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Air pollution, children's academic achievement and the potential mediating role of preterm birth

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ABSTRACT

Introduction: Previous research has observed relationships between higher prenatal exposure to air pollutants and neurodevelopmental and academic outcomes later in childhood. Identifying intermediate outcomes mediating this relationship would inform prevention and intervention efforts. We aimed to investigate if previously observed associations between prenatal exposure to common urban air pollutants, diesel and perchloroethylene, and performance on third grade standardized tests were mediated through increased risk of preterm birth.
Methods: Data from the 1994–1998 birth cohorts within the New York City Longitudinal Study of Early Development were included in this analysis. Exposure was determined by linking the mother's residence at the time of delivery to the U.S. EPA's 1996 National Air Toxic Assessment of estimated ambient concentrations of diesel and perchloroethylene. Children's third grade standardized math and language tests were used as the markers for academic achievement. Missing data on covariates were imputed, while participants with missing information on gestational age and test scores were excluded. Linear regression models and causal mediation analysis were used to examine potential mediation by preterm birth.
Results: In total, 187,723 and 196,122 participants were included in language and math analyses, respectively. Children with exposure to the fourth quartile of diesel or perchloroethylene had approximately 0.03 (95%CI: 0.02, 0.04) lower math z-scores when compared to individuals with exposure in the first quartile, although there was no consistent decreasing trend in math z-scores over increasing quartiles of diesel or perchloroethylene. We did not find evidence of mediation by preterm birth or exposure–mediator interaction in our models.
Conclusion: We did not find evidence that observed relationships between exposure to common urban air pollutants and test z-scores in childhood were mediated through an increased risk of preterm birth. This suggests other pathways between early exposure to air pollution and neurodevelopment should be investigated with causal mediation approaches.

1. Introduction

Air quality in urban areas is affected by emissions from various human-made sources, including mobile, stationary, and indoor sources. These emissions include both traffic-related air pollutants (TRAP) and air pollutants from other sources. Prenatal exposures to high levels of criteria pollutants, both from TRAP and non-TRAP sources (Karner et al., 2010), have been associated with poorer neurodevelopmental outcomes (Guxens et al., 2014; Ha et al., 2019). While a number of studies have demonstrated that exposure to air pollutants in the prenatal period is negatively associated with neurodevelopment or early childhood behavior (Cowell et al., 2015; Guxens et al., 2014; Ha et al., 2019; Perera et al., 2006; Ren et al., 2019; Sentís et al., 2017; Xu et al., 2016), others have found null or weak associations with these outcomes (Harris Maria et al., 2015). Some differences in findings across the studies could be explained by differences in pollutants that were investigated, such as focusing only on TRAP or non-TRAP pollutants.

We previously explored whether combined prenatal exposure to both TRAP and non-TRAP pollutants was associated with children's academic achievement in third grade (Stingone et al., 2016). In that work, diesel particulate matter (PM) was used as a marker of TRAP. Diesel fuel is used by a number of highway vehicles and engines, and its emissions are a mixture of solid and gaseous materials. The diesel particulate matter refers to the solid visible emissions, composed of black carbon and other

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organic compounds such as polycyclic aromatic hydrocarbons, benzene, formaldehyde, acetaldehyde, acrolein, and 1,3-butadiene(Wichmann 2007). Perchloroethylene is a non-TRAP chlorinated solvent that is used in the dry-cleaning industry and is prevalent in urban communities. (EPA 2010; Stingone et al., 2016). When examining the combination of exposure to diesel PM and perchloroethylene, we observed that high exposure to both diesel PM and perchloroethylene was associated with lower math scores among third graders in New York City when compared to children with low exposure to both pollutants (Stingone et al., 2016). This was consistent with previous literature suggesting exposure to air pollutants can hinder neurodevelopment and subsequent academic outcomes. However, the mechanisms of these observed relationships have not been explored.

One possible mechanism for the association between prenatal exposure to air pollution and neurodevelopmental outcomes could be through the greater risk of adverse birth outcomes such as preterm birth (Darrow et al., 2009; Li et al., 2017; Liu et al., 2019). Preterm delivery, defined as a birth before the completion of 37 weeks of gestation, is a significant adverse birth outcome associated with both air pollution exposure in pregnancy and adverse neurodevelopmental outcomes. Preterm birth and other adverse birth outcomes have been shown to be associated with higher air pollution exposure during pregnancy (Darrow et al., 2009; Li et al., 2017; Rich et al., 2009; Tan et al., 2017). Previous studies have demonstrated that preterm birth is associated with lower intellectual (Chan et al., 2016) and educational outcomes in early childhood (Lipkind et al., 2012; Morse et al., 2009; Quigley et al., 2012; Williams et al., 2013). A previous study among a cohort of children living in New York City found that children born preterm had higher odds for needing special education and lower standardized test scores compared to full-term children (Lipkind et al., 2012). These associations are biologically plausible as it is hypothesized preterm birth can alter dynamic brain growth and development, which normally occurs during the last trimester (Baron et al., 2012).

Given the association between air pollutants and preterm birth, as well as preterm birth with intellectual and educational attainment, we aimed to investigate if the associations between high perinatal exposure to diesel emissions and perchloroethylene and standardized test scores in the third grade are mediated through preterm birth. We were specifically interested in assessing natural direct effects, characterized as the change in standardized test z-scores associated with changing the exposures levels of diesel PM and perchloroethylene from high to low, while controlling preterm birth at the levels naturally observed at high or low levels of diesel PM and perchloroethylene. We specifically chose to estimate natural direct/indirect effects to describe the mechanism of mediation, as opposed to hypothesizing intervening directly on the mediator for all participants (controlled direct effects) or hypothesize a stochastic intervention (stochastic effects)(Rudolph et al., 2019). Due to the skewed nature of air pollution exposures and to facilitate mediation analyses, we dichotomized our air pollution exposures as high/low. We were also interested in assessing natural indirect effects (NIE), characterized as change in standardized test z-scores while setting exposure to pollutants as exposed, and intervening to change preterm births to full-term births. There are multiple existing public health and clinical interventions to reduce preterm birth (Ricklan et al., 2021). We aimed to investigate whether an intervention to reduce the risk of preterm birth would also impact the relationship between elevated exposure to air pollution and neurodevelopment, as interventions to reduce air pollution exposure are often difficult to make at the individual level and require societal intervention. Assessing the potential for mediation by preterm birth would inform if the potential harmful effects of exposure to diesel PM and perchloroethylene could be mitigated to some extent by interventions to reduce the risk of preterm births.

2. Methods

2.1. Study population

The Longitudinal Study of Early Development is an administrative data linkage of public health records from children living in New York City in the United States (Pfeiffer et al., 2012). Researchers at the New York City Department of Health and Mental Hygiene (NYCDOHMH) linked data across NYCDOHMH's birth and death certificate registries, the Early Intervention program, the Lead Poisoning Prevention Program registry, and the New York City Department of Education's administrative, special education and testing databases (Pfeiffer et al., 2012). Our sample included children born from 1994 to 1998 (1) who were born in New York City and whose mother's primary residence was in New York City; (2) whose address provided on the birth record was successfully geocoded; and (3) were enrolled in third grade at a public elementary school in New York City by 2007 (Stingone et al., 2016). The data systems to create LSED did not change over the study years. Thus, no harmonization across individuals from different birth years was required to create the LSED database. Children who had been born as part of multiple births were excluded, as were those who had congenital anomalies reported on their birth record. We also excluded children who did not have information on preterm birth, the mediator of interest in this study. Thus, a subset of the participants of the original study (Stingone et al., 2016) were included in the current analysis (Supplementary Fig. 1). This project was reviewed and approved by the NYCDOHMH and the Columbia University Institutional Review Boards.

2.2. Assessment of outcomes

We used math and English Language Arts (ELA) standardized test scores as a measure of children's academic attainment and a distal marker of children's neurodevelopment and cognition. The math and ELA standardized tests were administered to all children enrolled in third grade in public schools in New York City and were designed based on the third-grade academic curriculum to assess children's performance. The tests' scores range from 330 to 770 on a continuous scale. To use z-scores as our primary outcomes, we standardized test scores by birth year within the full New York City population as a proxy for the year the test was administered to ensure consistency in scores across the years. Because of the deidentification practices, the school year was removed from the LSED database. However, in New York City children are enrolled in school based on birth calendar year, thus we do not anticipate major differences between school year and birth year.

2.3. Assessment of exposures

Details about the assessment of diesel PM and perchloroethylene were described previously (Stingone et al., 2016). Ambient estimated concentrations of diesel PM and perchloroethylene were obtained from the 1996 U.S. EPA's National Air Toxic Assessment (NATA) (EPA 2016), the time period closest in time to our study population. NATA evaluates ambient air toxics in the United States periodically based on emissions inventories and simulation techniques (EPA 2016) These assessments are typically released every three years, with some exceptions. Estimates of diesel PM and perchloroethylene were assigned certainty ratings of "medium" or higher for both emissions certainty and modelling certainty by the NATA assessment. This suggests the modeled estimates are generally reflective of concentrations in the ambient air(EPA 2016). We linked the 1996 NATA estimates of diesel PM and perchloroethylene concentrations in New York City census tracts and assigned them to each child based on the census tract corresponding to the address provided at delivery. We categorized the raw concentrations of diesel PM and perchloroethylene to quartiles to account for skewed distributions of chemicals and minimize the influence of outliers. The majority of exposure variability is within the highest quartile. The categorization of

the exposure and comparison between highest and lowest quartiles provided meaningful contrast between high and low levels of exposure while facilitating the analysis of direct and indirect effects in mediation analysis.

2.4. Assessment of mediator and confounders

Preterm birth, the mediator of interest in our analysis, was defined as a birth that occurred before the 37th week of gestation. We used preterm birth as opposed to the continuous metric of gestational age as most clinical interventions focus on the prevention of preterm birth, not the general extension of gestation among all births. In order to assess the natural direct and indirect effects of diesel PM and perchloroethylene on math and language scores, strong assumptions about presence of confounding must be met (VanderWeele and Vansteelandt 2009). These assumptions include a) no unmeasured exposure–outcome confounders, b) no unmeasured mediator–outcome confounders, c) no unmeasured exposure–mediator confounders, and d) no mediator–outcome confounder affected by exposure (VanderWeele and Vansteelandt 2009). We identified the potential confounders through meticulous assessment of literature and used directed acyclic graphs (DAGs) to evaluate their role as potential confounding variables (Supplementary Fig. 2, Supplementary Text Box 1). We included maternal education, occupation, marital status, race/ethnicity, nativity, and season of birth as potential confounders of the diesel PM/perchloroethylene and preterm birth. The confounders for the exposure–mediator–outcome model were: maternal education, occupation, marital status, race/ethnicity, nativity, primary financial means for delivery charges, possession of medical risk factors (gestational or chronic diabetes, pregnancy-associated or chronic hypertension, preeclampsia, eclampsia, previous preterm or small-for-gestational-age delivery), tobacco or alcohol use, age, and total number of live births; and child's lead exposure (represented by the maximum venous blood lead concentration recorded in the lead poisoning prevention registry). Race/ethnicity was considered a confounder as a proxy for racism and other social factors that lead to disproportional exposure to environmental air pollutants and disparities in both adverse birth outcomes and neurodevelopment.

Maternal education was operationalized as a continuous variable by the number of years of education received. The borough of residence at delivery was also considered to control for neighborhood specific characteristics. Season of birth was calculated based on the date of birth and divided into December through February, March through May, June through August and September through November. Alcohol and tobacco use were considered present if the mother reported any consumption of these products on the birth certificate.

The neighborhood deprivation index (NDI) was defined and calculated for each census tract to adjust for neighborhood-level socioeconomic factors that could co-vary with ambient levels of diesel PM, perchloroethylene, and preterm birth as well as neurodevelopmental outcomes. The method used to calculate the NDI has been described elsewhere (Messer et al., 2006; Savitz et al., 2013). Briefly, this variable is calculated based on a principal component analysis of variables within the US Census data including "percent with college degree, percent unemployment, percent management/professional occupation, percent residential crowding, percent below 200% of the federal poverty line, percent of households receiving public assistance and percent of individuals identifying as a non-White race, and percent of linguistically isolated households." (Messer et al., 2006; Savitz et al., 2013).The resulting NDI is a standardized metric, with a mean of 0 and a standard deviation of 1. Higher values indicate greater neighborhood-level deprivation. (Messer et al., 2006; Savitz et al., 2013).

We created a joint variable representing maternal race/ethnicity and nativity comprising the following categories to ensure adequate sample size within the categories of this variable: US-born White non-Hispanics, US-born Black non-Hispanics, US-born Hispanics, foreign-born White non-Hispanics, foreign-born Black non-Hispanics, foreign-born Hispanics, and all Asians. We collapsed the category of foreign-born and US-born participants identifying as Asian or other race, due to the low number of observations in the US-born Asian and "other" race categories. We performed sensitivity analyses to assess the effect of this categorization choice by rerunning analyses including only foreign-born Asians and excluding the other, smaller groups.

2.5. Missing data

We used the MICE package in R (version 3.6.0) to impute missing values for lead concentration (21.84%), mother's total live births, and maternal occupation using single-chain MARKOV chain Monte Carlo methodology, as previously described (Yuan 2011). We used maternal nativity, maternal occupation, maternal race and ethnicity, marital status, maternal education, NDI, the season of birth, maternal age, age of residential building, borough of residence, and birthweight to impute the missing values using 20 iterations between each imputation to run five imputations.

2.6. Statistical analysis

We assessed the effects of diesel PM and perchloroethylene on test scores separately to account for the potential of different mediating relationships with preterm birth. We fit general linear models to assess the relationship between exposure to quartiles of diesel PM and perchloroethylene and test z-scores. We further assessed the relationship between exposure to these pollutants and the odds of preterm birth using logistic regression models. Finally, we used the mediation package in R (Tingley et al., 2014) to assess whether the association between the pollutants and math and ELA test z-scores is mediated by preterm birth. Natural direct and indirect effects, were calculated by comparing the fourth quartile to the first quartile of exposure as the reference category. In addition, the proportion of the total effect of pollutants on z-scores mediated by the preterm birth, was calculated after 1000 Monte Carlo draws for quasi-Bayesian estimation. The robust standard errors and 95% confidence intervals for natural direct and indirect effects were estimated. We identified the confounders from the constructed DAGs and methods to balance bias and variance of subsequent effect estimates (Greenland et al., 2016). Finally, we assessed exposure–mediator interaction, as well as borough of mother's residence during pregnancy in the pollutants-outcome model. An a priori alpha level of 0.05 was chosen to assess the presence of statistical interaction.

Our primary mediation analysis only includes children in the top and bottom quartiles of exposure. To use the full study population, we conducted a sensitivity analysis where we dichotomized exposures at the 75th percentile to examine if our models were sensitive to excluding individuals in the two middle quartiles. The 75th percentile was chosen due to the skew in the exposure data. All analyses were repeated using this new exposure contrast.

3. Results

3.1. Sociodemographic characteristics of study population

Demographic characteristics of the study population with complete data on both tests, overall and stratified by birth outcome, are presented in Table 1. There were a number of demographic differences between children born term versus preterm. Unmarried women made up a greater proportion of women who delivered at full-term when compared to women who delivered pre-term. Children who were born preterm tended to have lower math and ELA test z-scores compared to those who were born term. The neighborhood deprivation index score was also higher among children born preterm than their full-term counterparts. Pregnancy complications were more commonly reported for children born preterm than children born full-term. The mothers of participants

Table 1
Sociodemographic characteristics of study participants before imputation of missing variables [*n* (%) or mean (std)], New York City Longitudinal Study of Early Development Birth Years 1994–1998.

	Overall (*n* = 187,401)	Term Birth (≥37 weeks) (*n* = 171,828)	Preterm birth (<37 weeks) (*n* = 15,573)
Maternal age at birth (years)	27.3 (6.42)	27.3 (6.38)	27.7 (6.82)
Total live deliveries including current pregnancy			
Maternal education (years)	11.8 (2.6)	11.9 (2.7)	11.7 (2.5)
Neighborhood deprivation index	0.0 (1.0)	0.0 (1.0)	0.2 (1.0)
Perchloroethylene (μg/m^3)	0.8 (0.4)	0.8 (0.4)	0.8(0.4)
Diesel particulate matter (μg/m^3)	9.5 (8.7)	9.5 (8.7)	9.5 (8.2)
Math z-score	0.0 (1.0)	0.1 (1.0)	−0.2 (1.0)
Language z-score	0.0 (1.0)	0.0 (1.0)	−0.2 (1.0)
Highest venous blood lead level test (μg/dL)	4.7 (3.5)	4.7 (3.5)	4.8 (3.6)
Borough of residence (%)			
Manhattan	27174 (14.5)	24730 (14.4)	2444 (15.7)
Bronx	37913 (20.2)	34523 (20.1)	3390 (21.8)
Brooklyn	67310 (35.9)	61597 (35.8)	5713 (36.7)
Queens	44947 (24.0)	41657 (24.2)	3290 (21.1)
Staten Island	10057 (5.4)	9321 (5.4)	736 (4.7)
Maternal occupation (%)			
Works at home only	90578 (48.3)	82996 (48.3)	7582 (48.7)
Student	16389 (8.7)	14982 (8.7)	1407 (9.0)
Not employed	18392 (9.8)	16697 (9.7)	1695 (10.9)
Other occupation	57592 (30.7)	53048 (30.9)	4544 (29.2)
Missing	4450 (2.4)	4105 (2.4)	345 (2.2)
Maternal marital status—Unmarried	78563 (41.9)	73344 (42.7)	5219 (33.5)
Nativity & race			
Foreign-born Black non-Hispanic	22523 (12.0)	20491 (11.9)	2032 (13.0)
Foreign-born White non-Hispanic	10051 (5.4)	9522 (5.5)	529 (3.4)
Foreign-born Hispanic	39328 (21.0)	36818 (21.4)	2510 (16.1)
US-born Black Hispanic	40282 (21.5)	35667 (20.8)	4615 (29.6)
US-born White Hispanic	21796 (11.6)	20414 (11.9)	1382 (8.9)
US-born Hispanic	32276 (17.2)	29140 (17.0)	3136 (20.1)
Asian and Other (US- or foreign-born)	21145 (11.3)	19776 (11.5)	1369 (8.8)
Season of birth			
Winter	46199 (24.7)	42255 (24.6)	3944 (25.3)
Spring	46594 (24.9)	42757 (24.9)	3837 (24.6)
Summer	47918 (25.6)	43920 (25.6)	3998 (25.7)
Fall	46690 (24.9)	42896 (25.0)	3794 (24.4)
Any complication during delivery—Yes (%)	65137 (34.8)	57634 (33.5)	7503 (48.2)
Alcohol use during pregnancy Yes (%)	1071 (0.6)	791 (0.5)	280 (1.8)
Reported tobacco use during pregnancy Yes (%)	10727 (5.7)	9163 (5.3)	1564 (10.0)
Quartiles of Diesel PM			
1st quartile (<6.35 μg/m^3)	46988 (25.1)	43412 (25.3)	3576 (23.0)
2nd quartile (6.35–7.67 μg/m^3)	46939 (25.0)	42717 (24.9)	4222 (27.1)
3rd quartile (7.67–9.74 μg/m^3)	46702 (24.9)	42853 (24.9)	3849 (24.7)
4th quartile (9.74–140 μg/m^3)	46772 (25.0)	42846 (24.9)	3926 (25.2)
Quartiles of perchloroethylene (%)			
1st quartile (<0.58 μg/m^3)	46869 (25.0)	43345 (25.2)	3524 (22.6)
2nd quartile (0.58–0.68 μg/m^3)	46974 (25.1)	42764 (24.9)	4210 (27.0)
3rd quartile (0.68–0.84 μg/m^3)	46740 (24.9)	42972 (25.0)	3768 (24.2)
4th quartile (0.84–9.20 μg/m^3)	46818 (25.0)	42747 (24.9)	4071 (26.1)

with complete data on preterm birth and ELA and math scores tended to have a higher level of education (11.8 vs. 10.2), be exposed to lower concentrations of diesel PM and perchloroethylene and be more likely to work outside the home (Supplementary Table 1) than children with incomplete data. Distributions of diesel PM and perchloroethylene in the analytic population are provided in Supplementary Table 2. After imputation and excluding observations with missing values on mediators and outcomes, a total of 187,723 and 196,122 individuals were included in the ELA and math models respectively.

3.2. Association of pollutants with Children's test Z-scores

For both diesel PM and perchloroethylene, we found that individuals with exposure in the fourth quartile had 0.03 lower math z-scores (95% CI: 0.02, 0.04) than children in the first quartile of exposure, although there was no consistent decreasing trend in math z-scores over increasing quartiles of diesel or perchloroethylene (Table 2). Results for ELA scores were consistent across pollutants with a null relationship observed between higher exposure and ELA test scores. The results did not change when we repeated the analysis on complete cases (Supplementary Table 3), or when we excluded Asians born in the US and the participants identified as other races rather than combine them with Asians born outside the US into a single category (data not shown).

3.3. Mediation analysis

The results of mediation analysis are illustrated in Fig. 1. Final models did not include interaction terms, as we did not find any evidence of interaction between exposure and mediator. Within all mediation models, the exposure contrast examined was comparing the highest quartile to the lowest quartile of exposure. The natural indirect effects were consistently null when examining the different pollutants and both ELA and math test scores. Our findings did not change in

Table 2
Estimated effect of neighborhood pollutant concentrations on math and language test z-scores, calculated from linear models New York City Longitudinal Study of Early Development Birth Years 1994–1998.

	Coefficient[a,b]	Language z-score		Math z-score	
		Estimates	(95% CI)	Estimates	(95% CI)
Model 1 [c]	Diesel PM 1st quartile			REF	
	Diesel PM 2nd quartile	0.02	(0.01,0.03)	0.01	(0.00, 0.02)
	Diesel PM 3rd quartile	0.01	(0.00, 0.02)	0.01	(0.00, 0.02)
	Diesel PM 4th quartile	0.01	(0.00, 0.02)	−0.03	(-0.04, −0.02)
Model 2 [c]	Perchloroethylene 1st quartile			REF	
	Perchloroethylene 2nd quartile	0.04	(0.03, 0.05)	0.02	(0.01, 0.03)
	Perchloroethylene 3rd quartile	0.03	(0.02, 0.04)	0.03	(0.02, 0.04)
	Perchloroethylene 4th quartile	0.01	(0.00, 0.02)	−0.03	(-0.04, −0.02)
	Observations	187723		196122[c]	

Abbreviations: CI (Confidence Interval); PM (Particulate Matter); REF (Reference Category).

[a] Estimates represent change in the outcomes' z-scores associated with exposure level compared to reference.

[b] All the models were adjusted for neighborhood deprivation index, maternal education, child's highest venous lead level, maternal occupation status, maternal marital status, and maternal nativity/race/ethnicity.

[c] Separate linear models were fit for diesel particulate matter and Perchloroethylene as exposure variables. The sample size for these models varied as the language and math test z-scores were not imputed in corresponding models.

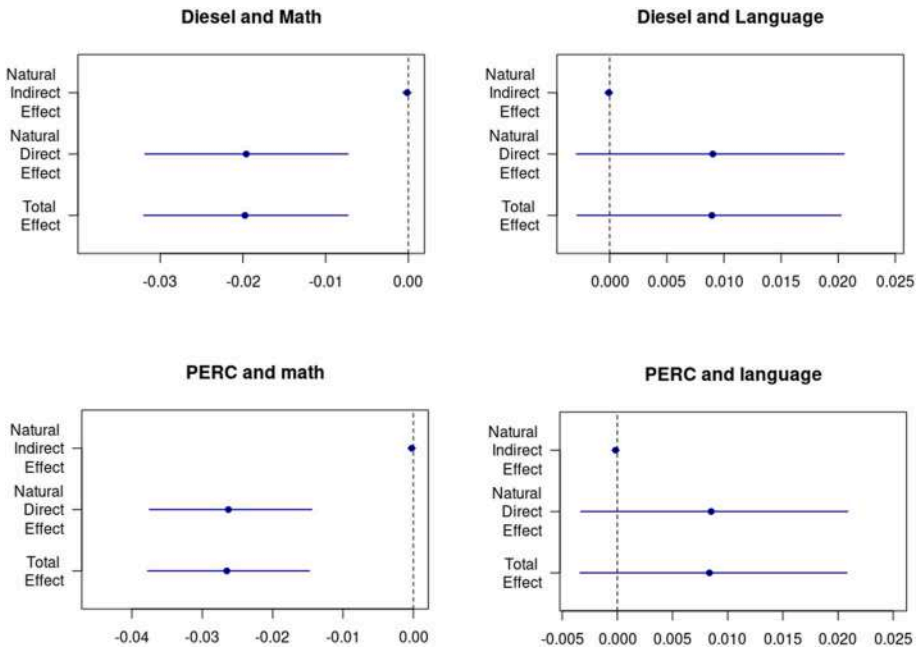

Fig. 1. Estimated natural direct, indirect and total effects and 95% confidence intervals for estimated effects of highest quartile of diesel PM and Perchloroethylene compared to lowest quartile on math and language test z-scores, New York City Longitudinal Study of Early Development Birth Years 1994–1998. Dotted line indicates null value. All models were adjusted for neighborhood deprivation index, maternal education, child's highest venous lead level, maternal occupation status, maternal marital status, maternal nativity/race/ethnicity, season of birth, maternal age, complications during labor, maternal total live births. Abbreviations: PERC: Perchloroethylene.

complete case analysis (Supplementary Fig. 3) or when we included only Asians born outside the US and excluded Asians born in the US and participants identified as other races from the combined category due to their small sample size (Supplementary Fig. 4). The proportion mediated did not exceed 1% in any of the models.

In a sensitivity analysis, we repeated the mediation analysis, excluding children who participated in the early intervention program in New York City. We hypothesized that children who were identified and subsequently received developmental interventions through the early intervention program would have higher academic test scores. However, the findings were not different from the results presented. Also similar to our primary results, we did not see evidence of mediation when we dichotomized exposures at the 75th percentile (Supplementary Fig. 5). However, the estimates for the direct effects were slightly larger in magnitude.

We further explored if exposure was different among the children with missing information on preterm birth (n = 1995; Supplementary Table 1) and with missing data on standardized test scores (Supplementary Table 1), suggesting the potential for selection bias. There were 11,729 children with missing data on ELA tests. These children were exposed to higher levels of air pollutants and were more likely to be born to a mother who was foreign-born Hispanic. (Supplementary Table 1).

4. Discussion

In this study, we sought to determine if the association between exposure to common urban air pollutants and standardized test scores is mediated by preterm birth. This research built upon existing literature documenting relationships between air pollution, preterm birth and neurodevelopment as measured by school achievement (Darrow et al., 2009; Lipkind et al., 2012; Stingone et al., 2016). However, we did not find any evidence of mediation by preterm birth in our analysis.

Consistent with the previous analysis(Stingone et al., 2016), we did observe small effects of exposure to the highest quartile of pollutants on the math z-scores. Given our sample size, we should have been able to see some level of mediation, even with the small main effect, if it were present in the data. As shown in in Table 1, we did not see a strong association between exposure to air pollutants and preterm birth in our sample. This likely explains the lack of mediation observed. Because of the right skewness of the exposure data, the majority of the variability of exposure was in the highest quartile. This could explain why a consistent decreasing trend in math z-scores was not observed over increasing quartiles of exposure. The observed association, although small in absolute magnitude, is likely meaningful at the population level, as it could shift the population distribution of the test outcomes resulting in a larger number of individuals within the lower tail (Rose 2001).

Our results are supported by a small number of studies exploring the potential mediation of the relationship between air pollutants and neurodevelopment through birth outcomes. Perera et al. (2006) found that the association of prenatal exposure to polycyclic aromatic hydrocarbons with neurodevelopmental outcomes was not mediated by weight or head circumference at birth. (Perera et al., 2006). Another study found that gestational age did not mediate the association between criteria air pollutants and autism spectrum disorders (Becerra et al., 2013). The studies mentioned above assessed mediation by a difference method, where the exposure coefficient in the model with the mediator is compared to the exposure coefficient in the model without the mediator and a reduction of the estimate in the former model compared to the latter indicates mediation (VanderWeele and Vansteelandt 2009; VanderWeele 2016). In contrast, we conducted a causal mediation analysis that allowed for exposure and mediator interaction and causal interpretation of the effect estimates (Tingley et al., 2014; VanderWeele and Vansteelandt 2009; VanderWeele 2016). Although using different methods, we also did not find evidence of mediation of the relationship between urban air pollutants and a marker of neurodevelopment through preterm birth. As acknowledged above, the relationship between the specific air pollutants studied and preterm birth was not strong in our sample. The use of study populations which experience greater risks of adverse birth outcomes associated with air pollutant exposure or a focus on pollutants that are more strongly associated with preterm birth may help address remaining questions about the role of adverse birth outcomes in neurodevelopmental deficits associated with air pollution.

Aside from triggering preterm birth, oxidative stress in the brain is another possible mechanism for the association of these pollutants with neurodevelopmental outcomes (Calderón-Garcidueñas et al., 2002). It has been suggested that the pollutants can translocate through the olfactory nerve to the brain (Oberdorster et al., 2004) and result in oxidative stress, as they do in other tissues (Beck-Speier et al., 2005; Calderón-Garcidueñas et al., 2002; Suglia et al., 2008). Studies

conducted on animal models also suggest possible neuroinflammation as a result of long exposure to diesel PM (Gerlofs-Nijland et al., 2010; Levesque et al., 2011). Moreover, exposure to fine and ultra-fine particles in the prenatal period may be associated with systemic cytokine response in the mother, which could impair fetal development but not contribute to a shortened gestation (Block and Calderón-Garcidueñas 2009; Kim et al., 2014; Peters et al., 2006).

