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Project Summary

New Approaches to Quantitating the Pulmonary Effects of Inhaled Pollutants

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A variety of non-invasive and other techniques was developed to study effects of inhaled pollutants on the lung. In the area of airway mechanics, a diameter gauge was developed to make continuous measurements of large airways caliber. The gauge provides an electrical output. Rapid methods for non-invasively measuring respiratory mechanics using forced random noise excitation at the mouth were developed and validated. The resulting respiratory impedance data were applied to appropriate models to obtain values for parameters such as "central" and "peripheral" airways resistance.

In the area of respiratory epithelial function, a non-traumatic technique was developed to measure transepithelial potential difference across respiratory (nasal and airways) epithelium. Tracheal epithelial permeability was measured *in vivo*, demonstrating increased permeability and decreased perselectivity in guinea pigs exposed to 4 ppm, 1 ppm and 0.3 ppm O_3.

In the area of pulmonary vasculature, a rapid non-invasive multi-gas rebreathing technique was developed to measure lung water and was used to develop an O_3-induced pulmonary canine model of delayed pulmonary edema using 1 ppm O_3

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction and Summary

The study focused on developing new approaches—conceptual and methodologic—to the study of pulmonary responses to pollutants, with an emphasis on techniques with possible human applications.

A non-invasive method of estimating the mechanical impedance of the respiratory system to air flow using excitation with a forced random noise signal applied via a mouthpiece (humans) or endotracheal tube (animals) was developed, validated, and integrated into an on-line system using a minicomputer. Useful data are obtainable between 2 - 35 Hz in animals and 5 - 35 Hz in humans. The impedance vs. frequency data were fitted to a series resistance-compliance-inertance model when the real component of the impedance (effective resistance) was not frequency dependent, and fitted to a five-parameter model including two resistances ("central" and "peripheral"), two compliances ("shunt" and "peripheral"), and an inertance when the effective resistance showed negative frequency dependence. Other, simpler, approaches were also developed for fractionating frequency-dependent ef-

fective resistance into central and peripheral components. All approaches use algorithms which require less than two minutes real time. The methods and analyses were successfully applied to various studies in animals, in young children, asymptomatic cigarette smokers and in patients with COPD, in subjects with asthma before and after brochodilator Rx, and in normals and mild asthmatics with methacholine challenge (studies carried out at EPA labs). In general, the techniques have been shown to be sensitive to presence of early disease (e.g., asymptomatic cigarette smokers) and to yield reproducible impedance patterns. The analysis of frequency dependence of effective resistance has produced novel approaches to studying the axial localization of airway changes, and a good correlation between the derived parameter, effective resistance extrapolated to a frequency of 1 Hz, and plethysmographically measured R_{aw} has been demonstrated.

A novel method for directly and continuously measuring the internal diameter of large airways was developed and evaluated. This device is positioned within the lumen and converts internal diameter into an electrical signal. It has a flat frequency response from DC to 8 Hz and exhibits excellent stability and signal to noise ratio. The device was used to examine quantitatively the smooth muscle response to regional arterial perfusion of the canine trachea with acetylcholine, and is currently being used to investigate the effects of zone inhalation on tracheal smooth muscle responsiveness.

Many of these studies focused on techniques designed to study the physiological function of respiratory epithelium *in vivo* and acute effects of ozone inhalation on large airways epithelium.

A technique was developed to measure the *in vivo* transepithelial electrical potential difference (PD). A fluid-filled perfused catheter lightly touching the epithelium serves as a bridge to an agar bridge to a calomel half cell. The reference electrode is a #19, agar-filled needle inserted into the subcutaneous tissue. With proper filtering of the signal, stable signals can be obtained. The PD values are ~30 mV (lumen negative) in canine and human trachea; they decrease as more distal bronchi reaching a value of 5 - 10 mV in segmental bronchi in both species, are explored. Guinea pig trachea has a PD of about 13 mV while rabbit trachea has about 20 mV. More recently, human nasal epithelial PD has been studied and this technique will soon be applied in a collaborative study of pollutant inhalation with the EPA/HERL Clinical Studies Group. Exposure of conscious guinea pigs or of anesthetized intubated dogs to 1 ppm O_3 and 4 ppm O_3 for 2 - 3 hours failed to cause any acute change in tracheal PD values. Since PD represents the ratios between ion-transport generated current (I) and the passive electrical conductance (G) of the tissue, the lack of PD alteration following O_3 exposure does not prove that no change in bioelectric properties has occurred.

In vivo permeability of respiratory epithelium by measuring early appearance rates in arterial blood of molecular probes simultaneously instilled onto the surface of guinea pig trachea via a fine catheter was examined. The probes used were ^{14}C-mannitol (MW-180 daltons), ^{3}H-dextran (MW-10,000 daltons) and horseradish peroxidase (HRP) (MW-40,000 daltons). Data indicate substantial increase in airways epithelial permeability and loss of permselectivity immediately following conclusion of exposure to either 4 ppm or 1 ppmO_3 for three hours. The effect persists at least twenty-four hours but is absent at seven days after exposure.

