

ehp

**ENVIRONMENTAL
HEALTH
PERSPECTIVES**

ehponline.org

**Prenatal Exposure to Airborne Polycyclic Aromatic
Hydrocarbons and Children's Intelligence at Age 5 in a
Prospective Cohort Study in Poland**

**Susan Claire Edwards, Wieslaw Jedrychowski,
Maria Butscher, David Camann, Agnieszka Kieltyka,
Elzbieta Mroz, Elzbieta Flak, Zhigang Li,
Shuang Wang, Virginia Rauh, and Frederica Perera**

**doi: 10.1289/ehp.0901070 (available at <http://dx.doi.org/>)
Online 20 April 2010**



NIEHS

National Institute of
Environmental Health Sciences

National Institutes of Health
U.S. Department of Health and Human Services

Prenatal Exposure to Airborne Polycyclic Aromatic Hydrocarbons and Children's Intelligence at Age 5 in a Prospective Cohort Study in Poland

Susan Claire Edwards ¹, Wieslaw Jedrychowski ², Maria Butscher ³, David Camann ⁴, Agnieszka Kieltyka ², Elzbieta Mroz ², Elzbieta Flak ², Zhigang Li ^{1,5}, Shuang Wang ^{1,5}, Virginia Rauh^{1,6}, Frederica Perera ^{1*}

¹ Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University, New York, NY; ² Department of Epidemiology and Preventive Medicine, Jagiellonian University, Krakow, Poland; ³ Polish-American Institute of Pediatrics, Jagiellonian University, Krakow, Poland; ⁴ Department of Analytical and Environmental Chemistry, Southwest Research Institute, San Antonio, TX; ⁵ Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY; ⁶ Department of Population and Family Health, Mailman School of Public Health

* Corresponding author

Columbia University, 100 Haven Avenue, Tower III, suite 25F, New York, NY10032, USA, phone: (212) 304-7280, fax: (212) 544-1943

Acknowledgements

We gratefully acknowledge Jennifer Arney for her assistance in the preparation of this manuscript.

Page 2

Prenatal airborne PAH and child IQ at age 5 years

Article descriptors: Children’s health, neurodevelopment

Air pollution, child, development, environmental, ETS, *in utero*, intelligence, prenatal, Poland, Raven

This study received funding from the NIEHS (1R01ES010165-01), the Gladys T. and Roland Harriman Foundation, and anonymous private donors. We would like to thank the U.S. Centers for Disease Control and Prevention in Atlanta, GA, for their analysis of cotinine, metals, and PAH metabolite levels in samples from this cohort.

There are no competing interests.

PAH	polycyclic aromatic hydrocarbon
ETS	environmental tobacco smoke
NYC	New York City
IQ	intelligence quotient
RCPM	Raven Coloured Progressive Matrices
WPPSI-R	Wechsler Preschool and Primary Scale of Intelligence–Revised
TONI-3	Test of Nonverbal Intelligence-Third edition
HOME Inventory	Home Observation for Measurement of the Environment Inventory

Page 3

Abstract

Introduction

Materials and Methods

Krakow Study Population

Prenatal Interview

Personal Air Monitoring

Biological Sample Collection and Analyses

Neurodevelopmental Testing

Model and covariates

Results

Discussion

References

Tables

Abstract

Background: This prospective cohort study of Caucasian mothers and children in Krakow, Poland, evaluated the role of prenatal exposure to urban air pollutants in the pathogenesis of neurobehavioral disorders.

Objectives: The objective of this study was to investigate the relationship between prenatal polycyclic aromatic hydrocarbon (PAH) exposure and child intelligence at age 5 years, controlling for potential confounders suspected to play a role in neurodevelopment.

Methods: A cohort of pregnant, healthy, non-smoking women was enrolled in Krakow, Poland, between 2001 and 2006. During pregnancy, participants were invited to complete a questionnaire and undergo 48-hour personal air monitoring to estimate their babies' exposure, and to provide a blood sample and/or a cord blood sample at the time of delivery. Two hundred and fourteen children were followed through age 5 years when their nonverbal reasoning ability was assessed using the Raven Coloured Progressive Matrices (RCPM).

Results: We found that higher (above the median of 17.96 ng/m³) prenatal exposure to airborne PAHs (range: 1.8-272.2 ng/m³) was associated with decreased RCPM scores at age 5 years, after adjusting for potential confounding variables (N=214). Further adjusting for maternal intelligence, lead or dietary PAHs did not alter this association. The reduction in RCPM score associated with high airborne PAH exposure corresponded to an estimated average decrease of 3.8 IQ points.

Conclusions: These results suggest that prenatal exposure to airborne PAHs adversely affects children's cognitive development by 5 years of age, with potential implications for school performance. They are consistent with a recent finding in a parallel cohort in New York City.

Introduction

Polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene, are ubiquitous air pollutants released to ambient and indoor air from combustion sources, such as coal burning power plants, diesel and gasoline-powered vehicles, home heating and cooking, and are present in tobacco smoke and charred foods (ATSDR 1995). Coal burning power plants, home heating, traffic emissions, and second hand smoke are the main contributors to airborne PAH levels in Poland (Choi et al. 2006).

