493 OZONE-INDUCED 4-HYDROXYNONENAL FORMATION IN MOUSE SURFACE LINING FLUIDS

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Inspired O_3 is absorbed within the lung via a reaction-dependent process which, due to reaction substrate availability, is most likely localized to the pulmonary surface lining layer (SLL). O_3 reacts with unsaturated fatty acids (UFA) to produce distinct aldehydic products. However, alternative reaction pathways produce free radicals which initiate peroxidative processes and can generate hydoxylated aldehydes. To evaluate the formation of SLL-derived UFA products, we exposed mice (C57BL/6) *in vivo* and mouse bronchoalveolar lavage (BAL) *in vitro* to O_3 and assayed BAL fluid (BALF) and cells for 4-hydroxynonenal (HNE)-protein adduct formation via Western blotting using a polyclonal antibody against HNE adducts to his, lys, cys. *Results:* (1) *In vivo* air controls showed no adducts in BALF or cells. (2) 1.2 ppm O_3 (3 hr) *in vivo* resulted in BALF adducts (25–27 & 40–45 kD) with minimal detection in cells. (3) *In vitro* BAL exposure (0.6 ppm O_3, 30 min) produced HNE in BALF (50–55, 70–80 kD) but little in cells. (4) Direct HNE (1 μM) addition to BAL generated BALF adducts similar to *in vitro* exposures but also produced cellular adducts. *Conclusions:* The results suggest that substantial O_3 chemistry occurs within the SLL extracellular space. HNE formation may contribute to O_3-induced toxicity. Differences in adduct distribution between O_3 exposures and HNE addition may result from experimental discrepancies between well-mixed BAL and the native SLL. (CIAR 90-23, & NIH ES04952, ES04804, T32ES07254).

494 STOICHIOMETRY OF LINING LAYER NONANAL PRODUCTION IN O_3 EXPOSED ISOLATED LUNGS

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O_3 is absorbed from inspired air due to chemical reactions with constituents of the lung surface lining layer (SLL). While the preferential targets for O_3 reactive absorption have not been extensively defined, the prevalence of unsaturated fatty acids should facilitate direct reaction with the SLL. Previous *in vitro* and *in vivo* studies have demonstrated that aldehydes, specific to O_3-fatty acid reactions, are produced as a consequence of exposure. To investigate the stoichiometry between pulmonary O_3 absorption and nonanal production (derived from oleic acid), we exposed isolated lungs and subsequently quantified nonanal in bronchoalveolar lavage (BAL). Isolated lungs (unperfused; $V_2 \cong 2.4$ ml, f = 50/min; 37° C) were employed to enable control of O_3 delivery rate, measurement of O_3 absorption, and to limit perfusion-based removal of products. Aldehydes were quantified using O-pentafluorobenzylhydroxylamine HCL derivatization and gas chromatography/ECD. *Results:* A linear (r = 0.99) dose/response was observed. (1) 0.26 ppm, 60 min (74 nmol O_3 dose) = 69 nmol O_3 uptake & 126 pmol nonanal production. (2) 1.0 ppm, 30 min (154 nmol dose) = 141 nmol uptake & 243 pmol nonanal. (3) 1.0 ppm, 60 min (304 nmol dose) = 278 nmol uptake & 380 pmol nonanal. (4) Hexanal and heptanal were also detected. The results suggest that direct O_3/fatty reactions occur within the SLL. O_3-specific aldehydes as measured represent only a small fraction of the absorbed dose. (CIAR 90-23, HEI 91-7, NIH ES04952 & T32ES07254).

495 OZONE DEGRADES IMPORTANT LUNG PHOSPHOLIPIDS AND PROTEINS TO SUBSTANCES THAT INDUCE DNA SINGLE STRAND BREAKS

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We have previously shown that ozone (O_3) can degrade free arachidonic acid (AA) to the genotoxic agent hydrogen peroxide (H_2O_2). However, in the lung, the vast majority of unsaturated fatty acids (UFAs) such as AA are esterified in phospholipids located intracellularly and in epithelial lining fluid (ELF). Albumin is the most abundant protein in human ELF. Upon exposure to O_3, these biomolecules may be degraded to H_2O_2. In these studies, the effect of O_3 exposure on the degradation of arachidonyl-containing phosphatidylcholine (PC) (used as a model phospholipid), and human serum albumin were determined. Additionally, the effect of the ozonized PC on the induction of DNA single strand breaks (ssb) was examined. PBS vehicle, 30 or 60 μM PC, and 0.5 mg/ml albumin were exposed to 0.4 ppm O_3 (2 hr) and generated 3, 13, 19, and 44 μM H_2O_2, respectively. H_2O_2 formation was also exposure time-dependent. Incubation of human lung fibroblasts (CCD-32Lu) at 4°C (1hr) with 30 μM ozonized PC (0.4 ppm, 2 hr) or 25 μM H_2O_2 induced approximately 65 and 185 rad equivalents of DNA ssb, respectively. These data suggest that some proteins and UFAs esterified to phospholipids can be degraded by O_3 to genotoxic species. The production of H_2O_2 from free and esterified UFAs as well as some proteins may contribute to the possible lung tumorigenic potential of O_3. [Supported in part by CR81763 and ES04951. This is an abstract of a proposed presentation and does not necessarily represent EPA policy.]

