United States
Environmental Protection
Agency

Health Effects Research
Laborarory
Research Triangle Park NC 27711

Research and Development

EPA-600/S1-81-021 May 1981

EPA

Project Summary

Tracheal Organ Culture as Air Pollution Damage Indicator

Leonard J. Schiff

This report presents the results of a study conducted to determine the effects of various energy-related effluents on respiratory tract epithelial tissue. Measurement of mucociliary activity and characterization of the morphological alterations induced by such effluents was carried out in hamster tracheal organ culture. Different combinations of *in vivo* and *in vitro* exposure and/or maintenance were used to determine the relationship between *in vivo* exposure danger and adverse effects observed in organ tissue.

The pollutants assayed included particulate effluents from mobile and stationary sources of both conventional and advanced energy processes. Included were fly ash (from coal-fired and oil-fired sources), cigarette smoke condensate, and diesel fuel exhaust extract, with benzo(a)pyrene serving as a positive control.

Both acute (72-hr) and long-term (14-day) studies permitted assessment of tissue specific effects. In addition to acute and long-term toxicity studies, testing was conducted to determine the effects of selected particulate effluents on the pathogenesis of viral infections.

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

The Health Effects Research Laboratory of the U.S. Environmental Protection Agency is presently involved in research to evaluate the health effects of human exposure to airborne environmental pollutants. Past research has established a link between exposure to air pollution and an increased frequency and severity of acute respiratory disease. Under certain exposure conditions, environmental pollutants may impair the function of important defense mechanisms of the respiratory tract epithelial tissue. Pathologic consequences could include shifts in normal cell populations, structural abnormalities of cells and cell organelles, and changes in normal cell repair processes. These adverse effects could render the tracheobronchial epithelium more susceptible to respiratory infections, as well as more vulnerable to non-infectious pollutant agents.

Many of the most dangerous particulate emissions commonly found in the ambient air are produced by conventional and advanced energy processes, such as the combustion of coal and oil. Thus, examination of the health effects resulting from fugitive emissions from these processes is becoming increasingly important.

Summary

In support of EPA's research program in this area, IIT Research Institute conducted a study to obtain data on hazardous energy-related pollutants suspected to interfere with normal respiratory function. Although examination of the

health effects of potential environmental pollutants has traditionally been based on chronic rodent bioassays, these bioassays are complex and require control of many variables over several years' time. Thus, IIT proposed to utilize *in vitro* methods in hamster tracheal organ culture to provide a more rapid, sensitive, and relevant test system for screening a variety of hazardous pollutants. In order to test the suitability of the *in vitro* method as a rapid screening model, both *in vivo* and *in vitro* studies of pollutant exposure effects on tracheal mucociliary transport parameters and cytopathology were conducted and the results compared.

The primary pollutant used in the study was coal-fired fly ash, obtained from a coal-fueled power plant using Eastern Kentucky coal. Additional particulate pollutants tested in tracheal organ culture were oil-fired fly ash, diesel fuel exhaust extract, and cigarette smoke condensate. These air pollutants contain a variety of chemical carcinogens, the most commonly recognized of which is the well known carcinogen benzo(a)pyrene. In order to establish the relative potency of the various test substances, the effect of benzo(a)pyrene on tracheal organ culture was compared to the effects observed for the other pollutants. Thus, benzo(a)pyrene served as a positive control for the study.

Cytotoxicity resulting from exposure to test compounds was expressed as alterations in ciliary activity and cell morphology. Both the short-term and long-term toxic effects of the pollutants on these factors were investigated. The primary indicator of pollution damage used was cilia beating frequency. Morphological changes were detected using light microscopy and scanning electron microscopy.

Suckling and adult golden Syrian hamsters were employed in the *in vivo* studies. In addition, whole tracheal explants from donor Fischer 344 rats and Alexander B10 F_1D hamsters were inserted with fly ash pellets and transplanted through subcutaneous grafting to recipient rats and hamsters. For *in vitro* studies, tracheal ring explants and whole tracheal explants were prepared and maintained in culture. Tracheal organ culture techniques were developed to maintain differentiated respiratory tract epithelial tissue.

