Article Title:

Using urinary biomarkers to evaluate polycyclic aromatic hydrocarbon exposure in 126 preschool children in Ohio

Journal Name:

International Journal of Environmental Health Research

Corresponding Author:

Marsha K. Morgan, US EPA's National Exposure Research Laboratory, Research Triangle Park, NC, United States; *morgan.marsha@epa.gov, phone: +1 919.541.2598*

Co-authors:

Paul A. Jones, US EPA's National Exposure Research Laboratory, Research Triangle Park, NC, United States, *jones.paul-A@epa.gov: phone: +1 919.541.1516*

Jon R. Sobus, US EPA's National Exposure Research Laboratory, Research Triangle Park, NC, United States, *sobus.jon@epa.gov, phone: +1 919.541.2332;*

Jane C. Chuang, *Battelle (retired), Columbus, OH 43201, United States; ccjane20@hotmail.com*

Nancy K. Wilson; *Battelle (retired), Durham, NC 27713, United States, Current contact: Wilson Associates, 6319 Mimosa Drive, Chapel Hill, NC 27514; njkwilson@nc.rr.com*

Acknowledgements:

''The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research described here under Contract #68-D-99-011 to Battelle. It has been subjected to Agency review and approved for publication."

Using urinary biomarkers to evaluate polycyclic aromatic hydrocarbon exposure in 126 preschool children in Ohio

Limited data exist on exposures of young children to polycyclic aromatic hydrocarbons (PAHs) in the United States (US). The urinary metabolite of pyrene, 1-hydroxypyrene (1-OHPyr), is widely used as a biomarker of total PAH exposure. Our objectives were to quantify urinary 1-OHPyr levels in 126 preschool children over a 48-hour period and to examine associations between selected sociodemographic/lifestyle factors and urinary 1-OHPyr levels. Monitoring was performed at 126 homes and 16 daycares in Ohio in 2001, and questionnaires and urine samples were collected. The median urinary 1-OHPyr level was 0.33 ng/mL. In a multiple regression model, sampling season (p=0.0001) and natural log (ln) transformed creatinine concentration (p=0.0006) were highly significant predictors of ln-transformed 1-OH-Pyr concentration; cooking appliance type (p=0.096) was a marginally significant predictor of ln(1-OHPyr). These children had higher median urinary 1-OHPyr levels compared to other US children ($\leq$ 0.15 ng/mL) in previously published studies, which suggests possible geographical differences in PAH exposure.

Keywords: biomarker; pyrene; exposure; 1-hydroxypyrene; Ohio

Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of over 100 chemicals that are produced from incomplete combustion of organic materials and are ubiquitous environmental contaminants worldwide (Phillips 1999; Mucha et al. 2006; Martinez-Salinas et al. 2010). Common sources of non-occupational exposures to PAHs are grilled and broiled foods, tobacco smoke, ambient air pollution, automobile exhaust, and wood-burning stoves and fireplaces (Mucha et al. 2006; Lee et al. 2009). In the United States (US), at least 20 different PAH's, including Group 1 (human carcinogens), and Group 2A (probable human carcinogens), and 2B (possible human carcinogens) PAHs, have been detected in soil, dust, air, hand wipes, and/or food samples collected at children's residences and daycare centers (Chuang et al. 1999; Wilson et al. 2000, 2001, 2003; IARC 2010; IRIS 2014). Research has indicated that inhalation and dietary ingestion of PAHs are major exposure routes for children in these settings (Chuang et al. 1999; Wilson et al. 2000.).

Once absorbed into the body, the lipophilic PAHs are rapidly metabolized in the liver (half-lives < 24-hour) and are primarily excreted as conjugated hydroxy-PAH metabolites in urine or feces (Buckley and Lioy 1992; Li et al. 2008). Monohydroxy-PAH metabolites (e.g., 1-hydroxypyrene [1-OHPyr]) are commonly measured in urine to assess implied human exposure to a number of PAHs, including benz[*a*]anthracene, benzo[*a*]pyrene, chrysene, indeno[1,2,3-*cd*]pyrene, and pyrene (Grainger et al. 2006). In addition the pyrene metabolite, 1-OHPyr is

commonly used as a surrogate urinary biomarker of total PAH exposure in humans, since PAHs generally occur as mixtures in the environment, and pyrene is typically a major component of these mixtures (Jacob and Seidel 2002; Huang et al. 2006; Mucha et al. 2006).

