EFFECT OF PRENATAL PAH EXPOSURE ON BIRTH OUTCOMES AND NEUROCOGNITIVE DEVELOPMENT IN A COHORT OF NEWBORNS IN POLAND. STUDY DESIGN AND PRELIMINARY AMBIENT DATA

WIESŁAW JĘDRYCHOWSKI¹, ROBIN M. WHYATT², DAVID E. CAMANN³, ULKA V. BAWLE², KOSTIA PEKI², JOHN D. SPENGLER⁴, THOMAS S. DUMYAHN⁴, AGNIESZKA PENAR¹ AND FEDERIKA F. PERERA²

¹ Department of Epidemiology and Preventive Medicine
Collegium Medicum
Jagiellonian University
Kraków, Poland

² Center for Children’s Environmental Health
Columbia University
New York, USA

³ Southwest Research Institute
San Antonio, USA

⁴ Department of Environmental Health
School of Public Health
Harvard University
Boston, USA

Abstract. Preliminary data suggest an association between infant mortality rates and several measures of ambient air pollution, including dustfall and polycyclic aromatic hydrocarbons (PAH). The effects of airborne PAH components on fetal growth and early childhood development are of primary interest since we have previously demonstrated that these pollutants were associated with significant decrements in birthweight, length and head circumference in Polish newborn babies. The undertaken research combines a state-of-the-art environmental monitoring and molecular approaches with comprehensive neurodevelopment assessments. A further innovation is the incorporation of biomarkers (lead and antioxidants in cord blood) to control for potential confounding of exposure-effect. The mean overall concentrations of specific PAH compounds in Kraków were highest for benzo(b)fluoranthene (23 ng/m³), benzo(a)anthracene (16 ng/m³), indeno(1,2,3-cd)pyrene (14 ng/m³), chrysesene (13 ng/m³) and benzo(a)pyrene (12 ng/m³). In general, concentrations of the PAH compounds measured by personal monitoring were considerably higher among the residents of the higher polluted area of the city. The comparison between the Kraków and New York City monitoring ambient data demonstrates that the concentrations and proportions of specific compounds in the total PAH mixture differ widely across these cities. Thus, one may expect that the PAH-related health risks would differ between the two areas not only because exposures are different, but also because the PAH profile differs. Presumably, this different profile is a consequence, at least in part, of variations in the sources of fossil and solid fuels used in production of energy. In Poland, solid fuels such as coal is used to a much greater extent than in New York City, where fossil fuels are the universal source of energy production. Auto emissions for example, are low in benzo(a)pyrene, whereas such emissions from refuse burning are high.

Key words:
Prenatal PAH exposure, Health outcomes, Newborns

INTRODUCTION

Higher incidence of congenital malformations in the provinces of Poland with marked environmental pollution [1] and an increase in rates of newborns with low birthweight in larger and polluted cities have been observed over the years 1985–1990 [2]. In Poland, the increased perinatal mortality of newborn babies noted over the period 1985–1995 was mainly due to a significant increase in per-
Preliminary data suggest an association between infant mortality rates and several measures of ambient air pollution, including dustfalls and polycyclic aromatic hydrocarbons (PAH) [4,5]. An excess prevalence of low birthweight and premature births has been found in a polluted district of the Czech Republic (Teplice), compared to Prachatice, where pollution was low [6]. The observed adverse effects were more common in infants conceived in the winter months and whose mothers were smokers. A study carried out in Beijing found an association between maternal exposure to total suspended particulate (TSP) and sulfur dioxide (SO₂) during the third trimester and decreased birthweight [7]. Another Beijing study, showed that preterm deliveries were connected with TSP and SO₂ [8]. Finally, in the USA, postneonatal infant mortality has been associated with particulate matter as well [9].

Our first study also suggested that PAHs in air pollution adversely affected birthweight, length and, especially, head circumference [10]. A possible mechanism for the latter is the induction of apoptosis following DNA damage by PAHs. This is an important finding, because a number of studies have reported that reduction in head circumference at birth or during the first year of life correlates with lower IQ, as well as with poorer cognitive functioning and school performance in childhood [11–13].

Sources of PAH exposure
Polycyclic aromatic hydrocarbons, a ubiquitous group of environmental pollutants, are formed during incomplete combustion or pyrolysis of organic material used in energy production (oil, gas, coal, wood). They are found in automobile exhaust, industrial effluents, cigarette smoke, and in charcoal-broiled foods. PAHs are complex mixtures of hundreds of chemicals, including derivatives of PAHs, such as nitro-PAHs and oxygenated products, and also heterocyclic PAHs.