Cilia beating frequency was measured for each tracheal explant at four separate areas on the lumen side, and the average recorded as beats per minute. The cilia beating frequency of whole tracheas was observed by focusing on the mucosa through the explant. After an initial incubation period, the baseline cilia beating frequency for each explant was determined.

In vivo inhalation exposure to respirable-size coal-fired fly ash was accomplished using suckling and adult hamsters. The hamsters were exposed to fly ash in a 432-liter capacity plastic chamber at a mass concentration of 2 mg/m^3 for 3 h/day, 5 days/week for 2 weeks. The chamber was maintained at 25-29°C and 30-55% RH. Mass concentration of the particle aerosol in the exposure chamber was monitored using an aerosol, smoke, and dust photometer. In addition, periodic determination of the aerosol concentration was also made using a condensation nuclei counter. The desired mass concentration for the trials was 2000 μg/m^3 of the fly ash particles; the actual concentration was 1987 μg/m^3.

In vivo exposure of hamsters to coal-fired fly ash resulted in a reduction of cilia beating frequency and cytological alterations in the tracheal mucociliary epithelium compared to ambient air controls. The tracheal epithelium of animals experiencing from five to ten 3-h exposures showed large areas of based cell hyperplasia and stratification. The recovery time from damage to the ciliated epithelium was directly proportional to the number of 3-h exposures.

Whole tracheas from adult B10 F_1D Alexander hamsters were grafted subcutaneously (heterotopic tracheal transplants). Epithelial changes after 2 months in untreated tracheas and tracheas with beeswax (cholesterol [1.9] pellets only) were essentially normal, resembling that of the host's own trachea. Pellets containing coal-fired fly ash induced mild focal hyperplasia and occasional areas of squamous metaplasia. In addition, presarcomatous lesions of connective and cartilagenous tissue was observed.

For *in vitro* inhalation exposure studies, fly ash (coal-fired and oil-fired) was suspended in medium at 5, 10, 50, 100, and 500 μg/ml. Tracheal ring explants were exposed to the fly ash for 1 or 3 h/day, 5 days/week for 2 weeks. Culture dishes were placed in a controlled atmosphere chamber and maintained in 5% CO_2 and balanced air. The chamber was then placed on a platform which rocked at 10 cycles/min, causing the medium containing the fly ash agents to flow continuously over the epithelial surface.

At various intervals, alterations in ciliary activity and cytology were documented by light microscopy and scanning electron microscopy. Tracheal cultures exposed for 1 or 3 h/day showed a decrease in ciliary activity that paralleled cytopathological changes following seven exposures to 50 μg oil-fired fly ash/ml or higher. Morphological alterations consisted of diffuse basal cell hyperplasia and stratified metaplastic epithelium. Whole suckling hamster tracheas in organ culture exposed for 1 or 3 h/day to 10 and 50 μg coal-fired fly ash/ml produced cornifying epidermoid metaplasia after seven exposures. The most characteristic finding of surface cells was a number of broad metaplastic areas with keratin for formation.

Oil-fired fly ash produced a more pronounced effect than coal-fired fly ash. Complete cessation of ciliary activity occurred after seven exposures to 50 μg oil-fired fly ash/ml. The epithelium of cultures exposed to 10 μg/ml or greater of oil-fired fly ash showed pathological alterations proportional to the concentration of fly ash and the period of time exposed. Histological sections of treated explants showed distinct cytopathologic changes which preceded ciliostasis.