Many studies indicate that the fetus and infant are more sensitive than adults to environmental toxicants including PAHs, lead, pesticides, and environmental tobacco smoke (ETS), because detoxification and DNA repair systems are immature and rates of cell proliferation are increased (NRC 1993b; Perera et al. 2005; Whyatt and Perera 1995; World Health Organization 1986). The central nervous system is particularly vulnerable during prenatal development (Rodier 2004). PAHs readily cross the placenta (Neubert and Tapken 1988; Perera et al. 2003)

PAHs have been shown to be neurodevelopmental toxicants in experimental studies (Saunders et al. 2006; Sram and Binkova 2000; Wormley et al. 2004). Although the precise mechanisms by which they might affect the developing brain are not known, suggested mechanisms include endocrine disruption (Archibong et al. 2002; Bui et al. 1986; Takeda et al. 2004), binding to placental growth factor receptors (Dejmek et al. 2000), binding to the human Ah receptor to induce P450 enzymes (Manchester et al. 1987), DNA damage resulting in activation of apoptotic pathways (Metzer et al. 1995; Nicol et al. 1995; Wood and Youle 1995), and oxidative stress (Saunders et al. 2006). In addition, prenatal PAH exposures may affect

epigenetic programming with neurological consequences (Barker 2004; Perera et al. 2009a; Schwartz 2004; Wilson and Jones 1983).

A prospective cohort study of African-American and Latina mothers and children in New York City (NYC) that parallels the present study has reported that prenatal exposure to airborne PAHs is significantly associated with developmental delay at age 3 years as measured by the Bayley Scales of Infant Development (NRC 1993a; Perera et al. 2006) and reduced IQ at age 5 years by the Wechsler Preschool and Primary Scale of Intelligence–Revised (WPPSI-R) (Perera et al. 2009b). In light of this evidence of neurodevelopmental effects in a multi-ethnic NYC population, the present analysis evaluated the effects of prenatal airborne PAH exposure on a measure of child intelligence in a Caucasian population.

Materials and Methods

Krakow Study Population: This study is part of an ongoing, longitudinal investigation of the health effects of prenatal exposure to outdoor and indoor air pollution on infants and children in Krakow, Poland. As described previously (Jedrychowski et al. 2004), eligibility criteria included: ≥ 18 years of age non-smoking women with singleton pregnancies, no current occupational exposure to PAHs or any other known developmental toxicants, no history of illicit drug use, pregnancy-related diabetes, or hypertension, and registering at a prenatal healthcare clinic in Krakow, where they will have lived for at least a year preceding screening. A total of 505 pregnant (8 to 13 weeks) women fulfilled these criteria.

Full enrollment required providing prenatal questionnaire data, complete prenatal air monitoring data, and a blood sample at delivery from the mother and/or her newborn child. A total of 358 women were fully enrolled by these criteria, of whom 344 had valid airborne PAH

Page 7

data (meeting quality control criteria). We excluded 10 mother-child pairs whose cord or maternal blood cotinine levels registered higher than 25 ng/mL, above which active smoking during pregnancy is suspected (Vartiainen et al. 2002). Of the remaining 334 children, 214 children reached the age of 5 years by August 2009 and had complete Raven Coloured Progressive Matrices (RCPM) test results. Written informed consent was obtained from all mothers on behalf of themselves and their child. The study was approved by the ethics committee of Jagiellonian University, and the Institutional Review Board of the New York Presbyterian Medical Center.

Prenatal Interview: A 45-minute questionnaire was administered by a trained interviewer during the second or third trimester of pregnancy to obtain demographic information, health and environmental data from the mothers. The questionnaire elicited information on ETS exposure during pregnancy (presence/absence of smokers in the household during pregnancy), dietary PAHs (frequency of consumption of broiled, fried, grilled or smoked meat during pregnancy), and socio-economic information related to income and education. Postnatal follow-up interviews were then administered to mothers every 6 months after birth to determine any changes in residence, exposure to ETS, and other health or environmental conditions.

Personal Air Monitoring: To assess exposure to airborne PAHs, the women were personally monitored over a 48-hour period during the second (N=253) or third (N=100) trimester of pregnancy. During the day, they carried small backpacks holding personal air monitors and kept the monitors by their beds at night (Jedrychowski et al. 2004). As previously described (Camann and Whyatt 2001; Tonne et al. 2004), the polyurethane foam cartridges were analyzed at Southwest Research Institute in San Antonio, Texas for concentrations of eight carcinogenic PAHs: benz(a)anthracene, chrysene/iso-chrysene, benzo(b)fluoranthene,

Page 8

benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, and benzo-(g,h,i)perylene. For quality control, each monitoring result was assessed as to accuracy in flow rate, time, and completeness of documentation. Only samples meeting quality control criteria were included in the analysis. Each PAH measured by personal air monitoring was detectable in 100% of personal air samples with a wide range of concentrations. As the eight airborne PAHs were highly intercorrelated ($0.95 < \text{Spearman's } r\text{'s} < 0.99$), and to be consistent with the NYC and other studies (Perera et al. 2003), these PAHs were summed to provide a measure of total airborne PAHs, hereafter "PAHs".

The use of personal monitoring was validated in this cohort in a subset of women (N=80) who were simultaneously monitored for personal, indoor and outdoor airborne PAHs. All three measurements were found to be highly correlated (pair-wise Spearman's coefficients ≥ 0.84 , $p < 0.01$) (Choi et al. 2008), which supported the use of personal monitoring to integrate indoor and outdoor exposure.

Biological Sample Collection and Analyses: After delivery, a cord blood sample was collected from the umbilical cord vein, and a venous blood sample obtained from the women. Plasma cotinine and lead levels in cord and maternal blood and levels of 24 urinary PAH metabolites in child urine collected at the third year follow-up were measured at the U.S. Centers for Disease Control and Prevention using high-performance liquid chromatography atmospheric-pressure ionization tandem mass spectrometry (cotinine) (Bernert et al. 1997), inductively coupled plasma mass spectrometry (lead) (Date and Gray 1989), and a combination of enzymatic deconjugation, automated liquid-liquid extraction, and gas chromatography/isotope dilution high resolution mass spectrometry (PAH metabolites) (Li et al. 2006), respectively. The PAH metabolites were creatinine-adjusted to control for dilution.

