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Progress Report

**Molecular Epidemiology of Severe Ambient Air Pollution on Women and
the Developing Fetus**
DE-FG02-93ER61719

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I. Specific Aims

The research goal is validation of a number of promising biomarkers in two groups of Polish women and their newborn infants: 73 mother/child pairs from Krakow, a city with elevated air pollution and 90 pairs from Limanowa, a less polluted area. Specifically, we are evaluating:

- 1) the relationship between ambient exposures and the following markers of biologically effective dose and biologic response: PAH-DNA adducts by enzyme-linked immunosorbent assay (ELISA), aromatic-DNA adducts by ^{32}P -postlabeling and the frequency of gene mutation at the hprt locus in T-lymphocytes;

- 2) the relationship between markers of biologically effective dose (adducts) and markers of biologic response (gene mutation);

- 3) the comparison of the same biomarkers in maternal and fetal/newborn tissues to assess possible differences in response and susceptibility;

- 4) the ability of genetic/metabolic markers (CYP1A1 mRNA, CYP1A1 enzyme activity, CYP1A1 MspI polymorphism and glutathione-S-transferase (GSTM1) genotype) to modify markers of biologically effective dose and biologic response, including an estimate of host versus environmental contributions;

- 5) the relationship between biomarkers (PAH-DNA adducts and serum cotinine) to birth weight.

These specific aims remain essentially unchanged from those stated in our original application.

II. Studies and Results

Progress made toward our research goals during the past year has been rewarding.

A. Laboratory analyses are proceeding on schedule. Analysis of adducts formed between polycyclic aromatic hydrocarbons (PAH-DNA adducts) by ELISA has been completed on 135/163 maternal and 135/163 infant cord blood samples (including 112 mother/infant pairs). Analyses included triplicates of each sample per assay and duplicate assays when quantity of DNA permitted, which was the case for approximately 60% of samples. Duplicate samples were highly correlated ($p < 0.001$).

Laboratory analysis of PAH-DNA adducts by ELISA in placental tissue is in progress. Prior to DNA isolation, placental tissue from the four quadrants and the center of the placenta was homogenized to assure a representative sample. DNA was isolated from placental tissue in Dr. S. Garte's laboratory, New York University Medical Center (at no cost to grant). Placental tissue DNA has also been isolated in Dr. R. Santella's laboratory. All analyses of placental PAH-DNA adduct levels by ELISA are being conducted by the same laboratory technician in Dr. Santella's laboratory. PAH-DNA adduct levels in the two separate DNA samples isolated from the same placental tissue were available for 58% of the placental samples. There was no correlation between adduct levels in duplicate placental samples ($p = 0.98$). Lack of

correlation may be due to localization of P450 enzymes resulting in variability in metabolism of PAHs across the placenta.

Analysis of 7-ethoxyresorufin-O-deethylase (EROD) activity has been completed on 160/163 placental samples by Dr. A. Gladek-Yarborough, in Dr. Santella's laboratory. We originally proposed to measure aryl hydrocarbon hydroxylase (AHH) activity but decided to measure EROD activity because the assay is faster, does not require extraction with organic solvents and, most importantly, appears to be more sensitive than AHH activity¹. In addition, Dr. S. Garte (New York University Medical Center) has measured CYP1A1 mRNA levels by Northern blot in 155/163 placental samples at no cost to the grant. Results will enable evaluation of effects of environmental exposures on both CYP1A1 gene induction and enzyme activity, allowing a comparison between induction and activity.

Laboratory assays for serum cotinine have been completed by T. Cooper (New York State Psychiatric Institute) on 161 maternal and 163 cord blood samples to provide an internal dosimeter of cigarette smoke exposure (active and passive). This assay was conducted at no cost to the grant.

Work on additional biomarkers is in progress. Lymphocyte samples have been shipped to Dr. J.P. O'Neill (Vermont Cancer Center) for analysis of hprt mutation frequency, including 212 samples from mother/child pairs and 10 unpaired samples for which the corresponding maternal or fetal sample was not available. Analysis of the 10 unpaired samples has shown the cryopreserved cells to be viable and able to proliferate into colonies. Analysis of paired maternal/fetal samples has commenced.