In vitro exposures of hamster tracheal epithelium to cigarette smoke condensate (CSC) and diesel fuel exhaust extract (DFEE) were performed to elucidate functional and morphological changes. Early expression of the cytotoxicity of both materials was indicated by altered ciliary activity and cytopathology. Controls exposed to DMSO (DFEE solvent) and acetone (CSC solvent) showed changes in the mucosa at a concentration of 0.25% and 0.2% nine days after initial exposure. At 50 and 100 μg cigarette smoke condensate or diesel fuel exhaust extract, histopathological alterations included stratified metaplastic epithelium (33%) and epidermoid metaplasia (20-25%). At these concentrations, ciliary activity and cytopathology were not altered. Ring explants exposed to 1 and 3 μg benzo(a)pyrene/ml, the prototype carcinogenic polycyclic aromatic hydrocarbon and a positive control for the organ culture bioassay, showed epithelial changes progressing to stratified metaplasia. These changes were reflected as an alteration in cytopathology and a decrease in beating frequency.

In separate experiments, *in vivo* and *in vitro* tests were used to determine the interactions of fly ash and influenza virus (e.g., what effect the virus had on the expression of fly ash damage).

In vivo experiments to study the interaction of fly ash and influenza A/PR-8 virus were conducted using adult male golden Syrian hamsters. In the first experiment, influenza virus replication was measured in tracheal epithelium after aerosol infection. Virus aerosol infection of hamsters was immediately followed by exposure to fly ash in the second experiment. The third experiment was conducted similarly to the second, except that fly ash exposure was delayed until one day after virus infection. In the last experiment, hamsters were exposed to fly ash prior to infection by the virus.

Results from the four *in vivo* experiments indicated that hamsters exposed to a pollutant during an on-going infection (i.e., infected by aerosolized influenza virus and 24 h later exposed to 1 mg fly ash/m^3 for 3 h/day, 5 days/week for 2 weeks) produced greater cytotoxic effects than hamsters exposed to fly ash for 2 weeks prior to virus infection. Toxicity was mainly expressed as cytopathological and histopathological alterations along the luminal surface.

Tracheal cultures from hamsters infected with influenza virus aerosol and then exposed *in vitro* to 10 or 50 ug coal-fired fly ash/ml showed morphological alterations that were similar to those observed in the *in vivo* experiments. In comparison to *in vitro* exposure to fly ash alone, histological changes in the virus-fly ash treatment group were less severe. Initial changes were characterized by focal loss of cilia and sloughing of superficial epithelium. Moderate epithelial vacuolation was observed. Only occasional areas of stratified metaplastic epithelium appeared, indicating the virus retards metaplastic changes. These changes were the same whether exposed immediately after infection or 24 h post-infection. Maximum infectious virus titers in fly ash treatment groups were reached earlier than in the virus infected control explants.

Conclusions

The results of this study indicate that cilia beating frequency and cytopathology alone are not adequate as pollution damage indicators. To detect the full extent of cytological damage, these indicators should be used in conjunction with light microscopy and scanning electron microscopy examinations.

Toxicity testing results showed that hamster tracheal organ cultures can be used to study acute effects of fly ash on respiratory ciliated epithelium, and that the toxic effects produced by the two fly ash species are the result of different mechanisms of inducing injury. The toxicity of oil-fired fly ash was found directly proportional to the concentration and duration of exposure. Morphological observations indicated that cytotoxic concentrations of oil-fired fly ash were below the level that produced ciliostasis. In addition, the transformation of tracheobronchial epithelium to epidermoid metaplasia by coal-fired fly ash was shown to be similar in response to the effects of carcinogens, vitamin A deficiency, and nutritional influences *in vitro*. Results also indicated a correlation between fly ash-induced changes in tracheobronchial epithelium of hamsters exposed *in vivo* (2 mg/m^3) and effects observed in hamster tracheal epithelium exposed in organ culture (5-10 μg/ml).

The organ culture assay permitted toxicity ranking of the pollutants tested. In order of decreasing toxicity, they were: oil-fired fly ash, cigarette smoke condensate, coal-fired fly ash, and diesel fuel exhaust extract.