Several studies have reported measureable levels of urinary monohydroxy-PAH biomarkers in US children, ages 2-19 years old (Chuang et al. 1999; Northridge et al. 1999; Wilson et al. 2000, 2003; Grainger et al. 2006; Li et al. 2008). However, only a few studies have examined the influence of sociodemographic or lifestyle factors on urinary monohydroxy-PAH biomarker levels in exposed children (Chuang et al. 1999; Huang et al. 2006; Suwan-ampai et al. 2009). Chuang et al. (1999) reported that urbanicity and smoking households were not significantly associated with the sum of hydroxy-PAH levels in 24 preschool children from low-income families in North Carolina in 1994-1995. However, Huang et al. (2006) reported that exposure to environmental tobacco smoke and age group were significant predictors ($p \leq 0.05$) of 1-OHPyr concentrations in 814 children ($\geq$ 6 years old) from the 1999-2000 National Health and Nutrition Examination Survey (NHANES), a US general population study. In a later analysis of NHANES 1999-2002 data, Suwan-ampai et al. (2009) also showed that environmental tobacco smoke exposure, age group, and poverty income ratio were significant predictors ($p \leq 0.05$) of urinary 1-OHPyr levels in 2,120 children. These studies provide evidence that certain sociodemographic and lifestyle factors likely increase children's exposures to PAHs in their everyday environments. However, more research is needed since data are limited on the driving factors that influence the non-occupational exposures of young children to PAHs in the US.

For this investigation, 1-OHPyr was utilized as a biomarker of total PAH exposure. The objectives of this study were to quantify urinary levels of 1-OHPyr in 126 preschool-aged children over a 48-hour monitoring period six counties in Ohio and to examine associations between selected sociodemographic or lifestyle factors and urinary 1-OHPyr levels.

Materials and methods

Study cohort

The Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study investigated the exposures of 257 preschool children (ages 2–5 years old) and their adult caregivers (living in the same households) to over 40 chemicals commonly found in their

everyday surroundings. An in-depth description of the study design and sampling methodology can be found elsewhere (Wilson et al. 2004). Briefly, the CTEPP study participants were recruited from six counties in North Carolina and from six counties in Ohio. The overall CTEPP study participants were 129 children and their adult caregivers from North Carolina and 128 children and their adult caregivers from Ohio. About half of the children attended daycare during the day in each state. Field sampling activities were performed at the children's homes and daycare centers between July 2000 and March 2001 in North Carolina and April 2001 and November 2001 in Ohio.

As part of the CTEPP study, several monohydroxy-PAH metabolites were measured in the children's urine samples to assess PAH exposures in these settings. Biomarkers of benz[*a*]anthracene (i.e., 1-hydroxybenz[*a*]anthracene and 3-hydroxybenz[*a*]anthracene), benzo[*a*]pyrene (i.e., 3-hydroxybenzo[*a*]pyrene), chrysene (i.e., 3-hydroxychrysene and 6-hydroxypyrene), and indeno[1,2,3-*cd*]pyrene (i.e., 6-hydroxy indeno[1,2,3-*cd*]pyrene) were found to be below the analytical limits of detection (0.2 ng/mL) in the majority of children's urine samples (> 5%). While only monitored in samples from the Ohio component of the study (due to an expansion of the analytical method), 1-OHPyr levels were detectable in most of the Ohio children's urine samples (79%) and are therefore used here as a quantitative surrogate for total PAH exposure. Our final dataset consisted of records for 126 preschool children from the Ohio component of the study. Two additional children were excluded from this dataset as they had either missing questionnaire data or urinary biomarker data.

Human subjects protection

The CTEPP study was classified as an observational exposure measurements study as defined in 40 Code of Federal Regulations in Part 26.402 (US ECFR, 2014). The CTEPP study protocol and procedures to obtain the informed consent of the adult participants and the assent of their children were reviewed by and received approval from an independent institutional review board in 1999 and has complied with all requirements of the Common Rule involving the added protections of study children (Subpart D). Adult participants signed informed consent forms for themselves and their children before participating in this study. Participants were also assigned new identification numbers in the CTEPP study database, so they cannot be identified by themselves, the public, or in this manuscript.

Questionnaires and urine sample collection

Adult caregivers (i.e., parents and daycare teachers) filled out five different types of hardcopy questionnaires during the 48-hour monitoring period at home and/or at daycare. The questionnaires were used to collect diverse types of information on the study children including personal characteristics (e.g., age, sex, income status, and race), daily activities (e.g., time spent outdoors), and other exposure-related information such as traffic conditions, household smoking habits, and household cooking methods/habits.

Spot urine samples were collected from each child over the 48-hour monitoring period. Three spot urine samples (first morning void, after lunch and before bedtime) were collected from the children by the adult caregivers each sampling day when at home. For children at daycare centers, the teachers collected one spot urine sample from the participating children in their classrooms after lunch each sampling day. Spot urine samples from the children were collected by placing a plastic bonnet under the toilet seat and then pouring the urine into a 120 mL polypropylene jar with lid. All urine samples were immediately placed into coolers with blue ice by the adult caregivers over the 48-hour monitoring period. Field technicians picked up the coolers from the children's homes or daycares and transported the coolers at reduced temperatures in motor vehicles to the Battelle laboratory in Columbus, Ohio. Urine samples were stored in laboratory freezers at $\leq$ -10^{o} C until chemical analyses.

