evaluate the effects of ETS exposure during perinatal development in site-specific lung subcompartments of non-human primates. Aged and diluted sidestream smoke was used to expose timed pregnant monkeys were exposed to ETS for six hours a day, five days a week, from 50 days gestational age (DGA) to 2.5 months postnatal age (PNA)(total suspended particulate, 0.99 ± 0.06 mg/m3; nicotine, 257 ± 91 µg/m3 and carbon monoxide, 4.4 ± 0.5 ppm) (n = 4 controls and 4 ETS). At 2.5 months PNA, infant monkeys were deeply anesthetized and microdissection techniques were used to isolate the airway tree and lung parenchyma. Microsomal fractions were prepared for the measurement of cytochrome P450 (CYP) 1A1 and 2E1 activities; cytosolic fractions were prepared for the determination of NO, MDA, GST, GSH, GSSG and GRR. Striking increases in microsomal CYP 1A1 activity were observed in all airway subcompartments of animals exposed to ETS compared to filtered air controls; these increases varied from 81- to 193- fold over control. In the parenchyma, CYP1A1 activity was increased 167-fold (480 pmol/min/mg protein) in monkeys exposed to ETS compared to control (2.9 pmol/min/mg protein). Increases of 2- to 6-fold for CYP 2E1 activities were observed in all airway subcompartments and parenchyma, although these increases were not statistically significant. Exposure to ETS significantly decreased NO in distal bronchioles and lung parenchyma. Exposure to ETS also elevated GSSG and GRR in the parenchyma. No significant changes were found in GST activity, MDA or GSH levels. We conclude that exposure to ETS during lung development dramatically increases pulmonary CYP1A1 activity and induces oxidative stress in a site-specific manner in the lungs of infant monkeys.

940 DIFFERENTIAL DISPLAY AND MICROARRAY DETECTION OF GENES UP-REGULATED IN NAPHTHALENE-TOLERANT MICE.

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Naphthalene (NAP) is a volatile aromatic hydrocarbon produced by incomplete combustion and is found in both ground water and air. Murine Clara cells are highly susceptible to NAP administered either *via* parenteral or inhalation routes. However, after repeated exposures to NAP, the susceptibility of target cells diminishes dramatically. There is significant protection of the lung from a large challenge dose of NAP following the last of seven daily intraperitoneal (IP) injections of 200 mg/kg NAP. In the current study, differential display and microarrays have been utilized to detect genes up-regulated in response to NAP tolerance. mRNA was isolated from microdissected airways of NAP-tolerant and corresponding vehicle treated control mice. cDNA libraries were ligated with a standard oligonucleotide before undergoing subtractive hybridization. The unhybridized cDNA remaining after removing hybrid sequences between treated and control cDNA represent genes expressed in the treated but not control library. Subtracted and control clones were arrayed on nylon membranes for differential screening and 14 differentially expressed clones of up-regulated genes were detected, purified, sequenced and confirmed by gene-chip expression analysis. Preliminary identification of genes that are differentially expressed and may play roles in the mechanism of NAP tolerance include those involved in cellular metabolism and catabolism (adenine-monophosphate-deaminase, kinase, phosphatase, ornithine transcarbamylase, RNA helicase, protease, malate dehydrogenase) in the cell cycle (cyclin, splicing factor Srp20), and in apoptosis (Siva gene, cytochrome c oxidase). In addition, various house keeping genes (actin), and stress response genes (heat shock, ATP and GTP binding protein) were upregulated as were genes associated specifically with the Clara cell (Clara cell secretory protein). Supported by NIEHS 04699 and 04311.

941 *IN VITRO* METHODS FOR ASSESSING THE CYTOTOXICITY AND METABOLISM OF THE METABOLICALLY ACTIVATED LUNG TOXICANT, 1-NITRONAPHTHALENE (NN).

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A number of chemically diverse agents including aromatic hydrocarbons such as naphthalene and NN, furans such as 4-ipomeanol and chlorinated ethylenes such as dichloroethylene produce selective toxicity to airway epithelial cells in rodent lungs. All of these agents require metabolic activation by the cytochrome P450 monooxygenases and orthologues of the P450's thought to be involved in their metabolic activation exist in primate lungs (2F for naphthalene and NN, 4B for 4-ipomeanol and 2E for dichloroethylene). Comparative microsomal metabolism studies have generally demonstrated substantial differences in the rates of metabolism of these substrates (rodent >>> primate) but the possibility remains that P450 monooxygenases are highly localized in primate lungs thus leading to low apparent activities. There are no studies evaluating the toxicity of these agents in primate lung tissue. Accordingly, we have developed methods for assessment of metabolism and toxicity of these agents using NN as a model in the rat. Rat lungs were filled with Hanks buffer containing varying concentrations of NN (0-2 mM) and were incubated for time periods up to 2.5 hrs at 37o C. Medium was removed and ethidium homodimer (1 µM in medium) was infused for 10 min to stain membrane permeable cells. Lungs were fixed, counterstained with Yo-Pro dye to label all nuclei and ethidium positive cells were imaged by confocal fluorescence microscopy. These studies showed that NN toxicity was concentration and time dependent and that injury reflected that observed after administration of the compound in vivo. We conclude that after inflation with medium, lungs remain viable for up to 2.5 hrs, a time sufficient to observe cytotoxicity in the presence metabolically activated substrate. Similar approaches with primate tissue should yield information on the susceptibility to toxicants like NN. Supported by NIEHS 04311 and 08408.

