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# Project Summary

# Final Report on CR 807392: U.S. EPA Cooperative Agreement with the University of North Carolina at Chapel Hill Center for Environmental Medicine

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**This Cooperative Agreement between the HERL (US EPA) and the Center for Environmental Medicine (CEM) of the University of North Carolina (UNC) School of Medicine was intended to:**

**1. Enhance interactions between EPA and UNC scientists.**

**2. Support visiting scientists onsite for longer periods of time, develop joint research conferences, and facilitate adjunct appointments to the University faculty for EPA scientists.**

**3. Perform collaborative research in areas relevant to EPA's mission, especially in the air program; but also in other areas (e.g., neurotoxicology, indoor air).**

**4. Undertake research that could not be readily accomplished by EPA with its existing personnel — both in the area of development and application of techniques, and in the performance of research.**

**5. Provide access to diseased as well as normal human subjects, including children, in order to study "sensitive" as well as "normal" populations challenged with inhaled pollutants in highly controlled environmental exposure chambers.**

**This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for presentation and publication.**

***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).***

## Discussion

This Cooperative Agreement between the Environmental Protection Agency's (EPA) Health Effects Research Laboratory (HERL) and the Center for Environmental Medicine (CEM) of the University of North Carolina (UNC) School of Medicine was intended to accomplish five principal results:

1. Provide EPA scientists with a ready means of access to University faculty and facilities and to enhance the intensity and frequency of interactions between EPA and UNC scientists.

2. Enrich the scientific milieu for EPA scientists by helping to recruit and support post-doctoral research fellows, inviting a series of visiting scholars for seminars and discussion of ongoing work, supporting visiting scientists onsite for longer periods of time, developing joint research conferences, inviting EPA scientists to participate in UNC teaching and research programs, and facilitating adjunct appointments to the University faculty for EPA scientists.

3. Perform collaborative research in areas relevant to EPA's mission, especially in the air program; but also in other areas (e.g., neurotoxicology, indoor air).

4. Undertake research that could not be readily accomplished by EPA with its existing personnel — both in the area of development and application of techniques, and in the performance of research.

5. Provide access to diseased as well as normal human subjects, including children, in order to study "sensitive" as well as "normal" populations challenged with inhaled pollutants in highly controlled environmental exposure chambers.

In conducting this project, UNC recognized the importance of EPA-HERL's research program to its own mission, and benefitted from the access to University faculty of the HERL scientific staff, to EPA research facilities, and to EPA funding support.

At the inception of the Cooperative Agreement in 1980, major attention was focussed on the "criteria" pollutant gases — $O_3$, $NO_2$, $SO_2$ and CO, and to particulate-gas interactions. Furthermore, the research was conducted almost exclusively with human subjects because of the immediate applicability of such research to the development and justification of national air quality standards.

The Cooperative Agreement with the UNC CEM has provided important support in the following areas of published collaborative work with the HERL's Clinical Research Branch (CRB) which have had a major impact on the understanding of the quantitative aspects of health effects of exposure to the criteria air pollutants:

## *Ozone ($O_3$)*

It is now clearly established that adult normal subjects develop symptoms and impaired pulmonary function from exposure to as little as 0.12 ppm $O_3$, provided that the subjects perform exercise during the exposure so as to increase their ventilation. Normal children, 8-11 years old, are similarly susceptible to ozone. These effects are caused by an involuntary inhibition of deep inspiration, probably neurally mediated. There is enormous intersubject variability in the intensity of the response to $O_3$. This variability is *not* attributable to a lack of reproducibility of the $O_3$ effect in a given subject. Nor does it appear to be attributable to age, sex, race or atopic status. The reasons for extreme variability of individual susceptibility among normal individuals remain to be elucidated and this problem constitutes an element of the present research program.

Patients with chronic, irreversible, obstructive lung disease are not highly sensitive to respiratory effects of ozone. Patients with mild asthma are probably not especially sensitive to ozone inhalation.

Concurrent exposure to ozone and low concentrations (100 $\mu g/m^3$) of acid sulfate aerosols does not markedly enhance the ozone effect in normal subjects, although there is a trend in that direction.

We have shown that $O_3$ inhalation causes inflammation in human airways. Ozone exposure also acutely increases respiratory epithelial permeability in human subjects. These studies are particularly important for two reasons:

1. They provide a basis for correlating animal and human toxicology.

2. They provide a basis for making judgments as to the relevance to health of the acute reversible changes in lung function provoked by controlled ozone exposure in environmental chamber studies.

