135.1

Role of macrophage scavenger receptors (SRs) for unopsonized particles: Effect of LPS activation. A. Palecanda*, K. O'Driscoll*, G. Kraal* and L. Kobzik*. Physiology Program, Harvard School of Public Health, Boston, MA, USA. *Faculty of Medicine, Vrije Universiteit, Amsterdam, The Netherlands.

Macrophage SRs, SR-AI/II and MARCO, contribute to normal host defense by binding unopsonized bacteria or environmental particles. We sought to investigate if macrophage activation can modulate these important functions. We measured the effect of LPS on expression and particle binding function of SR-AI/II and MARCO in the mouse macrophage cell line, J774A.1. LPS substantially increased MARCO expression as detected by immunofluorescent labeling with specific mAb, ED31 (fold increase, mean fluorescence: 5.4 ± 2.1, n = 4), whereas a high normal level of SR-AI/II expression detected with mAb 2F8 remained unchanged. Both untreated and LPS-activated J774A.1 cells bound unopsonized TiO_2 particles and heat killed *Escherichia coli (E. coli)*. The anti-MARCO mAb, ED31, inhibited TiO_2 and *E.coli* binding by LPS-activated J774A.1, but not in untreated cells (which express little MARCO) (Table). In contrast, anti-SR-AI/II mAb, 2F8, inhibited TiO_2 binding by both untreated and LPS-activated cells. Anti-SR-AI/II mAb also blocked untreated J774A.1 cell binding of *E.coli* by 44 ± 9%. Despite robust SR-AI/II expression after LPS treatment, anti-SR-AI/II had minimal effect on activated cell binding of *E.coli*:

% Inhibition by:

	Anti- SR-AI/II mAb		Anti-MARCO mAb	
	- LPS	+ LPS	- LPS	+ LPS
TiO_2	39 ± 5	28 ± 6	≤ 0	27 ± 2
E. coli	44 ± 9	8 ± 5	≤ 0	39 ± 10

These studies suggest: 1) Both SR-AI/II and MARCO can mediate uptake of unopsonized bacteria and inert particles, 2) MARCO may show selective greater affinity for bacteria, and 3) LPS-mediated activation of J774A.1 macrophages modulates function and expression of SR-AI/II and MARCO. The role of these receptors in normal or activated primary macrophages is being investigated. NIEHS 0002, 08129.

135.2

Macrophages Generate H202 after Exposure to Carbon Particles + Benzo[a]Pyrene.

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Environmental particulate matter (EPM) is a major by-product from the combustion of fossil fuels. We had previously demonstrated that a) exposure of macrophages (Mø) to a model of EPM, benzo[a]pyrene adsorbed on carbon black, (CB+BP) induces the secretion of TNF-a (AJP 275:L942-L949) and b) CB+BP were phagocytized at a slower rate than CB alone. Little is known about the production of ROS in EPM-induced injury. In this study we used luminol and lucigenin-derived chemiluminescence to detect H202 and O2- generated in Mø exposed to carbon black (CB) and CB+BP. Mø exposed to CB and CB+BP separately generated H202 after 15 and 30 min of exposure respectively, the latter exposure sustaining its levels for up to 8h. There were no significant increases in O2-. Once the CB+BP is internalized, the BaP is released from the particle, biotransformed into more reactive intermediates, among which is the mitochondrial redox-cycler, BP 1,6 quinone (BP 1,6 Q). Mø treated with exogenous BP 1,6Q generated increases in both O2- and H202, 1.5x and 14x respectively. Inhibition of the mitochondrial electron transport chain (METC) complex (I) with rotenone prior to exposure to BP 1,6Q was effective in reducing O2- to control levels whereas H202 levels were increased by 8x. However, inhibition of complex III with Antimycin A superinduced both O2- and H202 levels to 9x and 70x respectively presumably by the back-up of more free electrons. Taken together, our results suggest that phagocytosis of CB+BP induces Mø to generate H202 and the sustained levels of both O2- and H202 is attributed to the redox-cycling action of BP1,6Q.

135.3

COMPOSITIONAL HETEROGENEITY INFLUENCES INDUCTION OF LUNG INFLAMMATION BY RESIDUAL OIL FLY-ASH.

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Occupational residual oil fly ash (ROFA) exposure results in variable lung toxicity. ROFA composition appears to mediate these effects. The elemental composition of individual ROFA particles should be highly variable but little data are available. Objectives of this study were to: 1) analyze compositional differences of two ROFAs by water-soluble metal component analysis; 2) differentially assess lung inflammation in rats instilled with these ROFAs; 3) determine compositional heterogeneity of individual ROFA particles that may be associated with inflammation. Soluble metals were measured 1 or 24 hr post-suspension by ICP-MS; ROFA1 had comparatively higher levels of soluble vanadium and zinc while ROFA2 was higher in nickel. Rats were intratracheally-instilled with saline or ROFA (2.5 mg/kg body weight) suspended in saline and bronchoalveolar lavage (BAL) was performed at 4, 24, and 48 hr later. Both ROFA samples produced significant ($p < 0.05$) inflammation marked by neutrophil influx at all timepoints. However, ROFA1 induced greater neutrophil influx and significant increases in BAL eosinophils suggesting that inflammatory responses may be related to particle elemental composition. Preliminary individual particle analyses using a JOEL JSM-5600 SEM with an Oxford ISIS 310 microanalysis system showed substantial compositional variation between particles. These results suggest that particle composition modulates biological responses and that individual particles from a single ROFA may exert greater influence over this response. Supported by ES-00002, HL-54958, ES-08129, and ES-05947.