Urine sample analyses and quality assurance

At the Battelle laboratory, spot urine samples collected at the daycare centers were pooled over the 48-hour monitoring period into one sample per child. At the homes, spot urine samples were pooled over the 48-hour monitoring period into one sample for each child, except for those that had a recent pesticide application within seven days at their residences. For these 15 children, their urine samples were not pooled and each collected sample was analyzed separately.

The method used for the extraction and analysis of 1-OHPyr in the urine aliquots has been described earlier in Morgan et al. 2004. Briefly in 2001, a 1 mL aliquot was used from each pooled and non-pooled urine sample for chemical analyses. Each urine aliquot was hydrolyzed with 100 μL of hydrochloric acid in a vial and heated at 80^{o}C in an oven for one hour. Next, 1 mL of chlorobutane and 1 mL of 20% sodium chloride was added to the vial and centrifuged. The extracts

were then silylated with 100 µL N-(tert-butyldimethylsilyl)-N-methyltrifluoro-acetamide, heated at 70°C for 1 hour, and then transferred to a GC vial. The urine extracts were analyzed for 1-OHPyr by a gas chromatograph/mass selective detector (GC/MS) (Hewlett-Packard 6890/5973A) in the selected ion monitoring mode with an autosampler. The separation was performed using a DB-5 column (60 m x 0.32 mm, 0.25 um film thickness) that was coated with 5% phenyl methylsilicone, and helium was used as the carrier gas. The operating conditions for the GC/MS were set at 90°-290°C at 8°C per minute. We defined the limit of quantification (LOQ) as the "minimum concentration at which a chemical can be quantitatively measured in a sample with acceptable accuracy and precision" (Morgan et al. 2004). For 1-OHPyr, we estimated the LOQ by using the lowest calibration standard (2 ng/mL) with a signal-to-noise ratio above 2. The LOQ was calculated for each urine sample using the lowest calibration standard solution and sample size (Morgan et al. 2005). The LOQ for 1-OHPyr was 0.4 ng/mL. The estimated limit of detection (LOD) was one-half the reported LOQ for this analyte (i.e., 0.2 ng/mL).

For creatinine analysis, an additional 10 mL aliquot of urine was used from each pooled sample. Non-pooled urine samples were not analyzed for creatinine concentrations as they typically had small sample volumes. These urine aliquots were shipped to the Ohio State University Clinical Laboratory (Columbus, Ohio, USA) and analyzed for creatinine concentrations using the Jaffe Picric Colorimetric Method (Morgan et al. 2004).

Quality control samples (e.g., blanks, duplicates, and spikes) were used to assess the overall quality of urine sample collection and analyses. Field blanks and laboratory blanks for 1-OHPyr were all below the LODs in urine. Relative percent differences between duplicate samples (aliquots of the same sample) were less than 4% in urine for 1-OHPyr. For analytical duplicates (repeat analysis of the sample extract), relative percent differences were less than 7% for 1-OHPyr in urine. The matrix spikes for 1-OHPyr had a mean recovery of 92% in urine.

Statistical analyses

All statistical analyses were performed using SAS version 9.4 (SAS Cary, North Carolina). Descriptive statistics (sample size, detection frequency, mean and standard deviation, geometric mean [GM], percentiles [25^{th}, 50^{th}, 75^{th}, and 95^{th}], and range) were calculated for urinary 1-OHPyr concentrations as unadjusted (ng/mL) and creatinine-adjusted (ng/mg) values. Urine sample measurement values below the LOD were replaced by the $\mathrm{LOD}/\sqrt{2}$ (Verbovsek 2011). For

children that attended daycare during the day, we used the average 1-OHPyr concentration value of their pooled urine sample measurements collected at home and at daycare. In addition, for the children that had a recent pesticide application at home, we used the average 1-OHPyr concentration value of their non-pooled urine sample measurements (*up to six per person*). Since creatinine correction is a common method for adjusting for variable dilutions in spot urine samples, we have included these data in our analysis. Creatinine concentrations were calculated in the urine samples using the following equation: Creatinine-adjusted value (ng/mg) = 100 mL/dL x urine concentration (ng/mL) / creatinine concentration (mg/dL) (Morgan et al. 2004). However, this correction method may not be a reliable adjustment measure for children because factors such as age, diet, lean muscle mass, and physical activity have been shown to impact the variability of creatinine excretion over time (Boeniger et al. 1993: O'Rourke et al. 2000; Barr et al. 2005).