Studies of the effect of influenza A/PR-8 virus on the expression of fly ash damage demonstrated that tracheal explants infected 24 h prior to coal-fired fly ash exposure produce a significantly greater response than explants exposed immediately after infection. These findings may have bearing on the hypothesis that influenza may stimulate the transformation of tracheobronchial epithelium to epidermoid metaplasia by (1) altering the permeability of target cells, so that the penetration of the fly ash is facilitated, (2) increasing the mitotic activity of the cells providing a favorable condition for an attack by the aromatic hydrocarbons and trace elements that constitute the soluble fraction of the fly ash, or (3) interfering with the detoxification mechanisms of the target cells, leading to an increased persistance of the aromatic hydrocarbons.

Environmental exposure to airborne particulates has been linked to chronic respiratory disease, such as chronic bronchitis. Upon inhalation, pollutants are deposited to a large extent in the trachea where they are removed by mucociliary mechanisms, or phagocytized. The effects of the various pollutants on cells of the tracheobronchial epithelium were in many instances complex. Each of the real-world pollutants assayed produced a unique response, probably related to chemical composition and size of the particles. It is recognized that smaller particles are phagocytized by mucosal cells while the larger particles provoke hyperplastic and metaplastic changes. One might speculate that exposure to the particulate pollutants affects the structure and functional continuity of the epithelium, thereby contributing to chronic bronchitis.

Recommendations

Organ cultures of hamster trachea consisting in part of mucociliary epithelium have permitted study of the effects of toxic agents on this tissue. The ring and whole tracheal organ culture preparations were found to be well suited to evaluate a number of parameters on viable explants, as well as fixed tissue. These cultures were maintained for weeks and monitored while being exposed for a specific period under completely defined conditions. The ability to prepare a large number of tracheal cultures permitted multiple replicate exposures at each experimental point and a wide range of concentrations. Our results demonstrated that changes in any single parameter were insufficient indications of the toxic effects of exposure to a test substance, and we therefore recommend that cilia beating frequency data be correlated with morphological and biochemical parameters.

The increasing number of pollutants being emitted into the environment and the backlog of potentially hazardous chemicals used in industry make a rapid, reproducible, and relatively inexpensive test system for evaluating toxicity a pressing need. Hamster tracheal organ cultures provide the necessary criteria to facilitate identification of these toxic agents. Additional investigations are therefore needed to determine the response of mammalian respiratory tract tissue to a variety of complex mixtures.

The studies should be expanded using organ cultures of tracheal epithelium and heterotopic tracheal grafts as a bioassay for respiratory tract carcinogenicity to evaluate environmental pollutants as potential carcinogens. The criteria for assessment would include histologic and cytologic changes, DNA synthesis, and tumor production. In this

☆ U.S. GOVERNMENT PRINTING OFFICE 1981757-012/7095

way, the questions concerning the interrelationships between differentiation, cell population kinetics, and various stages in carcinogenesis could possibly be clarified.

Leonard J Schiff is with Life Sciences Research Division, IIT Research Institute, Chicago, IL 60616.
***Judith A. Graham** is the EPA Project Officer (see below).*
The complete report, entitled "Trachael Organ Culture as Air Pollution Damage Indicator," (Order No. PB 81-168 999; Cost: $9.50, subject to change) will be available only from.
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone. 703-487-4650
The EPA Project Officer can be contacted at·
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

Postage and
Fees Paid
Environmental
Protection
Agency
EPA 335

Official Business
Penalty for Private Use $300

United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Research Triangle Park NC 27711

Research and Development

EPA-600/S1-81-022 May 1981

EPA

Project Summary

Effects of Etiologically Defined Respiratory Infections on Lung Function and Its Growth in an Area of Low Air Pollution - Spirometry in Young Children When Illness-Free

Albert M. Collier, Gerald L. Strope, Ronald W. Helms, Lisa Morrisey Lavange, Wallace A. Clyde, Jr., Russel Pimmel, Floyd W. Denny, Frederick W. Henderson, and Gerald W. Fernald