496 NO_2-INDUCED SECONDARY MEMBRANE OXIDATION: POTENTIAL ANTIOXIDANT AND OXYGEN-DEPENDENCE

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NO_2-induced pulmonary injury is governed by the initial reactions between NO_2 and surface lining layer (SLL) target molecules. Since NO_2 *per se* is eliminated upon absorption, it is likely that SLL-derived products initiate the cytotoxic cascade. Previous studies demonstrated that SLL ascorbate (AA) and glutathione (GSH) are preferential NO_2 reactive absorption targets. We used a 3-compartment model to represent the lung surface and investigate the potential for these targets to initiate exposure-induced secondary oxidative processes. We covalently adhered red blood cell membranes (RBCM) to a glass substratum. Exposures (10 ppm NO_2, 20 min) were conducted by covering RBCM with model SLLs and cyclically tilting dishes to produce a thin film but preclude direct RBCM contact with the gas phase. RBCM oxidation was determined via thiobarbituric acid reactants (TBARS). *Results:* (1) NO_2 + 10 μM GSH, 25 μM AA, or rat lung lavage fluid significantly elevated TBARS production. (2) Air-only (+GSH or AA), or NO_2 (–GSH or AA) produced negligible TBARS. (3) NO_2 in N_2 (+GSH) reduced TBARS compared to air. (5) Superoxide dismutase (SOD) (+GSH) eliminated TBARS. *Conclusions:* GSH and AA may function as prooxidants during NO_2 exposure. The O_2-dependence and SOD effect suggest that $GS^{\bullet}$, produced from NO_2 + GSH, reacts to form $GSSG^{\bullet-}$ which reduces O_2 to $O_2^{\bullet-}$. Potentially, the extracellular production of $O_2^{\bullet-}$ within the SLL may function as an unrecognized mechanism of NO_2 toxicity. (CIAR 90-23, NIH ES04952 & T32ES07254).

497 REGULATION OF TYPE II PNEUMOCYTE PROLIFERATION FOLLOWING ACUTE EXPOSURE OF RATS TO OZONE

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Ozone is a highly reactive oxidant that causes necrosis of Type I epithelial cells in the proximal alveolar regions and proliferation of Type II pneumocytes which act as progenitors for Type I cells. In the present studies we analyzed mechanisms regulating Type II cell proliferation. Female SD rats were exposed to 2 ppm ozone or air for 3 h and the lungs isolated 12, 24 and/or 48 h later. Ozone inhalation resulted in proliferation of Type II cells which was evident at 12 h postexposure, as measured histologically and immunohistochemically by proliferating cell nuclear antigen expression. Type II cells isolated from ozone treated animals also incorporated significantly more ^{3}H-TdR than cells from control animals. Alveolar macrophages (AM) from both air and ozone exposed animals were found to be mitogenic towards Type II cells. AM from ozone treated rats caused a significantly greater increase in Type II cell ^{3}H-TdR incorporation. This effect required cell-cell contact indicating the involvement of cell surface molecules in the signaling pathway. These data suggest that AM participate in the repair of the alveolar epithelium after ozone-induced lung injury. (Supported by NIH Grant ES04738).

498 INFLAMMATORY AND MORPHOLOGICAL RESPONSES OF LACTATING AND POSTLACTATING RATS FOLLOWING OZONE EXPOSURE: A COMPARISON OF CONCENTRATION AND INHALED DOSE