The distributions of the 1-OHPyr concentrations for children were found to be non-normal using the Shapiro-Wilk normality test (GraphPad Software version 5.04, San Diego, CA). Therefore, urinary 1-OHPyr concentrations were log-transformed (ln) to normalize the distributions. Two-sample t-tests or an analysis of variance (ANOVA) were used to assess the bivariate associations between ln-transformed urinary 1-OHPyr levels in children and selected sociodemographic factors (i.e., age group, sex, urbanicity, family income status, race, study site locations, sampling seasons, and traffic conditions) and lifestyle factors (i.e., time spent outdoors, household smoking habits, cooking appliances, and cooking habits). A multiple regression model was used to collectively examine relationships between ln(urinary 1-OHPyr levels) in children (dependent variable) and sociodemographic and lifestyle factors (independent variables) that had a p-value of $\leq$0.200 in the above bivariate analyses. Separate models were not used for children that attended daycare compare to those that stayed at home during the day since their GM urinary 1-OHPyr levels were not significantly different between these locations in the bivariate analyses. In this regression model, the summer and fall seasons were combined into one category as these covariates had similar geometric mean urinary 1-OHPyr levels (GM=0.37 and 0.40) compared to the spring season (GM=0.24)." In addition, ln-transformed creatinine concentration (mg/dL) was included as an independent variable; this allows adjustment of the ln-transformed 1-OHPyr concentrations for variable urine dilutions (Barr et al. 2005). The multiple regression analyses were performed using a sequential, step-wise backward elimination process (PROC REG) in SAS. Independent variables were excluded from the final model with p-values $\geq$0.15.

Results

Table 1 shows summary statistics of the unadjusted (ng/mL) and creatinine-adjusted (ng/mg) 1-OHPyr levels (not ln-transformed) for the preschool children. The 1-OHPyr metabolite was detected in 79% of the children's unadjusted urine samples. The median unadjusted urinary 1-OHPyr level for children was 0.33 ng/mL, and the highest value was 2.7 ng/mL. For the creatinine-adjusted values, the children's median urinary 1-OHPyr concentration was 0.42 ng/mg, and the maximum value 4.1 ng/mg.

Table 2 presents the bivariate associations between selected sociodemographic or lifestyle factors and urinary 1-OHPyr levels in children. The results showed that urinary 1-OHPyr concentrations (log-transformed) were significantly different ($p<0.05$) across the three sampling seasons (spring, summer, or fall) for the children, with spring having the lowest biomarker levels. In addition, the children's urinary levels of 1-OHPyr were significantly higher ($p=0.003$) for those living in homes with gas cooking appliances (GM=0.41 ng/mL) compared to electric cooking appliances (GM=0.29 ng/mL).

Results of our final reduced regression model showed that sampling season and ln(creatinine concentration) were highly significant predictors of ln(1-OHPyr), and cooking appliance type was a marginally significant predictor of ln(1-OHPyr) (Table 3). These three variables collectively explained 27% of the variability in 1-OHPyr levels in the children's urine samples. Sampling season and cooking appliance type accounted for 15% of the variability of the children's 1-OHPyr levels. In particular, the children had significantly ($p=0.0001$) higher urinary 1-OHPyr concentrations in the combined summer/fall season compared to the spring season. In addition, the children had marginally ($p=0.096$) higher levels of 1-OHPyr for those living in homes with gas cooking appliances compared to electric cooking appliances.

Discussion

In the US, few studies have been conducted that have quantified the levels of 1-OHPyr in the urine of children, particularly young children (Chuang et al. 1999; Northridge et al. 1999; Wilson et al. 2003; Huang et al. 2006; Li et al. 2008). Chuang et al. (1999) reported arithmetic mean 1-OHPyr levels of 0.13±0.25ng/mL (range = 0.009-1.23 ng/mL) for 24 children, ages 2-4 years, from low-income families in Durham, North Carolina in 1994-1995. In another smaller study, Wilson et. al.

(2003) showed that nine preschool children, ages 2-5 years old, had arithmetric mean levels of 0.08 ng/mL (range = 0.02-0.16 ng/mL) in central North Carolina in the summer of 1997. In the 1999-2000 NHANES, Huang et al. (2006) reported that the median urinary 1-OHPyr level was 0.09 ng/mL in 310 children, ages 6-11 years old. In a later survey (2001-2002), Li et al. (2008) reported lower median urinary 1-OHPyr concentrations (0.06 ng/mL) in 387 children, ages 6-11 years old. However, the urinary 1-OHPyr levels of NHANES children have generally increased over the last decade with the highest median levels (0.15 ng/mL) reported in the most recent 2007-2008 survey (CDC, 2013). In comparison to our study conducted in 2001, the Ohio preschool children had at least three times higher median levels of 1-OHPyr (0.33 ng/mL) compared to the NHANES (1999-2002). In addition, our children's median urinary 1-OHPyr levels were at four times higher (1.4 pmol/mL) compared to urinary 1-OHPyr levels (0.33 pmol/mL) of 26 children, 12-14 years old, from an exposure study of diesel exhaust conducted in Harlem, New York in 1997. This above information suggests that there may be geographical differences in children's exposure to pyrene, and likely to other PAHs, in the US. This is supported by Morgan et al. (2004) showing that the median levels of nine, 4-6 ring PAHs (*pyrene not measured*) were at least three times higher in the soil and carpet dust samples at CTEPP children's homes and daycares in Ohio compared to North Carolina in 2000-2001. Lewis et al. (1999) also found substantially higher levels of B2 PAHs in carpet dust samples from homes in Columbus, Ohio (mean = 72,000 ng/g) compared to homes in Seattle, Washington (mean = 11,000 ng/g) in 1992. The authors suggests that the use of fossil fuels for heating was likely a major source of the PAHs in the carpet dust from the homes in Ohio (Lewis et al. 1999). Other studies from Afganistan, Germany, South Korea, and Ukraine have also reported various median urinary 1-OHPyr levels of 3.2, 0.14, 1.6, and 0.31 ng/mL, respectively, in nonoccupationally-exposed children, ages 3-14 years old (Mucha et al. 2006; Wilhelm et al. 2008; Lee et al. 2009; Hemat et al. 2012). These above studies showed that children were exposed to pyrene, and likely to other PAHs, in their everyday environments worldwide. This is supported by published research reporting measureable levels of PAHs in a number of environmental media around the world (Van Wijnen et al. 1996; Lewis et al. 1999; Rey-Salgueiro et al. 2009; Martinez-Salinas et al. 2010).