The proportions of different PAHs detected in different emissions sometimes differ widely from each other and from PAH profiles found in ambient air. It is assumed that the profiles of PAHs in ambient air do not seem to differ very much from one area to another, although large variations may be seen under special conditions. As a rule, the majority of PAHs is preferentially attached to particles and only a minor fraction, depending on the temperature, exists as volatiles.

Given the present body of knowledge, regulatory guidelines use benzo(a)pyrene (BaP) concentrations as an indicator of the carcinogenic PAHs in ambient air. Current annual mean outdoor concentrations of BaP in major European urban areas are in the range of 1–10 ng/m³. In the rural areas, the concentrations are less than 1 ng/m³ [14,15]. Up to now, no specific public health policy guidelines recommended for other specific PAH compounds in outdoor air have been established.

Major indoor sources of PAH compounds are the combustion of wood or other fuel for residential heating (e.g. kerosene heaters, wood or coal stoves, fireplaces), environmental tobacco smoke (ETS), and fumes from cooking, grilling, and frying. The unvented heating gas appliances can increase PAH concentrations in indoor air as well. Major outdoor sources of PAH emissions, such as automobiles and power plants greatly affect indoor PAH concentrations.

Food is also considered to be the major source of human PAH exposure, owing to PAH formation during cooking or from atmospheric deposition of PAHs on grains, fruits and vegetables. The relative contribution of airborne PAH pollutants to food levels via fallout has been well-characterized [16].

The biological properties of the majority of PAH compounds are yet unknown. The PAH benzo(a)pyrene is metabolized to a mixture of epoxides. This represents a biological activation of the parent compound to produce the diol-epoxide metabolite, a potent mutagen and carcinogen (Fig. 1). Despite the intensive work that has been done on airborne organic particulates, especially PAHs, it is estimated that over 90% of the atmospheric organic pollutants have never been determined. Animal studies data indicate that several PAHs may induce a number of adverse effects, such as immunotoxicity, genotoxicity, carcinogenicity and reproductive toxicity affecting male and female offspring (Fig. 2).

Benzo(a)pyrene has been the most intensively studied PAH in experimental animals. It produces tumors of
many different tissues, depending on the species tested and the route of application. In addition to their ability to bind to and damage DNA, PAHs such as BaP are capable of disrupting the endocrine system by altering metabolic pathways of natural hormones or otherwise interfering with their activity [17,18]. One mechanism for endocrine toxicity by PAHs is likely to be mediated by PAH activation of Ah receptors and subsequent induction of specific cytochrome P450 enzymes that modify the synthesis and metabolisms of endogenous hormones. Laboratory studies have found an association between transplacental exposure to certain PAHs and adverse reproductive outcomes. Pregnant animals exposed to BaP and other PAHs showed an increase in stillbirths, reabsorption and congenital abnormalities, as well as decreases in fetal weight [17, 19–22].

Main goals of the Kraków study
The effects of airborne PAH components on fetal growth and early childhood development are of primary interest since we have previously demonstrated that these pollutants were associated with significant decrements in birthweight, length and head circumference in Polish newborns [10]. This was the first such observation in humans that was consistent with experimental data on PAHs [23–25], and suggested that airborne PAHs adversely affect fetal growth. However, in our prior study on PAH-related fetal growth retardation, we neither addressed possible implications of PAH components for child neurodevelopment functioning or possible confounding by nutritional factors, nor estimated the relative impact of ambient PAHs, PM$_{2.5}$ and ETS and their possible combined effects. Therefore, given the potential public health implications of our initial findings, and their relevance to other populations worldwide, we believed that further study was warranted.

The undertaken research is novel in that there have been no prospective molecular epidemiologic studies of this scope, combining a state-of-the-art environmental monitoring and molecular approaches with comprehensive neurodevelopment assessments. A further innovation is the incorporation of biomarkers (lead and antioxidants in cord blood) to control for potential confounding of exposure-effect. Lead is a known developmental toxicant and potentially important confounding variable. The need to control for nutritional status was indicated by our pilot data showing variable levels of micronutrients in a sample of cord blood from the initial cohort.