135.4

EFFECTS OF ASPHALT FUME CONDENSATE (AFC) EXPOSURE ON THE PULMONARY CYTOCHROME P450 (CYP450) SYSTEM.

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Exposure to asphalt fumes is a concern due to the presence of polycyclic aromatic hydrocarbons (PAH) in asphalt. Bioactivation of many PAH requires metabolism by the CYP450 system. The objective of this study was to evaluate the effects of exposure of rats to AFC on the pulmonary CYP450 system. Rats were intratracheally instilled with saline, 0.1, 0.5, or 2 mg AFC for 3 consecutive days and sacrificed the following day. Total microsomal protein and CYP2B1 and CYP1A1 isozyme levels were determined. The activities associated with CYP2B1 and CYP1A1 were measured by the O-dealkylation of 7-pentoxyresorufin (7-PR) and 7-ethoxyresorufin (7-ER), respectively. AFC exposure did not cause significant changes in total microsomal protein content. CYP2B1 and CYP1A1 levels in AFC-exposed lungs were not significantly different from control as determined by Western blot analysis. However, microsomes from AFC-exposed rats exhibited a concentration- dependent increase in the activity of 7-PR and 7-ER O-dealkylase. These results demonstrate that AFC exposure did not alter total microsomal protein or CYP1A1 and CYP2B1 levels in the lung. However, AFC increased CYP2B1 and CYP1A1 activities in lung microsomes, suggesting that AFC exposure may significantly alter metabolism of PAH by the CYP450 system in the lung.

135.5

p53 IN NASAL BIOPSIES OF CHILDREN CHRONICALLY AND SEQUENTIALLY EXPOSED TO AIR POLLUTANTS. Calderon-Garciduenas L, Rodriguez-Alcaraz A, Mora-Tascareno A, Garcia R, Osnaya N, Villarreal-Calderon A, Carson J, Devlin RB,Van Dyke T. Curriculum in Toxicology, Univ of North Carolina at Chapel Hill,NC; Instituto Nacional de Pediatria,Mexico City;Soc Mex ORL y CCC;Dept of Pediatrics UNC; EPA, RTP,NC27711;Biochemistry and Byophysics Department,UNC,Chapel Hill,NC

Background: Air pollution is a serious health problem in Mexico City. South West Metropolitan Mexico City (SWMMC) atmosphere is a complex mixture of air pollutants, including ozone, particulate matter,aldehydes, and volatile organic compounds. Children in SWMMC are exposed chronically and sequentially to numerous toxicants and potential nasal carcinogens, and they exhibit significant nasal damage. Objective: to assess p53 accumulation by immunohistochemistry in nasal biopsies of children ages 6-12 we evaluated 111 biopsies from 107 children (83 exposed SWMMC children and 24 control children residents in a low polluted Caribbean island).A complete clinical history and physical examination , including an ENT exam were done.RESULTS:Control children gave no upper or lower respiratory symptomatology the three months previous to the study;their biopsies exhibited normal ciliated respiratory epithelium and were p53 negative. Exposed children: epistaxis,nasal obstruction and crusting were common.Irregular areas of whitish-gray depressed mucosa were seen in 25% of children, in association with flattened turbinates; sinusitis was documented in 24%. Exposed biopsies displayed basal cell hyperplasia, ciliated cells with shortened cilia,neutrophilic infiltrates, squamous metaplasia, and mild dysplasia.Four of twenty-one children with gross abnormal mucosa by ENT exam exhibited strong transmural nuclear p53 staining in their nasal biopsies. CONCLUSION: In the context of lifetime exposures to toxic and potentially carcinogenic pollutants, p53+ nasal induction in children could represent: A).A checkpoint response to toxic exposures, setting up a selective condition for p53 mutation or B). A p53 mutation has already occurred as a result of such selection.Ozone, particulate matter and aldehydes are likely playing a crucial role in the nasal pathology.[This is an abstract of a proposed presentation and does not necessarily reflect EPA policy].NEIHS training Grant T32ES0 7126.

135.6

PULMONARY CYTOKINE PRODUCTION OF SIDESTREAM CIGARETTE SMOKE IN MICE WITH DIETARY ALPHA-TOCOPHEROL SUPPLEMENTATION

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The effects of sidestream cigarette smoke (SSCS) with dietary α-tocopherol supplementation were studied on lung function, pulmonary cytokines, and pathological evaluation. Forty-six C57BL/6 mice (half each gender, 7 months old) were randomly assigned to three groups: control (room air), SSCS exposure, and SSCS with α-tocopherol supplementation. SSCS (2 standard research cigarettes per day, 1R4) or room air was delivered by the nose-only IN-Tox exposure system for 45 days. SSCS exposure induced increases of IL-1β and IL-6 in bronchoalveolar lavage (BAL) and TNF-α and IL-6 in lung tissue, which were accompanied by decreases of pulmonary dynamic compliance and increases of pulmonary resistance and histopathological alterations. Dietary α-tocopherol supplementation significantly prevented the SSCS-induced lung dysfunction, with reduction of cytokine levels, IL-1β in BAL and TNF-α in lung tissue. Our data demonstrates that dietary intake of α-tocopherol may improve lung dysfunction of the SSCS-induced local host defense mechanisms, in part, through cytokine regulation.

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ABSTRACTS

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