Neurodevelopmental Testing: At the 5 year follow-up point, a trained research worker administered to each child the Raven Coloured Progressive Matrices (RCPM), a widely-used age-adjusted nonverbal test of reasoning ability and intelligence based on figural materials or patterns (Raven et al. 1998). This instrument, which is listed as a cognitive test by the Agency for Toxic Substances and Diseases Registry, provides information on functioning in a number of cognitive domains such as visual-perceptual, language, praxis (performance), reasoning and concept formation. Because it is reported to have minimal cultural bias and to be capable of evaluating the intellectual status of children exposed to toxic chemicals (Sattler 1986; Sizemore and Amler 1996), the RCPM has been used in a number of studies of environmental exposures in children in Poland and elsewhere (Szustrowa 1992). Both the RCPM and the Wechsler Preschool and Primary Scale of Intelligence–Revised (WPPSI-R)—which was administered to the NYC cohort—are correlated with the standard test of intelligence called the Wechsler Intelligence Scale for Children (Raven et al. 1998). RCPM standard scores can be converted to intelligence quotient (IQ) scores for estimates of intelligence levels (Counter et al. 2005; Raven et al. 1998), though they should not be interpreted as a measure of global intelligence, but of nonverbal intelligence (Counter et al. 2009). Here, in order to compare the Krakow and the NYC study results, measured RCPM scores were converted to IQ score according to the RCPM manual (Raven et al. 1998).

Maternal intelligence was assessed using the Test of Nonverbal Intelligence-Third edition (TONI-3), a language-free measure of general intelligence considered to be relatively free of cultural bias (DeMauro 2000).

Model and covariates: Two hundred and fourteen participants were included in the primary analysis presented here. A subset of 171 of the participants had available data on

maternal intelligence (TONI-3) which was included as a covariate in a separate model. Missing covariates or test information occurred as a result of loss to follow-up or lack of a biological specimen for biomarker analysis.

Multiple linear regression models were used to estimate the association between prenatal airborne PAH exposure and the RCPM score (modeled as a continuous variable) as a measure of child IQ. Total airborne PAH exposure was modeled both as a dichotomous measure (cut at the median=17.96 ng/m³ and referred to as “high/low” in the tables) and as a continuous measure. The continuous PAH data were log transformed [“Ln(PAH)” in the tables] because the distribution of airborne PAH concentrations was markedly skewed

A number of covariates were selected *a priori* for inclusion in the model based on the literature and our own prior data. These included maternal report of ETS exposure in the household during pregnancy, sex of the child, and maternal education (completed/did not complete 12 years of schooling). The latter was used as a proxy for socio-economic status (Jedrychowski et al. 2009b). Because maternal intelligence (continuous TONI-3) is a known correlate of child cognitive development (Kagan and Moss 1959; Noble and McCandliss 2005) but was only available in a subset of the children (N=171), it was included with the other covariates in a secondary analysis.

Lead and dietary PAHs were not significant predictors of RCPM (p<0.1 in univariate regression analysis) and thus were not included in our final model. Because prenatal PAH exposure was previously found to be associated with reduced birth weight and head circumference in this cohort (Choi et al. 2008), we evaluated their potential as intermediate variables by including them in separate models. We also adjusted for postnatal PAH exposure using individual creatinine-adjusted PAH metabolites in urine collected at age 3 years, and a

Page 11

measure of postnatal residence change as an indicator of possible change in exposure to airborne PAHs after birth. We further adjusted for maternal report of postnatal exposure to ETS in the home. We also checked for effect modification by trimester of PAH monitoring, PAH monitoring season or season of birth by including terms for the interaction between PAH and trimester of PAH monitoring, between PAH and PAH monitoring season and between PAH and season of birth in separate models.

All effect estimates and p-values (α set at 0.05) were generated using SAS (version 9.1.0.3; SAS Institute Inc., Cary, NC).

Results

The average concentration of total airborne PAHs (of adequate monitoring quality) in personal air samples was 39.5 ± 48.1 ng/m³, with a median of 17.96 ng/m³ (N=344). Among all the children tested for RCPM at the 5 year follow-up (N=329), the average RCPM score was 21.8 ± 4.1 .

Table 1 compares the basic demographic characteristics of fully enrolled subjects having prenatal airborne PAH monitoring data and RCPM data at age 5 years vs. fully enrolled subjects having PAH monitoring data but no RCPM. The characteristics of the two groups did not differ significantly, except for newborn birth weight and mother's age. On average, the children with RCPM data were 133.2g lighter at birth, and mothers were older by 1 year. The differences in the means are modest, but statistically significant.

Correlations between airborne PAHs and ETS exposure, between airborne PAHs and dietary PAHs, and between airborne PAHs and maternal or cord cotinine were examined using Spearman rank-order correlation. None was found to be significant, either in the Krakow cohort

as a whole or the subset studied here (N=214) (Table 2). As expected, ETS exposure during pregnancy was significantly correlated with maternal and cord cotinine levels both in the entire cohort and in the present subset, supporting self-reported ETS as a reliable measure of ETS exposure. ETS exposure and dietary PAHs were correlated in the present subset (n=214) but not in the larger cohort.

High prenatal airborne PAH exposure levels were associated with a significant, albeit modest, reduction in child intelligence in models with either dichotomous or Ln-transformed continuous variables for PAH exposure (Table 3). The estimated effect of prenatal airborne PAH was significant after adjusting for prenatal ETS in the home and other potential confounders. The inverse relationship between airborne PAH level and RCPM score remained after adjusting for trimester of monitoring (second or third).