Another novel aspect of the research is our ability to assess the relative contributions of PAH (a constituent of PM$_{2.5}$), non-PAH components of PM$_{2.5}$ and ETS to neurodevelopmental impacts, as well as the contribution of indoor PAHs (ETS and indoor heating and cooking) versus ambient PAHs. This is important because ETS is both a major indoor source of PAHs and is thought to be an independent risk factor for growth and developmental impairment, acting via other non-PAH constituents. Similarly, PM$_{2.5}$ contains a number of other toxic compounds that may contribute to adverse birth outcomes, including metals such as lead and cadmium, and other organics in addition to PAHs.

Specific aims of the study
The aims are:
1) to test the hypothesis that prenatal exposure to airborne polycyclic aromatic hydrocarbons adversely affects fetal growth, after controlling for non-PAH components of par-
ticulate matter < 2.5 μm (PM$_{2.5}$), environmental tobacco smoke, nutritional status and other potential confounders; 2) to explore whether non-PAH components of PM$_{2.5}$ and ETS have independent effects on birth outcomes and childhood growth and development, after controlling for PAH, and to explore possible interactions between PAH, PM$_{2.5}$ and ETS; and 3) to estimate the relative contribution of ambient PAH pollution versus ETS and other indoor PAH sources to (a) personal PAH exposure and PAH-DNA adducts; and (b) impairment of fetal growth and early child development.

**STUDY DESIGN AND METHODS**

By design, the study is a birth cohort prospective study where the exposed and control cohorts are being established on the basis of data collected by interviewing pregnant women and by performing environmental measurements over the pregnancy period. The health outcomes in the babies are being assessed at the delivery and in the course of the follow-up over at least one-year period. In total, we will be enrolling 480 non-smoking pregnant women in order to achieve a final number of 400 infants aged 12 months. The Kraków women who have resided for the past year within a 0.5-km radius of the ambient air monitoring stations with either the highest (the Śródmieście district) or lowest (the Krowodrza district) PAH levels are eligible for the study. Women are being recruited toward the end of the 1st trimester of pregnancy (between the 8th and 13th week of gestation) continuously throughout the year to maximize the variability in ambient pollution levels. The cohort will be divided evenly between the high and low pollution areas. Women are being recruited through prenatal clinics in the high and in the low pollution areas. Women attending the prenatal clinics in the high pollution area deliver at the Municipal Hospital for Obstetrics in the center of the city, while those in the low pollution area deliver at the Obstetrical Clinic at the University Hospital. Enrollees are restricted to women >18 years of age to maximize the homogeneity of the sample. Specifically, we are restricting maternal age for the purpose of eliminating adolescent mothers, who are known to show a greater likelihood of low birthweight delivery. This restriction also introduces some control for variations in maternal growth status, so that all pregnant women will be fully-grown. Information on parity and previous low birthweight or preterm delivery is gathered and these factors are accounted for in the statistical analyses. Women with pregnancy-related diabetes or hypertension are excluded to eliminate the effect of these conditions on fetal development. Current smokers (i.e., women who smoked during pregnancy) are also excluded from the study because active smoking may confound the association between PAHs and exposure and fetal development. Women with exposure to ETS, however, are included in the study sample, but those with a history of using illicit drugs or at risk of occupational exposure to PAHs or other developmental toxicants (coke oven, chemical or rubber workers) are excluded. We include both term and preterm deliveries, but exclude multiple births (to minimize confounding of birth size).

The woman visiting the prenatal clinic because of her pregnancy receives a letter of introduction explaining the purpose of the study. Following a letter of introduction, the research assistant contacts the women either by phone or in person. Then, a short screening questionnaire is administered to determine whether the woman meets the eligibility criteria. Subsequently, face-to-face standardized interviews based on the questionnaire are being collected, including information on:

- demographic data,
- socioeconomic status (SES) (education, income levels and information indicative of living standard such as possession of a color TV, VCR, microwave, computer, automobile, size and quality of household, ownership of a summer house),
- residential history,
- active and passive smoking history,
- home characteristics,
- daily activity patterns, occupational history,
- alcohol and caffeine use during each trimester of pregnancy,
dietary consumption of PAH-containing foods (charcoal broiled, fried, and broiled meats, smoked foods etc.)
- current vitamin use,
- maternal age and height, and
detailed information about the current pregnancy, including estimated last menstrual period (LMP), weight and weight gain.

