease. Interleukin (IL)-6 is a well known proinflammatory cytokine that has been implicated in the asthmatic inflammatory response. Airway epithelial cells have the ability to secrete several cytokines, including IL-6. We hypothesize that the airway epithelial cells are playing an immunoregulatory role to inhaled allergens by secreting cytokines such as IL-6 that influence the immune system, creating an allergic response to the agent. To determine whether airway epithelial cells release IL-6 upon exposure to occupational allergens, we measured IL-6 levels in the supernatants of cultured BEAS-2B cells by ELISA. Cells were cultured in a defined Ham's F-12 medium plus growth factors, then exposed to either basal Ham's F-12 medium as control, 0.3 um TDI or 10 ug/ml $NiSO_4$ for 30, 60, 90 and 180 minutes. The control cells constitutively released IL-6 protein. The occupational allergen TDI caused a 2 to 3 fold increase over control at 30 and 60 minutes while $NiSO_4$ caused a 2 to 3 fold increase at 30, 60 and 90 minutes. These results suggest that IL-6 is quickly released from BEAS-2B cells in response to these chemical stimuli, suggesting that airway epithelial cells may contribute to the genesis of local inflammatory responses and the pathogenesis of occupationally induced asthma. Supported by HL0734-14 and Johns Hopkins Center for Alternative to Animal Testing (CAAT).

704 DIESEL EXHAUST PARTICLES STIMULATE THE SECRETION OF INTERLEUKIN-1, BUT NOT TUMOR NECROSIS FACTOR-ALPHA, IN RAT ALVEOLAR MACROPHAGES

H-M Yang[1], J Y C Ma[2], V Castranova[2], and J K H Ma[1]. [1]*School of Pharmacy, West Virginia University,* [2]*DRDS, NIOSH, Morgantown, WV*

Exposure to diesel exhaust particles (DEP) in animals results in persistent lung inflammation. This study investigated the effects of DEP on release of proinflammatory cytokines, interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), by rat alveolar macrophages (AM) *in vitro*. AM were isolated from male Sprague-Dawley rats (~300 g) and incubated with 0, 5, 10, 20, 50 or 100 μg DEP/10^6 AM/ml at 37°C in 5% CO_2. Methanol was used to extract organic compounds from DEP. In concurrent experiments, the effects of washed DEP and methanol DEP extracts of equivalent concentrations were also tested. Twenty-four hours after exposure, AM supernatants were collected and IL-1 and TNF-α activities were determined, respectively, by a mouse thymocyte proliferation assay and a L929 cytotoxicity assay. Exposure to either DEP, washed DEP or methanol DEP extracts did not significantly affect AM viability, as indicated by the results of trypan blue exclusion and LDH release. DEP (at 50 and 100 μg) and methanol DEP extracts (at 20 and 100 μg) significantly increased IL-1 release from AM, while washed DEP was ineffective. In contrast, neither DEP, washed DEP nor methanol DEP extracts stimulated TNF-α production. These results show that DEP can directly activate rat AM to release IL-1. This effect may be attributed to the organic components associated with DEP. Since DEP did not stimulate TNF-α production, IL-1 may play the more important role than TNF-α in DEP-induced inflammation.

705 TEMPORAL EXPRESSION AND CELLULAR DISTRIBUTION OF PULMONARY FIBRONECTIN GENE INDUCTION FOLLOWING EXPOSURE TO AN EMISSION SOURCE PARTICLE

W Y Su[(1)], U P Kodavanti[(2)], R H Jaskot[(2)], D L Costa[(2)] and K L Dreher[(2)]. *(1) Duke Univ. Med. Ctr. Durham, NC & (2) EPA, PTB, RTP, NC*

Fibronectin (Fn) is an extracellular matrix protein involved in a variety of cellular functions including inflammation, cell proliferation, and fibrosis. Previous studies from this laboratory have demonstrated an increase in Fn mRNA in rat lungs after exposure to an emission source particle, known as residual oil fly ash (ROFA). To understand the temporal and spatial distribution of Fn gene expression in ROFA-induced lung injury, we examined Fn mRNA expression by *in situ* hybridization in rat (Sprague-Dawley) lung during the acute phase of lung injury occurring 6 hr to 72 hr following intratracheal instillation of ROFA, 8.3 mg/kg body weight. The pattern of Fn mRNA expression was compared with histopathological changes. Fn mRNA was undetectable in rat lungs treated with saline as well as in lung tissue examined at 6 hr after ROFA treatment. However, at 24 hr after ROFA instillation, Fn mRNA was induced in airway epithelial cells, especially at terminal bronchioles. Histopathology showed peribronchial and perivascular inflammation as well as focal edema. At 48 hr after ROFA exposure, diffusive inflammation in the alveolar region with limited expression of Fn mRNA was evident occurring mainly in proliferating alveolar type 2 epithelial cells. At 72 hr after exposure, extensive Fn mRNA expression was seen in proliferating fibroblasts as well as in hyperplastic and hypertrophic epithelial cells within fibrotic lesions, while the intensity of expression in airway epithelial cells was decreased. Therefore, Fn mRNA induction in the lung following ROFA exposure was associated with inflammatory and fibrotic lesions indicating its possible role in the initiation of fibrogenesis. (This abstract does not necessarily reflect EPA policy).