Our study had several strengths. This research leveraged an existing administrative data linkage to efficiently investigate the role of preterm birth in the relationship between air pollutants and subsequent academic achievement. This administrative linkage provides population-based data on a large, diverse group of young children. The mediation of the association between exposure to diesel and perchloroethylene and test z-scores by preterm birth has not been assessed in any other cohort of this size, adding to the novelty of the present study. The children's academic achievement was assessed using routinely-given standardized tests, allowing for aggregation across multiple years. The use of DAGs to identify the minimally sufficient adjustment sets coupled with the use of causal mediation methods facilitates our ability to interpret our results within a causal framework.

The study had some limitations as well. The study population is from an older cohort and exposure levels have changed. Although the concentrations of diesel and perchloroethylene have changed over time (Kheirbek et al., 2013), overall patterns within NYC have stayed generally consistent. Globally the exposure to ambient air pollutants remains high (World Health Organization2016), supporting the need for work in this area and relevance for looking at time periods in the US with higher exposure levels. Although we adjusted our models for potential confounding by including covariates identified as part of our DAG's minimally sufficient adjustment set, we could not rule out the possibility of residual confounding due to unmeasured variables. For example, parenting behaviors, particularly in early childhood, could vary by neighborhood and could also affect children's educational outcomes (Ronfani et al., 2015). The impact of other spatially-varying factors was not considered. For example, neighborhood noise has been found to covary with ambient air pollution(Kheirbek et al., 2014)and to impact cognitive test scores in animal models(Barzegar et al., 2015). Adjusting for the NDI may account for some of these spatially-varying factors, as they could lie on the same backdoor path. Finally, the impact of other TRAP or non-TRAP co-pollutants that covary with diesel PM concentrations could also impact both mediator and outcome (Jedrychowski et al., 2015; Stingone et al., 2016; Suglia et al., 2008).

This analysis only included the children who had available measures of preterm birth and standardized test scores. We acknowledge that missing information on the mediator, and particularly on ELA tests, could have affected our findings. Our sensitivity analysis illustrated that children of foreign-born Hispanic mothers had both higher exposure to air pollutants and were more likely to be missing data on ELA tests. This could lead to selection-bias in our results, and potentially contribute to an underestimation of the associations as individuals with higher levels of exposure were excluded from final models. It also highlights another potential limitation of our study: the use of standardized tests as a marker for neurodevelopment and academic success. Achievement on these tests is affected by other factors beyond a child's individual aptitude, including their learning environments both in and out of school and impacts of biases within the test itself. For example, previous research in NYC has shown that children from linguistically-isolated households often have lower scores on language tests (Steifel, 2003). We do not have information on many of these factors that could influence test performance and may be more common among individuals exposed to higher levels if air pollution, so residual confounding remains a possible explanation for our findings.

Another possibility for selection bias could be due to the exclusion of children who did not attend public schools in NYC in the analytic sample. The children in public and private schools could differ in various sociodemographic characteristics including race and household income (Domanico 2020). Children who moved out of the city prior to school enrollment may be sociodemographically different in ways that could be associated with both exposure and outcome. Additionally, the study population included children who were born in 1994–1998. We did not observe considerable sociodemographic differences across individuals stratified by birth year (Supplementary Table 4). This supports our assumptions of similar confounding structure across the population, despite different birth years.

Exposures to air pollutants were assessed by the estimated concentrations of these pollutants for the census tract in which the mothers were reported to live at the time of delivery (EPA 2016). Research is mixed about the critical window of exposure in relation to preterm birth, but it is possible that examining exposure early in pregnancy may lead to different findings of mediation by preterm birth. Most studies of residential mobility during pregnancy suggest that impacts on exposure are limited, as people tend to move short distances and/or to areas with similar exposure levels(Bell and Belanger 2012; Chen et al., 2010). The use of area-based measures can lead to misclassification, as other locations visited by pregnant women and time spent indoors are not accounted for within the exposure assignment. In contrast to personalized estimates of pollution exposure, the use of ambient estimates as a proxy for exposure is not affected by personalized behaviors and thus not affected by confounding due to those behaviors (Weisskopf and Webster 2017).

4.1. Conclusion

In this study, we did not find evidence for a mediating effect of preterm birth on the association between diesel PM/perchloroethylene and performance on children's standardized math and ELA tests in third grade. This suggests other pathways between early exposure to air pollution and neurodevelopment should be investigated with mediation. The observed association between diesel PM/perchloroethylene and children's math z-scores, albeit small, could be meaningful at the population level as it could result in larger number of children at the lower tail of the population distribution of math standardized tests. Given the racial and socioeconomic disparities in exposure to air pollutants, it is of great importance to improve our understanding of the pathways connecting early-life exposure to adverse school outcomes, in order to identify early points of intervention.

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Appendix A. Supplementary data

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Association between prenatal cadmium exposure and child development: The Japan Environment and Children's study

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ABSTRACT

Cadmium is a heavy metal that can be found in soil, air, food, and water. Cadmium has toxic effects on the kidneys, bones, and respiratory system. Prenatal exposure to cadmium has been found to affect the mental development of children, but inconsistent results have been found in different studies. Therefore, it is unknown that prenatal cadmium exposure associated with child development after birth. To elucidate whether cadmium affect the child development or not, we analyzed nation-wide cohort study data, the Japan Environment and Children's Study. Prenatal cadmium concentrations in blood from mothers in the second or third trimester were determined by inductively coupled plasma mass spectrometry. Child development was evaluated using "Ages and Stages" questionnaires. The association between cadmium and child development were investigated by performing logistic regression analyses, multinomial logistic regression analyses and generalized linear mixed model using the child development parameters as dependent variables and the cadmium concentrations in maternal blood as the independent variable. There were significant associations between the cadmium concentration and child development at 6 months, 1 year, and 1.5 years after birth. However, the effect had disappeared at 2 years after birth or later. The number of developmental delays was positively associated with the cadmium concentration after adjusting individual difference. The results indicate that prenatal exposure affects child development, but the effect decreases with age.

1. Introduction

Cadmium (Cd) is a heavy metal that can be found in soil, air, water, and cigarettes (Faroon et al., 2012; Järup and Åkesson, 2009). It is well known that Cd has toxic effects on the kidneys, bones (e.g., itai-itai disease), the respiratory system, and the female reproductive system (Reeves and Chaney, 2008; Rzymski et al., 2015). The main sources of Cd in Japan include cigarettes and food (particularly rice and root vegetables). The half-life of Cd is > 10 years, so juvenile exposure to Cd may lead to effects on the reproductive system in adulthood and on progeny (Johnson et al., 2003; Satarug and Moore, 2004). Cd accumulates in the bones, so Cd may be released from the bones of the mother and be transferred to the embryo through the blood–placenta barrier. High Cd concentrations have been found in the hair of infants with mothers occupationally exposed to Cd (Huel et al., 1984). The results of the studies mentioned above indicate that exposure of a woman at any time before or during pregnancy may affect the woman's fetus or infant.

Cd has recently been found to affect neural development in animals. For example, prenatal, neonatal, and juvenile exposure to Cd was found to be related to altered operant paradigm behavior and memory in mice (Ali et al., 1986; Barański, 1986; Grover et al., 1991; Newland et al., 1986; Pelletier and Satinder, 1991). In neurochemical studies, Cd was found to alter the kinetics of Ca^{2+}-mediated neurotransmitter (e.g., dopamine and serotonin) release in synaptic nerve terminals (Andersson et al., 1997; Antonio et al., 1998; Lancaster and Nicoll, 1987). Precise neurotransmitter release is important for the correct wiring of neural circuits during neural development, so these results indicate that exposure to Cd could alter the functioning of the brain by affecting

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neurotransmitter release.

Taking together of previous studies, In utero accumulation of Cd, the effects of Cd on neural development in children led us to hypothesize that prenatal exposure to Cd can alter neural development in children. A few studies of the relationships between exposure to heavy metals and cognition in children have been performed but the results of different studies were inconsistent. In some studies it was found that exposure to Cd in early pregnancy is negatively associated with cognition (Jeong et al., 2015; Kim et al., 2013). However, in other studies it was found that prenatal exposure to Cd was not negatively related to cognition (Forns et al., 2014). It is therefore still unclear whether exposure to Cd affects child development. To attempt to resolve this, we investigated the relationship between the Cd concentration in maternal blood during pregnancy and child development using samples and data collected as part of the Japan Environment and Children's Study (JECS), which is a birth cohort study.

2. Methods

2.1. Data sources and study population

The aim of the JECS, which is an ongoing prospective birth cohort study that began in 2011, is to evaluate the effects of various environmental factors on the health and development of children (Kawamoto et al., 2014; Michikawa et al., 2018). The Japan Environment and Children's Study protocol was reviewed and approved by the Ministry of the Environment's Institutional Review Board on Epidemiological Studies and the Ethics Committees of all participating institutions (No.100910001). The Japan Environment and Children's Study was conducted in accordance with the Declaration of Helsinki and other relevant national regulations and guidelines. Written informed consent was obtained from all participants involved in the study. The study presented here used a JECS dataset (jecs-ta-20190930) that was released in October 2019. The dataset does not contain any identifying patient information.

We issued a questionnaire to each participant of the study in the first trimester of pregnancy either in person or by post when the participant was recruited after explaining the purpose and practicalities of the study. Follow-up questionnaires were issued in person or by post in the second/third trimester, shortly after childbirth, and 1 month postpartum at the times of scheduled in-patient hospital checkups. Subsequent follow-up questionnaires were issued by post 6, 12, 18, 24, 30, and 36 months postpartum. In total, 104,062 fetal records were registered in 19 prefectures in Japan (Aichi, Chiba, Fukushima, Fukuoka, Hokkaido, Hyogo, Kanagawa, Kochi, Kumamoto, Kyoto, Miyagi, Miyazaki, Nagano, Okinawa, Osaka, Shiga, Toyama, Tottori, and Yamanashi) between January 2011 and March 2014. We excluded 7896 fetal records because of stillbirth and missing data about maternal prenatal Cd concentration data and analyzed the data for 96,165 participants.

2.2. Child development measurements

Data from "Ages and Stages" questionnaires (ASQ-3) completed at 6 months, 1 year, 1.5 years, 2 years, 2.5 years, and 3 years after birth as part of the JECS questionnaires were used to indicate child development (Squires et al., 2009). Cutoff values for the Japanese version of the ASQ-3 were used (Mezawa et al., 2019, Supplemental Table 1). The ASQ-3 scores were divided into five categories (communication, gross motor function, fine motor function, problem solving, and personal–social). Delayed development was defined as the score for more than one category being lower than the cutoff value. For sub-group analysis, we defined a score below the cutoff value for a category in the Japanese version of the ASQ-3 as indicating delayed child development.

2.3. Confounding factors for the questionnaire data

A number of possible confounding factors for the data for the mothers were used. These were the mother's age, mother's BMI (less than 25 or more than 25), marriage status (Married or unmarried), number of deliveries (1st delivery or not), gestation weeks, smoking status (no smoking, exo-smoking, current smoking), medical history (ADHD, learning disorder, autism spectrum disorder, diabetes, and other developmental disorder; yes or no), autism spectrum quotient (AQ10; Total Score <7 or Total Score $\geqq 7$) (Baron-Cohen et al., 2001; Kurita et al., 2005), gestational diabetes (yes or no), house income (less than 4 million yen, 4–6 million yen, more than 6 million yen), education (less than 12 years, 12–16 years, more than 16 years) and heavy metal concentration (lead [ng/g] and mercury [ng/g]). A number of possible confounding factors for the data for the children were used. These were multiparity (yes or no), sex (male or female), birth weight (less than 2500 g or more than 2500 g), and going to nursery (yes or no). These confounding factors were selected after assessing the results of previous studies of the effects of Cd on child development (Forns et al., 2014; Jeong et al., 2015; Kim et al., 2013; Ma et al., 2021).

2.4. Blood sample collection and Cd concentration measurements

The blood samples were collected and analyzed following protocol. A 33 mL blood sample were collected from each pregnant woman at the second or third trimester of pregnancy (mid–late term). The Cd concentration in each blood sample was determined using a method described in a previous publication (Nakayama et al., 2019). Sodium ethylenediaminetetraacetic acid was added to each blood sample, then the sample was transferred to a central laboratory and stored at $-80\ ^{\circ}C$ until it was analyzed. A standard solution containing all of the target elements in 0.14 M nitric acid was prepared. The final Cd concentration in the standard was 20 ng/g. An internal standard (containing indium, thallium, and yttrium each at a concentration of 250 ng/g in 0.14 M nitric acid) was prepared. A 200 μL aliquot of a blood sample was mixed with 3800 μL a solution containing 2% v/v butan-1-ol, 0.1% tetramethylammonium hydroxide, 0.05% w/v polyoxyethlene octylphenyl ether, and 0.05% w/v ethylenediaminetetraacetic acid, then the mixture was vortexed before being subjected to inductively coupled plasma mass spectrometry using an Agilent 7700 instrument (Agilent Technologies, Santa Clara, CA, USA). Seronorm Trace Element Whole Blood L-1 (ref. 210105, lot. 1003191; Sero AS, Billingstad, Norway) and blood samples donated by the Japanese Red Cross were used as a quality control samples. The method detection limit for Cd was calculated using Currie's method. There were no sample below detection limit for Cd.

2.5. Statistical analysis

The adjusted odds ratio (aOR) was calculated by performing binomial and multinomial logistic regression analyses. Briefly, a model was constructed by performing binomial and multinomial logistic regression analyses using delayed development as the dependent variable and the confounding factors as independent variables. The model was then constructed using delayed development as the dependent variable and the confounding factors that were significantly associated with delayed development as independent variables. For the binomial logistic regression analysis, multiplicity issues were avoided by correcting the p-values using the Bonferroni correction method. Significance was defined as $p < 0.0026$, and the 99.7% confidence intervals were calculated. For the multinomial logistic regression analysis, significance was defined as $p < 0.05$, and the 95% confidence intervals were calculated.

The relationship between the Cd concentration in blood and delayed development was assessed using a generalized linear mixed model using the Poisson link function to consider the individual difference due to repeated measurement. The generalized linear mixed model included a random effect to take differences between participants into account. The

number of delayed categories was the dependent variable and confounding factors significantly associated with delayed development were the independent variables. The coefficients, standard errors of the coefficients, and p-value for the generalized linear mixed model were calculated. Significance was defined as $p < 0.05$.

The statistical analyses were performed using R software version 4.1.1 with the AER, tidyverse, nnet, and glmmML packages (Kleiber and Zeileis, 2008; R Core Team, 2021; Venables and Ripley, 2002; Wickham et al., 2019).

3. Results

3.1. General characteristics of participants

The general characteristics of the participants and the child development status results are summarized in Tables 1 and 2 and Supplemental Table 2. A delay in one child development category or more was defined as a delay in child development. Of children 6 months, 1 year, 1.5 years, 2 years, 2.5 years, and 3 years old participating in the study, 21.1%, 14.1%, 12%, 12.7%, 14%, and 15.3%, respectively, were classed as suffering delayed development. To determine the consistency of developmental delays over time, Fleiss's Kappa was calculated. Because Fleiss's Kappa was 0.28 ($p < 0.001$), the consistency of developmental delay was fair.

3.2. Association with child developmental delay

Multivariate logistic regression analysis was performed to investigate the relationship between the Cd concentration in maternal blood and delayed child development. The quartiles of the prenatal Cd concentrations in blood were used (minimum 0.0951 ng/mL, first quartile 0.496 ng/mL, second quartile (median) 0.663 ng/mL, third quartile 0.905 ng/mL, maximum 5.33 ng/mL, mean 0.75 ng/mL). The blood samples from six women contained Cd concentrations higher than the reference value (5 ng/mL). An association was found between the prenatal Cd concentration in blood (third quartile $< x <$ maximum) and child development 6 months, 1 year and 1.5 year after birth (aOR at 6 months 1.10, 99.7% CI 1.01–1.20; aOR at 1 year 1.13, 99.7% CI 1.02–1.24; aOR at 1.5 years 1.15, 99.7% CI 1.03–1.28). Interestingly, the association was not found at 2 years after birth or later (Table 3).

3.3. Association with specific category of child development

Multivariate logistic regression between the Cd concentration in blood and the child development category results for the results to 1.5 years after birth was performed to identify which child development categories were affected by the Cd concentration. The results are shown in Table 4. The Cd concentration in blood was found to be associated with gross motor function between 1 and 1.5 years after birth (aOR at 1 year 1.25, 99.7% CI 1.07–1.45; aOR at 1.5 years 1.19, 99.7% CI 1.01–1.40). The Cd concentration was also associated with problem solving at 1 year (aOR 1.22, 99.7% CI 1.05–1.42). There were trends between the Cd concentration and gross motor or problem solving at 6 months and 1.5 years, but the associations were not significant. These results indicated that prenatal exposure to Cd affects child development in various categories.

3.4. Association with delayed category number of child development

Multinomial logistic regression analysis was performed to assess the relationships between the numbers of delayed categories and the Cd concentrations in blood. The higher Cd concentration in blood was associated with multiple child development categories but not one particular category at 1 year, and 1.5 years (Table 5). A generalized linear mixed model was used to describe the relationships between the delayed category numbers and the Cd concentrations in blood

Table 1
Participant characteristics.

		Number	Percentage of total
Total		96165	100%
Mothers' Parameters			
Mean age (years ± SD)		30.7 ± 5.05	
Mean number of previous deliveries (mean ± SD)		0.87 ± 0.9	
The mean days of fetus period (days ± SD)		274.5 ± 10.8	
Mean blood lead concentration (ng/mL ± SD)		6.35 ± 2.8	
Mean blood mercury concentration (ng/mL ± SD)		4.2 ± 2.5	
Smoking at MT1	No	54968	57.2
	Ex	34965	36.4
	Current	4547	4.7
	NA	1685	1.8
Education	≤ 12 years	34391	35.8
	12–16 years	39719	41.3
	≥16 years	20217	21.0
	NA	1839	1.9
Marriage	Married	90670	94.3
	Unmarried	4084	4.2
	NA	1411	1.5
Income	<4 million yen	35588	37.0
	4–6 million yen	29069	30.2
	≥ 6 million yen	23431	24.4
	NA	8077	8.4
Body Mass Index	BMI <25	85753	89.2
	BMI ≥ 25	10301	10.7
	NA	111	0.1
Mothers' Medical Histories			
ADHD	No	95142	98.9
	Yes	28	0.0
	NA	995	1.0
Learning disorder	No	95156	99.0
	Yes	14	0.0
	NA	995	1.0
Autism spectrum disorder	No	95151	98.9
	Yes	19	0.0
	NA	995	1.0
Other disorder	No	95157	99.0
	Yes	13	0.0
	NA	995	1.0
Autism spectrum quotient	<7	89926	93.5
	≥7	2469	2.6
	NA	3770	3.9
Diabetes	No	95048	98.8
	Yes	122	0.1
	NA	995	1.0
Gestational diabetes	No	93319	97.0
	Yes	2622	2.7
	NA	224	0.2
Children's Parameters			
Mean birthweight (g ± SD)		3015.4 ± 421.6	
Parity	Singleton	94454	98.2
	Multiplet	1711	1.8
Sex	Male	49257	51.2
	Female	46892	48.8
	Unknown	3	0.0
	NA	13	0.0
Method for delivery	Natural vaginal delivery	54583	56.8
	Others	41139	42.8
	NA	443	0.5
Going to nursery school at 6 months old	No	83238	86.6
	Yes	6282	6.5
	NA	6645	6.9
Going to nursery school at 1 year old	No	63159	65.7
	Yes	23335	24.3
	NA	9671	10.1

(continued on next page)

Table 1 (*continued*)

		Number	Percentage of total
Going to nursery school at 2 years old	No	41115	42.8
	Yes	40875	42.5
	NA	14175	14.7
Going to nursery school at 3 years old	No	28244	29.4
	Yes	48444	50.4
	NA	19477	20.3

SD: Standard deviation; NA: Not answered.

containing individual difference. The results are shown in Table 6. The Cd concentration in maternal blood was significantly positively associated with the number of delayed categories, even taking the differences of individual specificity. These results indicated that prenatal exposure to Cd affects child development for at least 1.5 years after birth.

3.5. Sensitive analysis

The cutoff values for the Japanese version of the ASQ-3 presented in a previous publication (Mezawa et al., 2019) were used in this study. Because Mezawa reported that cutoff value was defined using 2 × the standard deviation (SD), delayed children who were less than 24 months was estimated about 2000 children in this study. However, more than 2000 children were defined delayed children in this study (Table 2). Therefore, the cutoff value may have been overestimated. We may therefore have overestimated the number of children with developmental delays, which may have led to false-positive associations between the Cd concentration and developmental delay categories. We assessed whether the number of children with developmental delays was overestimated by performing logistic regression analysis using altered cut off values for the ASQ-3 subcategories. We used the mean − (2 × SD) as the cut off value for each category (Supplemental Table 2). Similar results were found from this analysis (aOR for gross motor function at 1 year 1.19, 99.7% CI 1.03–1.38; aOR for problem solving at 1 year 1.21, 99.7% CI 1.04–1.41; aOR for gross motor function at 1.5 years 1.19, 99.7% CI 1.01–1.40) to the analysis using the original cutoff values. These results indicated that overestimating the number of children with delayed development did not give strongly false-positive results.

In our study, the covariates adjustment varied for different ages. Some of these covariates may be on the causal pathway. To assess the difference of covariates, we used logistic regression analysis using all covariates for sensitive analysis. Similar results were found from this analysis (Supplemental Table 4) compared with the results of selected covariates. These results indicated that there were no significant effect of covariates selection on the causal pathway.

4. Discussion

Exposure to Cd, such as through occupational exposure or exposure to polluted soil, to give Cd concentrations >5 ng/mL in blood can cause negative effects on the kidneys, bones, and respiratory system (Reeves and Chaney, 2008; Rzymski et al., 2015). Prenatal exposure of a fetus to Cd has been found to be associated with atopic dermatitis (Tsai et al., 2021). Recently, it was reported that prenatal Cd exposure cause poor cognition, but data relating to this are inconsistent. It is therefore unclear whether exposure of a fetus to Cd during pregnancy will affect the development of the child. To elucidate whether cadmium affect the child development or not, we analyzed nation-wide cohort study data. A correlation was found between prenatal Cd concentrations in maternal blood and delayed child development for 1.5 years after birth (Tables 3–6). However, the effect had disappeared at 2 years after birth or later (Table 3). Therefore Prenatal exposure to Cd in Japan therefore temporarily affects early child development.

Relationships between Cd concentrations in maternal blood at 8 weeks gestation and mental deficiency, decreased intelligence, and decreased Apgar scores have been found in previous studies (Kippler et al., 2012; Mokhtar et al., 2002). Some animal research relating to Cd-related behavioral changes has used operant paradigms (Ali et al., 1986; Barański, 1986; Grover et al., 1991; Newland et al., 1986; Pelletier and Satinder, 1991). The Cd concentration in maternal blood at the first trimester (Cd > 1.49 μg/L) was found to be negatively associated with cognition of the infant in an epidemiological study performed in South Korea (Jeong et al., 2015). The Cd concentration in maternal blood collected at delivery (Cd > 1.24 μg/L) from a Chinese cohort was found to be negatively associated with social skills and the brain-derived neurotrophic factor concentration in cord serum (Wang et al., 2016). These results indicated that exposure of a fetus to Cd may alter brain function by altering neural circuits or the release of neurotransmitters such as brain-derived neurotrophic factor. However, in another study, no significant association was found between exposure of a fetus to 1.4–1.5 μg/L of Cd during early pregnancy and child development at 6 months (Kim et al., 2013). No significant association was found between exposure of a fetus to 0.53 μg/L of Cd in the first and third trimesters and the cognitive score at the age of 4 years in a Spanish cohort study (Forns et al., 2014). We found a correlation between prenatal exposure to Cd at Cd concentrations >0.91 ng/mL in maternal blood at the second or third trimester of pregnancy and early child development, including mental and social development. However, this effect was not stable and disappeared as the age increased (Tables 3–5). These inconsistent results may be explained by the Cd concentrations found in the different studies. The Cd concentrations were lower in the blood samples collected from the participants of the Spanish study than in the blood

Table 2
Child development status.

Category		6 months		1 year		1.5 years		2 years		2.5 years		3 years	
		N	%	N	%	N	%	N	%	N	%	N	%
Communication	Normal	80204	99.33	76653	99.85	70791	97.68	72971	95.88	70446	94.88	72891	95.87
	Delayed	543	0.67	117	0.15	1680	2.32	3132	4.12	3799	5.12	3140	4.13
	Missing data	15418		19395		23694		20062		21920		20134	
Gross Motor	Normal	72213	89.44	72274	94.13	68966	95.14	71415	93.84	70867	95.41	72604	95.36
	Delayed	8526	10.56	4505	5.87	3524	4.86	4688	6.16	3407	4.59	3536	4.64
	Missing data	15426		19386		23675		20062		21891		20025	
Fine Motor	Normal	76316	94.77	72092	93.95	69181	95.49	74232	97.63	69415	93.91	69940	92.22
	Delayed	4208	5.23	4642	6.05	3265	4.51	1805	2.37	4499	6.09	5903	7.78
	Missing data	15641		19431		23719		20128		22251		20322	
Problem Solving	Normal	71807	88.93	72567	94.65	68965	95.76	72485	95.51	69647	94.00	69725	92.44
	Delayed	8937	11.07	4098	5.35	3052	4.24	3404	4.49	4442	6.00	5706	7.56
	Missing data	15421		19500		24148		20276		22076		20734	
Personal–social	Normal	77495	96.10	75530	98.69	70545	97.38	73682	96.97	71519	96.45	73363	96.57
	Delayed	3144	3.90	1004	1.31	1899	2.62	2304	3.03	2633	3.55	2607	3.43
	Missing data	15526		19631		23721		20179		22013		20195	

Cutoff values are indicated in Supplemental Table 1.

Table 3
Perinatal effect of cadmium on child development.

Cadmium concentration	Adjusted OR	Lower 99.7% CI	Upper 99.7% CI
6 months *development*[a]			
<1st quartile (0.496 ng/L)	ref		
1st quartile < x < 2nd quartile (0.663 ng/L)	1.02	0.94	1.11
2nd quartile < x < 3rd quartile (0.905 ng/L)	1.05	0.97	1.14
3rd quartile < x < maximum (5.33 ng/L)	1.10*	1.01	1.20
1 year development[b]			
<1st quartile	ref		
1st quartile < x < 2nd quartile	1.02	0.93	1.12
2nd quartile < x < 3rd quartile	1.08	0.98	1.19
3rd quartile < x < maximum	1.13*	1.02	1.24
1.5 years development[c]			
<1st quartile	ref		
1st quartile < x < 2nd quartile	1.03	0.92	1.14
2nd quartile < x < 3rd quartile	1.06	0.95	1.18
3rd quartile < x < maximum	1.15*	1.03	1.28
2 years development[d]			
<1st quartile	ref		
1st quartile < x < 2nd quartile	0.96	0.86	1.06
2nd quartile < x < 3rd quartile	1.04	0.93	1.15
3rd quartile < x < maximum	1.08	0.97	1.20
2.5 years development[e]			
<1st quartile	ref		
1st quartile < x < 2nd quartile	0.96	0.86	1.06
2nd quartile < x < 3rd quartile	1.00	0.90	1.10
3rd quartile < x < maximum	1.05	0.94	1.16
3 years development[f]			
<1st quartile	ref		
1st quartile < x < 2nd quartile	1.00	0.91	1.11
2nd quartile < x < 3rd quartile	1.04	0.94	1.14
3rd quartile < x < maximum	1.07	0.97	1.19

OR, odds ratio; CI, confidence interval; ref, reference.
a: Adjusted for age, marriage, smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, household income, delivery method, heavy metal concentration(lead, mercury). N = 65,882.
b: Adjusted for age, marriage, medical history (ADHD), smoking, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, delivery method, gestational diabetes, heavy metal concentration (mercury). N = 68,358.
c: Adjusted for age, smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's medical history (diabetes), mother's BMI, delivery method, gestational diabetes, going to nursery school, heavy metal concentration(mercury). N = 62,182.
d: Adjusted for age, medical history (autism, others), smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, household income, mother's medical history (diabetes), mother's BMI, delivery method, gestational diabetes, going to nursery school. N = 62,045.
e: Adjusted for age, marriage, medical history (ADHD), smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, household income, mother's medical history (diabetes), mother's BMI, delivery method, gestational diabetes, going to nursery school, heavy metal concentration(mercury). N = 59,046.
f: Adjusted for age, marriage, medical history (ADHD, others), smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, household income, mother's medical history (diabetes), mother's BMI, delivery method, gestational diabetes, going to nursery school, hevy metal concentration(lead, mercury). *N* = 60,119.
* significantly different, $p < 0.003$, logistic regression analysis.

samples collected from the participants of the Chinese, Korean, and Japanese studies. A lower dose of Cd (e.g. 0.53 μg/L of Cd from Forns's study) may not affect neural development. We found no relationship between low Cd concentrations (at least less than 0.91 ng/ml) in maternal blood and child development (Table 3). However, as was found in previous studies, we found that higher Cd concentrations in maternal blood were associated with child development. In Kim's study, although

Table 4
Perinatal effect of cadmium on child development sub-categories.