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Ozone (O_3) has been shown to elicit an inflammatory response and induce morphological changes in the lung. We have previously shown that rats late in pregnancy and throughout lactation had enhanced inflammatory responses following acute exposure to a high concentration (1.0 ppm) of O_3 compared to those of age-matched, virgin female and postlactating rats. This study examined the inflammatory and morphological responses in lactating and postlactating rats following an acute exposure to a range of O_3 concentrations. Adult, primiparous Sprague-Dawley CD VAF rats at day 13 of lactation or day-10 postlactation were exposed in barometric plethysmographs to 0, 0.3, 0.5, or 1.0 ppm O_3 for 6 hours. Tidal volume and breathing frequency were measured

at regular intervals throughout exposure for calculation of inhaled dose. The rats were killed 18 hours after exposure, the right lung was lavaged, and the left lung was fixed for histopathology. Lactating rats had increases in total protein and neutrophils recovered in bronchoalveolar lavage fluid (BALF) compared to postlactating rats. Severity and size of lesioned areas and number of free inflammatory cells in the lung parenchyma were greater in lactating rats and were concentration dependent for both groups. Alveolar septal tip thicknesses in the periacinar regions were increased at 1.0 ppm only, and there was no difference between groups of rats. Lactating rats had greater minute volumes than postlactating rats at all O_3 concentrations. The results of this study show that lactating rats are more sensitive than postlactating rats in selected responses to acute exposures of O_3 and suggest that inhaled dose explains a portion of the difference between these groups of animals. (Supported by NIEHS Grants ES05939 and ES00260).

499 ADAPTATION OF RABBIT AIRWAYS AND PULMONARY MACROPHAGES TO SULFURIC ACID AND OZONE AFTER 20 DAY *IN VIVO* EXPOSURES

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Previously, we have shown that ozone (O_3) and sulfuric acid (SA) induce airway hyperreactivity and modify the pharmacologic regulation of H_2O_2 production by pulmonary macrophages (PM) of rabbits following a 5d, 3h/d exposure. In this study, rabbits were exposed to either 50 or 75 $\mu g/m^3$ SA, 0.1 or 0.3 ppm O_3, or a combination of O_3 and SA for 20 d, 3 hr/d. *In vitro* bronchial responses to cumulative doses of acetylcholine or histamine were not altered by pollutant exposures. This contrasts with results from 5d exposures of increased reactivity at both O_3 concentrations, and at 125 $\mu g/m^3$ SA. β-adrenergic modulation of H_2O_2 production by PM, measured by chemiluminescence, was significantly altered only by 50 $\mu g/m^3$ SA, which increased production. In contrast, H_2O_2 production was reduced by both 0.1 and 0.3 ppm O_3 and also by 50 $\mu g/m^3$ acid in 5d exposures. Pharmacologic regulation of H_2O_2 production by isoproterenol was modified only after exposure to 50 $\mu g/m^3$ SA, while after 5d exposures pharmacologic regulation was altered by 0.1 ppm O_3 and 125 $\mu g/m^3$ SA, but not by 50 $\mu g/m^3$ SA. Responses to combined 20d pollutant exposures were generally additive, while 5d exposures produced concentration-dependent synergistic or antagonistic responses. This study indicates that airways and PM may adapt to insults by O_3 and high SA concentrations, while environmentally relevant SA concentrations may induce greater effects over a longer exposure period. Thus, acute health effects induced by these pollutants may be mitigated in prolonged pollutant episodes. However, an adaptational response is not necessarily protective but, rather, may mask a secondary phase of injury. (EPRI contract #RP2155-2).

500 ENHANCED PRODUCTION OF NITRIC OXIDE BY HEPATIC PARENCHYMAL AND NON-PARENCHYMAL CELLS FOLLOWING ACUTE INHALATION OF OZONE

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Treatment of rats with ozone (2 ppm; 3 hr) results in epithelial cell injury. This is associated with an influx of activated macrophages into the lung. These cells release increased quantities of reactive mediators and pro-inflammatory cytokines which have been implicated in tissue injury. Previously we demonstrated that acute exposure of rats to ozone also results in changes in the liver including increased protein synthesis and nitric oxide production by hepatocytes. In the present studies we determined if hepatic endothelial cells and Kupffer cells were also sensitized to produce increased amounts of nitric oxide following exposure to ozone. Cells were isolated from rats 48 hrs after exposure to air or ozone by liver perfusion with collagenase and protease followed by centrifugal elutriation. Following ozone inhalation both cell types were found to produce increased amounts of nitric oxide when compared to control. Endothelial cells were more sensitive to the effects of inhaled ozone than were macrophages. These data demonstrate that brief inhalation of ozone limited to the lung induces alterations in liver parenchymal and non-parenchymal cells. Our observations of increased TNFα and IL-1 staining suggest that the extra-pulmonary effects of ozone are mediated by cytokines. (Supported by NIH grants ES04738 and ES07148).