942 USE OF SIZE-SELECTED FIBERS TO EVALUATE THE CONTRIBUTION OF LENGTH *vs* CHEMISTRY IN FIBER CYTOTOXICITY.

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Studies have shown that asbestos can lead to lung disease. Therefore, substitutes have been developed that differ chemically from asbestos. However, fiber length as well as chemical composition may be an important factor in pathogenicity. The objective of this study was to investigate the role of length *versus* chemistry by monitoring the cellular effects of *in vitro* exposure to different length glass fibers (7 and 17µm) or three types of fibers (glass, chrysotile, or ceramic) of the same length. A dielectrophoretic classifier was used to separate fibers into specified length categories. Primary rat alveolar macrophages obtained by bronchoalveolar lavage were exposed to various concentrations of fibers. Cytotoxicity and inflammatory potency were assessed by lactate dehydrogenase and tumor necrosis factor α (TNF-α) release, respectively. Data show that 7µm glass fibers (100 µg/ml) caused 6% cell death while 17µm glass fiber caused 31% cell death (Blake et. al., 1998). We found that long glass fibers (at a cell:fiber ratio of 1:5) were twice as potent as short glass fibers in stimulating TNF-α production. Data indicate that MAP kinases, p38 and ERK, play a role in this TNF-α production. Long glass fibers were twice as potent as short fibers in activating p38 and ERK. Therefore, glass fiber length is an important factor in cytotoxicity and alveolar macrophage activation. To investigate the contribution of fiber chemistry to cytotoxicity, alveolar macrophages were exposed to chrysotile, glass, and ceramic fibers of similar dielectrophoretic size cuts (17µm target). Chrysotile appeared to exhibit the greatest cytotoxicity, *i.e.*, 100 µg/ml chrysotile, glass, and ceramic fibers caused 30, 19, and 7% cell death, respectively. In conclusion, our results suggest that both length and chemistry play a role in cytotoxicity to alveolar macrophages. In contrast to in vivo exposures, our *in vitro* system failed to demonstrate the high cytotoxicity of ceramic fibers.

943 BIODEGRADABILITY OF INHALED *p*-ARAMID RESPIRABLE FIBER-SHAPED PARTICULATES (RFP): *IN VITRO* CELLULAR STUDIES DEMONSTRATING SHORTENING OF RFP.

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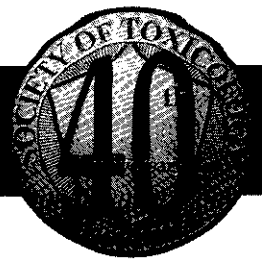
Biopersistence, or alternatively, biodegradability (*i.e.*, low biopersistence) represents an important concept in fiber toxicology. To investigate the mechanisms of RFP biodegradation, we have hypothesized that 1) lung fluids activate p-aramid RFP following deposition, and the RFP are then vulnerable to enzymatic attack in the lungs; and 2) pulmonary phagocytes contribute to the biodegradation process. To test the hypothesis, p-aramid RFP or cellulose RFP were incubated with saline or lung fluids and then processed by one of 2 simulated lung digestion techniques. In addition, L2 rat lung epithelial cells, primary rat alveolar macrophages, and co-cultures of L2 cells and macrophages were incubated with p-aramid RFP and evaluated 1 hr, 24 hrs, 1 week, and 2 weeks post *in vitro* exposure. The results demonstrated that mean lengths of p-aramid RFP processed with KOH and evaluated by SEM were 13.4 um; in contrast, mean lengths of p-aramid RFP samples, incubated in lung fluids and treated with the enzyme method were 8.8 um. The enzyme digestion method had no discernible effect on shortening of cellulose RFP, indicating that the results with p-aramid were specific. *in vitro* cellular studies demonstrated a shortening of the p-aramid RFP in macrophages (10.9 um) and co-cultures (11.8 um) but not L2 lung epithelial cells (15.5 um) at 24 hrs and 1 week postexposure. Our data indicate that components of lung fluids coat and catalyze the p-aramid, thereby predisposing the RFP to enzymatic cleavage. Moreover, the pulmonary macrophage may play a significant role in the biodegradation process.



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Preface

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An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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