	Adjusted OR	Lower 99.7% CI	Upper 99.7% CI
6 months development			
Communication[a]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	1.09	0.73	1.64
2nd quartile < x < 3rd quartile	1.09	0.73	1.64
3rd quartile < x < maximum	1.24	0.82	1.87
Gross motor function[b]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	1.02	0.92	1.13
2nd quartile < x < 3rd quartile	1.03	0.93	1.14
3rd quartile < x < maximum	1.11	1.00	1.24
Fine motor function[c]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	1.02	0.88	1.19
2nd quartile < x < 3rd quartile	1.11	0.96	1.29
3rd quartile < x < maximum	1.08	0.93	1.27
Problem solving[d]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	1.04	0.93	1.15
2nd quartile < x < 3rd quartile	1.08	0.97	1.20
3rd quartile < x < maximum	1.10	0.98	1.22
Personal–social[e]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	0.98	0.83	1.16
2nd quartile < x < 3rd quartile	1.15	0.98	1.36
3rd quartile < x < maximum	1.12	0.94	1.33
1 year development			
Gross Motor[f]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	1.02	0.88	1.18
2nd quartile < x < 3rd quartile	1.12	0.97	1.30
3rd quartile < x < maximum	1.25*	1.07	1.45
Fine Motor[g]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	0.99	0.86	1.13
2nd quartile < x < 3rd quartile	1.03	0.90	1.18
3rd quartile < x < maximum	0.99	0.86	1.15
Problem Solving[h]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	1.08	0.93	1.26
2nd quartile < x < 3rd quartile	1.16	1.00	1.35
3rd quartile < x < maximum	1.22*	1.05	1.42
1.5 years development			
Communication[i]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	1.04	0.83	1.32
2nd quartile < x < 3rd quartile	0.98	0.77	1.24

(*continued on next page*)

Table 4 (*continued*)

	Adjusted OR	Lower 99.7% CI	Upper 99.7% CI
3rd quartile < x < maximum	1.19	0.94	1.50
Gross Motor[j]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	1.01	0.86	1.19
2nd quartile < x < 3rd quartile	1.11	0.94	1.30
3rd quartile < x < maximum	1.19*	1.01	1.40
Fine Motor[k]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	1.04	0.87	1.23
2nd quartile < x < 3rd quartile	1.01	0.85	1.20
3rd quartile < x < maximum	1.09	0.92	1.29
Problem Solving[l]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	1.01	0.85	1.20
2nd quartile < x < 3rd quartile	1.11	0.93	1.32
3rd quartile < x < maximum	1.09	0.92	1.30
Personal–social[m]			
<1st quartile	Ref		
1st quartile < x < 2nd quartile	0.99	0.80	1.23
2nd quartile < x < 3rd quartile	0.95	0.76	1.18
3rd quartile < x < maximum	1.12	0.90	1.39

OR, odds ratio; CI, confidence interval; ref, reference.

a: Adjusted for age, medical history (autism), smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, heavy metal concentration (lead, mercury). N = 71,056.

b: Adjusted for age, marriage, smoking, number of pregnancy, AQ10, multiple births, gestation time, birth weight, child sex, delivery method, going to nursery school, heavy metal concentration(lead, mercury). N = 70,568.

c: Adjusted for age, marriage, smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, mother's education, household income, delivery method, going to nursery school. Heavy metal concentration(lead, mercury). N = 65,989.

d: Adjusted for age, marriage, smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, mother's education, household income, delivery method, gestational diabetes, heavy metal concentration(lead, mercury). N = 66,245.

e: Adjusted for age, marriage, medical history (other developmental disorders), smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's BMI, delivery method, going to nursery school, heavy metal concentration(lead, mercury). N = 70,457.

f: Adjusted for age, marriage, smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, household income, delivery method, going to nursery school, heavy metal concentration (mercury). *N* = 62,867.

g: Adjusted for age, smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's BMI, delivery method, gestational diabetes, going to nursery school, heavy metal concentration(lead). N = 67,207.

h: Adjusted for age, smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, mother's BMI, delivery method, medical history (diabetes), gestational diabetes, going to nursery school, heavy metal concentration (mercury). N = 66,936.

i: Adjusted for age, medical history (other developmental disorder), smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, household income, mother's BMI, delivery method, going to nursery school, heavy metal concentration(mercury). N = 58,881.

j: Adjusted for age, medical history (ADHD), smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, delivery method, medical history (diabetes), gestational diabetes, going to nursery school, heavy metal concentration(mercury). N = 62,659.

k: Adjusted for age, medical history (learning disorder), number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, house income, mother's BMI, delivery method, medical history (diabetes), gestational diabetes, going to nursery school, heavy metal concentration(mercury). N = 59,111.

l: Adjusted for age, smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, medical history (diabetes), mother's BMI, delivery method, gestational diabetes, going to nursery school, heavy metal concentration(mercury). N = 62,052.

m: Adjusted for age, smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, delivery method, medical history (diabetes), gestational diabetes, going to nursery school, heavy metal concentration (mercury). N = 62,629.

* significant difference, $p < 0.003$, logistic regression analysis.

they said there were no significant association between Cd and child development, there was a synergistic negative effect on child development when there was co-exposure of Pb and >1.51 μg/L Cd during pregnancy (Kim et al., 2013). Thus we concluded that higher prenatal doses of Cd may have an effect on infant development, but only in the early development stages.

It has previously been found that birth weight can be related to child development disorders (Orri et al., 2021). Prenatal exposure to Cd was found to affect the birth weight in Japanese studies (Inadera et al., 2020; Tsuji et al., 2018). These results indicated that Cd affects child development by decreasing the birth weight. However, we took the birth weight into account when performing multivariate logistic regression analysis and still found a separate significant effect of prenatal exposure to Cd on child development (Tables 3–6). In addition to our results, it has been found that Cd can block Ca^{2+} influx in neurons and Cd can bind to synaptotagmin-1, which is the main Ca^{2+} sensor involved in the release of neurotransmitters (Chow, 1991; Katti et al., 2017). These results indicate that Cd affects child development through different mechanisms to the birth weight, such as by affecting the release of neurotransmitters. Further studies should be focused on elucidating the molecular mechanisms involved.

There are various well known sources of Cd to humans, including cigarettes, food (particularly fish, rice, and root vegetables), and water (Faroon et al., 2012; Järup and Åkesson, 2009; Nakayama et al., 2019). Rice is an important part of the Japanese diet, so it is important to determine whether maternal and infant consumption of Cd in rice affects child health and development. We found that the Cd concentration in maternal blood was related to early child development (Tables 3–6). However, we could not find the significant association between maternal Cd concentration and child development after 2 years after birth. In this study, the consistency of child developmental delay over time was significantly fair but not so high (Kappa = 0.28, $p < 0.001$). These results suggests that prenatal exposure to Cd in Japan may weakly affect child development and the effect might be covered by other factors such as the increase of the age. The Japanese Ministry of Agriculture, Forestry and Fisheries found lower Cd concentrations in rice grown in 2009 than in rice grown in 1997 (Ministry of Agriculture, Forestry and Fisheries, 2021). Many Japanese people eat rice as the main part of every meal and root vegetables and fish as side dishes, and it has been suggested that Cd in the typical Japanese diet causes no or slight adverse health effects. The Japanese reference value for Cd in rice was decreased from 1 to 0.4 mg/kg in 2011. Pregnant women may therefore not need to take steps to avoid excessive Cd intake in food. However, cigarettes are a source of Cd, so pregnant women should avoid exposure to cigarettes smoke to prevent their fetuses being small for the gestation time, delayed development of their infants, and other negative effects (Rogers, 2008).

We found that the effects of prenatal exposure to Cd were not seen after 2 years old. Interestingly, it has previously been found that occupational exposure of pregnant women to organic solvents in cleaning

Table 5
Multinomial logistic regression analysis results for the relationships between the numbers of delayed categories and Cd concentrations in blood.

	Adjusted OR	Lower 95% CI	Upper 95% CI
6 months development (reference: no delay)[a]			
Number of delays = 1			
<1st quartile	ref		
1st quartile < x < 2nd quartile	1.01	0.95	1.08
2nd quartile < x < 3rd quartile	1.02	0.95	1.09
3rd quartile < x < maximum	1.09**	1.02	1.17
Number of delays = 2			
<1st quartile	ref		
1st quartile < x < 2nd quartile	1.03	0.93	1.14
2nd quartile < x < 3rd quartile	1.06	0.96	1.18
3rd quartile < x < maximum	1.08	0.97	1.20
Number of delays ≥ 3			
<1st quartile	ref		
1st quartile < x < 2nd quartile	1.04	0.90	1.21
2nd quartile < x < 3rd quartile	1.22**	1.05	1.41
3rd quartile < x < maximum	1.21*	1.04	1.40
1 year development (reference: no delays)[b]			
Number of delays = 1			
<1st quartile	ref		
1st quartile < x < 2nd quartile	1.04	0.97	1.12
2nd quartile < x < 3rd quartile	1.07	1.00	1.15
3rd quartile < x < maximum	1.13***	1.05	1.22
Number of delays = 2			
<1st quartile	ref		
1st quartile < x < 2nd quartile	0.95	0.82	1.10
2nd quartile < x < 3rd quartile	1.09	0.94	1.26
3rd quartile < x < maximum	1.08	0.93	1.26
Number of delays ≥ 3			
<1st quartile	ref		
1st quartile < x < 2nd quartile	1.03	0.82	1.29
2nd quartile < x < 3rd quartile	1.16	0.93	1.45
3rd quartile < x < maximum	1.19	0.94	1.50
1.5 years development (reference: no delays)[c]			
Number of delays = 1			
<1st quartile	ref		
1st quartile < x < 2nd quartile	1.03	0.95	1.12
2nd quartile < x < 3rd quartile	1.09*	1.00	1.18
3rd quartile < x < maximum	1.17***	1.07	1.27
Number of delays = 2			
<1st quartile	ref		
1st quartile < x < 2nd quartile	1.08	0.93	1.26
2nd quartile < x < 3rd quartile	1.04	0.89	1.22
3rd quartile < x < maximum	1.13	0.96	1.33
Number of delays = 3			
<1st quartile	ref		
1st quartile < x < 2nd quartile	1.08	0.89	1.30
2nd quartile < x < 3rd quartile	1.12	0.93	1.35
3rd quartile < x < maximum	1.27*	1.05	1.53

OR, odds ratio; CI, confidence interval; ref, reference.
a: Adjusted for age, marriage, medical history (ADHD, LD, autism, others), smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, household income, mother's BMI, delivery method, heavy metal concentration(lead, mercury). N = 65,865.
b: Adjusted for age, marriage, medical history (ADHD, LD, autism, others), smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, mother's BMI, delivery method, gestational diabetes, going to nursery school, heavy metal concentration(lead, mercury). N = 66,457.
c: Adjusted for age, medical history (ADHD, LD, others), smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, mother's education, medical history (diabetes), mother's BMI, delivery method, gestational diabetes, heavy metal concentration(lead, mercury). N = 63,139.
*, **, and *** indicate $p < 0.1$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

agents, cosmetics, detergent, dyes, gasoline, glue, grease remover, ink, paint, paint stripper, textile treatments, and varnish is associated with effects on early child behavior and that these effects may be attenuated over time (Costet et al., 2018). Prenatal exposure to xylene has been found to affect postnatal development and behavior in rats, and the effects were found to be attenuated over time (Hass et al., 1995). Infants are expected to be exposed very little to organic solvents after delivery, so the effects of exposure before delivery will be attenuated over time and/or compensatory neural development mechanisms may occur. Low levels of exposure to Cd after delivery and compensatory mechanisms may also affect the delays in child development caused by prenatal exposure to Cd. Further studies should be focused on elucidating the relationship between infant development and exposure to Cd at different times.

There were four main limitations to this study. First, we could not consider the exposure to Cd and other heavy metal after birth. It is possible that exposure to Cd and other heavy metal after birth may affect infant development. To address this, it will require heavy metal concentrations in blood samples or hair samples from infants to be determined and a statistical model containing terms for exposure to Cd and other heavy metals after birth to be developed. Second, we only considered Cd concentration in maternal blood and did not consider the urinary Cd, which is the long-term exposure indicator of Cd. If urinary Cd concentration was considered, we can see the estimation of Cd exposure from starting pregnancy and estimate the precise Cd effect on child development. To elucidate this, further study should be needed. Third, the outcome and confounding factor data were taken from questionnaires completed by the caregiver participating in the study. Observer bias may have affected the data, particularly because the ASQ-3 questionnaire was completed by the caregiver. For example, if a mother evaluates her child strictly, the child development may be underestimated, and if the mother evaluates the child gently, the child development may be overestimated. Objective records such as K-type development test results acquired by medically trained staff will be required to address this problem. Recently, Ma and colleagues reported the association between cadmium concentration and K-type development test (Ma et al., 2021). Compared with previous studies, our results considering about individual difference using GLMM were consistent with previous study. Taking together with previous studies, our results will reinforce the hypothesis about the cadmium effect on child neuronal

Table 6
Model of the association between cadmium concentrations during pregnancy and the number of delayed child development categories.

	Crude			Model #1			Model #2		
	Coefficient	SE	p value	Coefficient	SE	p value	Coefficient	SE	p value
Blood Cadmium Concentration[a]	0.16	0.019	<0.001	0.08	0.021	<0.001	0.08	0.022	<0.001

Model #1 was adjusted for age, marriage, smoking, household income, multiple births, and child sex.
Model #2 was adjusted for age, marriage, smoking, number of pregnancies, AQ10, multiple births, gestation time, birth weight, child sex, household income, mother's BMI, delivery method, and going to nursery school, heavy metal concentration(lead, mercury).
a: The cadmium concentration in blood was used as a continuous variable.
SE: standard error.

development. Fourth, missing data handling method was used pairwise deletion. In our study, because the missing value did not associate with other missing values, missing value developed by Missing At Random (MAR). We used logistic regression or GLMM to analyze the association between cadmium and child development. Logistic regression and GLMM estimate the parameter by maximum likelihood estimation method. Maximum likelihood estimation method was reported to produces no significant bias even if there were the data containing missing data produced by MAR (Little and Rubin, 2001; Rubin, 1976). Therefore, although missing data can affect the conclusion in our study, this bias of missing data may be very small. To completely avoid the bias of missing data, further epidemiological study to reduce the missing data such as the interview by other people but not self-administrated questionnaire by should be needed.

5. Conclusion

Prenatal exposure to Cd was found to temporarily affect child development after birth.

Declaration of competing interest

All authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113989.

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Associations between the built environment and emotional, social and physical indicators of early child development across high and low socioeconomic neighbourhoods

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ABSTRACT

Emerging evidence indicates that the built environment influences early child development. Access to, and the quality of, built environment features vary with the socioeconomic status (SES) of neighbourhoods. It has not yet been established whether the association between built environment features and early child development varies by neighbourhood SES. We sought to identify built environment features associated with neighbourhood-level variations in the early child development domains of physical health and wellbeing, social competence, and emotional maturity, and how these associations differ among high and low SES neighbourhoods where child development patterns follow expected outcomes ("on-diagonal" neighbourhoods) and where child development patterns differ from expected outcomes ("off-diagonal" neighbourhoods). This cross-sectional study analysed data from the Australian Early Development Census (AEDC) for children residing in 3839 neighbourhoods in the Perth and Peel metropolitan areas of Western Australia. Children's AEDC scores were aggregated at the area-level and merged with Geographic Information Systems derived measures of neighbourhood residential density, parks, walkability, community facilities and public transport. Multivariate logistic regressions modelled the odds of low and high SES neighbourhoods having a higher proportion of children developmentally "on-track" (scores in the 26th to 100th percentile of the AEDC) or "not on-track" (scores in the bottom 25th percentile of the AEDC) for each built environment feature. In high SES neighbourhoods, better development across all three domains was associated with greater residential density and improved access to parks, public transport, learning, childcare and health services. Conversely, in low SES neighbourhoods, greater residential density was associated with better physical, but poorer social and emotional development; increased traffic and street connectivity were associated with poorer physical and emotional development; shorter distances to parks, learning, childcare and health services were associated with poorer physical and emotional development; and more services and public transport stops were associated with poorer emotional development. The mixed findings in low SES neighbourhoods suggest that positive associations with built environment features seen in one domain of early child development may be negative in other domains. The reasons for the mixed findings in low SES neighbourhoods are likely multifactorial and may include parental neighbourhood perceptions, as well as quality and usage of built environment features. These findings can be used to inform state and local governments to establish child-friendly town planning and urban design features. Further research is needed to confirm the interplay between SES, early child development, the built environment and other unmeasured factors to better inform public health policy.

1. Introduction

The early years of a child's life provide significant opportunity to establish positive trajectories for health and development as children undergo considerable development of neural pathways (Shonkoff et al., 2012). Social ecological theory proposes that child development is the product of a combination of various influences on a child, ranging from interactions with immediate family members and the neighbourhood, to

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broader cultural and governmental influences (Bronfenbrenner, 1979). Features that may influence a child's developmental trajectory include parenting attributes, family size, parent employment status, community infrastructure, social cohesion, schooling, and built environment features (Christian et al., 2015). Development is also influenced by the complex interplay of genetic predisposition and environmental stressors (e.g., pollutants, lifestyle factors), known as the exposome (Maitre et al., 2021; Renz et al., 2017; Wild, 2005). Features of the environments in which children develop are shaped, in part, by individual, family, and area-level socioeconomic status (SES) (Chin-Lun Hung et al., 2015). Children who grow up in families or neighbourhoods of lower SES tend to exhibit poorer child development and school readiness (Roos et al., 2019).

Recognised domains of ECD include cognitive, language, speech, social, emotional and motor skill development (Janus and Offord, 2007). There is a plethora of literature on the interplay of parenting attributes, individual biomedical features and socioeconomic status on ECD (Goldfeld et al., 2015; Kershaw et al., 2007). However, the role of the built environment on ECD is an area of emerging research (Christian et al., 2015; Villanueva et al., 2016). The built environment consists of human influences on communities and neighbourhoods, including the design and construction of facilities, services, and the connections between these (Brooks-Gunn et al., 2010). Built environment characteristics vary across neighbourhoods of differing SES status in terms of quality and quantity (Flouri et al., 2016). Previous research demonstrates that physical, social and emotional domains of ECD have the strongest association with built environment features (Bell et al., 2020; Christian et al., 2017a).

Built environment features proposed to influence ECD include housing density and quality, access to services, playgrounds, parks and the outdoor home environment (Christian et al., 2015; Clair, 2018). The immediate home environment may facilitate opportunities for physical activity and play; larger yards are found to be associated with greater physical activity than smaller yards (Salmon et al., 2004), and improved housing quality is associated with better educational outcomes (Clair, 2018). Increased housing density and access to more parks have also been associated with increased physical activity in pre-school children (Ortegon-Sanchez et al., 2021; Roemmich et al., 2006).

Increased housing density often coexists with high traffic levels which makes a considerable contribution toward noise and air pollution (Kim et al., 2012). A European study examined four population-based birth-cohorts and found that antenatal exposure to increased street connectivity, and $PM_{2.5}$ (particulate matter <2.5 μm in diameter) were associated with poorer verbal abilities and poorer fine motor function respectively, at five years of age (Binter et al., 2022). Children are particularly vulnerable to environmental noise, which can affect their sleep, memory, comprehension, communication, task performance, reading skills, mood, and psychological stress responses (Clark and Paunovic, 2018). Walkable neighbourhoods with increased street connectivity, residential density, and mixed land use have been reported to counteract the negative effects from high traffic volumes in adults; however, it is unknown if this applies to young children (Giles-Corti et al., 2013).

Further investigation of the influence of the built environment on ECD can be facilitated by population-level ECD data that can be aggregated at the neighbourhood-level. In Australia, population-level ECD is monitored using the Australian Early Development Census (AEDC) (Australian Early Development Census, 2020). The AEDC is a national census of ECD completed by teachers every three years for children in their first year of full-time school. AEDC scores can be aggregated within geographic boundaries to provide a snapshot of ECD within neighbourhoods. When merged with data on the built environment, the AEDC provides a rich source of data for examining the influence of the built environment on ECD, and how developmental outcomes vary across neighbourhoods.

Interpretation of the influence of the built environment on ECD must occur within the context of other ECD determinants such as socioeconomic features. One approach is to consider "off-diagonal" neighbourhoods where children's outcomes do not follow expected trajectories for ECD and sociodemographic data (Goldfeld et al., 2017; Tanton et al., 2017). "Off-diagonal" neighbourhoods are those in which high proportions of children developmentally not on-track (those who scored in the bottom 25th percentile in the AEDC) are seen in high SES neighbourhoods, or high proportions of children developmentally on-track (those who scored in the 26th to 100th percentile in the AEDC) are seen in low SES neighbourhoods (Goldfeld et al., 2017; Tanton et al., 2017). Conversely, "on-diagonal" neighbourhoods follow expected patterns according to socioeconomic status, whereby lower proportions of children developmentally not on-track are seen in high SES neighbourhoods, and higher proportions of children developmentally not on-track are seen in low SES neighbourhoods. Comparing "on-diagonal" to "off-diagonal" neighbourhoods can help with isolating built environment features that may be associated with developmental outcomes, and to determine whether these features differ across high and low SES neighbourhoods (Kershaw et al., 2007; Goldfeld et al., 2017).

The aim of this study was to investigate the association between built environment features (residential measures, parks, walkability, community facilities and public transport) and variations in neighbourhood level measures of physical health and wellbeing, social competence and emotional maturity development of 5-year old children, as measured by the AEDC. By comparing "off-diagonal" neighbourhoods to "on-diagonal" neighbourhoods of a similar SES, we aimed to identify features of the built environment that are differentially associated with ECD within the context of broader socioeconomic factors. We hypothesised that the built environment features associated with better developmental outcomes would differ across high and low SES neighbourhoods.

2. Methods

2.1. Study design and participants

This cross-sectional linked data study investigated associations between built environment features and ECD. Data on ECD were obtained from the AEDC, and built environment features were created using geographic information systems (GIS); both datasets were collected in 2012. Data linkage was completed by the Western Australian (WA) Department of Health's Data Linkage Branch, whereby identifying information (e.g., names, addresses) from disparate data sets were matched, and then replaced with an encrypted key, to maintain individual confidentiality (Kelman et al., 2002). Individual consent was not required for the analysis of these linked population data (Kelman et al., 2002). Ethics approvals were obtained from the WA Department of Health Human Research Ethics Committee (reference removed for peer review) and the (Ethics Committee information removed for peer review).

The total linked cohort included 24,036 WA children who participated in the 2012 AEDC. During this year the AEDC had a response rate of 96.5% of the population of children in their first year of school (Australian Early Development Census, 2020). The average age of children was 5.4 years. Children classed as 'special needs' (i.e., children with diagnosed chronic medical, physical, or intellectually disabling conditions; n = 893) and those who were missing all AEDC domain scores (n = 84) were excluded. Children were retained in the study sample if they had a domain score for at least one of the developmental domains under investigation (physical health and wellbeing, social competence, emotional maturity). Individual-level AEDC data were aggregated into neighbourhoods, defined as children residing in the same Statistical Area Level 1 (SA1; n = 3839 SA1s). SA1s reflect local suburb delineations and have a population between 200 and 800 persons (average 400) (Australian Bureau of Statistics, 2017). GIS data were only available for the Perth and Peel metropolitan regions of Western Australia, requiring the exclusion of a further 15,150 children outside of

these areas. Lastly, SA1s with fewer than 10 children with AEDC data (n = 3278 SA1s) were excluded in the interest of maintaining participant confidentiality. The final sample included 561 SA1s that were eligible for selection for the on/off diagonal method.

2.2. Built environment features

Built environment features included residential measures (building footprint/lot ratio, dwelling density, single, duplex, triplex, quadruplex and greater than five dwellings), parks (pocket-small, medium-large and district-regional), walkability (traffic exposure, street connectivity and cul-de-sacs), community facilities (schools, child health centres, early childhood education and care centres, playgroups, services and convenience stores) and public transport (rail, school and standard bus stations). These features were derived using GIS data layers obtained and cleaned via the geospatial research team based at the Centre for the Built Environment and Health, at the University of Western Australia. Information about these measures have been published previously (Christian et al., 2017a) and are described in detail in Appendix 1.

2.3. ECD features

The AEDC is a teacher-completed instrument containing 104 items. Teachers score all children in their class on each item, based on classroom observations. These scores are then grouped into five developmental domains (physical health and wellbeing, social competence, emotional maturity, communication skills and general knowledge, and language and cognitive skills [school based]). The method of calculating AEDC domain scores is the intellectual property of McMaster University and details are not available for publication. This study focused on the physical health and wellbeing, social competence, and emotional maturity domains due to existing evidence of an association between these developmental areas and built environment features (Bell et al., 2020; Christian et al., 2017a). Examples of areas assessed for physical health and development include fine and gross motor skills, tiredness, hunger, illness, energy, self-care and personal hygiene. Social competence items include play and peer interactions, self-confidence, following instructions, listening, respecting and helping others. Emotional well-being items include self-control, eagerness to try new activities, tolerance of others, anxiety, aggression, sadness, inattention and impulsiveness. Further details of AEDC domains and survey items are published elsewhere (Australian Early Development Census, 2020; Brinkman et al., 2006). Domain scores are classified according to national percentiles developed in 2009 during the first national data collection (Australian Early Development Census, 2020). Children who score in the bottom 10th percentile are deemed 'developmentally vulnerable', those with scores between the 11th and 25th percentile 'developmentally at-risk', and children scoring in the 26th to 100th percentile 'developmentally on-track' (Australian Early Development Census, 2020). The AEDC is based on the Canadian Early Development Instrument (EDI), which has good inter-rater and test-retest reliability (Janus and Offord, 2007), and good construct and concurrent validity (Brinkman et al., 2006). For this study, children were classified as either 'developmentally on-track' or 'developmentally not on-track' (with the developmentally vulnerable and developmentally at-risk categories combined, to capture established and emerging vulnerabilities). The number of all children within each SA1 whose AEDC scores fell within the 'developmentally not on-track' and the 'developmentally on-track' categories on each of the three AEDC domains was calculated.

2.4. Sociodemographic features

Neighbourhood SES was measured using the Index of Relative Socio-economic Disadvantage (IRSD) (Australian Bureau of Statistics, 2013). The IRSD is an area-level measure that summarises socio-economic advantage and disadvantage using information collected from the 5-yearly Census of Population and Housing. Each SA1 is assigned an IRSD quintile; lower scores indicate greater disadvantage (Australian Bureau of Statistics, 2013).

Sociodemographic features included male gender, Aboriginal and Torres Strait Islander children (hereafter referred to as Aboriginal) and those with English as a second language (ESL). Data were obtained from the AEDC and reported as the number of children per SA1 with these characteristics.

2.5. On and off diagonal neighbourhoods

Applying a method developed by Tanton et al. (2017), neighbourhoods were divided into quintiles for both SES and level of developmental vulnerability for each of the three AEDC domains (Fig. 1). Groups A and B represent neighbourhoods where results did not follow expected outcomes for SES and vulnerability (off-diagonal) with group A representing neighbourhoods of low SES and low vulnerability (fewer children developmentally not on-track) and group B representing neighbourhoods of high SES and high vulnerability (more children developmentally not on-track). Groups C and D represent expected outcomes (on diagonal) with group C representing neighbourhoods of high SES and low vulnerability (fewer children developmentally not on-track) and group D representing neighbourhoods of low SES and high vulnerability (more children developmentally not on-track). Due to the reduced number of off diagonal, compared with on diagonal, neighbourhoods, groups A and B utilised adjacent quintiles to achieve statistical power (Appendix 2). A total of 260 unique SA1s were included in the analyses (136 low SES and 124 high SES neighbourhoods), many of which were selected for more than one AEDC domain. In the final models, there were 159 SA1s, 162 SA1s and 165 SA1s for physical health and wellbeing, emotional maturity and social competence models respectively.

In statistical analyses, group A was compared to group D (low SES neighbourhoods with low and high vulnerability, respectively) and group B was compared to group C (high SES neighbourhoods with high and low vulnerability, respectively).

2.6. Statistical analysis

Descriptive statistics were aggregated across all SA1s that were included in the analyses, and calculated at the SA1 level for low and high SES neighbourhoods separately. Multivariate logistic regressions were conducted using the events/trials method in SAS Version 9.4 (SAS Institute Inc., 2010). On-diagonal neighbourhoods were used as the reference group in all models, meaning that for low SES neighbourhoods, logistic regressions predicted the odds of neighbourhoods having more children developmentally on-track (as a proportion of all children within the SA1 with valid AEDC data), and for high SES neighbourhoods, logistic regressions predicted the odds of neighbourhoods having fewer children developmentally on-track (as a proportion of all children within the SA1 with valid AEDC data). Put simply, for low SES neighbourhoods, we assessed the odds of children being developmentally on-track (as opposed to not on-track), whereas for high SES neighbourhoods we assessed the odds of children being developmentally not on-track (as opposed to on-track). Built environment features were entered in models separately, adjusting for area-level counts of children who were Aboriginal, male, and spoke English as a second language.

3. Results

3.1. Descriptive statistics

3.1.1. Socio-demographic features

On average, there was a higher proportion of Aboriginal children and children with English as a second language across low, compared to high SES neighbourhoods (Table 1). The proportion of male children did not

			SES Quintiles [b]				
			Low SES [e]			High SES [f]	
			1st	2nd	3rd	4th	5th
AEDC Quintiles [a]	Low % vulnerability [c]	1st	Group A				Group C
		2nd					
		3rd					
	High % vulnerability [d]	4th					
		5th	Group D				Group B

[a] Australian Early Development Census
[b] Socioeconomic status
[c] Neighborhoods that are in the first and second quintile for vulnerability (less children developmentally not on track)
[d] Neighborhoods that are in the fourth and fifth quintile for vulnerability (more children developmentally not on track)
[e] Neighborhoods that are in the first and second quintile for SES (low SES)
[f] Neighborhoods that are in the fourth and fifth quintile for SES (high SES)

Fig. 1. *AEDC and SES Quintile Tabulation.*

vary across neighbourhood SES.