Prenatal ETS in the home was a significant predictor of RCPM score as shown in Table 3. Excluding prenatal ETS from the model did not materially alter the estimated effect size or p-value of PAH on child RCPM score [$\beta = -1.3$, $p=0.03$ in the PAH high/low model, and $\beta = -0.5$, $p=0.03$ in the Ln(PAH) model after excluding ETS (N=214)], indicating that prenatal ETS exposure is not a potential confounder of this association.

In the smaller subset having data for maternal intelligence (N=171), the estimated effect of PAH exposure remained consistent and significant [$\beta = -1.4$, $p=0.04$ for PAH high/low; $\beta = -0.6$, $p=0.05$ for continuous Ln(PAH)]. Prenatal exposure to ETS in the home and maternal intelligence were significant or borderline-significant covariates in this model (data not shown).

Neither lead nor dietary PAHs was a significant predictor of RCPM scores (<0.1) when included individually in the model. Lead (computed as a Ln-transformed variable) was not a significant predictor in the model after controlling for prenatal ETS exposure in the home, sex of

the child, and maternal education; and the effect of prenatal airborne PAH exposure remained significant with lead included in the model. The dietary route of exposure to PAHs was not a significant contributor to the effect of airborne PAHs, which remained significant after including dietary PAH in the model. The magnitude of the association between PAHs and RCPM was unchanged when either lead or dietary PAH exposure was included in the model.

Including birth head circumference or birth weight separately in the model did not alter the estimated effect of airborne PAH [adjusted for birth head circumference: $\beta = -1.4$, $p = 0.02$ for PAH high/low; $\beta = -0.6$, $p = 0.02$ for continuous $\ln(\text{PAH})$, $N = 214$; adjusted for birth weight: $\beta = -1.3$, $p = 0.03$ for PAH high/low; $\beta = -0.5$, $p = 0.02$ for continuous $\ln(\text{PAH})$, $N = 214$].

With respect to postnatal exposure to PAHs, we found no effect of postnatal urinary PAH metabolites on the magnitude of the effect of PAHs on RCPM. Twenty-three percent of families changed neighborhood of residence in the child's first three years of life, with a likely though unmeasured change in airborne PAH exposure. Adjusting for change in neighborhood of residence did not alter the strength of the inverse association found between prenatal airborne PAHs and RCPM score. Controlling for postnatal exposure to ETS in the home (22% of mothers reported exposure during at least one of the 10 follow-up interviews given between birth and the child's follow-up at age 5 years) did not alter the estimated effect of PAHs.

There was no evidence of an interaction between trimester of PAH monitoring, PAH monitoring season, or season of birth. The p-values of interaction terms were greater than 0.38 in the models with dichotomized PAH and greater than 0.25 in the models with log transformed PAH.

Finally, in order to better compare the Krakow cohort to the NYC cohort, we restricted analysis to the Krakow participants within the common PAH exposure range seen in Krakow and NYC (0.27-44.81 ng/m^3) and to the subset of women for whom data on maternal intelligence

were available (so that the models would be directly comparable). In this comparison, the estimated effect sizes in both the dichotomous and continuous models were similar to those observed in the entire Polish cohort (over the full exposure range: 1.8-272.2 ng/m³) (Table 4).

Discussion

We have found that higher prenatal exposure to airborne PAHs is associated with a modest and statistically significant reduction in scores on a test of non-verbal child intelligence in a sample of 5 year old children of non-smoking mothers from Krakow, Poland, after controlling for confounding variables. Children in the high exposure group (>17.96 ng/m³) had RCPM scores that were on average 1.4 points lower (p=0.02) compared to less exposed children (≤17.96 ng/m³), corresponding to an estimated 3.8 point average decrease in IQ points. The relationship between prenatal airborne PAH and intelligence at age 5 years remained significant after controlling for postnatal exposure to PAHs and ETS in the home.

Because the Home Observation for Measurement of the Environment (HOME) Inventory, a measure of the child's proximal caretaking environment which can confound a study on neurodevelopment (Bradley et al. 1996), is not widely used in Central Europe, it was not administered in the Polish cohort as in the parallel NYC cohort study; this constitutes a limitation of this study. However, we included maternal education in our models to partially account for the mothers' important role in stimulating the child (Kagan and Moss 1959; McAskie and Clarke 1976; Noble and McCandliss 2005; Jedrychowski et al. 2009a). Because of the concern that this dichotomized variable (completed vs. did not complete 12 years of schooling) may itself not be sensitive enough to control for differences in the home environment and account for potential confounding of PAH effects by maternal education, we also used 'total years of education

completed by the mother' as a continuous variable, and found no differences in the results. We also found that personal airborne PAH levels were not correlated with total years of education completed by the mother ($r = -0.038$, $p = 0.488$, $N = 333$), mitigating concern that unmeasured differences in socio-economic status may have confounded our findings on PAH exposure. Maternal intelligence was available for a subset of 171 women and was significantly associated with child IQ. After adjustment for maternal intelligence, the effect of prenatal PAH exposure was significant ($p = .0036$ for high/low PAH and $p = 0.039$ for continuous PAH).

Prenatal ETS in the home was significantly associated with child IQ but did not confound the association between airborne PAHs and child intelligence. The association between prenatal exposure to ETS and deficits in early cognitive functioning has been established previously (Eskenazi and Castorina 1999). Prior research in the same Krakow cohort showed that cotinine levels measured in newborns' plasma were significantly higher than mothers' blood, indicating that the fetus may be less able to detoxify this substance (Jedrychowski et al. 2007). Prenatal ETS was not correlated with airborne PAHs (Table 2), consistent with the analysis of Choi and colleagues which showed that all eight speciated PAHs monitored have outdoor sources identified with coal combustion, though some are also related to ETS (Choi et al. 2006).