An established oral reference dose (RfD) is currently available for pyrene, but not for total PAHs, in the United States Environmental Protection Agency's Integrated Risk Information System (IRIS 2014). The oral RfD for pyrene was based on noncarcinogenic effects (i.e., renal

toxicity) occurring in a subchronic oral bioassay in mice (IRIS, 2014). Assuming steady-state conditions, the maximum estimated daily intake dose of the CTEPP children (0.06 ug/kg-day) to pyrene was 500 times lower than the oral RfD of 30 ug/kg-day (IRIS 2014). This maximum daily intake dose was calculated by multiplying the highest unadjusted 1-OHPyr urine value (2.7 ng/mL) of a child by an estimated 24-hour urinary excretion rate of 22.4 mL/kg body weight (Miller and Stapleton 1989; Szabo and Fegyverneki 1995; Wilson et al. 2003). The excreted amount of 1-OHPyr was assumed to be equivalent to the intake amount of pyrene in the body.

We are unaware of any study conducted in the US that has examined the influence of sociodemographic or lifestyle factors on the urinary levels of 1-OHPyr in non-occupationally-exposed children (< 6 years). In perhaps the most comparable study, Chuang et al. (1999) found no significant associations between urbanicity or household smoking habits and the sum of urinary monohydroxy-PAH concentrations in 24 preschool children in North Carolina, with the lack of apparent association likely due to a small sample size. A few studies conducted in other counties have assessed the relationship between various sociodemographic and lifestyle factors and urinary 1-OHPyr concentrations in preschool children (van Wijnen et al. 1996; Tsai et al. 2003; Mucha et al. 2006; Freire et al. 2009). In a study conducted by Van Wijnen et al. (1996) in 1992, indoor sources of PAH's (i.e., household smoking habits and wood stoves and hearths) were significantly associated ($p < 0.05$) with higher urinary 1-OHPyr levels in 644 Dutch children, ages 1-6 years old. In another study, Tsai et al. (2003) showed that exposure to parental tobacco smoke (3-days prior to sample collection) was a major predictor of 1-OHPyr levels in 40 preschool children, ages 2-6 years old in southern Taiwan in 1999. In addition, Mucha et al. (2006) performed a study in two cities in Ukraine, Kiev and Mariupol, in 1998 and showed that city of residence was strongly associated ($p<0.001$) and second-hand smoke exposure was marginally associated ($p=0.07$) with the urinary 1-OHPyr levels of 90 three-year-old children. More recently, Freire et al. (2009) reported that predicted exposure to nitrogen dioxide was a strong predictor ($p=0.006$) and exposure to environmental tobacco smoke and cooking appliance were marginally significant predictors ($p\leq0.10$) of the urinary 1-OHPyr levels in 174 4-year-old Spanish children in 2005-2006. In the CTEPP study, our results showed that sampling season ($p=0.001$) and creatinine concentration ($p=0.0006$) were strong predictors and gas cooking appliance ($p=0.096$) was a marginally significant predictor of the children's 1-OHPyr levels. Our findings are supported by Tonne et al. 2004 that also showed seasonal differences in the levels of pyrene in personal ambient air

measurements collected from 348 participants in New York City, New York in 1997. The authors suggest that the substantially higher pyrene exposures in the summer compared to winter may have been due to increased temperature/volatilization of it from i.e., roads, vegetation, and soil. This above research indicates that certain sociodemographic factors (i.e., sampling season) and lifestyle factors (i.e., environmental tobacco smoke and wood/gas cooking appliances) can substantially influenced the variability of 1-OHPyr levels in children's urine. In addition, these factors suggests a linkage between children's exposures to pyrene via the inhalation of polluted air (including resuspended dust) and their urinary 1-OHPyr levels.