Medical information is being abstracted from the mothers’ and infants’ medical records following delivery. All measures of fetal growth are being obtained at this time. The maternal antenatal medical record is also being reviewed to identify any medical complications or events that were not recorded during the pregnancy contacts. The information includes: expected date of delivery, estimated gestational age, maternal height, pre-pregnancy weight, net weight gain during pregnancy, gender of the newborn, delivery date, birthweight, length, and head circumference, malformations of the infant, complications of pregnancy and delivery, and medications used during pregnancy. The design of the study is shortly presented in Tables 1 and 2.

Assessment of prenatal ambient and indoor PAH exposure
Monitoring of personal PAH inhalation exposure is carried out over a 48-h period during the 2nd trimester of pregnancy in all women initially enrolled. In a subsample of pregnant women, also PAH inhalation exposure is going to be measured in the first and third trimesters. From the monitoring data we are able to derive the following exposure variables: 1) each woman’s measured personal inhalation exposure to PAHs (ng/m³) and PM₁₀ (µg/m³) during the 2nd trimester as a continuous variable; and 2) her 1st and 3rd trimester-specific personal PAH and PM₁₀ inhalation exposure during pregnancy.

Once a woman is enrolled, a member of the air monitoring staff instructs her how to use the personal monitor, which is lightweight, quiet and worn in a backpack. The woman is asked to wear the monitor during the daytime hours for two consecutive days and to place the monitor near the bed at night. During the morning of the second day, the air monitoring staff member and interviewer visit the woman at home to change the battery-pack and administer the full questionnaire. They also check to see that the monitor has been running continuously and that there have been no technical or operating failures. A staff-member returns to the woman’s home in the morning of the third day to pick up the equipment.

Personal monitors draw air at a constant flow rate of 4 liters per min (LPM). The inlet of the pump is attached to a two-way flow splitter, consisting of tubing with different amounts of flow restriction for each of the paths. One path (with a flow rate of 2 LPM) is for the PAH sampler and
the other path (with a flow rate of 2 LPM) is for PM$_{2.5}$ sampler. Flow rates are calibrated (with filters in place) using a bubble meter prior to the monitoring, and are checked again with a change of the battery pack on the second day and at the conclusion of the monitoring. Pumps are monitoring air pollution continuously over a 48-h period. In addition to the personal monitoring, the ambient monitoring stations within Kraków are gathering continuous daily measurement data on ambient respirable particulate matter $<10\ \mu m$ in diameter (PM$_{10}$) and PAHs. Thus, we will be able to analyze the independent effects of these pollutants on the fetal and child development.

**Analysis of air samples**

**PAH components.** The polyurethane (PUF) sample cartridge is preceded by an impactor inlet with a 2.5 $\mu m$ cut at 2 LPM. Vapors and particles of $\leq 2.5\ \mu m$ in diameter pass through the impactor inlet and collect on a precleaned quartz microfiber filter (Palliflex Tissuquartz 2500 QAS, 25 mm diameter) and a precleaned PUF cylinder [26]. After sampling is completed, the field samplers are frozen and are shipped to South-West Research Institute in Texas on dry ice. Only the samples from mother/infant pairs with completed questionnaire, medical record data and collected biologic samples will be analyzed for BaP and the other PAHs. Determination of target PAHs (BaA, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, BaP, chrysene/iso-chrysene, dibenz(a,h)anthracene, indeno(1,2,3-cd)pyrene, and pyrene) in extracts is being performed using GC/MS as described previously [27].

**PM$_{2.5}$ particulate matter.** The personal environmental monitoring sampler (PEMS) [28–29] is used to measure particle mass. The PEMS is designed to achieve the particle target size of $\leq 2.5\ \mu m$ at a flow rate of 4.0 LPM with an array of 10 impactor nozzles. To modify the sampler to achieve the 2.5 $\mu m$ size cut at 2 LPM, 5 of the nozzles are blocked. This type of PEMS modification was used previously [30]. Particles are collected on the same type of Teflon membrane filter (37 mm Teflo™, Gelman Sciences) used in previous studies to measure PM$_{2.5}$. The combination of low pressure drop (permitting use of a low power sampling pump), low hygroscopicity (minimizing bound water interference in mass measurements), and low trace element background (improving analytical sensitivity) of these filters make them highly appropriate for personal particle sampling [31].