A total of 489 DNA samples (from maternal and infant WBC and placental tissue) have been shipped to Dr. K. Hemminki (Karolinska Institute). Laboratory analysis of aromatic-DNA adducts by ³²P-postlabeling is in progress. Analysis of CYP1A1 MspI polymorphism and GSTM1 genotype is also in progress in Drs. Lucier's and Bell's laboratory (NIEHS).

B. Data Analysis. Questionnaire data have been abstracted. Daily ambient monitoring data from Krakow for 1990 - 1992 have been collected from the Division of National Sanitary Inspection (15 monitoring stations) and the U.S. Environmental Protection Agency (5 monitoring stations). Each Krakow subject's exposure to ambient particulates < 10 µg (PM₁₀) has been estimated by taking the average of PM₁₀ measurements reported at the monitoring station closest to her residence for each of the past two years and the month prior to her delivery date. Questionnaire and ambient monitoring data are computerized and the database shaped for statistical analysis.

Analysis of CYP1A1 gene induction in placental tissue has been completed. After adjusting for age, diet, use of coal for residential heating and place of residence, the association between current smoking and CYP1A1 mRNA was highly significant ($p < 0.001$) as was the association between mRNA and serum cotinine (maternal

¹ Manchester, D., Biochemical Pharmacology, Vol.30 (7) 757-762, 1981.

and infant, $p < 0.001$). Among current smokers, serum cotinine predicted placental mRNA better than did daily consumption of cigarettes. Results indicate mRNA levels decline rapidly following smoking cessation. Among current smokers, an inverse correlation was seen with elapsed time since the last cigarette ($r = -0.52$, $p = 0.08$). However, levels did not return to baseline even after long-term cessation and were increased in ex-smokers who had quit prior to pregnancy ($P < 0.01$). A marginal increase in placental CYP1A1 mRNA with ETS exposure was evident. Within Krakow, there was a dose-response in mRNA with ambient pollution; the trend reached significance among cohort members who were not employed away from the home ($p = 0.01$). Our interpretation is that these unemployed subjects spent more time at their place of residence and thus the ambient monitoring data by place of residence were a better predictor of their exposure than for employed subjects. A significant increase in mRNA was also seen with consumption of broiled/smoked foods ($P < 0.01$). As expected, placental CYP1A1 mRNA levels were significantly correlated with placental EROD activity ($p = 0.001$).

Data analysis of PAH-DNA adducts is in progress. After controlling for diet and occupational exposures, maternal adduct levels were significantly increased among current smokers ($p = 0.007$) and with ETS exposure ($p = 0.01$). A dose-response in maternal adduct levels with increasing ambient air pollution was apparent among Krakow residents with the trend significant for cohort members not employed away from the home ($p = 0.02$). Adduct levels in infant cord WBC (7.9 ± 9.9 per 10^8 nucleotides) were higher than adduct levels in paired maternal WBC (5.9 ± 8.2 per 10^8 nucleotides).

Data analysis for serum cotinine is also progressing. Maternal and infant levels were significantly associated with current smoking and ETS exposure. Infant cotinine levels were significantly higher than maternal levels ($p < 0.001$, paired t-test), including for the subset of maternal/infant pairs for whom samples collection was within 1 ± 1.5 hours of each other.

III. Significance

The strength of this study is that multiple biomarkers that are biologically relevant to carcinogenesis are being evaluated in samples from mother/infant pairs. Findings are pertinent both to assessing maternal risks from ambient air and other environmental exposures as well as risks to the fetus from maternal exposures to PAHs and other transplacental carcinogens. The finding of higher PAH-DNA adduct levels in infant cord WBC than in paired maternal WBC -- despite evidence from experimental bioassays that transplacental exposure to PAHs is at least an order of magnitude lower than maternal exposure -- points to the possibility of increased susceptibility of the developing fetus to PAH-DNA adduct formation. The finding of significantly higher serum cotinine levels in infant cord blood than in paired maternal blood samples also suggests a reduced ability of the fetus relative to the mother to detoxify cigarette smoke constituents.