3.1.2. Built environment features

Table 1 also displays the descriptive statistics for built environment features. High SES neighbourhoods had marginally increased dwelling density compared with low SES neighbourhoods. There were slightly more parks in high SES neighbourhoods, compared with low SES neighbourhoods. However, the average distance to the closest park was further in high SES neighbourhoods, compared with low SES neighbourhoods. Low SES neighbourhoods had greater traffic exposure, reduced street connectivity and more cul-de-sacs compared with high SES suburbs. Access to community facilities including the number of, and distance to, schools, child health centres, early childhood education and care (ECEC) centres, and playgroups was largely equivalent across high and low SES neighbourhoods, with the exceptions of increased distance to closest playgroup and fewer services in low SES compared to high SES neighbourhoods. Compared to high SES neighbourhoods, low SES neighbourhoods had fewer public transport stops but the average distance to the closest stop was shorter.

3.2. Multivariate logistic regressions

3.2.1. Low SES neighbourhoods and odds of being developmentally on-track

Modelling of low SES neighbourhoods (Table 2) estimated the odds of children being developmentally on-track (i.e., the "unexpected" or "off-diagonal" outcome), with the reference group being low SES neighbourhoods with high proportions of children developmentally not on-track (i.e., the "expected" or "on-diagonal" outcome).

3.2.2. Physical health and wellbeing

Children living in low SES neighbourhoods had increased odds of being on-track on the physical health and wellbeing domain if their neighbourhoods had greater numbers of quadruplex dwellings (8% increased odds, 95% CI 1.01,1.16), higher counts of child health centres, services, and convenience stores (4%, 95% CI 1.00,1.07, 11%, 95% CI 1.02, 1.20, and 15% increased odds, 95% CI 1.08,1.23, respectively), but further distances to the closest park and ECEC centre (11%, 95% CI 1.05,1.18 and 86% increased odds, 95% CI 1.34,2.60, respectively). Lower odds of children in low SES neighbourhoods being on-track on the physical health and wellbeing domain were associated with increased numbers of single dwellings, total parks and medium-large parks (4%, 95% CI 0.94,0.99, 2%, 95% CI 0.95,0.99 and 9% lower odds, 95% CI 0.85,0.98, respectively), greater traffic exposure, street connectivity and cul-de-sacs (2%, 95% CI 0.97,0.99, 2%, 95% CI 0.97,0.99 and 14% lower odds, 95% CI 0.81,0.93, respectively), and more schools and playgroups within a 3 km radius (4%, 95% CI 0.92,0.99 and 10% lower odds, 95% CI 0.84,0.96, respectively).

3.2.3. Social competence

Children living in low SES neighbourhoods had increased odds of being on-track on the social competence domain if their neighbourhoods had greater numbers of cul-de-sacs (12% increased odds, 95% CI 1.04,1.20). Lower odds of children in low SES neighbourhoods being on-track on the social competence domain were associated with higher counts of triplex dwellings (1% reduced odds, 95% CI 0.98,0.99).

3.2.4. Emotional maturity

Children living in low SES neighbourhoods had increased odds of being on-track on the emotional maturity domain if their neighbourhoods were further away from the closest park, school, child health centre and ECEC centre (increased odds of 9%, 95% CI 1.03,1.16, 92%, 95% CI 1.44,2.57, 17%, 95% CI 1.02,1.34 and 43%, 95% CI 1.05,1.96 respectively). Lower odds of children in low SES neighbourhoods being on-track on the emotional maturity domain were associated with greater numbers of triplex, quadruplex and 5 or more dwellings (3%, 95% CI 0.96,0.99, 10%, 95% CI 0.85,0.95 and 8% lower odds, 95% CI 0.86, 0.99, respectively), greater total number of parks, and pocket-small parks (both 5% lower odds, 95% CI 0.92,0.97, and 0.92, 0.99, respectively), and more schools (9% lower odds, 95% CI 0.88,0.95), child health centres (4% lower odds, 95% CI 0.93,0.99), ECEC centres (4% lower odds, 95% CI 0.93,0.99), playgroups (10% lower odds, 95% CI 0.85,0.96), services (10% lower odds, 95% CI 0.83,0.98) and school bus stops (12% lower odds 95% CI 0.82,0.94).

3.2.5. High SES neighbourhoods and odds of being developmentally not on-track

Modelling of high SES neighbourhoods (Table 3) estimated the odds of children being developmentally not on-track (i.e., the "unexpected" or "off-diagonal" outcome), with the reference group being neighbourhoods with high proportions of children developmentally on-track (i.e., the "expected" or "on-diagonal" outcome).

3.2.6. Physical health and wellbeing

Children living in high SES neighbourhoods had increased odds of being not on-track on the physical health and wellbeing domain if their neighbourhoods had higher counts of medium-large parks and cul-de-sacs (7%; 95% CI 1.01,1.12 and 16% increased odds; 95% CI 1.06,1.27, respectively), and greater distances to the closest school, ECEC centre, and playgroup (39%; 95% CI 1.10,1.76, 34%; 95% CI

Table 1
Descriptive statistics for socio-demographic and built environment features in low and high SES neighbourhoods.

	Low SES SA1s (n = 136)		High SES SA1s (n = 124)	
	Mean % (SD)	% Range	Mean % (SD)	% Range
Socio-demographic features				
Proportion of Aboriginal children	8.17% (10.29)	0–50%	0.95% (3.05)	0–18%
Proportion of male children	49.52% (13.89)	10–83%	49.00% (15.30)	10–92%
Proportion of children with English as second language	17.56% (21.01)	0–90%	7.82% (9.56)	0–50%
Built environment features	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**
Residential Measures				
Building footprint/lot ratio[a]	32.16 (6.42)	6.75–46.12	36.87 (10.86)	1.03–54.84
Overall dwelling density [b]	4.40 (2.66)	0.06–13.66	4.64 (3.75)	0.02–17.51
Single dwellings[c]	14.61 (5.95)	0.39–31.48	16.03 (8.32)	0.06–40.93
Duplex dwellings[d]	6.54 (8.49)	0–39.20	7.58 (10.76)	0–54.20
Triplex dwellings[d]	2.99 (9.93)	0–64.70	2.06 (5.05)	0–34.20
Quadruplex dwellings[d]	1.06 (2.94)	0–15.80	1.15 (2.24)	0–10.20
Five or more dwellings[d]	1.73 (2.13)	0–9.20	2.31 (3.79)	0–16.30
Parks				
Total park count	12.27 (5.79)	0–29	12.80 (6.93)	0–30
Count of pocket-small parks	6.59 (4.80)	0–23	7.63 (4.73)	1–22
Count of medium-large parks	4.36 (2.22)	0–10	4.32 (2.79)	0–12
Count of district-regional Parks	1.42 (1.15)	0–5	1.39 (1.31)	0–5
Distance to closest park (100 m)	2.17 (2.35)	0–18.91	4.37 (8.27)	0–49.61
Walkability				
Traffic exposure (% high traffic roads)[e]	28.01 (12.53)	0–70.81	21.58 (12.56)	0–67.85
Street connectivity [f]	63.85 (21.20)	8.29–142.37	70.45 (26.71)	5.71–142.28
Cul-de-sacs [g]	40.08 (20.51)	3–108	28.95 (16.22)	1–94
Community Facilities				
School count within 3 km radius	5.83 (3.60)	0–17	5.53 (3.75)	0–14
Child health centre count within 10 km radius	8.81 (6.20)	0–24	9.07 (5.80)	1–20
Early childhood education & care centre count within 3 km radius	8.13 (4.49)	0–19	8.17 (5.70)	0–28
Playgroup count within 3 km radius	3.13 (2.59)	0–10	3.97 (3.35)	0–14
Distance to closest school (km)	0.96 (0.50)	0–2.91	1.08 (0.65)	0.08–2.87
Distance to closest child health centre (km)	2.20 (1.06)	0.39–5.28	2.86 (2.08)	0.27–9.56
Distance to closest early childhood education and care centre (km)	0.95 (0.49)	0–2.38	0.93 (0.58)	0.09–2.77
Distance to closest playgroup (km)	1.50 (0.70)	0.19–3.00	1.29 (0.74)	0.04–2.94
Count of services [h]	2.31 (0.03)	0–19	3.05 (3.56)	0–16
Count of convenience stores [i]	3.35 (3.03)	0–15	3.75 (4.42)	0–20
Public Transport				
Distance to closest standard transport stop (km)	0.46 (0.38)	0.01–4.08	0.56 (0.67)	0.06–3.91
Count of school bus stops within 1.6 km radius	0.92 (1.92)	0–9	2.03 (3.45)	0–16
Count of standard bus stops within 1.6 km radius	34.31 (17.29)	5–89	36.62 (22.86)	0–98
Count of rail stations within 5 km radius	3.04 (3.71)	0–14	4.33 (6.88)	0–33

Note. SES=Socio-Economic Status, SA1 = Statistical Area Level 1(i.e., neighbourhood). Descriptive statistics are aggregated across all SA1s included in subsequent analyses. All built environment features are measured at the SA1 level. All distance variables are measured from the SA1 centroid.

[a] Sum of SA1 building area/sum of SA1 lot area x 100.

[b] Count of residential dwellings per 10 m^2 of residential land area.

[c] Count of 100 dwellings per SA1; presented in a different format to other dwelling types due to the high count of single dwellings.

[d] Count of 10 dwellings per SA1.

[e] Percentage of total length of roads that are not main roads per SA1.

[f] Count of ≥3 way intersections per SA1.

[g] Count of 10 one-way nodes (cul-de-sacs) per SA1.

[h] Services include dry cleaner, post office, pharmacy, CD/DVD/Video store.

[i] Convenience stores include deli, general store, supermarket, produce market, seafood market, gas station, shopping centre, or other food market.

1.02,1.75, and 56% increased odds; 95% CI 1.22,1.99, respectively). Lower odds of children in high SES neighbourhoods being not on-track on the physical health and wellbeing domain were associated with increased overall residential density and percentage of the home lot that was building (7%; 95% CI 0.88,0.98 and 2% lower odds; 95% CI 0.97,0.99, respectively), greater numbers of duplex, quadruplex and 5 or more dwellings (4%; 95% CI 0.94,0.98, 8%; 95% CI 0.85,0.99 and 6% lower odds; 95% CI 0.89,0.98, respectively), greater traffic exposure (2% lower odds; 95% CI 0.97,0.99), and higher counts of pocket-small and district-regional parks (3%; 95% CI 0.93,0.99 and 23% lower odds; 95% CI 0.68,0.88, respectively). Decreased odds of children in high SES neighbourhoods being not-on-track for physical development were also associated with higher counts of schools, child health centres, ECEC centres and playgroups (8%; 95% CI 0.88,0.97, 6%; 95% CI 0.92,0.97, 5%; 95% CI 0.92,0.98 and 10% lower odds; 95% CI 0.85,0.95, respectively), and school bus stops and rail stations (11%; 95% CI 0.83,0.94 and 4% lower odds; 95% CI 0.93,0.99, respectively).

3.2.7. Social competence

Children living in high SES neighbourhoods had increased odds of being not on-track on the social competence domain if their neighbourhoods had greater distance to the closest school, child health centre, ECEC centre, and playgroup (67%, 95% CI 1.33,2.08, 8%, 95% CI 1.02,1.14, 32%, 95% CI 1.04,1.67 and 27% increased odds, 95% CI 1.02,1.14, respectively). Lower odds of children in high SES neighbourhoods being not-on-track on the social competence domain were associated with increased overall residential density (1% lower odds, 95% CI 0.97,0.99), and a greater number of child health centres, playgroups, bus stops and rail stations (3%, 95% CI 0.94,0.99, 5% 95% CI 0.90,0.99, 12%, 95% CI 0.81,0.96 and 4% lower odds, 95% CI 0.93,0.99, respectively).

3.2.8. Emotional maturity

Children living in high SES neighbourhoods had increased odds of being not on-track on the emotional maturity domain if their neighbourhoods had greater distances to parks, schools, child health centres,

Table 2
Logistic regressions predicting odds of children living within *low* SES neighbourhoods being *developmentally on-track*.

Built environment features	Physical Health & Wellbeing OR (95% CI)	p	Social Competence OR (95% CI)	p	Emotional Maturity OR (95% CI)	p
Residential Measures						
Building footprint/lot ratio[a]	0.99 (0.97, 1.02)	0.57	1.02 (0.99, 1.04)	0.07	0.99 (0.97, 1.01)	0.23
Overall dwelling density [b]	0.96 (0.90, 1.01)	0.12	1.01 (0.96, 1.07)	0.65	0.96 (0.91, 1.02)	0.18
Single dwellings[c]	**0.96 (0.94, 0.99)**	**0.01**	1.02 (0.99, 1.04)	0.16	0.99 (0.97, 1.02)	0.57
Duplex dwellings[d]	1.02 (0.99, 1.05)	0.09	1.00 (0.98, 1.02)	0.72	0.99 (0.97, 1.01)	0.37
Triplex dwellings[d]	1.02 (0.99, 1.04)	0.07	**0.99 (0.98, 0.99)**	**0.03**	**0.97 (0.96, 0.99)**	**<0.01**
Quadruplex dwellings[d]	**1.08 (1.01, 1.16)**	**0.02**	0.97 (0.93, 1.01)	0.17	**0.90 (0.85, 0.95)**	**<0.01**
Five or more dwellings[d]	1.02 (0.95, 1.10)	0.53	0.99 (0.92, 1.05)	0.66	**0.92 (0.86, 0.99)**	**0.03**
Parks						
Total park count[e]	**0.98 (0.95, 0.99)**	**0.04**	1.00 (0.98, 1.03)	0.95	**0.95 (0.92, 0.97)**	**<0.01**
Count of pocket-small parks[e]	1.00 (0.97, 1.03)	0.96	1.01 (0.98, 1.03)	0.64	**0.95 (0.92, 0.99)**	**<0.01**
Count of medium-large parks[e]	**0.91 (0.85, 0.98)**	**<0.01**	0.99 (0.92, 1.06)	0.82	0.95 (0.89, 1.01)	0.11
Count of district-regional Parks[e]	0.95 (0.83, 1.08)	0.41	0.93 (0.83, 1.04)	0.21	0.89 (0.78, 1.01)	0.08
Distance to closest park (100 m)[e]	**1.11 (1.05, 1.18)**	**<0.01**	1.06 (0.98, 1.15)	0.13	**1.09 (1.03, 1.16)**	**<0.01**
Walkability						
Traffic exposure (% high traffic roads)[a]	**0.98 (0.97, 0.99)**	**<0.01**	0.99 (0.98, 1.01)	0.07	**0.97 (0.96, 0.99)**	**<0.01**
Street connectivity [f]	**0.98 (0.97, 0.99)**	**<0.01**	1.00 (0.99, 1.01)	0.63	**0.99 (0.98, 0.99)**	**0.03**
Cul-de-sacs [g]	**0.86 (0.81, 0.93)**	**<0.01**	**1.12 (1.04, 1.20)**	**<0.01**	1.07 (0.99, 1.15)	0.08
Community Facilities						
School count within 3 km radius[e]	**0.96 (0.92, 0.99)**	**0.03**	0.98 (0.94, 1.04)	0.30	**0.91 (0.88, 0.95)**	**<0.01**
Child health centre count within 10 km radius[e]	**1.04 (1.00, 1.07)**	**0.04**	0.99 (0.97, 1.03)	0.90	**0.96 (0.93, 0.99)**	**0.02**
Early childhood education & care centre count within 3 km radius[e]	0.97 (0.93, 1.00)	0.05	0.98 (0.95, 1.01)	0.22	**0.96 (0.93, 0.99)**	**0.03**
Playgroup count within 3 km radius[e]	**0.90 (0.84 0.96)**	**<0.01**	0.98 (0.92, 1.03)	0.36	**0.90 (0.85, 0.96)**	**<0.01**
Distance to closest school (km)[e]	1.18 (0.91, 1.54)	0.21	1.08 (0.75, 1.57)	0.67	**1.92 (1.44, 2.57)**	**<0.01**
Distance to closest child health centre (km)[e]	0.91 (0.80, 1.04)	0.17	1.03 (0.89, 1.19)	0.69	**1.17 (1.02, 1.34)**	**0.03**
Distance to closest early childhood education and care centre (km)[e]	**1.86 (1.34, 2.60)**	**<0.01**	1.04 (0.73, 1.49)	0.82	**1.43 (1.05, 1.96)**	**0.02**
Distance to closest playgroup (km)[e]	0.85 (0.68, 1.06)	0.14	0.87 (0.69, 1.09)	0.22	1.06 (0.86, 1.30)	0.60
Count of services [h,e]	**1.11 (1.02, 1.20)**	**0.02**	0.99 (0.94, 1.06)	0.92	**0.90 (0.83, 0.98)**	**0.01**
Count of convenience stores [i,e]	**1.15 (1.08, 1.23)**	**<0.01**	1.04 (0.99, 1.10)	0.14	1.01 (0.95, 1.08)	0.72
Public Transport						
Distance to closest standard transport stop (km)[e]	1.22 (0.74, 2.01)	0.43	1.58 (0.94, 2.68)	0.09	1.04 (0.71, 1.51)	0.86
Count of school bus stops within 1.6 km radius[e]	1.01 (0.93, 1.09)	0.82	0.92 (0.77, 1.10)	0.37	**0.88 (0.82, 0.94)**	**<0.01**
Count of standard bus stops within 1.6 km radius[e]	1.00 (0.99, 1.01)	0.69	1.00 (0.99, 1.01)	0.88	1.00 (0.99, 1.01)	0.37
Count of rail stations within 5 km radius[e]	1.02 (0.97, 1.06)	0.47	0.99 (0.95, 1.03)	0.60	1.04 (0.99, 1.09)	0.11

Note. OR=Odds Ratio, CI=Confidence Interval, SES=Socio-Economic Status, SA1 = Statistical Area Level 1(i.e., neighbourhood). Reference group for logistic regressions is the count of all children within low SES neighbourhoods who are not developmentally on-track. Models adjusted for SA1 counts of male children, Aboriginal children, and children with English as a second language with valid Australian Early Development Census data. All built environment features are measured at the SA1 level. All distance variables are measured from the SA1 centroid. Results significant at $p < 0.05$ are indicated in bold.

[a] Per percentage point.

[b] Per increase in number of residential dwellings per 10 m^2 of residential land area.

[c] Per increase in 100 dwellings.

[d] Per increase in 10 dwellings.

[e] Per unit increase.

[f] Per increase in number of ≥3 way intersections.

[g] Per increase in 10 one-way nodes (cul-de-sacs).

[h] Services include dry cleaner, post office, pharmacy, CD/DVD/Video store.

[i] Convenience stores include deli, general store, supermarket, produce market, seafood market, gas station, shopping centre, or other food market.

playgroups and public transport stops (4%, 95% CI 1.01,1.06, 42%, 95% CI 1.16,1.76, 8%, 95% CI 1.01,1.15, 34%, 95% CI 1.07,1.67 and 31% greater odds, 95% CI 1.01,1.70, respectively). Lower odds of children in high SES neighbourhoods being not-on-track on the emotional maturity domain were associated with increased overall residential density (8% lower odds, 95% CI 0.87,0.97), greater numbers of single, triplex, quadruplex and 5 or more dwellings (3%, 95% CI 0.95,0.99, 10%, 95% CI 0.83,0.98, 13%, 95% CI 0.77,0.98 and 7% lower odds, 95% CI 0.88,0.99, respectively) and more schools, ECEC centres, playgroups and rail stations (7%, 95% CI 0.89,0.98, 4%, 95% CI 0.93,0.99, 10%, 95% CI 0.85,0.95 and 3% lower odds, 95% CI 0.94,0.99, respectively).

Table 3
Logistic regressions predicting odds of children living within *high* SES neighbourhoods being *developmentally not on-track*.

Built environment features	Physical Health & Wellbeing OR (95% CI)	p	Social Competence OR (95% CI)	p	Emotional Maturity OR (95% CI)	p
Residential Measures						
Building footprint/lot ratio[a]	**0.98 (0.97, 0.99)**	**0.02**	**0.99 (0.97, 0.99)**	**0.04**	0.99 (0.98, 1.01)	0.34
Overall dwelling density [b]	**0.93 (0.88, 0.98)**	**<0.01**	0.97 (0.92, 1.01)	0.16	**0.92 (0.87, 0.97)**	**<0.01**
Single dwellings[c]	0.99 (0.97, 1.01)	0.18	0.98 (0.96, 1.00)	0.07	**0.97 (0.95, 0.99)**	**<0.01**
Duplex dwellings[d]	**0.96 (0.94, 0.98)**	**<0.01**	0.98 (0.97, 1.00)	0.08	0.98 (0.96, 1.00)	0.07
Triplex dwellings[d]	0.96 (0.93, 1.00)	0.06	0.95 (0.90, 1.01)	0.12	**0.90 (0.83, 0.98)**	**0.02**
Quadruplex dwellings[d]	**0.92 (0.85, 0.99)**	**0.04**	0.91 (0.82, 1.01)	0.08	**0.87 (0.77, 0.98)**	**0.02**
Five or more dwellings[d]	**0.94 (0.89, 0.98)**	**<0.01**	0.95 (0.90, 1.01)	0.09	**0.93 (0.88, 0.99)**	**0.02**
Parks						
Total park count[e]	0.99 (0.97, 1.01)	0.42	0.99 (0.97, 1.02)	0.68	0.99 (0.97, 1.02)	0.46
Count of pocket-small parks[e]	**0.97 (0.93, 0.99)**	**0.04**	0.97 (0.93, 1.00)	0.06	0.99 (0.96, 1.03)	0.71
Count of medium-large parks[e]	**1.07 (1.01, 1.12)**	**0.02**	1.04 (0.99, 1.10)	0.10	1.05 (0.99, 1.11)	0.07
Count of district-regional Parks[e]	**0.77 (0.68, 0.88)**	**<0.01**	0.96 (0.85, 1.10)	0.58	0.99 (0.86, 1.13)	0.86
Distance to closest park (100 m)[e]	1.01 (0.99, 1.02)	0.54	1.01 (0.99, 1.02)	0.55	**1.04 (1.01, 1.06)**	**<0.01**
Walkability						
Traffic exposure (% high traffic roads)[a]	**0.98 (0.97, 0.99)**	**<0.01**	1.00 (0.99, 1.02)	0.53	1.01 (0.99, 1.02)	0.32
Street connectivity [f]	1.00 (0.99, 1.01)	0.24	0.99 (0.91, 1.04)	0.11	0.99 (0.99, 1.00)	0.06
Cul-de-sacs [g]	**1.16 (1.06, 1.27)**	**<0.01**	1.01 (0.91, 1.13)	0.83	1.07 (0.98, 1.17)	0.16
Community Facilities						
School count within 3 km radius[e]	**0.92 (0.88, 0.97)**	**<0.01**	0.96 (0.92, 1.01)	0.11	**0.93 (0.89, 0.98)**	**<0.01**
Child health centre count within 10 km radius[e]	**0.94 (0.92, 0.97)**	**<0.01**	**0.97 (0.94, 0.99)**	**0.03**	0.98 (0.95, 1.01)	0.10
Early childhood education & care centre count within 3 km radius[e]	**0.95 (0.92, 0.98)**	**<0.01**	0.98 (0.95, 1.01)	0.17	**0.96 (0.93, 0.99)**	**0.03**
Playgroup count within 3 km radius[e]	**0.90 (0.85, 0.95)**	**<0.01**	**0.95 (0.90, 0.99)**	**0.04**	**0.90 (0.85, 0.95)**	**<0.01**
Distance to closest school (km)[e]	**1.39 (1.10, 1.76)**	**<0.01**	**1.67 (1.33, 2.08)**	**<0.01**	**1.42 (1.16, 1.76)**	**<0.01**
Distance to closest child health centre (km)[e]	1.06 (0.99, 1.14)	0.08	**1.08 (1.02, 1.14)**	**0.01**	**1.08 (1.01, 1.15)**	**0.03**
Distance to closest early childhood education and care centre (km)[e]	**1.34 (1.02, 1.75)**	**0.04**	**1.32 (1.04, 1.67)**	**0.02**	1.17 (0.93, 1.47)	0.17
Distance to closest playgroup (km)[e]	**1.56 (1.22, 1.99)**	**<0.01**	**1.27 (1.02, 1.58)**	**0.03**	**1.34 (1.07, 1.67)**	**0.01**
Count of services [h,e]	0.94 (0.89, 1.01)	0.07	0.97 (0.92, 1.02)	0.23	1.00 (0.96, 1.04)	0.98
Count of convenience stores [i,e]	0.96 (0.92, 1.01)	0.09	0.98 (0.94, 1.02)	0.30	1.01 (0.90, 1.13)	0.84
Public Transport						
Distance to closest standard transport stop (km)[e]	1.09 (0.88, 1.35)	0.44	1.08 (0.85, 1.38)	0.53	**1.31 (1.01, 1.70)**	**0.04**
Count of school bus stops within 1.6 km radius[e]	**0.89 (0.83, 0.94)**	**<0.01**	0.80 (0.58, 1.11)	0.18	0.98 (0.92, 1.04)	0.44
Count of standard bus stops within 1.6 km radius[e]	0.98 (0.97, 0.99)	**<0.01**	**0.88 (0.81, 0.96)**	**<0.01**	1.00 (0.99, 1.01)	0.88
Count of rail stations within 5 km radius[e]	**0.96 (0.93, 0.99)**	**<0.01**	**0.96 (0.93, 0.99)**	**0.01**	**0.97 (0.94, 0.99)**	**0.03**

Note. OR=Odds Ratio, CI=Confidence Interval, SES=Socio-Economic Status, SA1 = Statistical Area Level 1(i.e., neighbourhood). Reference group for logistic regressions is the count of all children within high SES neighbourhoods who are developmentally on-track. Models adjusted for SA1 counts of male children, Aboriginal children, and children with English as a second language with valid Australian Early Development Census data. All built environment features are measured at the SA1 level. All distance variables are measured from the SA1 centroid. Results significant at $p < 0.05$ are indicated in bold.

[a] Per percentage point.
[b] Per increase in number of residential dwellings per 10 m^2 of residential land area.
[c] Per increase in 100 dwellings.
[d] Per increase in 10 dwellings.
[e] Per unit increase.
[f] Per increase in number of ≥3 way intersections.
[g] Per increase in 10 one-way nodes (cul-de-sacs).
[h] Services include dry cleaner, post office, pharmacy, CD/DVD/Video store.
[i] Convenience stores include deli, general store, supermarket, produce market, seafood market, gas station, shopping centre, or other food market.

4. Discussion

We sought to identify associations between built environment features and ECD and how this varied by neighbourhood SES. In high SES neighbourhoods, associations between the built environment and ECD were as expected, however, the findings for low SES neighbourhoods were mixed.

4.1. Low SES neighbourhoods and odds of being developmentally on-track

In low SES neighbourhoods, children had better physical development and poorer social and emotional development if their neighbourhood had higher residential density. Lower density can provide benefits such as reduced crime and noise however, these benefits are offset by

reduced walkability and access to services, in turn diminishing social interactions for young children (Binter et al., 2022). Prior research has shown that greater residential density is associated with reduced vehicle dependency, and therefore improved walkability (Christian et al., 2017b). However, concentrated poverty can lead to stigma, reduced self-esteem in adults and children, and a perceived lack of neighbourhood safety. This can negatively affect ECD by lowering social interaction and social cohesion within the community (Villanueva et al., 2019). An English study of mothers with young children found a negative relationship between neighbourhood satisfaction and housing density, particularly in low SES areas (Mcculloch, 2012). Further research is needed to unpack the mechanisms through which residential density differentially impacts ECD. Health Impact Assessments provide a practical framework to assist local governments to achieve multi-sector collaboration and development of locally tailored initiatives (Furber et al., 2011). For example, Health Impact Assessments can assist local governments to determine locally appropriate density limits for new or existing neighbourhoods, taking into account the mixed findings between ECD outcomes and density.

In low SES neighbourhoods, children had poorer physical and emotional development if their neighbourhood had higher levels of traffic and greater street connectivity. Higher traffic levels may diminish the transfer of walkability from adult to child, with research showing children spend less time outside if parental safety concerns exist regarding increased traffic (Bringolf-Isler et al., 2010; Eyre et al., 2014). Greater traffic exposure also results in increased road traffic noise, which has been associated with adverse mental health and neurodevelopment in children (Zare Sakhvidi et al., 2018). Where there were more cul-de-sacs, children had better social development. Cul-de-sacs support children's social development by allowing outdoor play near the home uninterrupted by traffic and creating an environment in which groups of children can exercise self-discipline due to awareness of parental surveillance, known as street panopticism (Hochschild, 2013). Outdoor play is important for both physical and psychosocial development in young children (Veitch et al., 2006). Low traffic environments, with reduced street connectivity and more cul-de-sacs, appear to have overall benefits for early child development in low SES neighbourhoods. However, town planners and developers should use these results cautiously and consider a whole of life approach in neighbourhood design, noting that increased street connectivity has walkability benefits in older populations (Christian et al., 2017b).

In low SES neighbourhoods, children had better physical development with more local services (i.e., dry cleaners, post offices, pharmacies and video stores) and convenience stores (i.e., delis, general stores, supermarkets, green grocers, seafood shops, gas stations, other food shops, and shopping centres). Having somewhere to walk to encourages people to use more active modes of transport and is key for improving the walkability of neighbourhoods (Giles-Corti et al., 2013; Villanueva et al., 2019). Conversely, higher counts of services and school bus stops was associated with poorer emotional development. It is possible that increased services and public transport may be associated with increased crime and anti-social behaviour. High-crime areas restrict social interaction and limit learning opportunities and relationship building in young children (Ellen and Turner, 1997). Neighbourhood urban design involves the adaptation of built environment features to meet community needs (Cozzolino et al., 2020). Urban design features can exist at the macro scale (for example, changes to infrastructure, zoning and transportation) or the micro scale (for example, changes to fixtures, facades, landscaping and lighting) (Australian Sustainable Built Environment Council, 2015). Crime prevention is associated with the development of 'defensible spaces' (places that allow greater surveillance, and a sense of ownership within a community), and the use of information and communications technology (for example, smart closed circuit television) (Chiodi, 2016; Cozens, 2002; Han et al., 2015). Defensible spaces encourage a collective responsibility to maintain a safe and thriving neighbourhood (Cozens, 2002; Newman, 1973) and the use of information and communications technology is associated with improved social interactions within low SES neighbourhoods (Chiodi, 2016), thus providing an opportunity for parents (and by proxy, their children) to develop social capital. Urban design features can influence the social, cultural, economic and health status of communities (Australian Sustainable Built Environment Council, 2015). Local governments should ensure collaboration between town planners, environmental health officers, parents and children, to design and retrofit child-friendly spaces. Investing in measures to improve ECD can have lifelong individual and community benefits (Muhajarine et al., 2006).