This longitudinal study was performed in a group of 3-12 year old children to document normal lung growth patterns as measured by spirometry. By clinical and laboratory parameters, these children were free of illness at the time of study and had been for the preceding 21 days. Spirometry was performed prospectively over a period of six years in 69 children (27 black females, 23 black males, 10 white females, and 9 white males) from a day care center. Eight-hundred fifteen spirometric tests were made on these children. Linear regression analyis by a method of weighted least squares was found to adequately describe the data over a height range of 100-150 cm and was performed on six spirometric parameters: forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), peak expiratory flow (PEF), forced expiratory flow during the middle half of the FVC ($FEF_{25-75}\%$), and maximum expiratory flows after 50% and 75% of the FVC have been exhaled ($\dot{V}max_{50}\%$ and $\dot{V}max_{75}\%$, respectively). Ninety-five percent confidence limits for the regression lines and 95% prediction intervals for individual observations were also computed. There were significant differences between the regression lines (considering slope and intercept) for all six parameters when black females were compared to black males, white females to white males, black females to white females and black males to white males, except for $\dot{V}max_{75}\%$ for the comparison of black females to black males and white females to white males. These slopes and intercepts were similar to those reported by others for children of similar age and height. The 95% confidence limits and 95% prediction intervals were proportional to height and were similar to estimates of parameter variability reported by others. This study demonstrates that spirometry can be performed reliably at an early age in a day care center population, that there are significant racial and sexual differences in spirometric volume and flow parameters and that the variability of these measurements is

proportional to height rather than being a constant.

This final report was submitted in fulfillment of Grant #R-804577 by the University of North Carolina under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from August 1, 1976 to April 30, 1980 and was completed as of April 30, 1980.

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

The growth of the lung in children is a dynamic process characterized by an increase in alveolar number followed by an increase in size of airways and air spaces in the lung. Other researchers have shown that the total number of airways is the same in children and adults. The total complement of bronchi al generations is present by the end of the sixteenth week of gestation and the spaces which will contain air begin to change to an alveolar pattern during the latter part of gestation During the first weeks of life, typical alveoli appear and lung growth for the first four years of life consists predominantly of an increase in alveolar number At this point, increase in lung size changes to a pattern of growth consisting of an increase in size of airways and alveoli The physiologic importance of this growth pattern has been demonstrated by other researchers who found that the conductance of peripheral airways (beyond the fifteenth bronchial generation) increases dramatically at about five years of age, while no change occurs in the central airways.

Most reference data for pulmonary function tests in children have been generated on newborns and on children over five or six years of age. Pulmonary function tests from infancy to five years of age have not been well documented and it is not known what effect the relative lower conductance of the peripheral airways might have on measurements of lung function. It is also not known what effects acute respiratory illnesses might have on measurements of pulmonary function and if these effects are more easily detected because conductance of the peripheral airways is lower. Previous research has shown that the peripheral fraction of total respiratory resistance measured by the forced random noise technique in a group of children, whose average age was 46 months, to be significantly greater than in a group of children whose average age was 61 months. (Conductance of the peripheral airways was less in younger children than in the older children.) Other researchers have demonstrated a greater number of significantly decreased spirometric parameters during acute upper respiratory illness in a group of children less than 84 months of age, as compared to a group of children greater than 84 months of age. These data suggest that there are differences in lung function during early life attributable to developmental phenomena in the airways and that these differences may be important in the manifestation of disease.

Additionally, data of pulmonary function in children have been collected in a cross-sectional fashion. No data exist which have been systematically generated in a longitudinal fashion. These data are important to determine if lung function development in children progresses in a predictable fashion much the same as height and weight development, or if the growth of the lung might progress in another as yet undetermined pattern.

This report outlines the analysis of the spirometry data collected over a six-year period at the Frank Porter Graham Child Development Center. Only information gathered on the children when they were well has been included in this analysis. The data have been analyzed in a cross-sectional fashion so that later analyses can be performed to determine what the relationships of each individual child's growth pattern is to the overall growth pattern. Because of the known effects of race and sex on lung function, the children have been separated into four groups for these analyses: black females and males and white females and males.