Investigations in other biological systems of the mechanisms of ozone effects are critical to regulatory decision-making for similar reasons. Thus, the project has shown that endothelial cells (in culture) lose their ability to produce prostacyclin from arachidonate-containing membrane lipids after a brief exposure to ozone. This effect appears to be due to specific inhibition of cyclooxygenase activity by a peroxide-dependent mechanism. Endothelial cells do not have vigorous antioxidant mechanisms and are particularly vulnerable to oxidant injury.

The physiological relevance of these *in vitro* findings is supported by the observation that following ozone exposure, the canine pulmonary vasculature *in vivo* exhibits enhanced susceptibility to vasoconstrictive stimuli. Similar effects are observed when prostacycline synthesis is specifically inhibited by pretreatment with the cyclooxygenase blocker, indomethacin.

The pulmonary alveolar macrophage is much more resistant to oxidant injury and displays a different repertoire of arachidonate metabolism than endothelial cells. Nevertheless, there appear to be major new lipid oxidation products as well as changes in arachidonate liberation and metabolism patterns when cultured pulmonary alveolar macrophages are exposed to ozone *in vitro*. Among other reasons, the author hopes to use this information to estimate the levels of ozone in the alveolar regions of the lung parenchyma during exposure to this gas.

Because epithelial cells are directly exposed to reactive inhaled gases, a special effort was made to develop effective methods to culture and study respiratory epithelial cells, including human airway cells.

A large amount of evidence indicates that ozone damages or modifies respiratory epithelial function. Thus, epithelial permeability to polar solutes is increased, active ion transport is increased, mucin secretion from surface epithelial cells occurs, inflammatory cells infiltrate the epithelium and appear on the airway surface, ciliated cells are damaged and alveolar type 1 lining cells are damaged.

The project studied twenty-four young adult male volunteers experimentally inoculated with type 39 rhinovirus to determine whether the course of viral infection was modified by exposure to moderate levels of ozone (0.3 ppm for 6 hours a day) over the five days following virus inoculation. No differences in rhinovirus titers in nasal secretions, recruitment of neutrophils into nasal secretions, levels of interferon in nasal lavage fluid, *in vitro* lymphocyte proliferative responses to rhinovirus antigen, or levels of convalescent serum neutralizing antibody to type 39 rhinovirus were demonstrated in relation to ozone exposure. The level and pattern of ozone exposure used in this experiment had no demonstrable adverse effects on the immune responses necessary to limit and terminate rhinovirus infection of the upper respiratory tract. Thus, this unique study was not able to demonstrate any effect of significant $O_3$ exposure on the development or course of a common rhinovirus upper respiratory infection. This finding does not however preclude the possibility that infections caused by other agents might be affected by $O_3$ exposure, or that a different sequence of viral infection and ozone exposure might produce a different outcome in the series of measurements made.

## *Nitrogen Dioxide ($NO_2$)*

In a carefully designed effort the current study was unable to confirm the findings of a 1976 study by Orehek et al., that claimed enhanced bronchial reactivity in asthmatic subjects following exposure to 0.1 ppm $NO_2$. Newly designed methodology was employed in this effort, both to assess the airways response and to provide quantitative exposures to inhaled aerosolized bronchoconstrictors. These techniques have been incorporated into the CRB's standard lung function testing procedures.

More recently, the CRB and others have been re-examining the question of $NO_2$ effects in asthmatics using higher $NO_2$ levels (up to 0.6 ppm) and in-

corporating exercise into the exposure protocols. Some evidence of effects of exposure to 0.3 ppm $NO_2$ has been obtained, but the reproducibility of these observations is not established. Nevertheless, these findings suggest that the potential toxicity of $NO_2$ for susceptible human subjects requires considerably more attention.