The study data had several limitations. The CTEPP study was conducted over a decade ago and may not reflect the current exposures of Ohio preschool children to pyrene. Also, the levels of pyrene were not measured in any of the environmental samples (soil, dust, air) and personal samples (food and hand wipes) collected in this study. These data would have helped to identify the major sources and routes of the children's exposures to pyrene in these settings. Since most of the children's urine samples were pooled over the 48-hour monitoring period, the short-term variability of 1-OHPyr levels could not be ascertained for this cohort of children. As PAHs often have short biological half-lives (< 24 hours), there may be considerable variability (within- and between-individual) in spot measurements of 1-OHPyr over this time period. Also, since the volume of the individual urine voids were not recorded in this study, urinary excretion rates could not be calculated which would have removed the impact of variable dilutions on analyte concentrations (Barr et al. 2005). In addition, only one analytical method was used to quantify the levels of 11 different chemical biomarkers in the urine samples which resulted in a higher LOD (0.2 ng/mL) for the monohydroxy-PAH metabolites compared to previously published US studies. In addition, the data could not be stratified by children's exposure to environmental tobacco smoke because cotinine was not measured in the urine samples. Finally, about 20% of the children in this study had 1-OHPyr concentrations in urine below the analytical LOD. Using single value imputation for these observations decreased the variability in the 1-OHPyr measurement distributions, which may have impacted final statistical parameter estimates.

Conclusion

Based on measured urinary 1-OHPyr levels, our study shows that these Ohio CTEPP preschool children were exposed to higher levels of pyrene, and likely to other PAHs, compared to other

children in previously published studies in the US. This finding suggests that there are geographical differences in young children's exposures to PAHs and that specific risk modifying factors (i.e., sampling season and type of cooking appliance) can increase their exposures to PAHs in these areas.

Conflict of interest

All authors declare that there are no conflicts of interest

References

Barr D, Wilder L, Caudill S, Gonzalez A, Needham L, Pirkle J. 2005. Urinary creatinine concentrations in the US population: implications for urinary biologic monitoring measurements. Environ Health Perspect. 113:192–200.

Boeniger M, Lowry L, and Rosenberg. 1993. J. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustment: A review. Am Ind Hyg Assoc. J 54: 615–627.

Buckley TJ, Lioy, PJ. 1992. An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. Brit J Ind Med. 49: 113–124.

Centers for Disease Control and Prevention (CDC). 2013. National Report on Human Exposure to Environmental Chemicals. Available online: http://www.cdc.gov/exposurereport/ (accessed on 4 June 2014).

Chuang JC, Callahan PJ, Lyu CW, Wilson NK. 1999. Polycyclic aromatic hydrocarbon exposures of children in low-income families. J Expo Anal Environ Epidemiol. 2:85–98.

Freire C, Abril A, Fernandez MF, Ramos R, Estarlich M, Manrique A, Aguirre A, Ibarluzea J, Olea N. 2009. Urinary 1-hydroxypyrene and PAH exposure in 4-year-old Spanish children. Sci Total Environ. 407:1562–1569.

Grainger J, Huang W, Patterson DG, Turner WE, Pirkle J, Caudill SP, Wang RY, Needham LL, Sampson EJ. 2006. Reference range levels of polycyclic aromatic hydrocarbons in the US population by measurement of urinary monohydroxy metabolites. Environ Res. 100:394–423.

Hemat H, Wittsiepe J, Wilhelm M, Muller J, Goen T. 2012. High levels of 1-hydroxypyrene and hydroxyphenanthrenes in urine of children and adults from Afghanistan. J Expo Sci Environ Epidemiol. 22:46–51.

Huang W, Caudill SP, Grainger J, Needham LL, Patterson DG. 2006. Levels of 1-hydroxypyrene and other monohydroxy polycyclic aromatic hydrocarbons in children: A study based on U.S. reference range values. Toxicol Lett. 163:10–19.

Integrated Risk Information System (IRIS). 2014. US EPA's Integrated Risk Information System Homepage. http://www.epa.gov/IRIS/ (accessed February 21, 2014).

International Agency for Research on Cancer (IARC). 2010. Monographs on the evaluation of carcinogenic risks to humans: Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures; volume 92; Lyon, France.

Jacob J, Seidel A. 2002. Review: Biomonitoring of polycyclic aromatic hydrocarbons in human urine. Journal of Chromatography B. 778:31–47.

Lee KH, Vermeulen R, Lenters V, Cho SH, Strickland PT, Kang D. 2009. Determinants of urinary 1-hydroxypyrene glucuronide in South Korean children. Int Arch Occup Environ Health. 82:961–968.

Lewis RG, Fortune CR, Willis RD, Camann DE, Antley JT. 1999. Distribution of pesticides and polycyclic aromatic hydrocarbons in house dust as a function of particle size. Environ Health Perspect. 107:721-726.

Li Z, Sandau CD, Romanoff LC, Caudill SP, Sjodin A, Needham LL, Patterson DG. 2008. Concentrations and profile of 22 urinary polycyclic aromatic hydrocarbon metabolites in the US population. Environ Res. 107:320–331.

Martinez-Salinas RI, Leal ME, Batres-Esquivel LE, Dominguez-Cortinas G, Calderon J, Diaz-Barriga F, Perez-Malonada IN. 2010. Exposure of children to polycyclic aromatic hydrocarbons in Mexico: assessment of multiple sources. Int Arch Environ Health. 83:617–623.