501 MODIFICATION OF INFLAMMATORY FUNCTIONS IN THE RAT FOLLOWING OZONE EXPOSURE

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Several cell types and their soluble mediators are involved in an inflammatory response in the lung following ozone (O_3) exposure. In this study, we have examined the effects of the combination of antibodies to two important cytokines, interleukin (IL)-1α and tumor necrosis factor (TNF)-α, on the *in vivo* and *in vitro* responses to inhaled O_3. Male, Sprague-Dawley rats (250–300 g) were exposed, nose-only, to 0.8 ppm O_3 for 3 hrs and the *in vivo* and *in vitro* parameters were measured 8–12 hrs following exposure. In *in vitro* studies, BALF macrophages harvested from O_3-exposed rats exhibited a significant increase in adherence to cultured lung epithelial cells (ARL-14) as compared to the adherence of macrophages isolated from air-exposed controls. Furthermore, this adherence was significantly reduced in antibody-treated cells as compared to cells treated with pre-immune rabbit serum. The *in vivo* studies revealed increased recovery of neutrophils in bronchoalveolar lavage fluid (BALF), elevated transport of ^{99m}Tc-diethylenetriaminepentaacetate across tracheal epithelium, and higher concentrations of total protein and albumin in BALF following O_3 exposure. However, these effects were not significantly altered by treatment with the anti-IL-1α/anti-TNF-α combination. From these studies we conclude that O_3, affects the early stages of the inflammatory response, particularly with respect to macrophage activation and adherence to epithelial cells, and that this early response may be mediated by IL-1 and/or TNF. Supported by NIEHS grant ES03521.

502 PULMONARY BIOCHEMICAL CHANGES AFTER EXPOSURE TO URBAN PATTERNS OF THE OXIDANT AIR POLLUTANTS: NITROGEN DIOXIDE, OZONE AND THEIR COMBINATION

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Exposure to individual oxidant air pollutants such as nitrogen dioxide (NO_2) and ozone (O_3) at environmental or occupational levels was shown to cause pulmonary injury in both animals and humans. However, in urban cities where pollution is highest, oxidants are present continuously at low levels, and peak only periodically either individually or in combination. In this study, we examined the pulmonary response to such an urban pattern of exposure. We exposed Swiss Webster mice (6 mice/group) continuously to 1.6 ppm NO_2 for 7 days, and superimposed intermittently (8 h/day) peaks of 4.8 ppm NO_2, 0.48 ppm O_3 or a combined NO_2 + O_3 peak. After 7 days of exposure, all mice were euthanized and their lungs analyzed for a number of established biochemical indices of pulmonary injury involved in detoxification and repair that included, enzymes of the pentose phosphate cycle, and protein and nonprotein bound sulfhydryl (glutathione) contents. We found that the continuous exposure to low-level NO_2 had no significant effect on the parameters measured. Similarly, exposure to single peaks of either NO_2 or O_3 superimposed on the continuous low-level NO_2 were also not significant. Only the exposure to low-level NO_2 combined with the NO_2 + O_3 peak had a significant effect. We conclude that the lack of effect when the animals were exposed to NO_2 or O_3 peaks superimposed on low-level NO_2, may be due to low toxicity or to an acquired resistance (adaptation) to injury induced by the continuous low-level NO_2 exposure. The significant response resulting from continuous exposure to low-level NO_2 combined with the superimposed intermittent NO_2 + O_3 peak reflects a synergistic effect of the combined gases.

503 EFFECTS OF TEMPERATURE ON THERMAL EMISSIONS CHARACTERIZATION AND SENSORY IRRITATION IN MICE USING ETHYLENE METHYL ACRYLATE (EMA) COPOLYMER

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Thermal emissions (TE) are produced when polymeric materials are heated during manufacture and use. EMA was tested in a modified DIN 53 436 apparatus to characterize the major chemical components of TE and to evaluate sensory irritation in mice. EMA was tested over a range of typical use conditions: 300, 400, 450, 490, and 525°F. Air flows of 8 L/min were used. Chamber concentrations at 300 to 450°F were relatively consistent for total hydrocarbons (THC) (11 ± 2 mg/m^3), nonvolatile aerosols (NVA) (4 ± 3 mg/m^3), acrylic acid (AA) (<0.2 mg/m^3), methyl acrylate (MA) (0.5 ± 1 mg/m^3) and formaldehyde (FA) (<0.6 mg/m^3). Respiratory rates (RR) at these temperatures were within normal limits. At 490°F, NVA, AA and FA emissions began to increase. At 525°F, THC and MA increased 2 to 3-fold (28 and 1.0 mg/m^3) while NVA and AA increased 10-fold (44 and 1.8 mg/m^3). FA levels increased to 3.8 mg/m^3. Corresponding RR decreases were 51 and 68% at 490 and 525°F. Maximal RR decreases were observed within 10 min of exposure initiation and were sustained for the duration of the 30 min exposure period. Groups at 490 and 525°F had recovered to 14 and 24% of pre-test within 10

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