The locus within the epithelium of this increased permeability is unclear. After 4 ppm O_3, there are marked changes in the ultrastructure of the intercellular "tight" junctions shown by freeze-cleave preparations of the tracheal epithelium. Cellular extrusion was also noted. These changes were not seen, however, after the 1 ppm O_3 exposure.

It would be valuable to study in a more controlled fashion the relation of epithelial bioelectric properties and specific transepithelial ion fluxes to the permeability alterations induced by O_3. A tubular (cylinder of trachea) version of the Ussing preparation is being developed for this purpose.

A correlation of *in vivo* permeability alterations to altered responsiveness of the airways to bronchoconstrictor drug challenge after O_3 exposure is also planned, to explore the hypothesis that O_3-induced increased epithelial permeability is responsible for greater access of inhaled aerosolized drugs to effector sites in the airway wall.

This project included a study of O_3-induced pulmonary edema in dogs using a multi-gas (C_2H_2, He and $C^{18}O$) rebreathing technique to repetitively measure lung water following the O_3 exposure. It was demonstrated in anesthetized, intubated dogs that after inhalation of only 1.0 ppm O_3 for three hours, a significant increase in lung water occurred at twenty-four hours but was not present either at forty-eight hours or immediately after the exposure. The induction of delayed edema in this model offers the opportunity to study "early" events which may play a pathogenetic role in the eventual development of edema. Although statistically significant reductions in cardiac output were found acutely after exposure to 0.3 ppm O_3, we could not demonstrate edema. The rebreathing measurements of lung water were confirmed by wet/dry lung weights whenever possible. This system will be further explored by adding an increase in pulmonary microvascular pressure at twenty-four hours after O_3 exposure in order to test the hypothesis that the alveolar-capillary membranes are injured even at low levels of O_3 exposure but that the injury remains sub-clinical and can be detected by increasing filtration pressure. Lung lymph flow and protein composition will also be examined in these studies.

The rebreathing technique was successfully applied to a study of effects of 0.75 ppm SO_2 inhalation on human subjects. No changes in lung water, D_LCO or FRC were observed in this EPA/HERL study conducted by the Clinical Studies Branch.

Finally, a tendency was observed for canine pulmonary vascular resistance to be increased by O_3 exposure. It was hypothesized that (similar to the effect of indomethacin, a prostaglandin synthetase inhibitor) O_3 might be responsible for inhibition of prostacyclin synthesis by blood vessels and thus enhance the normal pulmonary arteriolar constriction to alveolar hypoxia. This was studied in dogs and it was possible to demonstrate significant enhancement of hypoxic pulmonary vascular constriction by pretreatment with indomethacin. Similar changes were seen after O_3 exposure but did not reach the $p < .05$ level of statistical significance. Nevertheless, studies of O_3 interaction with arachidonate metabolism may be of interest.

Conclusions

Techniques for non-invasively studying and analyzing respiratory mechanics have been worked out and applied

successfully to a variety of experiments using human subjects. Some of these experiments were carried out in EPA's HERL Human Studies facility.

A diameter gauge was developed that is suitable for direct, continuous measurement of caliber of large airways to aid in studies of pollutant effects on airways mechanics and hyperresponsiveness to bronchoconstrictor agents.

An atraumatic technique for measuring the bioelectric properties of respiratory epithelium *in vivo* was developed and used to study O_3 inhalation effects on the trachea of animal models. The technique was extended to nasal epithelium which is readily accessible in human subjects.

Striking increases in permeability and loss of perm-selectivity of guinea pig tracheal epithelium were demonstrated following exposure to either 1 ppm or 4 ppm O_3. (A similar, but lesser, effect has now been demonstrated for 0.3 ppm O_3 exposure.) Similar techniques are being considered for development for human use.

A canine O_3- induced pulmonary edema model was characterized with as little as 1.0 ppm O_3 exposure. This model will be used to study the interaction between O_3 exposure and elevated pulmonary microvascular pressure which would be relevant to O_3 effects in patients with heart failure or other causes of elevated pulmonary capillary pressure.

During the course of this work a non-invasive, multi-gas rebreathing technique to repetitively measure D_LCO, FRC and lung water was developed and extensively validated. This technique was successfully applied to an EPA/HERL study of acute effects of inhalation of 0.75 ppm SO_2 in man.

There is evidence suggesting that prior O_3 exposure may enhance the pulmonary arteriolar vasoconstrictive response to alveolar hypoxia in dogs. The potential interaction between O_3 and arachidonate metabolism warrants exploration.

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The complete report, entitled "New Approaches to Quantitating the Pulmonary Effects of Inhaled Pollutants," (Order No PB 81-222 382; Cost: $6.50, subject to change) will be available only from:

National Technical Information Service
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