706 EARLY ALTERATIONS IN THE mRNA ABUNDANCE OF IL-1β, IL-1β, iNOS, MIP-2, $TGF\beta_1$, AND VEGF ASSOCIATED WITH ULTRAFINE PARTICLE EXPOSURE

C Johnston, J Finkelstein, R Gelein, R Baggs, P Mercer, N Corson, K Nguyen and G Oberdörster. *Depts. of Environm. Medicine & Pediatrics, University of Rochester, Rochester, NY*

Polytetrafluoroethylene PTFE (Teflon®]) fume consisting of ultrafine particles (~20 nm) has been described to induce acute effects in the respiratory tract such as hemorrhagic inflammation with extensive pulmonary edema. The ultrafine particles were generated by heating PTFE to ~460°C. Fischer-344 rats were exposed for 15 min. to a concentration of 5 x 10^5 particles/cm^3. Rats were examined 4 hrs. post-treatment. Cells in lung lavage from these rats consisted of 55–75% PMNs. Increases in message abundance for interleukin-1 α and β (IL-1α, IL-1β), inducible nitric oxide synthase (iNOS), and macrophage inflammatory protein-2 (MIP-2) were ~4, 8, 4 and 40-fold, respectively. Transforming growth factor beta-1 ($TGF\beta_1$) was unaltered and vascular endothelial growth factor (VEGF) mRNA was decreased by 80% as compared to sham-exposed controls. *In situ* hybridizations demonstrated that PMNs and macrophages as well as epithelial and endothelial cells were actively involved in message production. These results suggest inhaled ultrafine particles penetrate into and interact with alveolar epithelial cells which are activated to induce pro-inflammatory cytokines (IL-1α, IL-1β, MIP-2) and release NO- *via* induction of NOS. VEGF is down regulated and may affect the integrity of the capillary endothelium and result in increased permeability which may also facilitate MIP-2 initiated transmigration of PMNs into the alveolar interstitium. Support by grant NAGW-2356.

707 SILICA ACTIVATION OF MIP-2 AND CINC GENE EXPRESSION BY RAT LUNG EPITHELIAL CELLS: ROLE OF OXIDATIVE STRESS

K E Driscoll, B W Howard, J M Carter. *The Procter & Gamble Co. Cincinnati, OH*

Inflammatory mineral particles and fibers activate rat lung epithelial cells *in vitro* to express the neutrophil chemotactic cytokine, macrophage inflammatory protein 2 (MIP-2). Analysis of the 5' flanking region of the MIP-2 gene has demonstrated that the oxidant sensitive transcription factor, NFκB, plays a key role in upregulating MIP-2 expression in response to several agonists including endotoxin and TNFα. In the present studies, we investigated the potential role of oxidant stress and activation of NFκB in silica-induced increases in MIP-2 expression by lung epithelial cells. Briefly, cultures of the rat lung epithelial cell line, RLE-6TN, were exposed to doses of 2 - 20 μg/

Attachment B

Textbook of CLINICAL OCCUPATIONAL and ENVIRONMENTAL MEDICINE

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Attachment C

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Dermatitis among Automobile Production Machine Operators Exposed to Metal-working Fluids

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Short title: Dermatitis among machine operators

Key words: dermatitis, coolants, metal-working fluids, machine operators

Abstract:

This cross-sectional study was designed to assess differences in prevalence of contact dermatitis between machine operators exposed to metal-working fluids (MWFs) and unexposed assemblers and to assess potential risk factors for contact dermatitis among these machine operators. In their work, machine operators were exposed to either semi-synthetic or soluble oil MWFs. We evaluated 158 machine operators and 51 assemblers from one large automobile transmission plant using questionnaire, dermatologist examination of the skin, and dermal dosimetry to measure wetness and metal exposures. We found that machine operators had more combined (definite plus possible) dermatitis (27.2% vs. 13.7%, χ^2=3.9, p=0.05, 1df) compared with assemblers. Among machine operators, risk factors significantly associated with combined dermatitis were subjective assessment of wetness of the work, exposure to semi-synthetic as opposed to soluble oil MWF, current cigarette smoking, and increasing worker age. These risk factors suggested preventive and control measures including control of wet work, surveillance programs including early self-report of dermatitis, consideration of replacement of semi-synthetic with soluble oil MWFs, and strictly limiting smoking among machine operators exposed to MWFs.