In low SES neighbourhoods, higher counts of playgroups, ECEC centres, schools, and child health centres were associated with higher proportions of children having poor physical and emotional development. This finding was unexpected as better access to key learning, care and health services was expected to be associated with improved ECD outcomes. Other attributes of child-specific services that are important for ECD include the quality of the service and how it is used. For example, only 12% of Western Australian children from low SES areas attend a preschool program before their first year of full-time schooling (Steering Committee for the Review of Government Service Provision, 2014), despite having access to these close to home. It is therefore possible that our finding of poorer physical and emotional development in low SES neighbourhoods is related to home (family) rather than neighbourhood effects. Furthermore, there can be considerable variation in the quality of preschool education by way of provider type (school, non-profit, family-run and private programs), and carer qualifications can vary across differing programs. Parents in higher SES areas have greater ability to afford higher quality services with more qualified staff, compared to those in lower SES areas (Warren and Haisken-DeNew, 2013). Additionally, teachers are more likely to be under-supported in low SES areas with larger class sizes, inexperienced staff and limited resources such as books and toys, resulting in children receiving less attention and stimulation resulting in poorer school readiness (Bracey, 2003). One Canadian study found that although children of lower income families were less likely to attend preschool educational services than those of higher income families, attendance was associated with less developmental vulnerability (Laurin et al., 2015). Overall, the findings highlight that quality (and use), rather than just access, of early child services should be considered when examining the relationship between the built environment and ECD, particularly in low SES neighbourhoods.

Unexpectedly, children had poorer development across all three domains in low SES neighbourhoods with more parks. Since green spaces are associated with improved psychological health in children, particularly those of low socioeconomic status (Maas et al., 2009) we expected to see a positive relationship between ECD and park access in our study. Greenspaces are beneficial by reducing harmful environmental exposures (heat, air and noise pollution), for capacity building (by facilitating physical activity and social cohesion), and for capacity restoration (psychological stress recovery) (Markevych et al., 2017). Attention Restoration Theory explains that exposure to nature may improve a person's concentration by varying degrees depending on the extent of exposure, interest in green spaces and removal from one's usual environment (Kaplan and Kaplan, 1989). Attention Restoration Theory based interventions may assist young children to develop self-regulation skills which are critical for their development (Kaplan and Berman, 2010). However, studies have shown that parks in low SES neighbourhoods have greater levels of anti-social behaviour and lower levels of maintenance compared with parks in high SES neighbourhoods (Hughey et al., 2016) and this may make them less appealing places for parents and young children to experience. Similarly, a UK study found that parents living in low SES neighbourhoods perceived their neighbourhoods as unsafe, contributing to low levels of physical activity outside the home, despite the availability of parks and facilities within the neighbourhood (Eyre et al., 2014). These results warrant a call to action for town planners to examine solutions that may improve the

functionality and quality of green spaces and, ultimately, the perception of safety within these, to improve usage in low SES areas.

4.2. High SES neighbourhoods and odds of being developmentally not on-track

In high SES neighbourhoods increased residential density was associated with better physical and emotional development. These findings are consistent with prior research showing increased residential density is associated with reduced vehicle dependency and improved walkability (Christian et al., 2017b). These results differ from those seen in low SES neighbourhoods, where increased residential density was associated with better physical development but poorer emotional development. One hypothesis is the role of status and choice on home location. Unlike in low SES areas, in high SES areas it is likely a choice to live in higher density housing (as opposed to choosing lower density housing in cheaper neighbourhoods). Attainment and status in high SES neighbourhoods may improve parental satisfaction, consequently having a positive influence on parenting behaviours and ECD (Mcculloch, 2012). A similar explanation may account for our finding that better physical and social development was associated with smaller yard sizes in high SES areas. Typically, greater yard sizes have been associated with children being more physically active (Salmon et al., 2004). However in high SES neighbourhoods larger homes (and smaller yards) can be a marker of affluence (Boyce et al., 2006) whereby children have their own bedrooms, bigger bedrooms and additional spaces within the home to play, resulting in reduced crowding and consequently improved mental health and resilience in young children (Evans et al., 2002).

The association between ECD and parks in high SES neighbourhoods largely followed more expected patterns. Children had better physical development if there were more pocket/small parks and district/regional parks (but not medium-large sized parks) within the neighbourhood. Further, children had poorer physical and emotional development with greater distances to the closest park. Outdoor play is important for both physical activity levels and emotional wellbeing in children (Veitch et al., 2006). Prior research has shown that young children tend to be the most physically active users of parks (Evenson et al., 2015) however, this may not apply to parks of all sizes and further research should examine variation in usage patterns across differing park sizes. For example, medium-large parks may predominantly be used for structured sporting programs tailored toward older children and adults.

In high SES neighbourhoods, more ECEC centres were associated with better physical development, more schools were associated with better physical and emotional development, and more child health centres and playgroups were associated with better outcomes across all three domains. Closer ECEC centres were associated with better physical development, closer child health centres were associated with better emotional and social development, and closer schools and playgroups were associated with better child development across all three domains. That is, the more schools, child health centres, ECEC centres and playgroups, and the shorter distances to these, the better children's development was. These findings contrast those seen in low SES neighbourhoods whereby higher counts of playgroups, ECEC centres, schools and child health centres were associated with poorer physical and emotional development. This may reflect a higher quality and/or greater uptake of these services in high compared with low SES neighbourhoods. It is well established that attendance at playgroups and high quality preschool education is associated with global benefits in ECD (Sincovich et al., 2020, Warren and Haisken-DeNew, 2013). In Australia, parents can choose to send their children to preschool programs or ECEC centres - within the constraints of affordability, availability and practicality, with family SES being a strong predictor of ECEC centre quality (Cloney et al., 2016). Access and usage of child health centres is also important with improved access to health care associated with improved health outcomes in children (Leininger and Levy, 2015). These services benefit young children both directly, by providing screening and health prevention strategies, as well as indirectly, by addressing the physical and mental health of their carers (National Framework for Universal Child and Family Health Services, 2011).

Adults with access to public transport have better physical and social health (Mihaylova, 2021) and it is likely these benefits transfer to young children. In high SES neighbourhoods, higher counts of, and shorter distances to, public transport stops were associated with better child development across all three domains. This is different to low SES neighbourhoods, where more school bus stops were associated with poorer emotional development. Public transport promotes social inclusion by allowing access to employment, education, health care and community facilities (Tourism and Transport Forum, 2010) and increased uptake of public transport is associated with increased physical activity levels (Rissel et al., 2012). This paper highlights the importance of public transport infrastructure however, further research should consider why these results were not replicated in low SES areas, with consideration to the role car dependency plays in low SES neighbourhoods (Sipe and Dodson, 2008).

Unexpectedly, greater traffic exposure in high SES neighbourhoods was associated with improved physical development, unlike in low SES neighbourhoods which found increased traffic exposure associated with poorer physical and emotional development. Research should consider traffic factors in addition to volume (i.e. vehicle type and speed, and air and noise pollution) and how these may differ between high and low SES neighbourhoods. It is likely that parents perceive faster vehicular traffic (i.e. more highways and freeways) and larger vehicles (i.e. trucks), as being more unsafe and research has shown that children's outdoor physical activity is influenced by parental perceptions of road safety (Bringolf-Isler et al., 2010; Ortegon-Sanchez et al., 2021). The introduction of traffic safety attributes (for example, physical barriers and traffic calming measures) may ameliorate the effects of high traffic on ECD in low SES areas. A systematic review found safe traffic environments to be supportive of active travel behaviours in children (Melody et al., 2022). Reducing traffic volumes may also provide broader benefits such as reduced air pollution (Melody et al., 2022). Greater maternal $PM_{2.5}$ (particulate matter <2.5 μm in diameter) exposure during pregnancy has been associated with reduced fine motor function in children at five years of age (Binter et al., 2022) and closer proximity to major roads during early childhood has been associated with poorer cognitive development; however, the role of pollutants versus socioeconomic status requires further exploration (Harris et al., 2015). As expected, more cul-de-sacs were associated with poorer physical development in high SES neighbourhoods; cul-de-sacs can restrict walkability for adults (Giles-Corti et al., 2013), who are the decision makers for young children.

5. Study strengths and limitations

Strengths of this study include the use of population-level data on ECD and the wide range of objectively measured built environment features considered. Additionally, three domains of child development were considered concurrently. Furthermore, the analysis of being developmentally on-track or developmentally not on-track within high and low SES neighbourhoods separately, using an off-diagonal approach, allowed those doing better or worse than expected to be identified and the relationship with built environment measures examined. The off-diagonal approach is a novel concept which highlights built environment features that are associated with variations in ECD across neighbourhoods of high and low SES (Tanton et al., 2017).

Study limitations include the lack of individual level data (for example, individual/family SES measures such as educational attainment and household income, housing quality, physical activity, school and parental variables). As such, it was not possible to disentangle the effects of neighbourhood exposures from family/residential or school exposures. Further research should use individual level ECD and built

environment variables. In addition, future studies should consider objective markers of built environment feature quality, and how these may vary by neighbourhood SES. There were unmeasured built environment features that may further explain how the built environment influences ECD; these include industrial land (proximity and percentage), walkability indicators (for example, bicycling lanes, pedestrianised streets and physical barriers), and pollution (noise and air). Composite measures of the built environment that are relevant for early child development (similar to walking indices and walking for transport) (Koohsari et al., 2016; Ribeiro and Hoffimann, 2018) should also be developed. This study included children aged 5–6 years and assessed outcomes in the first year of full-time schooling; consequently, we cannot draw conclusions about the influence of built environment features in older or younger age groups. Several counterintuitive findings were seen in low SES neighbourhoods and it cannot be discounted that some of these may have been the product of chance, rather than meaningful effects. It is also possible that the counter-intuitive findings observed are explained by the transactional processes between neighbourhood-level social and cultural factors and the built environment. For example, collective cultural values may influence the ways in which groups of individuals interact with the environment, thus moderating the relationship between the built environment and early child development. Further, as the study was cross-sectional we could only identify associations and no causation can be derived from the results.

6. Conclusion

This study adds to the limited evidence base regarding the influence of the built environment on early child development, taking into account the role of neighbourhood SES. Built environment features were associated with developmental vulnerability, with differing patterns across high and low SES neighbourhoods. Means to address children being developmentally on-track or not in early childhood cannot apply a one size fits all approach and must be considered in the context of the local neighbourhood SES, as well as multi-jurisdictional policies that directly or indirectly influence how the neighbourhood influences ECD. Adjustments to the built environment (changes to urban planning, retrofitting built environment features and installing safety measures), may improve ECD, providing lifelong individual child benefits and community gains. The findings can serve to inform policy makers when determining the role of the neighbourhood built environment on child development. Further research is required to ascertain how quality, as well as quantity, proximity and use, of built environment features predicts ECD outcomes, and how the influence of built environment features on development varies over the life course.

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Declaration of competing financial interests

The authors declare they have no actual or potential competing financial interests.

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Appendix 1. Built Environment Features – Definition

Built Environment Features	Description
Residential Measures	
Building footprint/lot ratio	Sum of building area/sum of lot area x 100 (%) per SA1[a,b]
Dwelling density	Count of residential dwellings per ten square metres[c]
Dwelling type:	
Single dwellings	Count of every 10 single dwellings per SA1[a,c]
Duplex dwellings	Count of every 10 duplex dwellings per SA1[a,c]
Triplex dwellings	Count of every 10 triplex dwellings per SA1[a,c]
Quadruplex dwellings	Count of every 10 quadruplex dwellings per SA1[a,c]
>5 Dwellings	Count of every 10 groups of five or more dwellings per SA1[a,c]
Parks	
Total parks	Count of parks per SA1[a,b]
Pocket-small parks	Count of small sized (≤0.5 ha) parks per SA1[a,b]
Medium-large parks	Count of medium sized parks (>0.5 to ≤5 ha) per SA1[a,b]
District-regional parks	Count of large sized parks (>5 ha) per SA1[a,b]
Distance to closest park	Distance in 100 m units to the nearest park from SA1centroid[a,b]
Walkability	
Traffic exposure	Proportion of high traffic volume roads compared to all roads each SA1[a,d]
Street connectivity	Count of 3 way nodes (or greater) per SA1[a,d]
Cul-de-sacs	Count of 1 way nodes per SA1[a,d]
Community Facilities	
Schools	Count of kindy and pre-primary within 3 km of SA1 centroid[a,e]
Child health centres	Count of child health centres within 10 km of SA1 centroid[a,f]
Early childhood education and care (ECEC) centres	Count of child care centres within 3 km of SA1 centroid[a,g]
Playgroups	Count of playgroups within 3 km of SA1 centroid[a,h]
School distance	Distance in kilometers between SA1 centroid and closest kindy/pre-primary[a,b,e]
Child health centre distance	Distance in kilometers between SA1centroid and closest child health centre[a,b,f]
ECEC centre distance	Distance in kilometers between SA1 centroid and closest ECEC centre[a,b,g]

(*continued on next page*)

(*continued*)

Built Environment Features	Description
Playgroups distance	Distance in kilometers between SA1 centroid and closest playgroup[a,b,h]
Services	Count of different types of services per SA1 (range 0–4)[a] (Knuiman et al., 2014)
Convenience stores	Count of different types of convenience stores per SA1 (range 0–8)[a] (Knuiman et al., 2014)
Public Transport	
Distance to closest	Distance in kilometers between SA1 centroid and closest public transport stop[a,b,i]
School bus (1600 m)	Count of stops within 1600 m from SA1 centroid[a,i]
Standard bus (1600 m)	Count of stops within 1600 m from SA1 centroid[a,i]
Rail (5000 m)	Count of stations within 5000 m from SA1 centroid[a,i]

[a] SA1 = Statistical Area Level 1 (i.e., neighbourhood); these generally have a population of 200–800 people (average 400 people).
[b] Source: Centre for the Built Environment and Health (CBEH), The University of Western Australia.
[c] Source: Spatial cadastre database and Vesting Reserve report provided by Landgate, land use classification planning and land use codes from Valuer Generals Office.
[d] Source: Road network data from Landgate and Functional Road Hierarchy information from Main Roads Western Australia.
[e] Source: Western Australian Government Department of Education and Department of Planning.
[f] Source: Western Australian Government Department of Health.
[g] Source: Western Australian Government Department of Local Government and Communities.
[h] Source: Playgroup WA Inc.
[i] Source: Western Australian Public Transport Authority.

Appendix 2. AEDC and SES Quintile Cross Tabulation Tables; Number of SA1s by Physical, Social and Emotional Domains

			SES Quintiles [b]				
			Low SES[e]			High SES[f]	
			1st	2nd	3rd	4th	5th
AEDC Quintiles [a]	*Low % vulnerability* [c]	1st	15	15			46
		2nd	12				
		3rd					
	High % vulnerability [d]	4th					16
		5th	41			12	5

			SES Quintiles [b]				
			Low SES[e]			High SES[f]	
			1st	2nd	3rd	4th	5th
AEDC Quintiles [a]	*Low % vulnerability* [c]	1st	17	20			46
		2nd	8				
		3rd					
	High % vulnerability [d]	4th					16
		5th	41			12	5

			SES Quintiles [b]				
			Low SES[e]			High SES[f]	
			1st	2nd	3rd	4th	5th
AEDC Quintiles [a]	*Low % vulnerability* [c]	1st	15	15			39
		2nd	16				
		3rd					
	High % vulnerability [d]	4th					18
		5th	35			11	10

[a] Australian Early Development Census.
[b] Socioeconomic status.
[c] Neighbourhoods that are in the first and second quintile for vulnerability (less children developmentally not on-track).
[d] Neighbourhoods that are in the fourth and fifth quintile for vulnerability (more children developmentally not on-track).
[e] Neighbourhoods that are in the first and second quintile for SES (low SES).
[f] Neighbourhoods that are in the fourth and fifth quintile for SES (high SES).

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Attributable risk of household solid fuel use and second-hand smoke associated with under-5 mortality in 46 low- and lower-middle-income countries, 2010–2020

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A B S T R A C T

Background: Household solid fuel use (including indoor and outdoor) and second-hand smoke (SHS) are considered to be major contributors of under-5 mortality (U5M) in low- and lower-middle-income countries (LMICs). This study provides a comprehensive assessment of their odds ratios and attributable mortality in LMICs.
Methods: We used the Demographic Health Surveys data for under-5 children in 46 LMICs (n = 778,532) from 2010 to 2020. Mixed effect multilevel logistic regressions were conducted to estimate the pooled adjusted odds ratio (aOR) for U5M due to solid fuel use, SHS and their combination compared to no exposure to them in 46 LMICs. The attributable mortality of solid fuel use, SHS, and their combination were assessed for each LMIC.
Findings: The pooled aOR of solid fuel use and SHS for U5M was estimated to be 1.27 (95% Confidence Interval (CI): 1.19–1.36) and 1.13 (95%CI: 1.06–1.25), respectively, whereas those of their combination was 1.40 (95%CI: 1.31–1.50). U5M attributable to indoor and outdoor solid fuel use was the highest in Myanmar (18.0%) and the Gambia (16.5%), respectively, while those attributable to SHS was the highest in Indonesia (9.8%). U5M attributable to the combination of solid fuel use and SHS was the highest in Timor-Leste (22.7%).
Interpretation: The combined effect of exposure to solid fuel and SHS had a higher risk of U5M than the individual risk. The use of clean fuel and tobacco control measures should be integrated with other child health promotion policies.
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1. Introduction

Household solid fuel use and second-hand smoke (SHS) exposure are the world's major health and environmental problems – particularly for the poorest in the world due to a reliance on solid fuel for cooking and smoking inside the house (Our World in Data, 2019; Murray et al., 2020). Around 3 billion people still use solid fuel and kerosene in open fires and inefficient stoves in 2019 (World Health Organization, 2018; Bickton et al., 2020). Most of them are poor and live in low- and lower-middle-income countries (LMICs) (World Health Organization, 2018; Bickton et al., 2020; Fullerton et al., 2008). Over 80% of the world's 1.3 billion tobacco users live in LMICs and 1.2 million people die every year because of SHS exposure (Murray et al., 2020; World Health Organization, 2017; Yousuf et al., 2020; Reitsma et al., 2017).

Under-5 children are at risk of exposure to solid fuel and SHS because they spend most of the time inside the house (Bickton et al., 2020; Bruce et al., 2000; Bonjour et al., 2013). Smoke emitted from burning solid fuels contains complex mixtures of harmful pollutants (Our World in Data, 2019; World Health Organization, 2018; Samet et al., 1987). Exposure to these harmful household air pollutants adversely affects birth outcomes and under-5 children's health (Longo, 1977). Exposure to solid fuel doubles the risk of pneumonia and acute lower respiratory infection (ALRI), contributing to over 450,000 under-5 deaths according to Global Burden of Disease (GBD) data in 2019 (Murray et al., 2020; Paulson et al., 2021). SHS is the inhalation of other people's tobacco smoke from burning tobacco products, such as cigarettes, cigars or pipes (WHO Global Health Observatory (GHO), 2019). Tobacco smoke contains more than 7000 chemicals, including hundreds that are toxic

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(World Health Organization, 2017; WHO Global Health Observatory (GHO), 2019). Children are particularly vulnerable to the effects of SHS exposure, which has been linked to an increased risk of childhood illnesses including lower respiratory tract infections, asthma, wheezing, middle ear infections, sudden infant death syndrome (SIDS) and invasive meningococcal disease (Jones et al., 2011). Nearly half of all children around the world breathe air polluted by tobacco smoke and 65, 000 children die every year due to illnesses related to SHS (World Health Organization, 2019).

One of the targets for SDG 3 is to reduce the under-5 mortality rate to 25 deaths or below per 1000 live births by 2030 (Paulson et al., 2021). Substantial progress has been made toward achieving this target and the global under-5 mortality rate declined by 59% from 93 deaths per 1000 live births in 1990 to 39 in 2019 (Paulson et al., 2021). However, the burden of under-5 deaths remains unevenly distributed among countries and regions (Paulson et al., 2021). About 80% of under-5 deaths in 2019 occurred in LMICs where the solid fuel and SHS were the main sources of air pollution inside the house (Paulson et al., 2021). Thus, it is important to investigate the odds ratio and mortality attributable to solid fuel and SHS exposure among children in each LMICs to implement the country-specific policy and intervention to reduce under-5 mortality.

Some previous studies have examined the association between either household solid fuel exposure or SHS exposure and under-5 mortality (Bickton et al., 2020; Kleimola et al., 2015; Owili et al., 2017; Naz et al., 2017; Khan et al., 2017; Andriani et al., 2019). However, to our knowledge, none have examined yet how the interaction between solid fuel and SHS contributes to under-5 mortality. Therefore, we aimed to estimate the risks and attributable fractions from indoor solid fuel, outdoor solid fuel, SHS and combined solid fuel and SHS associated with under-5, infant and neonatal mortality in 46 LMICs.

2. Methods

2.1. Study population and data collection

This study is a secondary analysis based on the data from the Demographic and Health Surveys (DHS) conducted in 46 LMICs between 2010 and 2020. Briefly, DHS are cross-sectional nationally representative household surveys with the stratified two-stage sample design including a comprehensive list of questionnaires: (i) household questionnaire, (ii) women's questionnaire, (iii) men's questionnaire, (iv) biomarker questionnaire (United Sates Agency International Development, 2021a). The information related household solid fuel use and SHS, household characteristics and housing condition was extracted from household modules of DHS whereas the information about under-5 mortality was extracted from women modules of DHS (United Sates Agency International Development, 2021a).. This study is based on the most recent DHS data from 2010 to 2020 since the source of solid fuel and SHS is changing overtime along with the advancement in technology. The country classification of each LMIC was based on a 2020 list of countries by income by the World Bank. Countries with Gross National Income (GNI) per capita of less than 1045 USD were categorized as low-income countries, countries with GNI per capita between 1046 USD and 4095 USD were categorized as lower-middle-income countries (World Bank, 2022). The detailed description about country selection steps is presented in Appendix Fig. 1. The characteristics of DHS used in this study are presented in Appendix Table 1.

2.2. Exposure assessment

The analysis was based on two main exposures of interest: solid fuel use and SHS. Regarding fuel use, the results of responses to the question "What type of cooking fuel do you use?" were used (United Sates Agency International Development, 2021a). The responses were in the form of choices (United Sates Agency International Development, 2021a). DHS defined electricity, liquefied petroleum gas, natural gas, and biogas as clean fuel and petroleum, kerosene, coal, lignite, charcoal, wood, straw/shrubs/grass, agricultural crop, animal dung and others as solid fuel (United Sates Agency International Development, 2021a, 2021b). This study followed the DHS definition to categorize the study population by cooking fuel types (clean fuel or solid fuel) (Appendix Table 2). Clean fuel use was considered as a reference category for the analyses described below. Because of the significant impact of inhaling smoke from solid fuel when cooking indoors (Naz et al., 2017; Khan et al., 2017), the solid fuel use was evaluated in two categories (indoor use and outdoor use) based on the results of responses to the following DHS question, "Is the cooking usually done in the house, in a separate building, or outdoors?" (United Sates Agency International Development, 2021a). In this study, cooking in the house was regarded as indoor use while cooking in a separate building or outdoors were regarded as outdoor use (Bickton et al., 2020; Naz et al., 2017; Khan et al., 2017).

Table 1
Weighted distribution of, socioeconomic, demographic, household and individual variables in the study populations by the exposures of interests.

Characteristic	Under-5 children (N = 778,532)			
	Clean fuel N = 177,505 (22.8%) n (%)	Outdoor solid fuel = 361,239 (46.4%) n (%)	Indoor solid fuel N = 239,788 (30.8%) n (%)	Second-hand Smoke N = 268,594 (34.5%) n (%)
Mother's age at birth (mean (SD))	21.1 (0.02)	19.0 (0.05)	19.6 (0.1)	20.5 (0.05)
Child's sex				
Male	92,835 (52.3)	183,871 (50.9)	123,251 (51.4)	137,789 (51.3)
Female	84,670 (47.7)	177,368 (49.1)	116,537 (48.6)	130,805 (48.7)
Breastfeeding status				
Yes	84,492 (47.6)	160,751 (44.5)	107,185 (44.7)	109,586 (40.8)
No	93,013 (52.4)	200,488 (55.5)	132,603 (55.3)	159,008 (59.2)
Number of household member (mean (SD))	6.5 (0.04)	7.2 (0.06)	7.1 (0.04)	7.2 (0.03)
Place of residence				
Urban	108,101 (60.9)	78,389 (21.7)	35,249 (14.7)	64,731 (24.1)
Rural	69,404 (39.1)	282,850 (78.3)	204,539 (85.3)	203,863 (75.9)
Household wealth index				
Richest	73,132 (41.2)	37,207 (10.3)	11,510 (4.8)	29,814 (11.1)
Richer	55,914 (31.5)	59,604 (16.5)	24,938 (10.4)	42,438 (15.8)
Middle	32,483 (18.3)	76,582 (21.2)	44,361 (18.5)	53,450 (19.9)
Poorer	11,893 (6.7)	91,393 (25.3)	67,380 (28.1)	66,074 (24.6)
Poorest	4083 (2.3)	96,450 (26.7)	91,599 (38.2)	76,818 (28.6)
Parent's education status				
Higher	46,861 (26.4)	17,339 (4.8)	14,867 (6.2)	19,875 (7.4)
Secondary	89,640 (50.5)	96,089 (26.6)	72,176 (30.1)	101,528 (37.8)
Primary	24,318 (13.7)	103,314 (28.6)	66,901 (27.9)	74,669 (27.8)
None	16,685 (9.4)	144,495 (40.0)	85,844 (35.8)	72,520 (27.0)

N, weighted number of under-5 children; SD, standard deviation; n, weighted number calculated out of total N per variable.

Table 2
Association between child mortality exposed to solid fuel, second-hand smoke, and interaction between them in 46 countries.

Exposure	Under-5 mortality aOR (95% CI)	Infant mortality aOR (95% CI)	Neonatal mortality aOR (95%CI)
Clean fuel	1.00 (ref)	1.00 (ref)	1.00 (ref)
Solid fuel	1.27 (1.19–1.36) **	1.28 (1.20–1.37) **	1.31 (1.21–1.42) **
SHS			
No	1.00 (ref)	1.00 (ref)	1.00 (ref)
Yes	1.13 (1.06–1.25) *	1.15 (1.04–1.25) *	1.07 (1.01–1.20)*
Combined solid fuel and SHS	1.40 (1.31–1.50) **	1.43 (1.35–1.50) **	1.45 (1.38–1.55) **
Mother's age at birth	1.13 (1.06–1.21) *	1.14 (1.07–1.21) *	1.18 (1.05–1.24) *
Child's sex			
Male	1.00 (ref)	1.00 (ref)	1.00 (ref)
Female	0.80 (0.76–0.89) **	0.81 (0.77–0.89) **	0.79 (0.75–0.84) **
Breastfeeding status			
Yes	1.00 (ref)	1.00 (ref)	1.00 (ref)
No	1.08 (1.06–1.17) **	1.15 (1.09–1.26) *	1.18 (1.08–1.24) *
Number of household members	1.06 (1.03–1.15) **	1.07 (1.07–1.08) *	1.07 (1.06–1.08) **
Place of residence			
Urban	1.00 (ref)	1.00 (ref)	1.00 (ref)
Rural	1.09 (1.06–1.16) **	1.07 (1.03–1.12) **	1.04 (1.02–1.09) *
Household wealth index			
Richest	1.00 (ref)	1.00 (ref)	1.00 (ref)
Richer	1.20 (1.13–1.28) *	1.13 (1.07–1.20) **	1.08 (1.01–1.17) **
Middle	1.32 (1.23–1.38) *	1.22 (1.15–1.29) **	1.12 (1.04–1.21) **
Poorer	1.41 (1.33–1.52) *	1.28 (1.20–1.36) **	1.13 (1.04–1.22) **
Poorest	1.52 (1.43–1.62) *	1.35 (1.27–1.44) **	1.18 (1.09–1.28) **
Parent's education status			
Higher	1.00 (ref)	1.00 (ref)	1.00 (ref)
Secondary	1.28 (1.17–1.36) *	1.19 (1.12–1.27) *	1.14 (1.05–1.23) *
Primary	1.49 (1.39–1.60) *	1.39 (1.29–1.48) *	1.31 (1.20–1.42) *
None	1.64 (1.52–1.76) *	1.46 (1.36–1.56) *	1.37 (1.26–1.49) *
Random effects	Variance (SE)		
Community (PSU)	0.03 (0.01)	0.02 (0.01)	0.04 (0.02)
Country	0.41 (0.01)	0.39 (0.02)	0.45 (0.02)

aOR, adjusted Odds Ratio; CI, Confidence Interval; SHS, Second-hand Smoke; PSU,Primary Sampling Unit; SE, Standard Error **p-value<0.001,*p-value<0.05.

The DHS question used to obtain data for the SHS was based on the frequency of any household member smoking inside the house (i.e. "How often does anyone smoke inside your house?") which had four responses: "never", "daily", "weekly", "monthly, or less than once in a month". (United Sates Agency International Development, 2021a). Sensitivity analysis was conducted to examine if there is a dose-response relationship. If the household members were smoking daily, weekly, monthly, and less than once in a month, the under-5 children in those households were regarded as having exposure to SHS (Andriani et al., 2019).