Conclusions

Over the six year study period, 69 children were followed for an average of 3.5 years each and had an average of 3.14 measurements made per year. The children, in general, were 90 to 155 cm. tall and spanned an age range of 2½ to 12 years. Only data from the children whose heights were between 100 and 150 cm., however, were included in the regression analyses

The regression coefficients and standard errors of the six spirometric parameters computed by weighted least squares analysis for the four race/sex groups are shown in Table 1. The coefficients for the 95% confidence and 95% prediction limits are also listed. Plots of each discrete data point along with the regression lines and 95% confidence and prediction intervals for each parameter in each of the four race/sex groups reveal an excellent fit of the data to the regression lines. Comparisons of plots of the regressions for the four race/sex groups in general reveal that, for any given height, white children have larger lung function parameters than black children. The p-values for the tests of no difference comparing the regression lines (including slope and intercept) between the important race/sex groupings are shown in Table 2. All of these comparisons are significantly different except for $\dot{V}max_{75}\%$ for black females compared to black males and for white females compared to white males. The variability parameters (95% prediction limits) as computed in these analyses are similar to variability as computed by more classical methods (e.g., Mean ± 2SD) This suggests that making measurements in a longitudinal fashion does not reduce the expected variability, as demonstrated below.

1. The feasibility of obtaining reproducible spirometric tests of lung function in small children as young as three years of age
2. Significant racial and sexual differences in most spirometric parameters of lung function in young children.
3. That regression lines of spirometric parameters of lung function can be adequately described in young children by a linear regression over the height range of 100-150 cm.
4. That parameters of variability are proportional to height rather than being a constant over the region of interest.

Recommendations

To ascertain the effects of acquired inslut on the lung such as from air pollution on infectious agents, additional reference data from populations studied in an area with minimal air pollution, and from populations whose illness patterns have been closely documented, are needed. Until the effects of lower

Table 1. *Weighted Regression Coefficients[a] for Spirometric Parameters*

Parameter[d]	Black Females b[e]	Black Females m[f]	Black Females K[b] (x10³)	Black Females L[c] (x10²)	Black Males b	Black Males m	Black Males K (x10³)	Black Males L (x10²)
FVC, l	-1.966±0.084	0.0267±0.0008	0.415	0.371	-2.601±0.100	0.0324±0.0009	0.485	0.372
FEV_1, l	-1.533±0.076	0.0222±0.0007	0.378	0.334	-2.191±0.090	0.0281±0.0008	0.437	0.335
PEF, l/sec	-3.112±0.290	0.0531±0.0026	1.477	1.281	-4.913±0.343	0.0685±0.0031	1.692	1.284
FEF_{25-75}%, l/sec	-0.493±0.211	0.0195±0.0019	1.041	0.931	-1.650±0.246	0.0292±0.0022	1.231	0.933
$\dot{V}max_{50}$%, l/sec	-0.820±0.245	0.0245±0.0022	1.213	1.084	-1.888±0.291	0.0330±0.0026	1.478	1.087
$\dot{V}max_{75}$%, l/sec	0.243±0.177	0.0073±0.0016	0.865	0.781	-0.297±0.209	0.0119±0.0019	1.032	0.783

Parameter	White Females b	White Females m	White Females K (x10³)	White Females L (x10²)	White Males b	White Males m	White Males K (x10²)	White Males L (x10³)
FVC, l	-2.969±0.165	0.0365±0.0013	0.708	0.374	-2.442±0.152	0.0331±0.0013	0.663	0.374
FEV_1, l	-2.469±0.148	0.0313±0.0012	0.639	0.336	-1.676±0.136	0.0256±0.0011	0.581	0.336
PEF, l/sec	-3.846±0.569	0.0611±0.0046	2.393	1.288	-1.378±0.523	0.0402±0.0043	2.227	1.288
FEF_{25-75}%, l/sec	-1.644±0.414	0.0316±0.0033	1.739	0.936	-0.077±0.380	0.0174±0.0032	1.618	0.937
$\dot{V}max_{50}$%, l/sec	-2.111±0.482	0.0380±0.0039	2.026	1.090	-0.287±0.443	0.0209±0.0037	1.885	1.091
$\dot{V}max_{75}$%, l/sec	-0.256±0.347	0.0130±0.0028	1.485	0.786	0.466±0.319	0.0067±0.0026	1.390	0.786