Transplacental exposures to PAHs have been linked to decrements in head circumference, birth weight, and length (Dejmek et al. 2000; Perera et al. 2003; Whyatt et al. 1998). These decrements have potential longer-term implications for producing lower cognitive functioning and poorer school performance in childhood (Hack et al. 1991). However, in this analysis neither the newborn's head circumference nor birth weight predicted intelligence at 5 years of age.

Relying on a single measurement of prenatal air for our exposure matrix is limited. However, because measurements during the second and third trimesters were correlated (Choi et al. 2008), we considered the single monitoring timepoint to be a reasonable indicator of prenatal exposure via inhalation over the last two trimesters of pregnancy. Despite known seasonal variation in air pollution levels related to heating with coal in Krakow (Choi et al. 2008), the potential effect of season on our results was mitigated by the fact that monitoring was evenly distributed across seasons (N=86, 81, 85 and 92 mothers were monitored in spring, summer, fall and winter, respectively). We also considered the limitation of using a 48-hour monitoring period to represent a longer continuous exposure period. However, we found no effect modification by trimester of PAH monitoring, PAH monitoring season or season of birth on any of our findings. We also did not make the assumption that the second and/or third trimesters of pregnancy are the most vulnerable periods with respect to brain development. The first trimester may be equally or more important.

To our knowledge, there has only been one prior epidemiological study reporting an inverse association between prenatal exposure to PAH and child intelligence at age 5 years, and that is from our Center's ongoing cohort study in NYC (Perera et al. 2009b). By design, all variables were measured similarly in NYC and Krakow, except for the neurodevelopment assessment tool used (RCPM in Krakow vs. the WPPSI-R score in NYC). The NYC study included African-Americans and Dominican-Americans; the Krakow study, Caucasians. Data on the home caretaking environment were available only in New York City. A further limitation of the Krakow study is the fact that the data on maternal intelligence were only available for a subset of the participants.

Nonetheless, the results of the two cohort studies, one in African-Americans and Dominicans and the other in Caucasians, are generally consistent both across the full range of exposure and restricting to the common, lower range. To compare the two different studies, we standardized the exposure effect sizes (β /SE) and found that they were similar in the two cohorts at age 5 years: -2.06 (95% CI:-4.02,-0.1) on RCPM in Krakow (entire range) compared with -2.63 (95% CI: -4.59, -0.67) for Full Scale IQ in the NYC cohort (Perera et al. 2009b). Restricting to the common, lower exposure range, the standardized exposure effect size (β /SE) was -2.56 (95% CI: -4.52, -0.6) on RCPM in Krakow, indicating general consistency of results across these two different cohorts at age 5 years. Because the NYC and Krakow cohorts represent a wide range of exposures experienced in many other urban areas, study results in Krakow and NYC are relevant to other populations worldwide.

As noted, there are a number of limitations in this study. They include the single 48-hour prenatal monitoring, the lack of HOME Inventory data, and the fact that, although the prenatal ETS questionnaire data were validated by cotinine, the estimate of postnatal ETS was based on interviews alone.

In summary, these results indicate that airborne PAHs may adversely affect children's cognitive development at 5 years of age, with potential implications for school performance. The present findings are of concern because RCPM scores measured during the preschool period have been shown to correlate with academic achievement later in life (Balboni et al. 2009; Raven 2000). We are continuing to follow this cohort in order to determine longer-term effects of prenatal exposure on behavioral outcomes and academic readiness.

References

- Archibong AE, Inyang F, Ramesh A, Greenwood M, Nayyar T, Kopsombut P, et al. 2002. Alteration of pregnancy related hormones and fetal survival in F-344 rats exposed by inhalation to benzo(a)pyrene. *Reprod Toxicol* 16:801-808.
- ATSDR. 1995. Toxicological profile for polycyclic aromatic hydrocarbons (PAHs). Atlanta, GA: Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services, Public Health Service. Available: <http://www.atsdr.cdc.gov/toxprofiles/tp69.html> [accessed].
- Balboni G, Naglieri JA, Cubelli R. 2009. Concurrent and Predictive Validity of the Raven Progressive Matrices and the Naglieri Nonverbal Ability Test. *Journal of Psychoeducational Assessment* published on August 25, 2009 as doi: 10.1177/0734282909343763:
- Barker DJ. 2004. The developmental origins of adult disease. *J Am Coll Nutr* 23:588S-595S.
- Bellinger D, Leviton A, Wateraux C. 1989. Lead IQ and social class. *International journal of epidemiology* 18:180-185.
- Bernert JT, Turner WE, Pirkle JL, Sosnoff CS, Akins JR, Waldrep MK, et al. 1997. Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. *Clinical Chemistry* 43:2281-2291.
- Bradley RH, Corwyn RF, Whiteside-Mansell L. 1996. Life at home: same time, different places- an examination of the HOME inventory in different cultures. *Early Development & Parenting* 5:251-269.
- Bui QQ, Tran MB, West WL. 1986. A comparative study of the reproductive effects of methadone and benzo(a)pyrene in the pregnant and pseudopregnant rat. *Toxicology* 42:195-204.

Page 19

Camann DE, Whyatt RM. 2001. Retention and storage stability of pesticides and PAH in PUF air samples. In: Proceedings of the 11th Annual Meeting of International Society of Exposure Analysis, #172. Charleston, SC: International Society of Exposure Analysis.

Choi H, Jedrychowski W, Spengler J, Camann DE, Whyatt RM, Rauh V, et al. 2006.

International studies of prenatal exposure to polycyclic aromatic hydrocarbons and fetal growth. *Environ Health Perspect* 114:1744-1750.