The study will also help elucidate the role of placental CYP1A1 in modulating the transfer of carcinogens/mutagens from mother to fetus. CYP1A1 codes for a highly inducible enzyme system involved in the biotransformation of xenobiotics, such as PAHs, to phenolic products and epoxides, some of which are toxic, mutagenic and carcinogenic. As inducibility increases, so does metabolism of PAHs to reactive carcinogenic intermediates. Environmental and genetic determinants of this enzyme system in the placenta, which is at the interface between maternal and fetal circulation, may be an important factor in transplacental DNA damage. Our finding of a highly significant association between active cigarette smoking status and placental CYP1A1 mRNA levels is consistent with prior studies of placental AHH and EROD activity. However, the long-term persistence in mRNA induction following smoking cessation has not been seen previously and may be due to storage of PAHs from cigarette smoke in body lipids during periods of active smoking which are then released into the bloodstream during pregnancy. Ours is also the first finding of an association between either placental CYP1A1 gene induction or enzyme activity and dietary consumption of broiled and smoked foods. Although these results should be considered preliminary, they are biologically plausible. PAHs are products of combustion and pyrolysis and are common contaminants of cooked foods. The effect of ambient air pollution on CYP1A1 induction seen in the current study is consistent with results of a prior study that observed an association between placental AHH activity and ambient air pollution in Ankara, Turkey.

IV. Plans for the coming year.

PAH-DNA adduct levels by ELISA in duplicate DNA samples will be completed in Dr. Santella's laboratory for 163 placental samples.

Laboratory analysis for frequency of gene mutation at the hprt locus in T-lymphocytes will be completed in Dr. O'Neill's laboratory for the 212 samples from mother/infant pairs.

Laboratory analyses of aromatic-DNA adducts by the ³²P-postlabeling method will also be completed for 163 maternal and infant cord WBC samples and 163 placental samples in Dr. Hemminki's laboratory.

Data analysis will be completed for PAH-DNA adducts, aromatic-DNA adducts and frequency of hprt mutation as outlined in our competing application. The analyses will evaluate:

1. the relationship between ambient air pollution and other environmental exposure and biomarker levels in both maternal and fetal samples;
2. the comparison of biomarker levels in maternal to infant samples, including the role of placental CYP1A1 induction and enzyme activity to modulate this relationship;
3. the comparison of PAH-DNA and aromatic-DNA adducts in different specimens from the same donor (placental tissue versus cord blood).

Publication List

Perera, F.P. and Mooney, L.A. The role of molecular epidemiology in cancer prevention. *Cancer Prevention*, 1-15, 1993.

Perera, F.P. and Santella, R.M. Carcinogenesis. In: P.A. Schulte and F.P. Perera (Eds.) *Molecular Epidemiology: Principles and Practices*. New York: Academic Press, Inc., pp.277-300, 1993.

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OBJECTIVE: To pursue an academic career in applying mathematics and statistics to health science research.

EXPERIENCE:

June, 1993 **The Center for Social Sciences, Columbia University**
to Present: **Graduate Research Assistant** for the NIH and NSF project "Urban Areas and Youth". in charge of data management and all statistical programming. Excellent overall computer skills, and professional in using SAS and SPSS, and other specialized software. Experienced in longitudinal database management and various advanced statistical analyses, such as survival analysis and complex time variate models.

March, 1993 **Microcomputer & Media Center, Health Sciences Library, C.U.**
to **General Assistant**, mainly served as computer consultant to help users
August, 1993 with various software and provided other general public services.

September, 1991 **Department Of Mathematics, University Of Michigan, Ann Arbor, MI**
to **Teaching Assistant** for Linear Algebra and Calculus courses, also did all
December, 1992 computing work at a Math Lab using MS Excel and PCT_eX, etc.

EDUCATION:

January, 1993 **COLUMBIA UNIVERSITY, SCHOOL OF PUBLIC HEALTH**
to present M.S. degree in Biostatistics expected in Fall, 1994.
Finished comprehensive statistical courses, all graded at A level, most A⁺.

September, 1991 **UNIVERSITY OF MICHIGAN, GRADUATE SCHOOL OF ARTS & SCIENCES**
to Studied in Ph.D. program in Math and passed Qualifying Examination.
December, 1992 *Department of Mathematics, University of Michigan, Ann Arbor, MI 48109*

September, 1986 **UNIVERSITY OF SCIENCE AND TECHNOLOGY OF CHINA**
to B.S. degree in Mathematics
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