2.3. Outcome assessment

During the DHS survey, each mother was asked whether all her children born 5 years prior to the survey were alive or dead (United Sates Agency International Development, 2021a). If a child was dead, a follow-up question was on the child's age at death (United Sates Agency International Development, 2021a). Based on that, in this study, there were three outcome measures: the outcome variables were under-5 mortality (the number of deaths before the fifth birthday, 0–59 months), infant mortality (the number of deaths during the first year of life, 0–11 month) and neonatal mortality (the number of deaths during the first 28 days of life) (United Sates Agency International Development, 2021b; Croft Trevor et al., 2018).

2.4. Potential confounders

Household wealth index (categorized by default as "richest", "richer", "middle", "poorer" or "poorest") and parent's education (categorized by default as "higher", "secondary", "primary" or "no education") were included as potential confounders since these variables have been identified previously as markers to reflect socio-economic status (Kleimola et al., 2015; Owili et al., 2017; Naz et al., 2017; Khan et al., 2017; Andriani et al., 2019). DHS calculated the wealth index by principal component analysis based on the data collected in household questionnaire about the household's ownership of a number of consumer items such as a television and car; dwelling characteristics such as flooring material; type of drinking water source; toilet facilities; and other characteristics that related to wealth status (Rustein and Staveteig, 2014). The resultant wealth index scores were divided into quintiles of richest, richer, middle, poorer and poorest (Rustein and Staveteig, 2014).

Place of residence (categorized by default as "urban" or "rural" based on infrastructure and population size) was included as a marker of demographic factors because rural children have a higher risk of death from use of polluting fuel than urban counterparts (Kleimola et al., 2015; Andriani et al., 2019). Household and individual factors such as sex of the child (male or female), number of household members at the time of survey and mother's age at birth of deceased child were also considered as potential confounders. This is because it has been shown that more members in the household are associated with adverse child health and increased under-5 mortality (Nishimwe et al., 2021). Advanced or extremely young maternal age at birth is also associated with poor birth outcomes including under-5 mortality (Finlay et al., 2011; Fall et al., 2015). The number of household members and mother's age at birth were entered as continuous variables.

Breastfeeding status (categorized by default as ever breastfed: "yes" or "no") of children was also included as a potential confounder since breastfeeding is known to provide protection against infection and, therefore, may reduce the risk of under-5 mortality associated with HAP (Sankar et al., 2015). Since birth weight is likely on the causal pathway between exposure to HAP and under-5 mortality, it was not considered as a confounder in this study. Adjustment of that kind of intermediate variable would produce biased estimates that leads to underestimation of the strength of the effect of the exposure on the outcome under study (Whitcomb et al., 2009; Schisterman et al., 2009).

2.5. Statistical analysis

First, proportions of under-5 mortality, infant mortality and neonatal mortality during the five-year periods preceding the DHS surveys were calculated by using the data on the children's birth date, their survival status, and the dates of death or ages at death of deceased children following the direct estimation method provided in the guideline of the DHS program (United Sates Agency International Development, 2021b; Croft Trevor et al., 2018). The denominators were the total number of live births during the five-year periods preceding the DHS survey

(United Sates Agency International Development, 2021b; Croft Trevor et al., 2018). Then, prevalence estimates of exposure variables (indoor solid fuel, outdoor solid fuel, SHS and combined solid fuel (regardless of indoor or outdoor) and SHS) for each countries, outcome variables (under-5 mortality, infant mortality and neonatal mortality) and 95% of confidence intervals (CI) were calculated while adjusting for the cluster sampling survey design by using the "svy" command in STATA.

Second, pooled models for all 46-countries combined were constructed to estimate odds ratios for each exposure variable after being adjusted for confounders. Logistic regression models were estimated separately for each of the three outcome variables. Also, for each outcome, there were two types of model (Model 1 and 2). Model 1 considered the solid fuel (regardless of indoor or outdoor use), SHS and the interaction between solid fuel and SHS. Model 2 considered the indoor solid fuel, outdoor solid fuel, SHS, the interaction between indoor solid fuel and SHS; and the interaction between outdoor solid fuel and SHS. So, in total there were six models. For all analyses, the selection of variables was based on the backwards-stepwise method with a p-to-remove of >0.05 (Bursac et al., 2008). Backward-stepwise regression starts with all the candidate variables in the model and removes the least significant variables until all the remaining variables are statistically significant (Bursac et al., 2008).

The following model equation was applied to develop the mixed-effects multilevel logistic regression models:

$$y_{ijk} \sim \mathrm{Bin}\left(n_{ijk}, p_{ijk}\right)$$

$$\mathrm{logit}\left(p_{ijk}\right) = \alpha_{ijk} + \beta_1 X_{ijk1} + \beta_2 X_{ijk2} + \beta_3 X_{ijk1} X_{ijk2} + \beta_4 X_{ijk3} + \beta_5 X_{ijk4} + \beta_6 X_{ijk5} + \beta_7 X_{ijk6} + \beta_8 X_{ijk7} + \beta_9 X_{ijk8} + \beta_{10} X_{ijk9} + \mu_{0jk} + \rho_{0jk}$$

where y_{ijk} is the outcome (death or no death) of child i in j primary sampling unit (PSU) in k country, n_{ijk} is the total number of children in the family of child i in j PSU in k country, α_{ijk} is the probability of the outcome (death or no death) of a child i in j PSU from the country k.

In Model:1, β_1 is the coefficient for X_{ijk1} (solid fuel – no or yes), β_2 is the coefficient for X_{ijk2} (SHS – no or yes), β_3 is the interaction coefficient for solid fuel and SHS.

In Model:2, β_1 is the coefficient for X_{ijk1} (solid fuel – no, indoor solid fuel or outdoor solid fuel), β_2 is the coefficient for X_{ijk2} (SHS – no or yes), β_3 is the interaction coefficient for solid fuel and SHS (to be precise, separate slopes were estimated for indoor solid fuel and SHS; and outdoor solid fuel and SHS).

In both Model 1 and 2, β_4 is the coefficient for X_{ijk3} (mother's age at birth), β_5 is the coefficient for X_{ijk4} (child's sex), β_6 is the coefficient for X_{ijk5} (breastfeeding status), β_7 is the coefficient for X_{ijk6} (the number of household members), β_8 is the coefficient for X_{ijk7} (place of residence – urban or rural), β_9 is the coefficient for X_{ijk8} (household's wealth), β_{10} is the coefficient for X_{ijk9} (parents' education status) , μ_{0jk} is the random PSU effect assumed to have normal distribution with N (0, σμ (Murray et al., 2020)), ρ_{0jk} is the random country effect assumed to have normal distribution with N (0, σρ (Murray et al., 2020)).

Sensitivity analysis (mixed effect multilevel logistic regression model) was also conducted to examine if there is a dose-response relationship between SHS and under-5 mortality. SHS was categorized into never, daily, weekly or monthly including less than once in a month. "Never" had an exposure to SHS was regarded as reference.

The following model equation was applied to develop the mixed effects multilevel logistic regression models for a dose-response relationship between SHS and under-5 mortality:

$$y_{ijk} \sim \mathrm{Bin}\left(n_{ijk}, p_{ijk}\right)$$

$$\mathrm{logit}\left(p_{ijk}\right) = \alpha_{ijk} + \beta_1 X_{ijk1} + \beta_2 X_{ijk2} + \beta_3 X_{ijk3} + \beta_4 X_{ijk4} + \beta_5 X_{ijk5} + \beta_6 X_{ijk6} + \beta_7 X_{ijk7} + \beta_8 X_{ijk8} + \beta_9 X_{ijk9} + \mu_{0jk} + \rho_{0jk}$$

where y_{ijk} is the outcome (death or no death) of child i in j PSU in k country, n_{ijk} is the total number of under-5 children in the family of the child i in the j PSU in k country, α_{ijk} is the probability of the outcome (death or no death) of child i in the j PSU from the country k. β_1 is the coefficient for X_{ijk1} (SHS – never, daily, weekly or monthly including less than once in a month), β_2 is the coefficient for X_{ijk2} (solid fuel – no or yes), β_3 is the coefficient for X_{ijk3} (mother's age at birth), β_4 is the coefficient for X_{ijk4} (child's sex), β_5 is the coefficient for X_{ijk5} (breastfeeding status), β_6 is the coefficient for X_{ijk6} (the number of household members), α_7 is the coefficient for X_{ijk7} (place of residence – urban or rural), β_8 is the coefficient for X_{ijk8} (household's wealth), β_9 is the coefficient for X_{ijk9} (parents' education status), μ_{0jk} is the random PSU effect assumed to have normal distribution with N (0, σμ2), ρ_{0jk} is the random country effect assumed to have normal distribution with N (0, σρ2).

Finally, the population attributable fraction (PAF) was calculated to measure the proportion of under-5, infant, and neonatal mortality in LMICs that could theoretically be prevented by eliminating solid fuel and SHS exposure in households, assuming the other risk factors remain unchanged. The PAF was calculated by using the following formula (Murray et al., 2003).

$$\mathrm{PAF} = \sum p\ (\mathrm{RR}-1)/\ (1+\sum p\ (\mathrm{RR}-1))$$

where p is the prevalence of exposure from solid fuel use or SHS in each country and RR is the adjusted pooled relative risk of each exposure on outcomes estimated from the analyses above. The odds ratio is very similar to the risk ratio when the incidence of an outcome is low (<10%). (Zhang and Yu, 1998) In this study, the odds ratio was considered as risk ratio since the incidence of under-5, neonatal and infant mortality in those children without exposure to solid fuel and SHS is low (<10%).

2.6. Ethical considerations

As the de-identified data for the current study came from secondary sources whose data is publicly available, ethics approval for this study was not required.

3. Results

3.1. Descriptive characteristics of the study population

A total of 778,532 under-5 children out of 687,500 households from 46 LMICs were considered eligible for this study after excluding all observations with missing cooking fuel type (n = 809), or missing outcome data (n = 654) and twin births (n = 536). Out of these, there were 21,061 (2.7%) neonatal, 33,750 (4.0%) infant and 41,860 (5.2%) under-5 deaths (Appendix Table 3).

Table 1 shows the study population characteristics by different types of fuel (clean fuel, outdoor solid fuel and indoor solid fuel) and SHS exposure. Among 778,532 under-5 children, 361,239 (46.4%), 239,788 (30.8%) and 268,594 (34.5%) under-5 children had exposure to outdoor solid fuel, indoor solid fuel and SHS respectively (Table 1).

3.2. Variations in the prevalence of exposure to solid fuel and SHS among 46 low- and lower-middle-income countries

Prevalence of under-5 children's exposure to solid fuel (indoor and outdoor), SHS and combined solid fuel and SHS in 46 LMICs are presented in Appendix Figs. 2, 3, 4 and 5, respectively. The prevalence of under-5 children's exposure to indoor solid fuel and SHS was higher in countries from Asia and Oceania than the countries from Africa. On the other hand, the prevalence of under-5 children's exposure to outdoor solid fuel was higher in the countries from Africa. Then, the prevalence of under-5 children's exposure to combined solid fuel and SHS was

Table 3
Association between child mortality exposed to indoor solid fuel, outdoor solid fuel, secondhand smoke and interaction between them in 46 countries.

Exposure	Under-5 mortality aOR (95% CI)	Infant mortality aOR (95% CI)	Neonatal mortality aOR (95%CI)
Clean fuel	1.00 (ref)	1.00 (ref)	1.00 (ref)
Indoor solid fuel	1.42 (1.35–1.50) **	1.35 (1.27–1.45) **	1.35 (1.24–1.47) **
Outdoor solid fuel	1.21 (1.12–1.28) **	1.20 (1.12–1.31) **	1.28 (1.18–1.40) **
SHS			
No	1.00 (ref)	1.00 (ref)	1.00 (ref)
Yes	1.13 (1.06–1.21) **	1.15 (1.05–1.25) **	1.12 (1.07–1.21) *
Combined indoor solid fuel and SHS	1.51 (1.49–1.69) **	1.50 (1.38–1.60) **	1.51 (1.37–1.66) **
Combined outdoor solid fuel and SHS	1.35 (1.29–1.45) **	1.35 (1.25–1.40) **	1.38 (1.27–1.48) **
Mother's age at birth	1.13 (1.05–1.21) *	1.14 (1.07–1.21) *	1.18 (1.05–1.24) *
Child's sex			
Male	1.00 (ref)	1.00 (ref)	1.00 (ref)
Female	0.80 (0.76–0.89) **	0.81 (0.77–0.89) **	0.79 (0.75–0.84) **
Breastfeeding status			
Yes	1.00 (ref)	1.00 (ref)	1.00 (ref)
No	1.08 (1.06–1.17) **	1.15 (1.09–1.26) *	1.18 (1.08–1.24) *
Number of household members	1.06 (1.03–1.15) **	1.07 (1.07–1.08) *	1.07 (1.06–1.08) **
Place of residence			
Urban	1.00 (ref)	1.00 (ref)	1.00 (ref)
Rural	1.09 (1.06–1.16) **	1.07 (1.03–1.12) **	1.04 (1.02–1.09) *
Household wealth index			
Richest	1.00 (ref)	1.00 (ref)	1.00 (ref)
Richer	1.20 (1.13–1.28) *	1.13 (1.07–1.20) **	1.08 (1.01–1.17) **
Middle	1.32 (1.23–1.38) *	1.22 (1.15–1.29) **	1.12 (1.04–1.21) **
Poorer	1.41 (1.33–1.52) *	1.28 (1.20–1.36) **	1.13 (1.04–1.22) **
Poorest	1.52 (1.43–1.62) *	1.35 (1.27–1.44) **	1.18 (1.09–1.28) **
Parent's education status			
Higher	1.00 (ref)	1.00 (ref)	1.00 (ref)
Secondary	1.28 (1.17–1.36) *	1.19 (1.12–1.27) *	1.14 (1.05–1.23) *
Primary	1.49 (1.39–1.60) *	1.39 (1.29–1.48) *	1.31 (1.20–1.42) *
None	1.64 (1.52–1.76) *	1.46 (1.36–1.56) *	1.37 (1.26–1.49) *
Random effects	Variance (SE)		
Community (PSU)	0.03 (0.01)	0.02 (0.01)	0.04 (0.02)
Country	0.41 (0.01)	0.39 (0.02)	0.42 (0.01)

aOR, adjusted Odds Ratio; CI, Confidence Interval; SHS, Second-hand Smoke; PSU,Primary Sampling Unit; SE, Standard Error **p-value<0.001,*p-value<0.05.

higher in the countries from Asia and Oceania than in Africa. The prevalence estimates with CI for each country can be seen in Appendix Table 4.

3.3. Association between household air pollution exposure and children mortality

The pooled adjusted associations between solid fuel, SHS (by considering the interaction between solid fuel and SHS) and children mortality for all 46 countries combined (Model 1) are presented in Table 2. After adjusting for covariates, in reference to the household with clean fuel use and no exposure to SHS, the pooled adjusted odds ratio of solid fuel use for under-5, infant and neonatal mortality were 1.27 (95%CI: 1.19–1.36), 1.28 (1.20–1.37) and 1.31 (1.21–1.42) respectively. In reference to no exposure to SHS and the household with clean fuel use, the pooled adjusted odds ratio of exposure to SHS for under-5, infant and neonatal mortality were 1.13 (95%CI: 1.06–1.25), 1.15 (1.04–1.25) and 1.07 (1.01–1.20) respectively. Then the pooled adjusted odds ratio of combined solid fuel and SHS for under-5, infant and neonatal mortality were 1.40 (95%CI: 1.31–1.50), 1.43 (1.35–1.50) and 1.45 (1.38–1.55) respectively.

Table 3 presents the results of Model 2, which are the pooled adjusted associations between indoor solid fuel, outdoor solid fuel and SHS (by considering the interaction between indoor or outdoor solid fuel and SHS) and child mortality for all 46 countries combined.

After adjusting for the covariates, in reference to households with clean fuel use and no exposure to SHS, the pooled adjusted odds ratio of indoor solid fuel use for under-5, infant and neonatal mortality were

Table 4
Dose-response relationship between exposure to second-hand smoke and child mortality in 46 countries.

Exposure	Under-5 mortality aOR (95% CI)	Infant mortality aOR (95% CI)	Neonatal mortality aOR (95%CI)
SHS			
Never	1.00 (ref)	1.00 (ref)	1.00 (ref)
Daily	1.13 (1.06–1.20) **	1.15 (1.05–1.22) **	1.12 (1.05–1.31) *
Weekly	1.12 (1.02–1.20) **	1.11 (1.04–1.17) **	1.10 (1.03–1.29) *
Monthly or less than once a month	1.11 (1.07–1.14) **	1.09 (1.05–1.14) **	1.08 (1.05–1.13) **
Mother's age at birth	1.13 (1.05–1.21) *	1.14 (1.07–1.21) *	1.18 (1.05–1.24) *
Child's sex			
Male	1.00 (ref)	1.00 (ref)	1.00 (ref)
Female	0.80 (0.76–0.89) **	0.81 (0.77–0.89) **	0.79 (0.75–0.84) **
Breastfeeding status			
Yes	1.00 (ref)	1.00 (ref)	1.00 (ref)
No	1.08 (1.06–1.17) **	1.15 (1.09–1.26) *	1.18 (1.08–1.24) *
Number of household members	1.06 (1.03–1.15) **	1.07 (1.07–1.08) *	1.07 (1.06–1.08) **
Place of residence			
Urban	1.00 (ref)	1.00 (ref)	1.00 (ref)
Rural	1.09 (1.06–1.16) **	1.07 (1.03–1.12) **	1.04 (1.02–1.09) *
Household wealth index			
Richest	1.00 (ref)	1.00 (ref)	1.00 (ref)
Richer	1.20 (1.13–1.28) *	1.13 (1.07–1.20) **	1.08 (1.01–1.17) **
Middle	1.32 (1.23–1.38) *	1.22 (1.15–1.29) **	1.12 (1.04–1.21) **
Poorer	1.41 (1.33–1.52) *	1.28 (1.20–1.36) **	1.13 (1.04–1.22) **
Poorest	1.52 (1.43–1.62) *	1.35 (1.27–1.44) **	1.18 (1.09–1.28) **
Parent's education status			
Higher	1.00 (ref)	1.00 (ref)	1.00 (ref)
Secondary	1.28 (1.17–1.36) *	1.19 (1.12–1.27) *	1.14 (1.05–1.23) *
Primary	1.49 (1.39–1.60) *	1.39 (1.29–1.48) *	1.31 (1.20–1.42) *
None	1.64 (1.52–1.76) *	1.46 (1.36–1.56) *	1.37 (1.26–1.49) *
Random effects	Variance (SE)		
Community (PSU)	0.03 (0.01)	0.02 (0.01)	0.04 (0.02)
Country	0.41 (0.01)	0.39 (0.02)	0.42 (0.01)

aOR, adjusted Odds Ratio; CI, Confidence Interval; SHS, Second-hand Smoke; PSU, Primary Sampling Unit; SE, Standard Error, **p-value<0.001, *p-value<0.05.

1.42 (1.35–1.50), 1.35 (1.27–1.45) and 1.35 (1.24–1.47), respectively whereas the pooled adjusted odds ratio of outdoor solid fuel use for under-5, infant and neonatal mortality were 1.21 (1.12–1.28), 1.20 (1.12–1.31) and 1.28 (1.18–1.40), respectively. In reference to no exposure to SHS and the household with clean fuel, the pooled adjusted odds ratio of exposure to SHS for under-5, infant and neonatal mortality were 1.13 (1.06–1.21), 1.15 (1.05–1.25) and 1.12 (1.07–1.21), respectively. Then in reference to no exposure to solid fuel and SHS, the pooled adjusted odds ratio of exposure to combined indoor solid fuel use and SHS for under-5, infant and neonatal mortality were 1.51 (1.49–1.69), 1.50 (1.38–1.60) and 1.51 (1.37–1.66), respectively. On the other hand, the pooled adjusted odds ratio of exposure to combined outdoor solid fuel use and SHS for under-5, infant and neonatal mortality were 1.35 (1.29–1.45), 1.35 (1.25–1.40) and 1.38 (1.27–1.48), respectively.

3.4. Sensitivity analysis

The dose-response relationships between SHS and under-5, infant, and neonatal mortality are presented in Table 4. SHS was categorized into "never", "daily", "weekly", "monthly or less than once in a month". "Never" had an exposure to SHS was regarded as reference and it is observed that the pooled adjusted odds ratio of daily exposure to SHS for under-5 mortality, infant mortality and neonatal mortality were 1.13 (95%CI: 1.06–1.20), 1.15 (95%CI: 1.05–1.22) and 1.12 (95%CI: 1.05–1.31). The pooled odds ratio of weekly exposure to SHS for under-5 mortality, infant mortality and neonatal mortality were 1.12 (95%CI: 1.02–1.20), 1.11 (95%CI: 1.04–1.17) and 1.10 (95% CI: 1.03–1.29). The pooled odds ratio of monthly or less than once in a month exposure to SHS for under-5 mortality, infant mortality and neonatal mortality were 1.11 (95%CI: 1.07–1.14), 1.09 (95%CI: 1.05–1.14) and 1.08 (95%CI: 1.05–1.13).

3.5. Children mortality attributable to household air pollution

Under-5 mortality attributable to indoor solid fuel, outdoor solid fuel, SHS and combined solid fuel and SHS exposure in 46 countries are presented in Figs. 1–4, respectively. It can be seen that under-5 mortality attributable to indoor solid fuel use was higher in countries from the Asian region whereas those resulting from outdoor solid fuel use were higher in countries from the African region. However, those resulting from SHS were higher in countries from the Asian region compared to countries from African region. On the other hand, the under-5 mortality attributable to combined solid fuel and SHS exposure was higher in the countries from Asian and Oceania region than African region. The estimates with CI for each country can be seen in Appendix Table 5,6,7.

Under-5 mortality attributable to indoor solid fuel, outdoor solid fuel, SHS and combined solid fuel and SHS was the highest in Myanmar (18.0%), the Gambia (16.5%), Indonesia (9.8%), and Timor-Leste (22.7%), respectively. Similarly, infant and neonatal mortality attributable to indoor solid fuel, outdoor solid fuel, SHS and combined solid fuel and SHS were also the highest in Myanmar, the Gambia, Indonesia and Timor-Leste, respectively (Appendix Figs. 6–13).

4. Discussion

This comprehensive multi-country analysis of DHS data from 46 LMICs between 2010 and 2020, showed that solid fuel use had a significantly higher risk of children mortality than the risk from clean fuel use. This study employed an augmented solid fuel exposure by cooking place: indoor or outdoor. It was shown that indoor solid fuel use had a higher risk of children mortality than outdoor solid fuel use. It was also observed that SHS had a significantly increased risk of children mortality. Moreover, this study also showed that the combined effect of exposure to solid fuel and SHS had a higher risk of children mortality than the individual risk. Furthermore, children who had exposure to the either combined indoor solid fuel and SHS; or outdoor solid fuel and SHS had a significantly increased risk of mortality than those who had neither exposure to solid fuel (indoor or outdoor) nor SHS. Additionally, this study observed that children mortality attributable to indoor solid fuel and SHS were high in the countries from Asia. In contrast, children mortality attributable to outdoor solid fuel was high in the countries from Africa. The proportion of children mortality attributable to combined solid fuel and SHS were high in the countries from Asia. It could be due to the cooking and smoking habit or housing structure influenced by climate and economic conditions between countries from Asia and Africa (Reitsma et al., 2017; Bruce et al., 2000).

The significant positive associations between indoor solid fuel and children mortality observed in this study were consistent with several previous studies conducted in Sub-Saharan Africa (SSA), and some South Asian countries (Bickton et al., 2020; Kleimola et al., 2015; Naz et al., 2017; Khan et al., 2017). Previous studies suggest that not only the solid fuel use but also the place of solid fuel use can impact children mortality because cooking indoor with solid fuel drastically increases the airborne toxic pollutants' concentration in the household and ambient air (Bickton et al., 2020; Kleimola et al., 2015; Naz et al., 2017; Khan et al., 2017). Some previous studies from LMICs considered the

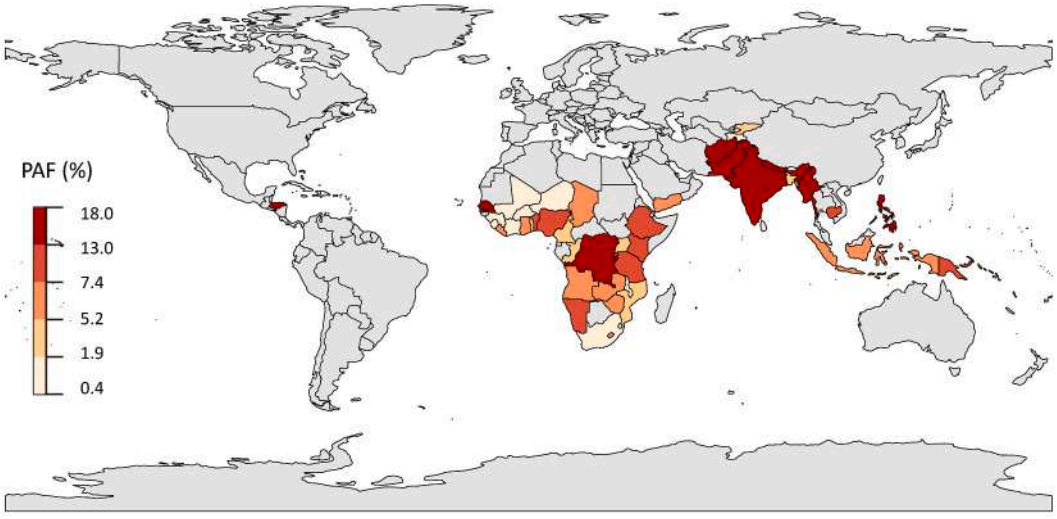

Fig. 1. Under-5 mortality attributable to indoor solid fuel exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%).

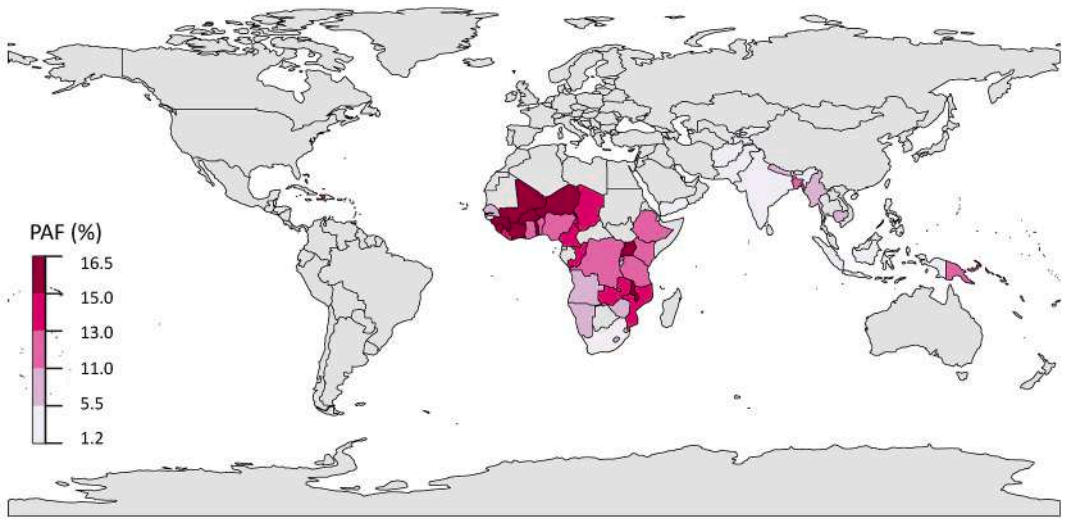

Fig. 2. Under-5 mortality attributable to outdoor solid fuel exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%).

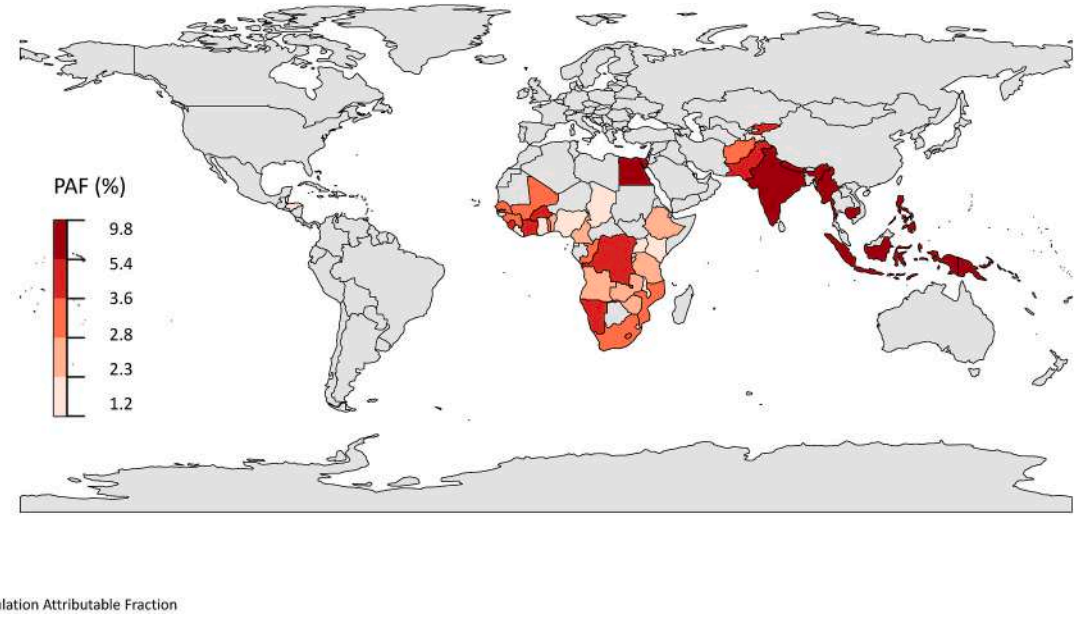

Fig. 3. Under-5 mortality attributable to secondhand smoke exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%).

kitchen location when investigating the association between solid fuel and under-5 mortality (Naz et al., 2016, 2017, 2018; Khan et al., 2017; Bassani et al., 2010). One study conducted in India found out that children who lived in houses with kitchens inside had higher risk of mortality compared to children who lived in houses with kitchens outside (Bassani et al., 2010). Findings from our study showed not only a greater risk of child mortality from households using solid fuel but also increased risk of child mortality where solid fuel was used indoors. Households using indoor solid fuel have a higher level of particulate matter (PM) concentration, and children are exposed to those pollutants from soild fuel burning as they spend many hours inside the house (Naz et al., 2016, 2017, 2018; Khan et al., 2017; Bassani et al., 2010).