**PRELIMINARY RESULTS OF THE PAH AMBIENT MONITORING IN KRAKÓW**

At the initial stage of the Kraków study, 135 pregnant women have been recruited to date. Of these, 83% were employed in offices and small businesses. The majority (71%) of the women reported that their apartments were equipped with municipal central heating; however, 17% reported that their dwellings were heated with electric stoves and 11% that gas-operated heating stove were used within the household. In 70% of households, gas appliances were used for water heating. Interestingly, about 70% of women reported that they used candles in the households and 23% that they used incenses. Both are potential sources of indoor PAHs.

For cooking, the majority (85%) of interviewed women used gas cookers and 13.8% used electric stoves. In about 3% of households, grilling of foods were reported; about 50% of respondents confirmed that they fried meals; and 18% of them did the baking at home over the last 48 h. These preliminary data suggest that the contribution of indoor sources of PAHs may be significant in our study.

Results of the personal exposures to PAH compounds over the period of 48 h were available for 30 women. The mean overall concentrations of specific PAH compounds in Kraków were highest for benzo(b)fluoranthene (23 ng/m$^3$), benzo(a)anthracene (16 ng/m$^3$), indeno(1,2,3-cd)pyrene (14 ng/m$^3$), chrysene (13 ng/m$^3$) and benzo(a)pyrene (12 ng/m$^3$). In general, concentrations of the PAH compounds measured by personal monitoring were considerably higher among the residents of the higher polluted area of the city (Fig. 3). Mean PAH concentrations did not differ between the women who reported ETS exposure both at home and in the occupational settings compared to women free from ETS exposure (Fig. 4).

Figure 5 gives the comparison between the levels of PAH exposure among the Kraków women and the levels of the
same PAH measured over 48-h by personal air monitoring during pregnancy in more than twelve hundred women residing in New York City (NYC). Exposures to each of the PAH were approximately tenfold higher among the Kraków women. Even in the lower polluted area in Kraków, the PAH levels were of several orders of magnitude higher than concentrations observed in NYC. The contribution of various compounds in the total PAH mixture in the Kraków air was also clearly different from that seen in NYC (Fig. 6). Specifically, among the Kraków women, the PAHs contributing the highest proportion to the total mixture were benzo(b)fluoranthene (23.6%) and benz(a)anthracene (15.8%). In NYC, the highest proportions were contributed by benzo(g,h,i)perylene (32.5%), benzo(b)fluoranthene (15.0%) and indeno(1,2,3-cd)pyrene (15.0%). The results of the personal PAH monitoring of the Kraków women indicate that BaP is not the most prominent PAH. The high concentrations of other compounds such as benzo(b)fluoranthene and benzo(a)anthracene were prevalent.

In summary, the comparison of the Kraków and NYC ambient data monitoring demonstrates that the proportions of specific compounds in the total PAH mixture differ widely across the cities. Thus, one may expect that the PAH-related health risks would differ between the two areas not only because exposures are different, but also because the PAH profile differs. Presumably, this different profile is a consequence, at least in part, of variations in the sources of fossil and solid fuels used in production of energy. In Poland, solid fuels such as coal is used to a much greater extent than in NYC, where fossil fuels are the universal source of energy production. Auto emissions for example, are low in BaP, whereas such emissions from refuse burning are high.

To date, a large body of evidence on toxicity and the occurrence of BaP have been assembled, but data are
less complete for other PAHs. Our preliminary results of personal air monitoring show high concentrations of benzo(b)fluoranthene and benzo(a)anthracene whose biologic properties are not precisely elucidated. In fact, little is known about the occurrence of many PAHs in various environmental settings, and the critical endpoints for health risk assessments are not well characterized. Additional efforts must be made to characterize environmental health hazards resulting from the complex mixture of PAHs. It is truism to say that the main goal of air pollutant measuring in ambient air lies in identifying and predicting undesirable health effects at the population level. Until recently, epidemiologic studies investigating the relationship between air pollution and adverse health effects have relied principally on crude description of outdoor air quality, including annual or daily averages for total suspended particulates or SO$_2$, to represent population exposures. It is clear that new epidemiologic studies of the health effects due to air pollutants must use sophisticated exposure techniques, which are currently available. This paper is an example how the noteworthy advances in instrumentation and techniques for monitoring air pollutants and assessing the total integrated exposure offer new added values for epidemiologic investigations.

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