[a] *Regression equations have the form y=mx + b, where y represents the parameters; m, the slope with units equal to those of the parameter divided by cm; b, the intercept with units identical to the parameter; and x, the height in cm.*
[b] *Ninety-five percent confidence limits (for the regression line) for any height between 100 and 150 cm can be computed using the form (mx + b) ± Kx, where K represents the 95% confidence coefficient.*
[c] *Ninety-five percent prediction limits (for an individual observation) for any height between 100 and 150 cm can be computed using the form (mx + b) ± Lx, where L represents the 95% prediction coefficient.*
[d] *Parameters are FVC (forced vital capacity), FEV_1 (forced expiratory volume in one second), PEF (peak expiratory flow), FEF_{25-75}% (forced expiratory flow during the middle half of the FVC), $\dot{V}max_{50}$%, (maximum expiratory flow after 50% of the FVC has been exhaled), and $\dot{V}max_{75}$% (maximum expiratory flow after 75% of the FVC has been exhaled).*
[e] *b = intercept ± S.E.*
[f] *m = slope ± S.E.*

respiratory illnesses during infancy on lung growth and development are better understood, populations whose entire life history of respiratory illness is known should be studied. Since lower respiratory illnesses during infancy may be related to altered lung function later in life, these illnesses need to be documented Documentation should not only include determination of the etiology of the illness and other clinical data but should also include attempts to characterize changes in lung function during these illnesses, as well as when illness free. This will necessitate the continued effort in developing tests of lung function which can be utilized in very young children. Also, reference data for tests of pulmonary function are needed which have been collected in a longitudinal fashion in illness-free, non-smoking individuals 13-25 years of age who are living in an area relatively free of atmospheric pollution.

Table 2. *P-Values for Tests of No Differences Between the Indicated Regression Lines*

Comparisons[a]	FVC	FEV_1	PEF	FEF_{25-75}%	$\dot{V}max_{50}$%	$\dot{V}max_{75}$%
BF vs BM[b]	0[c]	0	<0.001	<0.001	<0.002	<0.2
WF vs WM	0	0	<0.003	<0.001	0	<0.2
BF vs WF	0	0	<0.002	0	0	<0.001
BM vs WM	0	0	0	0	<0.001	<0.001

[a] *Comparisons are for the regression lines described in Table 2 between the important race/sex groupings.*
[b] *BF (Black females), BM (Black males), WF (White females) and WM (White males).*
[c] *0 indicates p-value less than 10^{-5}.*

☆ U.S. GOVERNMENT PRINTING OFFICE 1981-757-012/7099

Albert M. Collier, Gerald L. Strope, Ronald W. Helms, Lisa Morrisey Lavange, Wallace A. Clyde, Jr., Russel Pimmel, Floyd W. Denny, Frederick W. Henderson, and Gerald W. Fernald are with the University of North Carolina School of Medicine, Chapel Hill, NC 27514.

***Ralph W. Stacy** is the EPA Project Officer (see below).*

The complete report, entitled "Effects of Etiologically Defined Respiratory Infections on Lung Function and Its Growth in an Area of Low Air Pollution - Spirometry in Young Children When Illness-Free," (Order No. PB 81-173 338; Cost: $8.00, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

The EPA Project Officer can be contacted at:

Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

Postage and
Fees Paid
Environmental
Protection
Agency
EPA 335

Official Business
Penalty for Private Use $300