## *Carbon Monoxide (CO)*

This project's contributions have been in three major areas:

a. Evidence to support the use of the Coburn equation to infer the relation between inspired CO concentrations and the level of blood COHb. This evidence will be critical to the ultimate "translation" of health effects attributable to COHb into an ambient air quality standard.

b. A system for transient exposures of subjects to very high CO levels. This system is being used to study possible neurotoxic effects and to look for significant deviations of COHb kinetics from the Coburn equation under these extreme conditions. This work is relevant to EPA which has entered into an agreement with the Department of the Army to pursue these problems in inhalation toxicology.

c. Detailed studies of the effects of approximately 4% COHb and 6% COHb on myocardial function in patients with criteria for the presence of ischemic heart disease. The study utilized angina, ECG changes, and ventricular function as determined by gamma camera imaging as the dependent variables. The studies were carried out in double-blind fashion in the CRB laboratories by a UNC-CRB team. No changes were demonstrable at a 3.8% COHb level. Preliminary analysis suggests, however, that evidence of enhanced ischemia during exercise will be shown at the ~ 6% COHb level.

The possibility of COHb-induced arrhythmias is also being studied in patients with heart disease. The study of defined subpopulations of patients with ischemic heart disease and of patients with other forms of heart disease is projected.

## *Sulfur Dioxide ($SO_2$)*

This study has contributed to the recently-acquired, but now solid body of evidence showing that mild asthmatics are extraordinarily susceptible to bronchospasm during exercise in atmospheres containing as little as 0.25 ppm $SO_2$. The mechanisms underlying this interaction between $SO_2$ and exercise are not yet clear. The mechanisms of $SO_2$-induced bronchoconstriction appear to involve cholinergic mechanisms but also may involve chemical mediators, mast cells, and possibly neural mechanisms.

Interactions between $O_3$ and $SO_2$ in asthmatic subjects remain to be explored.

The study has also shown that $SO_2$ exposure causes ciliary damage in nasal epithelial biopsies from human subjects. Surprisingly, exposure to $O_3$ did not appear to cause similar defects.

In summary, the Cooperative Agreement has supported a body of collaborative research that has had important impact on the knowledge and understanding of the effects of controlled exposure to criteria air pollutants on normal humans and on a variety of well-characterized subpopulations. The resulting information is generally recognized as a significant contribution to regulatory authority within the Agency. Among the scientific community, there is no doubt that the scientific reputation of the Clinical Research Branch has been enormously enhanced, by recent accomplishments which include:

1) Development of nasal and bronchoalveolar lavage procedures to obtain material suitable for studies of lung cell biology in relation to inhalational toxicology. These procedures are now being used on an almost daily basis for a variety of protocols.

2) Development of cultured cell systems and methods of exposing such surfaces to gaseous substances. Study of respiratory epithelial cells, pulmonary vascular endothelial cells and pulmonary alveolar macrophages. Development of evidence of dose-related ozone effects on arachidonate metabolism of endothelial cells and alveolar macrophages.

3) Application of new technologies to examine respiratory epithelial function and its cellular basis in relation to inhalational toxicology as well as infections. Establishment of a morphology laboratory that performs sophisticated studies of respiratory structures in tissues obtained from human as well as animal sources.

4) Development of an animal model (the outbred Bio/Y mouse) to study the effects of air pollutants on lung collagen and elastin. Development of sensitive immunoassays for fragments of elastin and collagen for application to studies of acute lung injury.

5) Made available essential support in the form of space, equipment and professional personnel for development of EPA programs in lung cell biology and in particle deposition, detection and clearance.

6) Taken major steps in developing an indoor air pollution study program, with particular emphasis on health effects in children.

Finally, in terms of recruitment and retention of productive scientists: The Cooperative Agreement has been directed since its inception by Dr. Philip Bromberg, Professor of Medicine and Chief of the Division of Pulmonary Medicine, and by Dr. Albert Collier, Professor of Pediatrics and Chief of the Division of Pediatric Infectious Diseases. These individuals have not only devoted major fractions of their time and effort to the Cooperative Agreement, they have stimulated members of their own divisions to develop research relevant to the needs of the CRB and to collaborate more widely within the HERL. They have been widely recognized in the scientific community as leaders in the area of air pollution.

Thus, we believe that in all the following aspects:

1. development of fruitful interactions between UNC and EPA staff,
2. scientific productivity,
3. enhancement of the scientific reputation of the CRB,
4. broadening of its scientific base,
5. recruitment of scientists,
6. training, and
7. scientific enrichment,

the Cooperative Agreement has amply fulfilled its objectives. The UNC Center for Environmental Medicine looks forward to a continuing close association with multiple laboratories within HERL, and particularly with the CRB. We believe that this association will continue to be of the greatest value to both partners, and that it will constitute a model for successful interaction between a public university and a federal agency in the area of biological and clinical research.