Moreover, in this study, it was observed that SHS also had a significantly increased risk of children mortality compared with the risk from no exposure to SHS. These results were in line with previous studies from SSA and some South Asian countries (Owili et al., 2017; Andriani et al., 2019). Furthermore, children who had exposure to either combined indoor solid fuel and SHS; or outdoor solid fuel and SHS had significantly increased risk of mortality than those who had neither exposure to solid fuel (indoor or outdoor) nor SHS. Additionally, indoor solid fuel and SHS's combined effect had a significantly increased risk of children mortality than the combined effect of outdoor solid fuel and SHS.

This comprehensive study has several strengths. One of the main strengths of this study is the use of data driven systematic modelling to estimate the attributable risk of solid fuel use (indoor and outdoor) and the SHS on under-5 mortality in each 46 LMICs. This study was based on nationally representative DHS surveys that used standardized methods yielding an average response rate of 98% (United Sates Agency International Development, 2021a). The large study population was sufficient to separately analyze the effects of exposure to indoor solid fuel

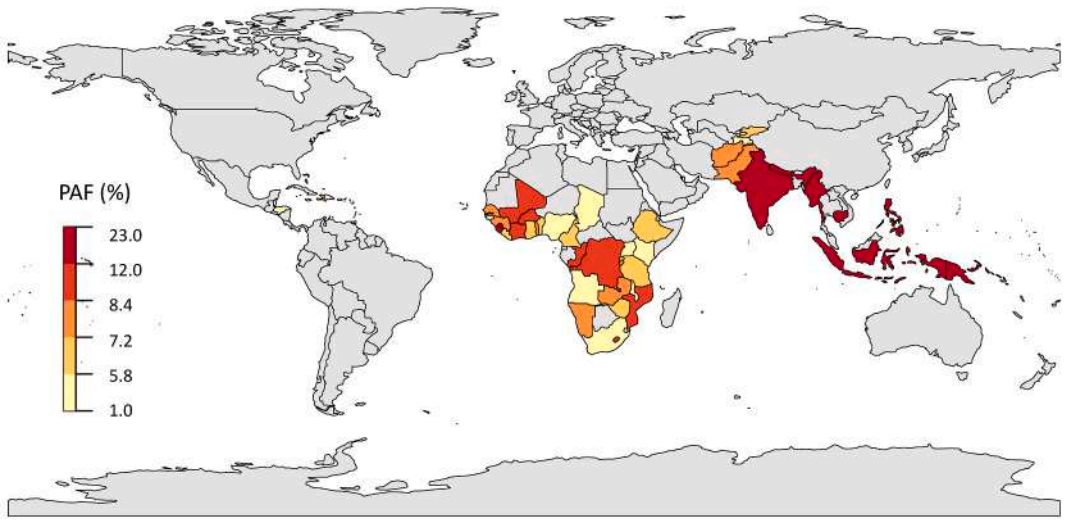

Fig. 4. Under-5 mortality attributable to solid fuel and secondhand smoke exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%).

use, outdoor solid fuel use and SHS. This is of particular importance as many previous studies only assessed solid fuel use or SHS. This study was able to assess the combined effect and individual effect of solid fuel use and SHS. The pooled method increased the study power and allowed us to safely generalize the findings to other populations with similar characteristics. Moreover, multilevel modelling was used, which took into account the hierarchical structure of the data and the variability within the country levels to better estimate the level of association of the study factors with the outcome.

However, this study also has some limitations. These limitations have been well-acknowledged in similar studies that used DHS data. Firstly, the main limitation is that this study used the extracted data from the retrospective cross-sectional surveys. Under-5 mortality data were retrospectively collected at the time of survey interview while the household characteristics were inferred for the deceased child. However, there is an assumption that children's exposure to solid fuel and SHS were not changing over time using extrapolated cross-sectional data (Kleimola et al., 2015; Owili et al., 2017). Still, this study could not ascertain the true measure of causal relationship. Therefore, the findings from this study should be interpreted with caution. Secondly, in order to evaluate the relationship between exposure to risks (solid fuel and SHS) and health outcomes in terms of odds ratios, this study used all-cause mortality reported by parents. There is always a methodological limitation to this approach, since it does not allow us to examine the relationship between the exposure variable and more precisely measured causes of death that might be directly related to the exposure. Cause of death data based on death certificates would allow a more accurate estimation of the odds ratio of exposure to solid fuel and SHS risk, but this is difficult due to the lack of such data in DHS (Amoroso et al., 2018). Thirdly, the nature of DHS questionnaire regarding household member smoking might be subjective to social desirability bias. For example, some household members might hesitate to admit about their smoking status which may lead to underestimate the prevalence of SHS (Owili et al., 2017; United Sates Agency International Development, 2021a). Fourthly, DHS only recorded the predominant type of fuel used for cooking in the household which may result in the misclassification of the fuel exposure, particularly if the household use both clean and solid fuel together. For example, some households from LMIC use solid fuel together with clean fuel in order to reduce the electricity bill (United Sates Agency International Development, 2021a, 2021b; Croft Trevor et al., 2018). Moreover, type of fuel use for heating inside the house during the winter season is not included in DHS survey yet. In some countries like Afghanistan, Tajikistan and Nepal, solid fuel is also used for household heating in the winter (Fullerton et al., 2008; Kleimola et al., 2015). Therefore, there might be underestimation in the prevalence of indoor solid fuel exposure in those countries. Finally, some potential confounding factors like childhood illnesses, nutritional status, ambient air pollution, meteorological factors and health seeking behaviors could not be considered in this study due to the missing or lack of information in DHS (Murray et al., 2020; Reitsma et al., 2017; Paulson et al., 2021; Croft Trevor et al., 2018).

5. Conclusion and policy implications

The findings from this study indicated that the prevalence of children's exposure to indoor solid fuel, outdoor solid fuel, SHS and combined solid fuel and SHS varied among LMICs. Children's exposure to solid fuel and SHS were significantly associated with the increased risk of children mortality. Moreover, the combined effect of exposure to solid fuel and SHS had a higher risk of children mortality than the individual risk of solid fuel and SHS. Furthermore, related to the variation in the prevalence of exposure, children mortality attributable to indoor solid fuel, outdoor solid fuel, SHS and combined solid fuel and SHS varied among LMICs. Policymakers in LMICs, where solid fuel and SHS as child health issues is relatively neglected, should integrate the use of clean fuel, improvement in household ventilation systems, implementation of eco-friendly stove and tobacco control measures with other child health promotion policies. Understanding the variation in the exposure level and nature of solid fuel and SHS among LMICs may help to address the targeted interventions to reduce under-5 mortality because solid fuel and SHS exposures are potentially modifiable and reducing those exposures could be a method for accelerating progress toward reducing under-5 mortality (Paulson et al., 2021). Reducing exposure to solid fuel and secondhand smoke offers LMICs cost-effective, popular and easily implemented strategies to significantly reduce infant and child mortality. If these countries can reduce exposure to the risk factors identified in this study, they can make significant progress towards SDG 3, and improve the health of all populations.

Contributors

KSL and SN conceived the study, KSL managed the data and conducted the statistical analysis in consultation with SN, SG and MH. All the authors made critical revisions to the manuscript for important intellectual content and gave final approval of the manuscript.

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Research in context

Evidence before this study

Although substantial progress has been made towards reducing the under-5 mortality rate to 25 deaths or below per 1000 live births by 2030, low- and lower-middle-income countries (LMICs) are still struggling to achieve this target. Existing evidence suggested that exposure to solid fuel or second-hand smoke (SHS) has been linked to an increased risk of childhood illnesses. We searched PubMed for published literature in English using the search terms "household air pollution", "solid fuel use", "second-hand smoke", "under-5 mortality", "population attributable fraction", "PAF", "low- and lower-middle-income countries" and "LMICs" up to October 30, 2021. We found some previous studies that have investigated the association between either solid fuel use or SHS and under-5 mortality. However, there have been few comprehensive multi-country assessments or studies that have examined how the interaction between solid fuels use and SHS affects under-five mortality.

Added value of this study

This study provides a comprehensive analysis of variation in attributable risk of solid fuel use and SHS associated with under-5 mortality among 46 low- and lower-middle-income countries. The large study population was able to separately analyze the effects of exposure to indoor solid fuel use, outdoor solid fuel use and SHS. This is of particular importance as many previous studies only assessed solid fuel use or SHS. This study was able to assess the combined effect and individual effect of solid fuel use and SHS. The findings from this study indicated that the prevalence of children's exposure to indoor solid fuel, outdoor solid fuel, SHS and combined solid fuel and SHS varied among LMICs. Children's exposure to solid fuel and SHS were significantly associated with the increased risk of children mortality. Moreover, the combined effect of exposure to solid fuel and SHS had a higher risk of children mortality than the individual risk. Furthermore, related to the variation in the prevalence of exposure, children mortality attributable to indoor solid fuel, outdoor solid fuel, SHS and combined solid fuel and SHS varied among LMICs.

Implications of all the available evidence

The findings from this study would inform the policymakers in LMICs, where solid fuel and SHS as child health issue is relatively neglected, to integrate the use of clean fuel, improvement in household ventilation systems and tobacco control measures with other child health promotion policies. Reducing exposure to solid fuel and secondhand smoke offers cost-effective, popular and easily implemented strategies for LMICs to significantly reduce under-5 mortality. If these countries can reduce exposure to the risk factors identified in this study, they can make significant progress towards SDG 3, and improve the health of all populations.

Declaration of competing interest

We declare no competing interests.

Acknowledgment

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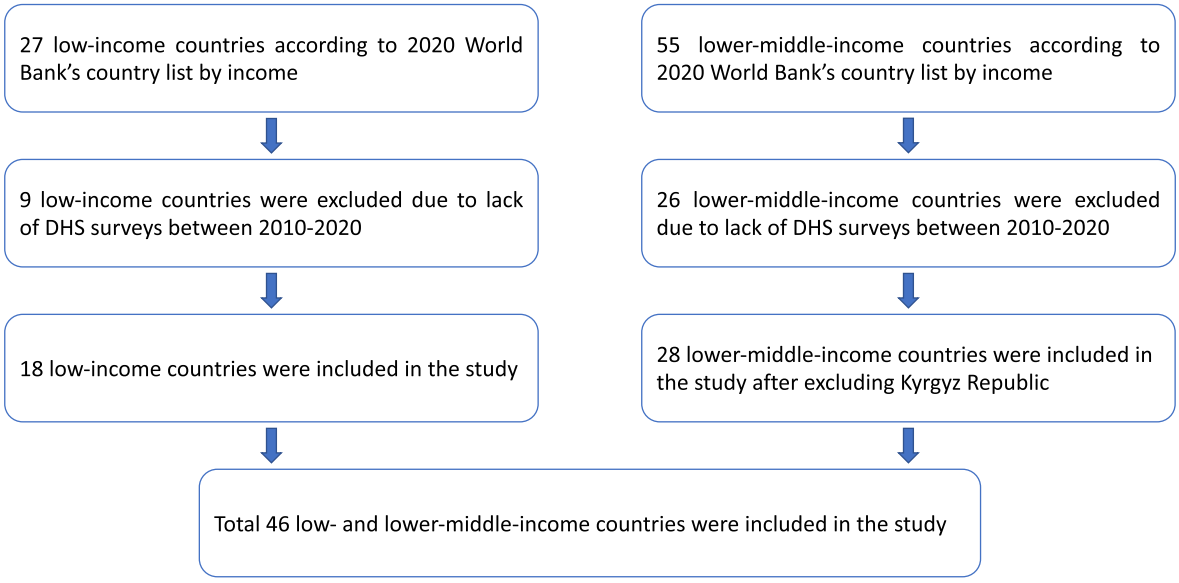
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113986.

Appendix



DHS survey was conducted in Kyrgyz Republic in 2012. However, this country was excluded from the study since it has already achieved Sustainable Development Goal (SDG) 3.2

Fig. 1. Selection of low- and lower-middle-income countries included in the study

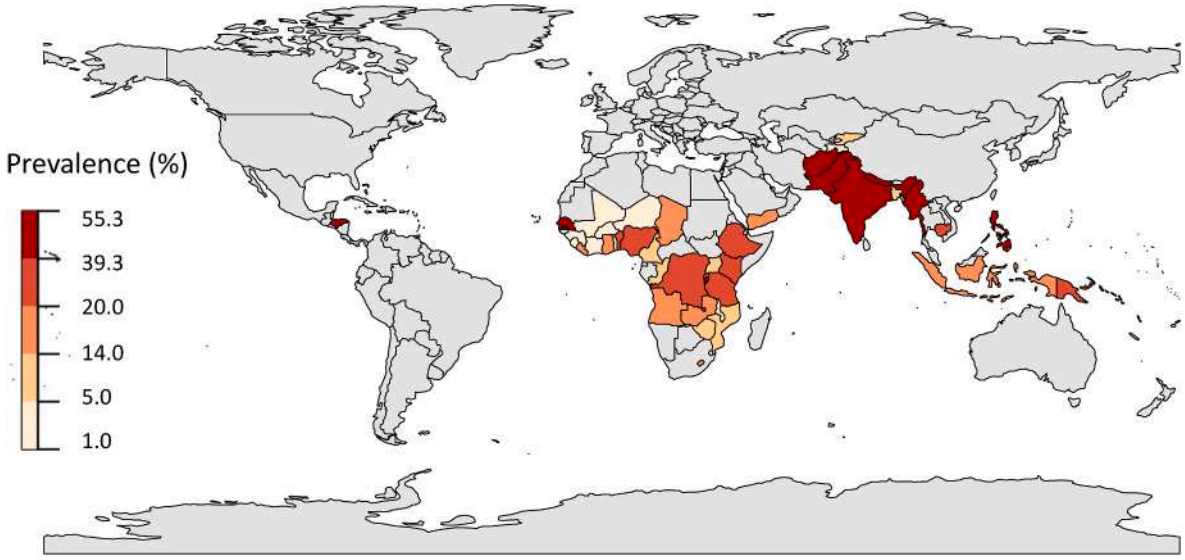


Fig. 2. Prevalence of exposure to indoor solid fuel among under-5 children between 2010 and 2020

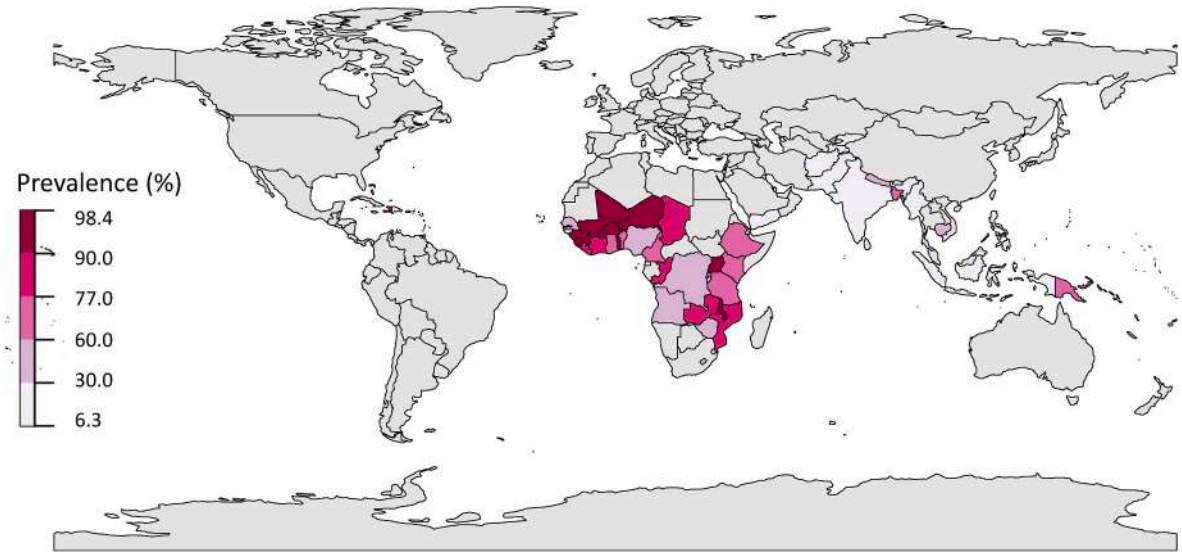


Fig. 3. Prevalence of exposure to outdoor solid fuel among under-5 children between 2010 and 2020

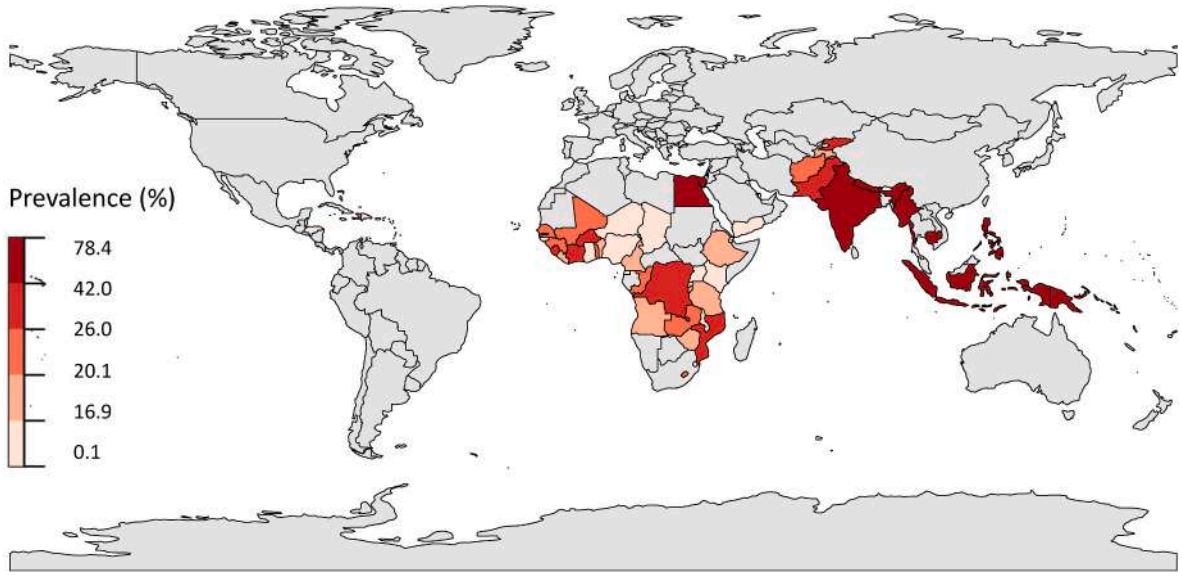


Fig. 4. Prevalence of exposure to secondhand smoke among under-5 children between 2010 and 2020

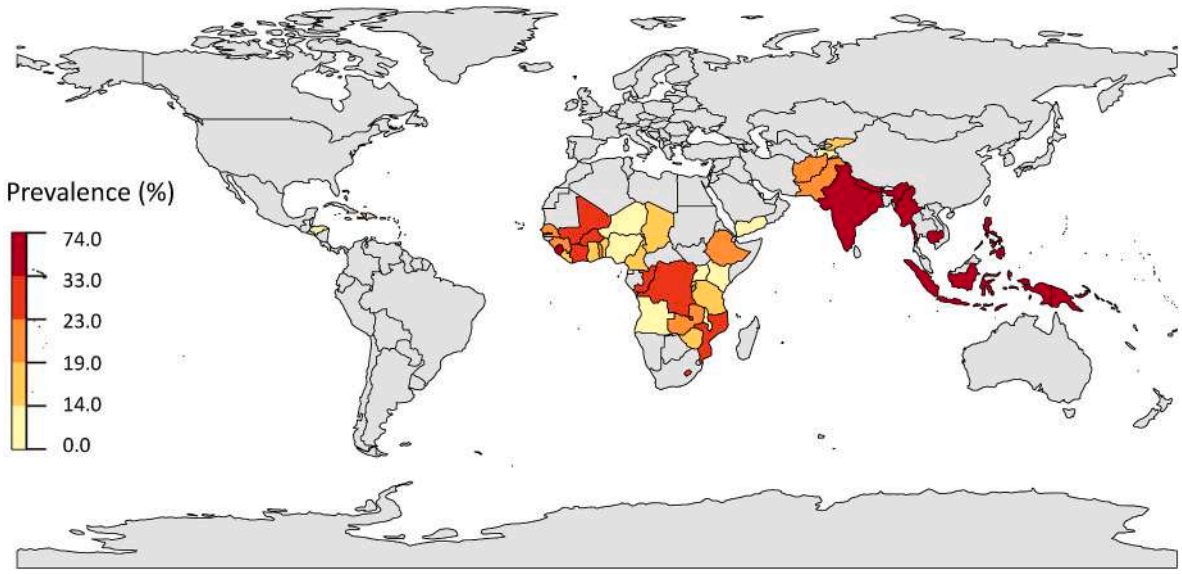


Fig. 5. Prevalence of exposure to solid fuel and secondhand smoke among under-5 children between 2010 and 2020

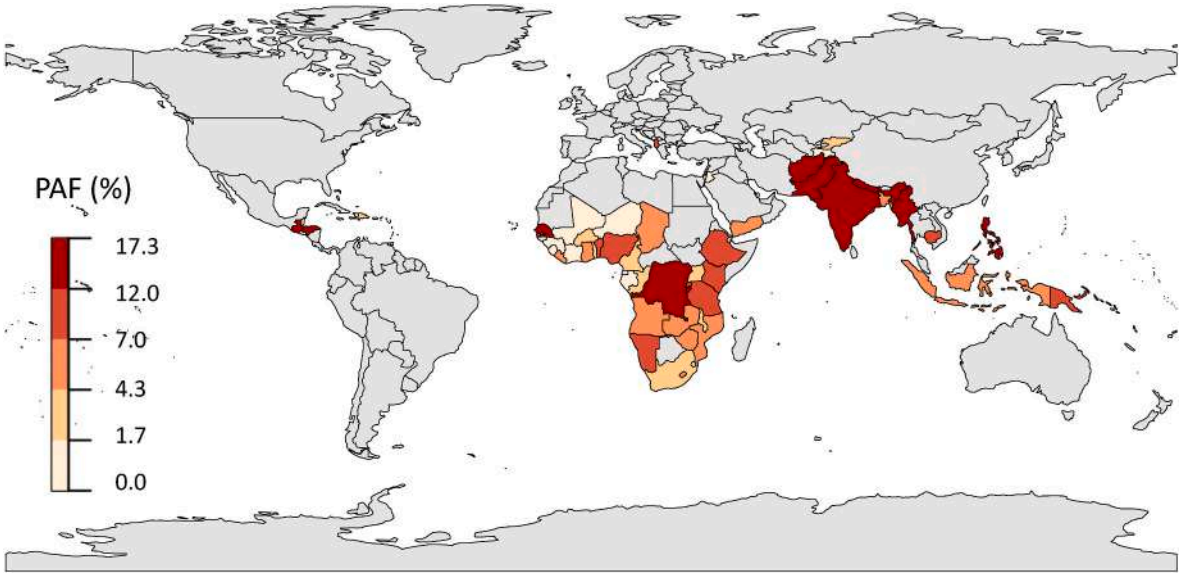


Fig. 6. Infant mortality attributable to indoor solid fuel exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%)

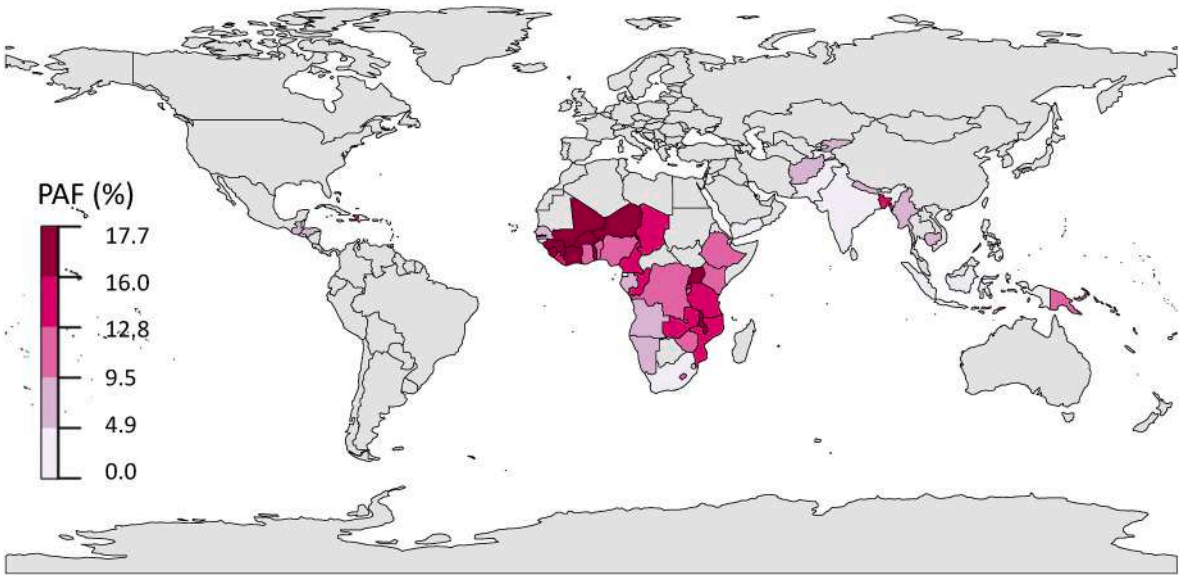


Fig. 7. Infant mortality attributable to outdoor solid fuel exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%)

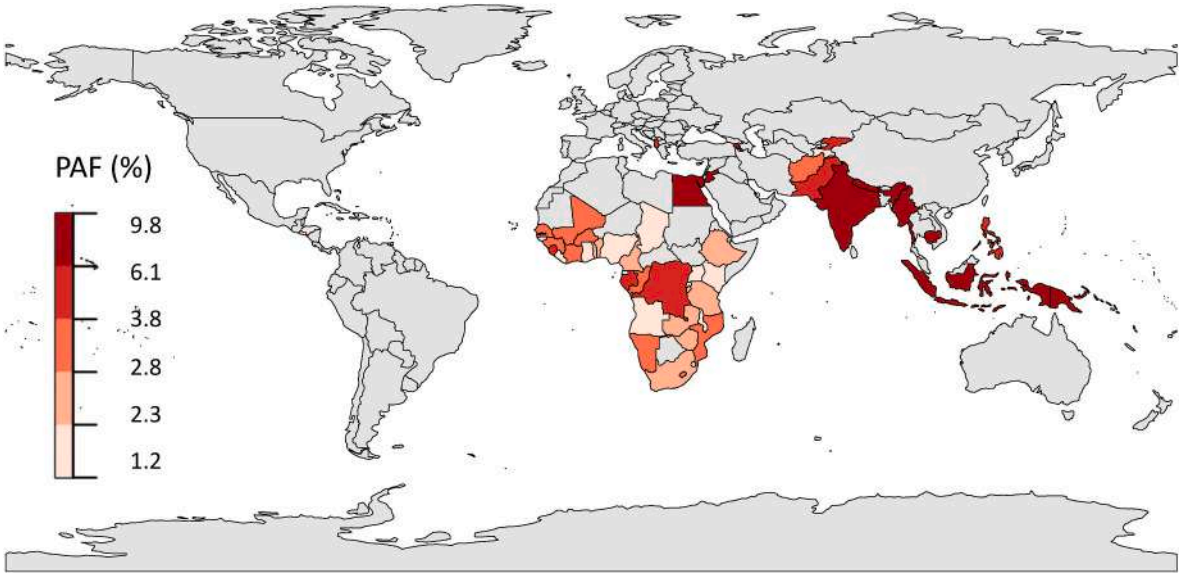


Fig. 8. Infant mortality attributable to secondhand smoke exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%)

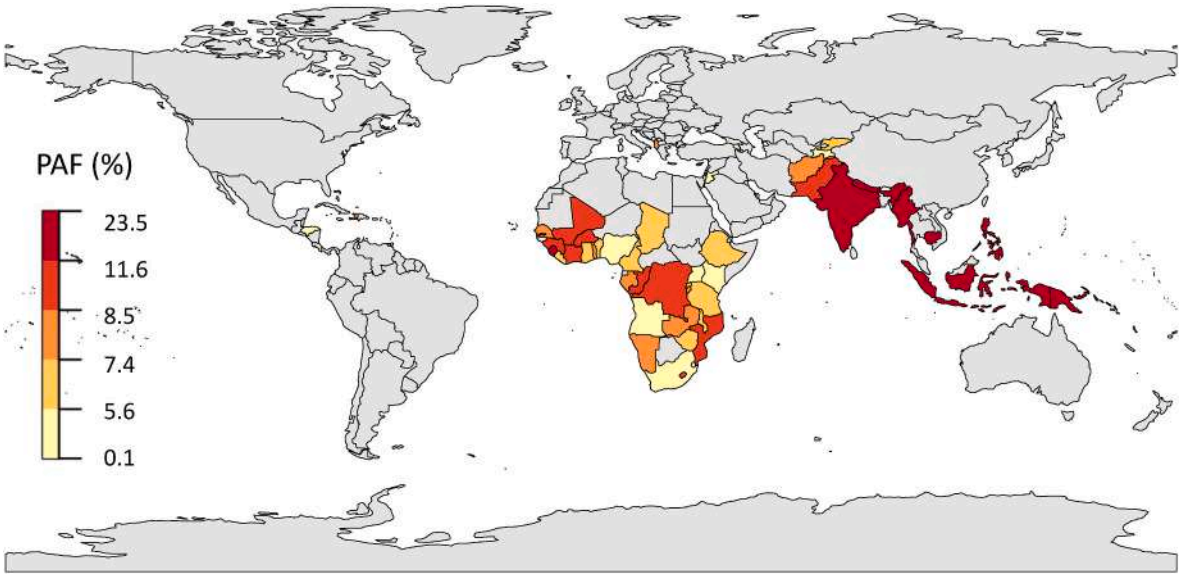


Fig. 9. Infant mortality attributable to solid fuel and secondhand smoke exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%)