Choi H, Perera FP, Pac A, Wang L, Flak E, Mroz E, et al. 2008 Estimating Individual-Level Exposure to Airborne Polycyclic Aromatic Hydrocarbons throughout the Gestational Period Based on Personal, Indoor, and Outdoor Monitoring *Environ Health Perspect* 116:1509-1518.

Counter SA, Buchanan LH, Ortega F. 2005. Neurocognitive impairment in lead-exposed children of Andean lead-glazing workers. *J Occup Environ Med* 47:306-312.

Counter SA, Buchanan LH, Ortega F. 2009. Neurocognitive screening of lead-exposed Andean adolescents and young adults. *Journal of Toxicology and Environmental Health, Part A* 72:625-632.

Date AR, Gray AL. 1989. Applications of inductively coupled plasma mass spectrometry. New York, NY: Chapman and Hall.

Dejmek J, Solansky I, Benes I, Lenicek J, Sram RJ. 2000. The impact of polycyclic aromatic hydrocarbons and fine particles on pregnancy outcome. *Environ Health Perspect* 108:1159-1164.

DeMauro GE. 2000. Review of the toni-2. Second edition. *Mental Measurements Yearbook*.

Eskenazi B, Castorina R. 1999. Association of prenatal maternal or postnatal child environmental tobacco smoke exposure and neurodevelopmental and behavioral problems in children. *Environmental health perspectives* 107:991-100.

Hack M, Breslau N, Weissman B, Aram D, Klein N, Borawski E. 1991. Effect of very low birth weight and subnormal head size on cognitive ability at school age. *The New England journal of medicine* 325:231-237.

Jedrychowski W, Bendkowska I, Flak E, Penar A, Jacek R, Kaim I, et al. 2004. Estimated risk for altered fetal growth resulting from exposure to fine particles during pregnancy: an epidemiologic prospective cohort study in Poland. *Environ Health Perspect* 112:1398-1402.

Jedrychowski W, Pac A, Choi H, Jacek R, Sochacka-Tatara E, Dumyahn TS, et al. 2007. Personal exposure to fine particles and benzo[a]pyrene. Relation with indoor and outdoor concentrations of these pollutants in Krakow. *Int J Occup Med Environ Health* 20:339-348.

Jedrychowski W, Perera F, Jankowski J, Mrozek-Budzyn D, Mroz E, Flak E, et al. 2009a. Gender specific differences in neurodevelopmental effects of prenatal exposure to very low-lead levels: The prospective cohort study in three-year olds. *Early Hum Dev* 85:503-510.

Jedrychowski W, Perera F, Jankowski J, Mrozek-Budzyn D, Mroz E, Flak E, et al. 2009b. Very Low Prenatal Exposure to Lead and Mental Development of Children in Infancy and Early Childhood. Krakow Prospective Cohort Study. *Neuroepidemiology* 32:270-278.

Kagan J, Moss H. 1959. Parental correlates of child's IQ and height: a cross-validation of the Berkeley growth study results. *Child development* 30:325-332.

Li Z, Romanoff LC, Trinidad DA, Hussain N, Jones RS, Porter EN, et al. 2006. Measurement of urinary monohydroxy polycyclic aromatic hydrocarbons using automated liquid-liquid extraction and gas chromatography/isotope dilution high-resolution mass spectrometry. *Anal Chem* 78:5744-5751.

Manchester DK, Gordon SK, Golas CL, Roberts EA, Okey AB. 1987. Ah receptor in human placenta: stabilization by molybdate and characterization of binding of 2,3,7,8-

Page 21

tetrachlorodibenzo-p-dioxin, 3-methylcholanthrene, and benzo(a)pyrene. *Cancer Res* 47:4861-4868.

McAskie M, Clarke A. 1976. Parent-Offspring resemblances in intelligence-Theories and evidence. *British Journal of Psychology* 67:243-273

Metzer R, Delgado JL, Herrell R. 1995. Environmental Health and Hispanic Children. *Environmental health perspectives* 103:25-32.

Neubert D, Tapken S. 1988. Transfer of benzo(a)pyrene into mouse embryos and fetuses. *Arch Toxicol* 62:236-239.

Nicol CJ, Harrison ML, Laposa RR, Gimelshtein IL, Wells PG. 1995. A teratologic suppressor role for p53 in benzo[a]pyrene-treated transgenic p53-deficient mice. *Nat Genet* 10:181-187.

Noble K, McCandliss B. 2005. Reading development and impairment: Behavioral, social, and neurobiological factors. *Journal of Developmental and Behavioral Pediatrics* 26:370-378.

NRC. 1993a. *Issues in Risk Assessment*. Washington, DC: National Academy Press.

NRC. 1993b. *Pesticides in the diets of infants and children*. Washington, DC: National Academy Press.

Perera F, Tang D, Whyatt R, Lederman SA, Jedrychowski W. 2005. DNA damage from polycyclic aromatic hydrocarbons measured by benzo[a]pyrene-DNA adducts in mothers and newborns from Northern Manhattan, the World Trade Center Area, Poland, and China. *Cancer Epidemiol Biomarkers Prev* 14:709-714.

Perera F, Tang WY, Herbstman J, Tang D, Levin L, Miller R, et al. 2009a. Relation of DNA methylation of 5'-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. *PLoS ONE* 4:e4488.

Perera FP, Li Z, Whyatt R, Hoepner L, Wang S, Camann D, et al. 2009b. Prenatal polycyclic aromatic hydrocarbon exposure and child intelligence at age 5. *Pediatrics* 124:e195-e202.

Perera FP, Rauh V, Tsai WY, Kinney P, Camann D, Barr D, et al. 2003. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multi-ethnic population. *Environ Health Perspect* 111:201-205.