Miller L.A., and Stapleton F.B. 1989. Urinary volume in children with urolithiasis. J Urol. 141(4):918–920.

Morgan M, Sheldon L, Croghan C, Chuang J, Lordo R, Wilson N, Lyu C, Brinkman M, Morse, N, Chou Y, Hamilton C, Finegold J, Hand K, Gordon S. 2004. A pilot study of children's total exposure to persistent pesticides and other persistent organic pollutants (CTEPP). EPA/600/R-041/193.

Morgan M, Sheldon L, Croghan C, Jones P, Robertson G, Chuang J, Wilson N, Lyu C. 2005. Exposures of preschool children to chlorpyrifos and its degradation product 3,5,6-trichloro-2-pyridinol in their everyday environments. J Expo Anal Environ Epidemiol. 15:297–309.

Mucha AP, Hryhorczuk D, Serdyuk A, Nakonechny J, Zvinchuk A, Erdal S, Caudill M, Scheff P, Lukyanova E, Shkiryak-Nyzhnyk Z, Chislovska N. 2006. Urinary 1-hydroxypyrene as a biomarker of PAH exposure in 3-year-old Ukrainian children. Environ Health Prospect. 114:603–609.

Northridge ME, Yankura J, Kinney PL, Santella RM, Shepard P, Riojas Y, Aggarwal M, Strickland P, the Earth Crew. 1999. Diesel exhaust exposure among adolescents in Harlem: A community-driven study. Am J Public Health. 89:998–1002.

O'Rourke M, Lizardi P, Rogan S, Freeman N, Aguirre A, Saint C. 2000. Pesticide exposure and creatinine variation among young children. J Expo Anal Environ Epidemiol. 10:672–681.

Phillips DH. Polycyclic aromatic hydrocarbons in the diet. Mutat Res. 443:139–147.

Rey-Salgueiro L, Martinez-Carballo E, Garcia-Falcon M, Gonzalez-Barreiro C, Simal-Gandara J. 2009. Occurrence of polycyclic aromatic hydrocarbons and their hydroxylated metabolites in infant foods. Food Chem. 115: 814-819.

Suwan-ampai P, Navas-Acien A, Strickland PT, Agnew. J. 2009. Involuntary tobacco smoke exposure and urinary levels of polycyclic aromatic hydrocarbons in the United States, 1999 – 2002. Cancer Epidemiol Biomarkers Prev. 18(3):884–93.

Suzuki K, Yoshinaga J. 2007. Inhalation and dietary exposure to polycyclic aromatic hydrocarbons and urinary 1-hydroxypyrene in non-smoking university students. Int Arch Occup Environ Health. 81:115–121.

Szabo L and Fegyverneki S. 1995 Maximum and average urine flow rates in normal children F the Miskolc nomograms. Br J Urol. 76(1):16–20.

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

Tsai HT, Wu MT, Hauser R, Rodrigues E, Ho CK, Liu CL, Christiani, DC. 2003. Exposure to environmental tobacco smoke and urinary 1-hydroxypyrene levels in preschool children. J Med Sci. 19:97–103.

US ECFR (United States Electronic Code of Federal Regulations); Government Printing Office. Available online: http://www.ecfr.gov (accessed 31 March 2014).

Van Wijnen JH, Slob R, Jongmans-Liedekerken G, van de Weerdt RH, Woudenberg F. 1996. Exposure to Polycyclic aromatic hydrocarbons among Dutch Children. Environ Health Perspect. 104:530–534.

Verbovsek T. 2011. A comparison of parameters below the limit of detection in geochemical analyses by substitution methods. Materials and Geoenvironment. 58(4):393–404.

Wilhelm M, Hardt J, Schulz C, Angerer J, on the behalf of the Human Biomonitoring Commission of the German Federal Environment Agency. 2008. New reference value and the background exposure for the PAH metabolites 1-hydroxypyrene and 1- and 2-naphthol in urine of the general population in Germany: Basis for the validation of human biomonitoring data in environmental medicine. Int J Hyg Environ Health. 211:447–453.

Wilson NK, Chuang JC, Lyu CW. 2000. PAH exposures of nine preschool children. Polycyclic Aromat Compd. 21:247–259.

Wilson NK, Chuang JC, Lyu CW. 2001. Levels of persistent organic pollutants in several child day care centers. J Expo Anal Environ Epidemiol. 11:449–458.

Wilson NK, Chuang JC, Lyu CW, Menton R, Morgan MK. 2003. Aggregate exposures of nine preschool children to persistent organic pollutants at day care and at home. J Expo Anal Environ Epidemiol. 13:187–202.

Wilson NK, Chuang JC, Iachan R, Lyu C, Gordon SM, Morgan MK, Ozkaynak H, and Sheldon LS. 2004. Design and sampling methodology for a large study of preschool children's aggregate exposures to persistent organic pollutants in their everyday environments. J Expo Anal Environ Epidemiol. 14:260–274.

Table 1. Urinary 1-hydroxypyrene concentrations in children over a 48-hour monitoring period

Urine	N[a]	%[b]	AM± SD[c]	GM[d]	Min	Percentiles[e]				Max
						25th	50th	75th	95th	
Unadjusted (ng/mL)	126	79	0.41±0.33	0.31	<0.2	0.20	0.33	0.55	0.86	2.7
Creatinine-adjusted (ng/mg)	111[f]	78	0.52±0.47	0.44	<0.2	0.26	0.42	0.66	1.1	4.1

[a]Number of children
[b]Percentage of urine samples with detectable levels of 1-hydroxypyrene
[c]Arthmetric mean and standard deviation
[d]Geometric mean
[e]Percentiles (25th, 50th, 75th, and 95th)
[f]Creatinine levels were not measured in the non-pooled urine samples of 15 children that had a recent pesticide application at home.

Table 2. Levels of 1-hydroxypyrene (ng/mL) in children by selected sociodemographic or lifestyle factor

Variables	N[a]	%[b]	GM[c]	95% CI[d]	P-value
Age group					
< 48 months	64	51	0.31	0.26-0.37	0.496
≥ 48 months	62	49	0.34	0.29-0.40	
Sex					
Male	63	50	0.33	0.27-0.39	0.863
Female	63	50	0.32	0.28-0.37	
Urbanicity (*county-level*)[e]					
Urban	109	87	0.32	0.28-0.36	0.276
Rural	17	13	0.38	0.26-0.57	
Family income status[f]					
Low-income	40	35	0.37	0.30-0.45	0.317
Middle/high-income	73	65	0.32	0.28-0.37	
Race[g]					
White	88	73	0.32	0.27-0.36	0.923
Black	32	27	0.31	0.25-0.40	
Site location[h]					
Home	57	45	0.33	0.28-0.38	0.917
Daycare	69	55	0.32	0.27-0.38	
Sampling season[i]					
Spring	42	33	0.24	0.20-0.28	**0.001[j]**
Summer	58	46	0.37	0.31-0.44	
Fall	26	21	0.40	0.31-0.52	
Time spent outdoors during 48-hour period					
≤ 2 hours/day	64	51	0.34	0.29-0.39	0.528
> 2 hours/day	62	49	0.31	0.26-0.37	
Freeway/heavy traffic near home					
Yes	80	63	0.31	0.27-0.36	0.239
No	46	37	0.36	0.29-0.43	
Traffic conditions during the day					
Low (< 1 car/minute)	63	50	0.30	0.26-0.35	0.473
Medium/High (≥ 1 car/minute)	63	50	0.35	0.29-0.41	
Exposure to cigarette smoke during 48-hour period[k]					
Yes	25	20	0.34	0.27-0.43	0.689
No	101	80	0.32	0.28-0.37	
Cooking appliance used at home during 48-hour period[l]					
Electric	81	66	0.29	0.25-0.32	**0.003**
Gas	42	34	0.41	0.33-0.52	
Grilling/broiling foods at home					
None	81	64	0.34	0.29-0.39	0.382
≥ 1 times/week	45	36	0.30	0.25-0.36	
Baking foods at home					
< 3 times/week	74	59	0.35	0.30-0.40	0.209
≥ 3 times/week	52	41	0.30	0.24-0.36	
Frying foods at home					
None	86	68	0.31	0.27-0.35	0.203
> 1 times/week	40	32	0.36	0.29-0.45	

[a]Number of children
[b]Percentage of children
[c]Geometric mean
[d]Confidence interval
[e]Urban counties (Cuyahoga, Licking, Franklin, and Hamilton) and rural counties (Defiance and Fayette).
[f]Missing data on income-class for 13 children
[g]Other races (3 Hispanic and 3 Asian/Pacific Island children) excluded in analyses due to small sample size
[h]Only children attended daycare
[i]Field sampling activities were performed at homes and at daycares between April 2001 and November 2001
[j]Statistically significant associations ($p \leq 0.05$) are in bold text
[k]Refers to household smoking habits
[l]Excluded three children that had 'other' appliance category selected

Table 3. Results from the final reduced regression model for urinary 1-OHPyr levels (ng/mL) in children[abc]

	β coefficient	SE[e]	95% CI[f]	P-value
Sampling season (spring)	-0.489	0.124	-0.732 to -0.246	**0.0001**
Cooking appliance (electric)	-0.207	0.123	-0.448 to 0.034	0.096
Creatinine level[d]	0.447	0.127	0.198 to 0.696	**0.0006**

[a]A total of 111 children were used in this analysis.
[b]Reference categories: sampling season (summer/fall) and cooking appliance (gas)
[c]The $r^2 = 0.27$
[d]Continous variable (log-transformed); units are mg/dL
[e]Standard error
[f]Confidence interval
[g]Statistically significant variables ($p \leq 0.05$) are in bold text