Perera FP, Rauh V, Whyatt RM, Tsai WY, Tang D, Diaz D, et al. 2006. Effect of prenatal exposure to airborne polycyclic aromatic hydrocarbons on neurodevelopment in the first 3 years of life among inner-city children. *Environ Health Perspect* 114:1287-1292.

Rauh VA, Whyatt RM, Garfinkel R, Andrews H, Hoepner L, Reyes A, et al. 2004. Developmental effects of exposure to environmental tobacco smoke and material hardship among inner-city children. *Neurotoxicol Teratol* 26:373-385.

Raven J. 2000. Manual for Raven's progressive matrices and vocabulary scales: research supplement no. 3. London: Oxford Psychologists Press Ltd.

Raven J, Raven JC, Court JH. 1998. Manual for Raven's progressive matrices and vocabulary scales: section 2 coloured progressive matrices. London: Oxford Psychologists Press Ltd.

Rodier PM. 2004. Environmental causes of central nervous system maldevelopment. *Pediatrics* 113:1076-1083.

Sattler J. 1986. *Assessment of Children*. 3rd edition ed. San Diego: Jerome M. Sattler, Publisher, Inc.

Saunders CR, Das SK, Ramesh A, Shockley DC, Mukherjee S. 2006. Benzo(a)pyrene-induced acute neurotoxicity in the F-344 rat: role of oxidative stress. *J Appl Toxicol* 26:427-438.

Schwartz J. 2004. Air pollution and children's health. *Pediatrics* 113:1037-1043.

Sizemore O, Amler R. 1996. Characteristics of ATSDR's adult and pediatric environmental neurobehavioral test batteries. . *Neurotoxicology* 17:229-236.

Sram RJ, Binkova B. 2000. Molecular epidemiology studies on occupational and environmental exposure to mutagens and carcinogens, 1997-1999. *Environmental health perspectives* 108 57-70.

Szustrowa TJA. 1992. Podrecznik do testu matrycy Ravena, wersja kolorowa (1956). Warszawa: Pracownia Testow Psychologicznych Polskiego Towarzystwa Pscychologicznego.

Takeda K, Tsukue N, Yoshida S. 2004. Endocrine-disrupting activity of chemicals in diesel exhaust and diesel exhaust particles. *Environ Sci* 11:33-45.

Tonne CC, Whyatt RM, Camann DE, Perera FP, Kinney PL. 2004. Predictors of personal polycyclic aromatic hydrocarbon exposures among pregnant minority women in New York City. *Environ Health Perspect* 112:754-759.

Vartiainen E, Seppala T, Lillsunde P, Puska P. 2002. Validation of self reported smoking by serum cotinine measurement in a community-based study. *J Epidemiol Community Health* 56:167-170.

Whyatt RM, Bell DA, Santella RM, Garte SJ, Jedrychowski W, Gladek-Yarborough A, et al. 1998. Polycyclic aromatic hydrocarbon-DNA adducts in human placenta and modulation by CYP1A1 induction and genotype. *Carcinogenesis* 19:1389-1392.

Whyatt RM, Perera FP. 1995. Application of biologic markers to studies of environmental risks in children and the developing fetus. *Environ Health Perspect* 103:105-110.

Wilson VL, Jones PA. 1983. Inhibition of DNA methylation by chemical carcinogens in vitro. *Cell* 32:239-246.

Page 24

Wood KA, Youle RJ. 1995. The role of free radicals and p53 in neuron apoptosis in vivo. *J Neuroscience* 15:5851-5857.

World Health Organization. 1986. Principles for evaluating health risks from chemicals during infancy and early childhood: The need for a special approach. In: *Environmental Health Criteria* 59. Geneva, Switzerland: World Health Organization.

Wormley DD, Ramesh A, Hood DB. 2004. Environmental contaminant-mixture effects on CNS development, plasticity, and behavior. *Toxicol Appl Pharmacol* 197:49-65.

Table 1: Krakow Cohort: Subset with airborne Polycyclic Aromatic Hydrocarbon (PAH) monitoring data and Raven Coloured Progressive Matrices (RCPM) data (219)^a vs. Subset with airborne PAH monitoring data but missing RCPM data (139)

Continuous Variables	p-value ^b	Subset with airborne PAH monitoring and RCPM		Subset with airborne PAH monitoring but missing RCPM	
		Mean ± SD	N ^c	Mean ± SD	N ^d
Total valid airborne PAH exposure (ng/m ³)	0.65	39.62 ± 48.49	219	39.39 ± 47.70	125
Dietary exposure to PAHs during pregnancy ^e	0.31	42.49 ± 5.65	219	43.14 ± 6.27	139
Cord blood cotinine level (ng/mL)	0.49	2.04 ± 11.52	219	3.29 ± 15.56	138
Cord blood lead level (µg/dl)	0.59	1.60 ± 1.19	210	1.51 ± 0.78	129
Cord blood mercury level (µg/dl)	0.43	1.07 ± 0.64	161	1.16 ± 0.79	106
Gestational age (days)	0.07	39.26 ± 1.73	219	39.51 ± 1.63	139
Birth weight (g)	0.01	3368.49 ± 508.38	219	3501.65 ± 474.22	139
Birth height (cm)	0.76	54.44 ± 3.06	219	54.54 ± 3.10	139
Head circumference at birth (cm)	0.21	33.81 ± 1.54	219	34.01 ± 1.47	139
Mother's age (years)	0.01	28.50 ± 3.59	219	27.49 ± 3.73	139
Maternal height (cm)	0.18	164.69 ± 5.71	219	165.52 ± 5.59	139
Maternal pre-pregnancy weight (kg)	0.95	58.44 ± 8.80	219	58.50 ± 9.32	139
Maternal test of non-verbal intelligence (TONI-3)	0.23	33.48 ± 9.07	176	31.42 ± 9.71	31

Maternal blood cotinine level at birth (ng/mL)	0.97	1.68 ± 10.09	219	2.99 ± 14.34	138
Maternal blood lead level at birth (µg/dl)	0.79	1.92 ± 0.83	209	1.86 ± 0.66	136
Categorical Variables	p-value ^f	Proportion	N	Proportion	N
Caucasian Race	1.00	1.00	219	1.00	139
Maternal education (proportion graduated high school, i.e. 12 years of schooling)	0.47	0.91	219	0.88	139
ETS in the home (yes=1, no=0)	0.52	0.22	219	0.25	139
Exposure to alcohol during pregnancy (yes=1, no=0)	0.18	0.60	219	0.67	139
Sex of the child (proportion female)	0.52	0.52	219	0.48	139

^a Among these 219 mother-child pairs with valid PAH monitoring data and complete RCPM data, 5 pairs had blood cotinine levels > 25ng/mL and were not included in the sample size of 214 presented in our final model (Table 3). Mother-child pairs included in Table 1 were not restricted based on blood cotinine level, as we thought this might bias our comparisons.

^b p-values were generated by two sample t-tests, except for cord blood cotinine level, maternal test of non-verbal intelligence, maternal blood cotinine level at birth, and gestational age, which were not normally distributed, and for which non-parametric Wilcoxon tests were therefore used.

^c If the sample size is <219, some subjects are missing data on the variable in question.

^d If the sample size is <139, some subjects are missing data on the variable in question. In the case of PAH exposure levels, only 125 of 139 had valid PAH data; i.e., meeting quality control standards.

Page 27

^e estimated by the frequency of intake of PAH-containing foods during pregnancy, as reported in the prenatal questionnaire by the mother

^f p-values were generated by Fisher Exact test

Table 2. Krakow Cohort: Spearman Correlations (correlation coefficient r , p -value (N))

	ETS	Maternal cotinine	Cord cotinine	Total airborne PAHs
Fully enrolled participants with adequate monitoring data				
Dietary PAH	0.09, 0.11 (344)	-0.02, 0.70 (343)	0.03, 0.60 (343)	0.06, 0.25 (344)
ETS		0.48, <0.01 (343)	0.47, <0.01 (343)	-0.05, 0.38 (344)
Maternal cotinine	--		0.85, <0.01 (343)	0.08, 0.15 (343)
Cord cotinine	--	--		0.03, 0.53 (343)
Subset of children included in the present analysis (N=214) ^a				
Dietary PAH	0.19, 0.01	-0.04, 0.57	-0.04, 0.54	0.05, 0.46
ETS		0.48, <0.01	0.46, <0.01	-0.05, 0.50
Maternal cotinine	--		0.99, <0.01	0.02, 0.75
Cord cotinine	--	--		0.04, 0.60

^a This subset excludes subjects with monitoring data not meeting quality control criteria, with maternal or cord cotinine levels > 25 ng/mL, and if any of the following data are missing: child RCPM score, prenatal ETS in the home, sex of child, or maternal education.

Table 3. Association Between Prenatal Exposure to Airborne PAHs and RCPM Score at Age 5 Years in the Krakow Cohort (N=214)^{a, b}

N=214	β	95% CI		p-value	N=214	β	95% CI		p-value
PAH (high/low)	-1.36	-2.48	-0.23	0.02	Ln(PAH)	-0.56	-1.00	-0.11	0.02
ETS in the home ^c	-1.83	-3.3	-0.36	0.02	ETS in the home	-1.86	-3.32	-0.39	0.02
sex of child ^d	0.57	-0.56	1.69	0.33	sex of child	0.54	-0.58	1.67	0.35
maternal education ^e	0.81	-1.32	2.93	0.46	maternal education	0.75	-1.38	2.87	0.50

^a This table presents two models side by side, one with dichotomous PAH (“high/low”) and one with continuous Ln-transformed PAH (“Ln(PAH)”). They both adjust for prenatal ETS in the home, sex of the child, and maternal education.

^b As noted in the text, after further including maternal intelligence (using continuous TONI-3 score) which was significantly associated with child intelligence in the model, the betas and p-values for PAH both high/low and Ln-transformed were similar and significant (N=171). Lead, dietary PAHs, birth weight, birth head circumference, individual creatinine-adjusted PAH metabolites from urine, maternal report of postnatal exposure to ETS in the home, and whether the child changed residence postnatally, were not significant predictors ($p < 0.1$) and were therefore not included in the final model presented here.

^c maternal report of smoke in the home during pregnancy: yes=1, no=0

^d female=1, male=0

^e graduated from high school, i.e. completed 12 years of schooling: yes=1, no=0

Table 4. Association Between Prenatal Exposure to Airborne PAHs and RCPM Score at Age 5 Years in the Krakow Cohort Including only Subjects Whose PAH Exposure was Within the Common Exposure Range Between the NYC and Krakow Cohorts (0.27-44.81 ng/m³)

N=150	β	95% CI		p-value	N=150	β	95% CI		p-value
PAH (high/low)	-1.62	-2.98	-0.26	0.02	Ln(PAH)	-1.24	-2.05	-0.43	<0.01
ETS	-2.07	-3.75	-0.38	0.02	ETS	-1.91	-3.58	-0.25	0.02
sex of child	0.63	-0.73	1.99	0.37	sex of child	0.73	-0.61	2.07	0.28
maternal education	0.77	-2.02	3.57	0.59	maternal education	1.00	-1.77	3.77	0.48