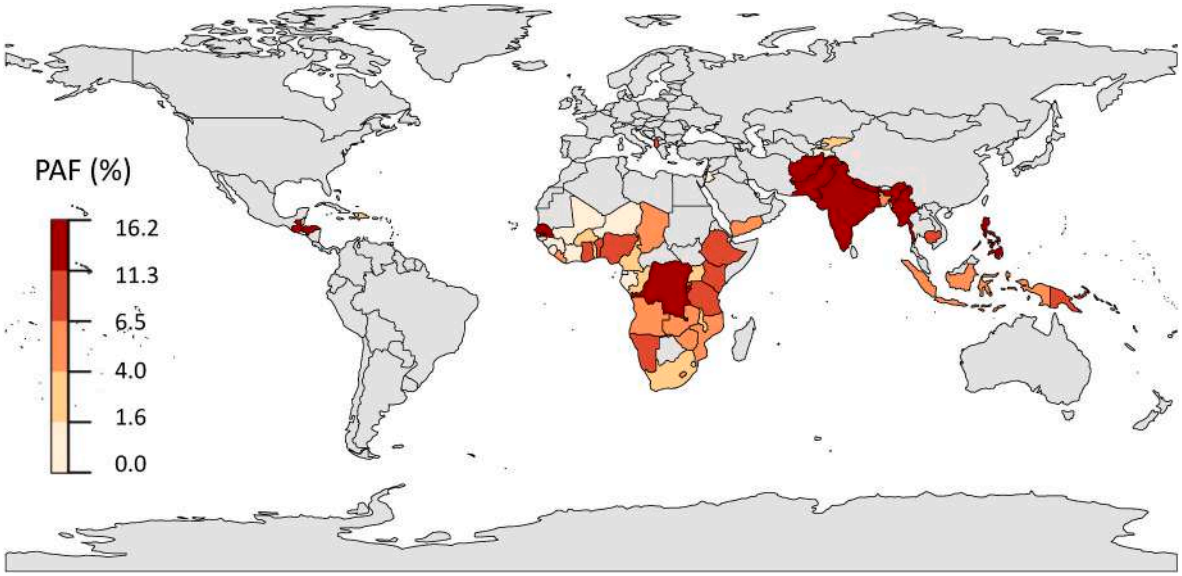


Fig. 10. Neonatal mortality attributable to indoor solid fuel exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%)

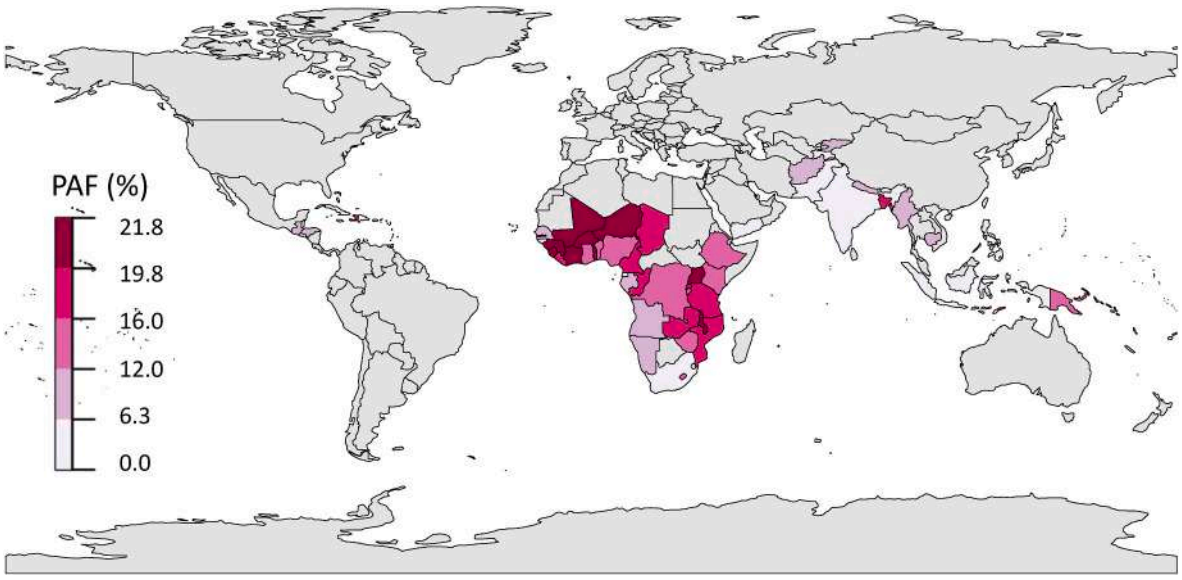


Fig. 11. Neonatal mortality attributable to outdoor solid fuel exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%)

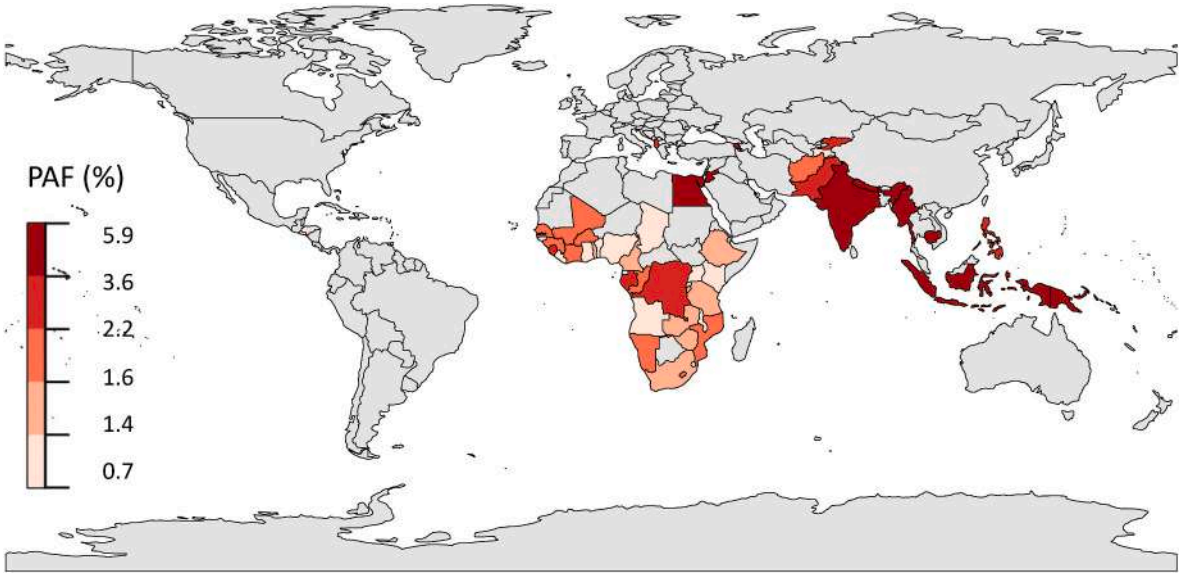


Fig. 12. Neonatal mortality attributable to secondhand smoke exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%)

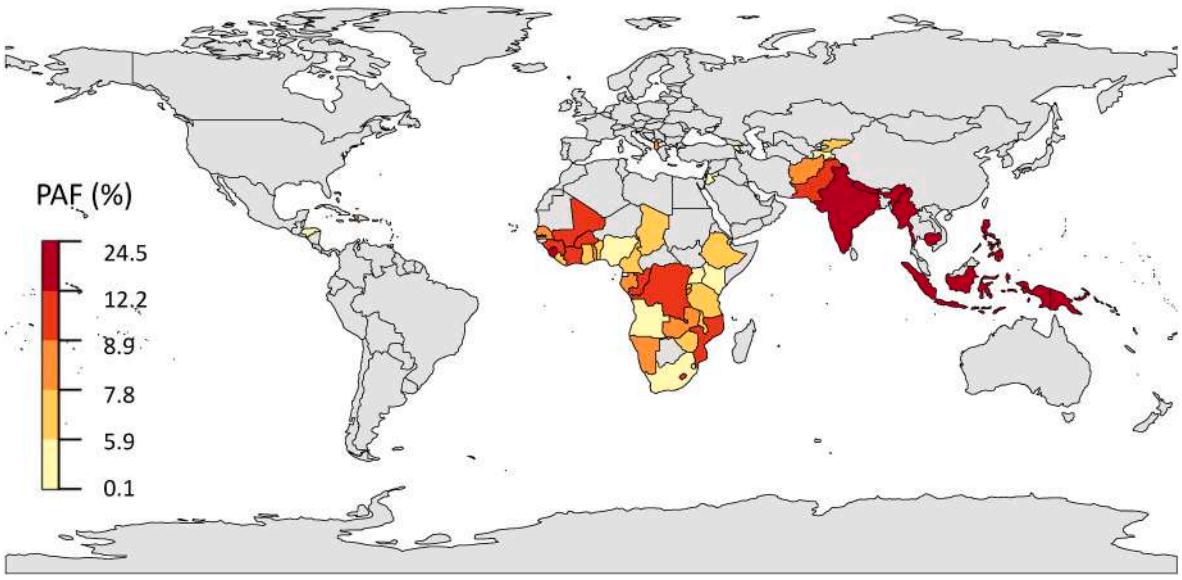


Fig. 13. Neonatal mortality attributable to solid fuel and secondhand smoke exposure among 46 low- and lower-middle-income countries between 2010 and 2020 (PAF-%)

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Exposure variability and determining factors of urinary metals for schoolchildren in Taiwan

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Determining variable

ABSTRACT

Urinary heavy metal levels in schoolchildren vary greatly over time, and research on the determinant variables explaining variance components in urinary metal exposure is limited. This study assessed metal concentrations and variability in the urine of schoolchildren and explored their important determining factors. We collected spot urine samples from schoolchildren (n = 321) living in urban, suburban, and rural areas during the warm and cold seasons. The toxic (As, Cd, Ni, Cd, and Pb) and essential (Co, Cu, Mo, and V) elements of urine samples were analyzed by inductively coupled plasma mass spectrometry. We assessed the within- and between-subject variability in urinary metal concentrations, calculated the "probability of overexposure (θ)," compared with the reference values. We evaluated the spatiotemporal and biological factors that determine average exposure by using a mixed-effects model. The within-subject variance accounting for 63.8%–95.4% of the total variance in exposures was predominant for selected urinary metals. Urinary As levels (subject-specific mean = 69.0 μg/g creatinine) in schoolchildren were remarkably high and presented ~99.9% of θ. After adjusting for the selected variables, we found that urban schoolchildren had significantly higher urinary metal levels (As, Cr, Co, and Ni) than suburban and rural schoolchildren. The urinary levels of most metals (except for Cu and Pb) increased during the cold season. Girls had higher urinary Cr, Co, Cu, Ni, and Pb levels than boys. Body mass index and urinary creatinine could also affect urinary metal levels. Those variables explained 15.8% (Pb), 11.6% (Cr), and 6.5% (Cd) of total variance in urinary concentrations. The repeated measurements of spot urine samples across seasons in individuals for long-term exposure estimates of metals were suggested. This biomonitoring survey of a large number of urine samples provided useful information on toxic metals and on the important determinants of exposure in schoolchildren.

1. Introduction

Heavy metals are ubiquitous in the environment and can be found in the air, soil, water, food, and even cigarette smoke. Exposure to heavy metals via the respiratory, dermal, and oral routes may cause adverse health effects depending on the dose (Al Osman et al., 2019). Children are susceptible to harmful substances because their immune systems and metabolic pathways are not yet fully developed. Children have greater exposure to substances on a bodyweight basis than adults because they have higher breathing rates and mouth contact; furthermore, children have much more time to develop chronic diseases than adults (Järup, 2003; Landrigan and Miodovnik, 2011).

Exposure to toxic elements (e.g., Cd and Pb) even at relatively low levels is associated with renal tubular damage, developmental neurotoxicity, and diminished intellectual capacity in children (Sanders et al., 2015; Gundacker et al., 2021; Shen et al., 2021). Although studies have reported that high As exposure from drinking water is associated with lower intelligence and adverse neurodevelopmental effects in children (Rocha-Amador et al., 2007; Wasserman et al., 2014), Khan et al. (2011) found that there was no association between these issues. The inconsistent findings are likely derived from exposure levels and uncertainties in the assessment of environmental exposures, such as in accounting for multiple exposures and adjusting for confounders.

Human biomonitoring is commonly used for exposure estimates in

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epidemiological studies on human subjects because it is easily accessible and noninvasive. It also enables the estimation of internal exposure (such as urinary metal levels) from all exposure routes (Barbosa Jr. et al., 2005; Skröder et al., 2017). One spot urine is usually used in large-scale monitoring investigations, such as US National Health and Nutrition Examination Survey (NHANES), Canadian Health Measures Survey (CHMS), German Environmental Survey 2014–2017 (GerES V), and population-based studies (Haines et al., 2017; Saravanabhavan et al., 2017; Pollock et al., 2021; Sanders et al., 2019; Vogel et al., 2021; Hahn et al., 2022), to determine an individual's and population's exposure to metals of interest. However, strong within-subject variability (creatinine-adjusted intraclass correlation coefficient (ICC) < 0.3) has been documented in the concentrations of urinary metals (such as Cr, Pb, Mo, Ni, Zn) from healthy adults in spot urinary samples collected during short and long periods (Wang et al., 2016; Paglia et al., 2017; Chen et al., 2019). Urinary metal concentrations in individuals may fluctuate widely over time because of several factors, such as age, sex, diurnal variation, lifestyle, diet, medication, metabolic/excretion rates, half-life of elements, and environmental exposures. It is not clear how such variables affect urinary metal exposure among schoolchildren. Several studies have indicated that seasonal and spatial variations play important roles in determining urinary metals in children (Caldwell et al., 2009; Roca et al., 2016; Kafaei et al., 2017), but the results have been inconsistent. In addition, determinant variables are rarely included in the model to explore the effects of urinary metal exposure. The clarification of exposure determinants of urinary metals can improve the exposure assessment design and attenuate exposure misclassification in future epidemiological studies.

This study aimed to measure urinary metal concentrations in schoolchildren in the urban, suburban, and rural areas of Taiwan across different seasons and time. We collected spatiotemporal variables and individual biological information to identify the significant factors associated with urinary metal levels and variability using a mixed-effects model.

2. Materials and methods

2.1. Study area and population

We recruited schoolchildren (age range: 9–12 years) from 2 schools in the Xizhi and Yonghe districts of Taipei (TP, urban cohort) in northern Taiwan, 2 schools in the Wuqi and Shalu districts of Taichung (TC, suburban cohort) in central Taiwan, and 2 schools in the Dacheng and Fangyuan districts of Changhua (CH, rural cohort) in central Taiwan. Taipei is the capital city of Taiwan and has a population of approximately 3.97 million people. A total of 5.25 million motor vehicles are registered in this metropolitan area. The selected schools in the suburban area are situated in the west coast of Taichang and are near a large coal-fired power plant and an integrated iron ore and steelmaking plant (~10 km radius). This suburban area has approximately 0.2 million people (~2500 person/km^2). The schools in the rural area are located in the agricultural town of southwest Changhua, which produces rice and vegetables, and is surrounded by a large-scale oil refinery and petrochemical complex. This rural area has approximately 50,000 people (~300 person/km^2).

A total of 114 schoolchildren from TP schools, 152 from TC schools, and 55 from CH schools and their parents agreed to participate in the cross-sectional study. Urinary samples from 321 children were collected across seasons for the 2014–2015 period, and 4 sampling campaigns for each child were performed. A total of 1176 out of 1284 spot urine samples were collected during the study period, of which 108 urine samples were missing or invalid. Urine samples for children were collected at school on Monday mornings (8–9 a.m.) and Friday afternoons (3–4 p.m.) for one week during the warm (May to June) and cold (January to December) seasons to capture daily and seasonal variations. Each campaign was performed simultaneously in the three study areas for one month. Four repeated measurements across seasons were used to present a long-term exposure estimate for the individual presumably. Information on sex and body mass index (BMI) was also collected from the school health offices. Information on consumption of food and water intake was not collected. Daily samples of ambient metals in fine and coarse particles for the same schools were collected during biomonitoring programs. Detailed information on the sampling and analyzing methods and QA/QC for particle-bound metals can be referred to our previous studies (Hsu et al., 2016, 2019). This study was approved by the Ethics Committee of the National Health Research Institutes (protocol no. EC1021001). Informed written consent (with a Chinese phonetic version) was obtained from each participant and their legal guardian.

2.2. Analysis of urinary metals

Each collected urine sample (50 ml) was stored at −20 °C until analysis. Nine metals (As, Cr, Co, Cu, Mo, Ni, Cd, Pb, and V) in the urine were analyzed using an Agilent 7700x inductively coupled plasma mass spectrometer (Agilent Technologies, CA, USA). Rh (5 μg/L) was used as the internal standard. For sample treatment, a 1 mL aliquot of urine was acidified with 9 mL of 1% HNO_3 (v/v; OptimaTM grade, Fisher Scientific, FairLawn, NJ, USA) at 4 °C for at least 24 h. Standard reference materials were purchased from High Purity Standards (North Charleston, SC, USA). The concentration ranges of the calibration curves of these metals ranged from 0 to 50 μg/L. We used a spiked pooled urine sample to assess the accuracy and precision of the method. The spike recovery values of the metal concentrations were 82.4%–101% (Table S1). The limits of detection (LODs) were 0.001–0.031 μg/L. Our laboratory regularly participates in the German External Quality Assessment Scheme with Seronorm L1 and L2 tests, which can generate reliable urinary metal concentrations. We used a fully automated clinical chemistry analyzer to measure the urine creatinine concentration (Mindray Medical International Ltd.). Urinary metal concentrations were expressed as uncorrected concentrations (μg/L) and creatinine-corrected concentrations and were calculated by dividing the uncorrected concentration by the urinary creatinine concentration (μg/g creatinine). Only 5% of urine samples in the concentration were below 0.3 g/L and above 3 g/L creatinine.

2.3. Statistics

Descriptive statistics were used to describe the data on nine urinary metals. The subject-specific values (i.e., arithmetic mean, AM; geometric mean, GM) derived from four urinary measurements per subject were used to present urinary metal exposures for children. The mixed-effects model with logarithmic-transformed urinary metal concentrations (μg/g creatinine) was used to estimate the within-subject (σ_W^2) and between-subject (σ_B^2) variance components. Intraclass correlation coefficients (ICCs) were computed to assess the reproducibility of repeated measurements from random-effects models with subject ID included as the random effect. The ICC is the ratio of the between-subject variance to the sum of the between-subject and within-subject variance (range from 0 to 1). The standardized criteria of the ICCs were poor reproducibility (ICC <0.40), fair to good reproducibility (0.40 ≤ ICCs <0.75), and excellent reproducibility (ICC ≥0.75) (Rosner 2015). Poor reproducibility indicates that nearly all variability in urinary metal concentrations is within-subject variability; fair to good reproducibility indicates moderate contrast in urinary metal concentrations among subjects; excellent reproducibility indicates that between-subject variability in urinary metal concentrations is dominant.

Mixed-effects models with the unbalanced covariance structure based on a restricted maximum likelihood estimation procedure (Searle et al., 1992) were used to examine the associations between selected variables (Table 1) and the log-transformed creatinine-corrected level of

Table 1
Definition of demographic variables and sampling time.

Variable (type)	Description	Number of subjects	Number of measurements
Location (categorical)	1: TP (urban)	114	435
	2: TC (suburban)	152	585
	3: CH (rural), Reference	55	156
Season (dichotomous)	1: Warm (May, June)	321	643
	2: Cold (December, January), Reference	321	533
Time of sampling (dichotomous)	1: Morning	321	586
	2: Afternoon, Reference	321	590
Sex (dichotomous)	1: Boy	159	588
	2: Girl, Reference	162	588
BMI (categorical)	1: BMI< 18.5	168	656
	2: BMI ≥24	37	122
	3: 18.5≤ BMI< 24, Reference	102	347

BMI: body mass index.

urinary metals. The determinants were treated as fixed effects in the model. The mixed-effects model was specified by:

$$\ln\ (\mathrm{Yij}) = \beta_0 + \beta_1 \mathrm{X}_{1ij} + \ldots.. + \beta_n \mathrm{X}_{nij} + \mathrm{b}_i + \varepsilon_{ij} \quad (1)$$

for $i = 1$–321 (subject) and $j = 1$–4 (repetitions of the ith measurement), where Y_{ij} is the log-transformed metal level; β_0 is the overall intercept for the group corresponding to the mean background level (log-transformed) of urinary metals when all factors are equal to zero; β_1 to β_n are fixed effects; X_{ij} is the variable for the ith subject on the jth day; b_i is the random effect for the ith subject corresponding to the discrepancy between the individual intercept and group intercept; β_0 and ε_{ij} are assumed to be independent and normally distributed with a mean of 0 and with respective variances of σ_B^2 and σ_W^2. The calculation was also performed with logtranformed concentrations of urinary metals.

To evaluate the potentially high exposure to heavy metals for the study population, we calculated the "probability of overexposure (θ)" compared with the reference value. This test can determine whether a subject has been exposed to the upper margin of background exposure of the selected metal. So far, there has been no exposure threshold of health effects for children. The reference value used in this study is the upper margin of background exposure in the 95th percentile as RV_{95} from nationally representative surveys for children. Here, we used urinary metals in the concentration with RV_{95} if available, i.e., As (17.7 μg/g creatinine), Co (1.70 μg/g creatinine), Mo (200 μg/g creatinine), Cd (0.157 μg/g creatinine), and Pb (1.14 μg/g creatinine) from the NHANES (https://www.hsdl.org/?view&did=821074), Cr (0.77 μg/g creatinine) from the GerES V (Vogel et al., 2021), and Cu (25 μg/L) and Ni (4.4 μg/L) from the CHMS (Saravanabhavan et al., 2017). The θ defines the likelihood that a randomly selected subject's arithmetic mean exposure is greater than the RV_{95}:

$$\theta = P\left\{\mu_{X(i)} > RV_{95}\right\} = 1 - \Phi\left\{\frac{ln(RV_{95}) - \mu_y - \frac{\sigma_w^2}{2}}{\sqrt{\sigma_B^2}}\right\} \quad (2)$$

where $\mu_{X(i)}$ is the mean exposure level of the urinary metal for the ith subject. $\Phi\{z\}$, μ_{yh}, σ_B^2, and σ_W^2 were obtained from the mixed-effects model. The overexposure was calculated for all subjects together and by area. Spearman rank correlation coefficients to examine the strength of correlations between air particle and spot urine samples in metal concentrations were calculated. Significance testing in comparisons between study areas for airborne metal concentrations was conducted based on the Mann-Whitney test and Kruskal-Wallis test. Statistical analyses were performed using IBM SPSS 22 software (SPSS Inc., IBM Company, Chicago, IL, USA).

3. Results

3.1. Exposure level and variability of urinary metals

Table 2 shows the descriptive statistics of subject-specific urinary metal concentrations with and without creatinine-corrected values among the 321 children. Most urinary metals in each sample of children were detectable and had values greater than the LOD. Only Cd, Cr, and V had concentrations below the LOD at 2.06%, 1.58%, and 1.74%, respectively. The geometric mean (GM) concentrations for As, Cr, Ni, Cd, and Pb were 58.4, 1.08, 3.54, 0.532, and 1.27 μg/g creatinine, respectively. The four essential elements (Co, Cu, Mo, and V) had GM concentrations ranging from 0.204 μg/g creatinine (V) to 87.5 μg/g creatinine (Mo). Table S2 also present the subject-specific AM and GM of urinary metal concentrations (μg/g creatinine) stratified by location, season, time, sex, and BMI. Detailed comparisons in urinary metal concentrations among variables were presented in the next section.

Table 3 shows the σ_W^2, σ_B^2, and ICC values for concentrations of creatinine-corrected urinary metals for selected schoolchildren across areas. The within-subject variance in urinary metals was higher than the between-subject variance. Fair to good reproducibility for creatinine-corrected Co (ICC = 0.551) in spot urine samples was obtained, and creatinine-corrected As, Cr, Cu, Mo, Ni, Cd, Pb, and V showed poor reproducibility (ICC = 0.046–0.311). When we evaluated ICCs in urinary metals stratified by sex, girl and boy schoolchildren had similar results (not shown). Table 4 shows the values of θ for urinary As, Cr, Co, Cu, Mo, Ni, Cd, and Pb in schoolchildren. Overall, the θ of As, Cr, and Cd were >99.9%, while Pb, Ni, and Cu were 87.1%, 84.5%, and 20.9%, respectively. Rural schoolchildren had a relatively higher θ for Cu and Pb than urban and suburban schoolchildren. The θ of Co and Mo was extremely low for all schoolchildren.

3.2. Factors associated with urinary metals

Table 5 shows the coefficients of the selected variables and variance components for creatinine-corrected urinary metal concentrations among schoolchildren using the mixed-effects model. Regarding location variables, urban schoolchildren (TP) had significantly higher (~20%–48%) levels of urinary As, Cr, Co, and Ni than rural schoolchildren. By contrast, rural schoolchildren (CH) had significantly higher concentrations (~25%–40%) of urinary Cd and Pb than urban and suburban schoolchildren. The location variable could explain 8.0% of the between-subject (σ_B^2) variability of the total variance for Pb in urinary levels and less than 3% for other metals. For the season variable, the concentrations of all selected urinary metals in schoolchildren were significantly lower in the warm season, except for Cu and Pb. For instance, schoolchildren had a lower urinary Cr level of 70% in the warm season than in the cold season but had a higher urinary Pb level of 16% in the warm season than in the cold season. For time of sampling variable, As, Cr, Cu, Ni, and Pb levels in the schoolchildren's urine samples in the morning were significantly higher than those in the afternoon, except for Mo. The temporal factors (season and time) explained 9.0% of within-subject variability (σ_W^2) of the total variance in urinary Cr and less than 3% for other metals. For sex, the levels of urinary Cr, Co, Cu, Ni, and Pb (μg/g creatinine) in boys were significantly lower than those in girls. Schoolchildren with a lower BMI (<18.5) may have higher urinary Mo, Cd, and Pb, and a higher BMI (≥24) resulted in higher urinary Cu. When creatinine was included as a model covariate instead of creatinine-corrected values to account for urine dilution, all selected urinary metal in concentrations (μg/L) were positively and significantly associated with urinary creatinine (except for Pb with a negative association) (Table S13). The above variables, including spatial and temporal variables and biological factors, explained 15.8% (Pb), 11.6% (Cr), 6.5% (Cd), 4.3% (Mo), 4.0% (Cu), 3.8% (As), 3.0% (Ni), 3.0% (V), and 1.2% (Co) of the total variance in urinary concentrations

Table 2
Descriptive statistics of subject-specific urinary metal concentrations among 321 children.

Metals		% < LOD	AM	SD	GM	IQR	P5	P95	Range
As	μg/g-creatinine		69.0	51.3	58.4	43.3	25.7	153	14.5–572
	μg/L	0	77.1	51.5	64.4	52.1	24.0	165	8.29–351
Cr	μg/g-creatinine		2.30	4.26	1.08	1.16	0.297	9.58	<0.001–35.5
	μg/L	2.06	1.86	2.84	1.03	1.21	0.251	7.79	0.026–29.1
Co	μg/g-creatinine		0.454	0.259	0.398	0.283	0.185	0.979	0.127–1.84
	μg/L	0	0.548	0.408	0.441	0.429	0.158	1.38	0.070–2.94
Cu	μg/g-creatinine		13.9	6.59	13.2	4.05	9.28	21.7	6.20–106
	μg/L	0.079	16.2	7.84	14.7	7.48	6.61	29.1	1.89–93.6
Mo	μg/g-creatinine		97.0	71.5	87.5	43.2	48.0	177	31.8–1148
	μg/L	0	115	104	96.2	67.9	34.0	242	18.5–1610
Ni	μg/g-creatinine		5.79	24.1	3.54	2.23	1.60	9.71	1.07–413
	μg/L	0.079	5.05	11.1	3.73	2.92	1.44	9.14	0.443–184
Cd	μg/g-creatinine		0.576	0.276	0.532	0.250	0.290	1.01	0.218–2.76
	μg/L	1.58	0.688	0.457	0.587	0.400	0.227	1.40	0.081–3.896
Pb	μg/g-creatinine		1.46	0.977	1.27	0.716	0.659	3.55	0.452–7.80
	μg/L	0	2.76	2.30	1.46	4.03	1.38	66.4	0.102–9.90
V	μg/g-creatinine		0.223	0.131	0.204	0.090	0.122	0.399	0.093–1.62
	μg/L	1.74	0.193	0.065	0.182	0.077	0.102	0.309	0.033–0.465

LOD: limit of detection (μg/L), AM: arithmetic mean, SD: standard deviation, GM: geometric mean, IQR: interquartile range, P5: 5th percentile, P95: 95th percentile, Range: minimum to maximum.

Table 3
The within (σ_W^2) and between (σ_B^2) subject variability and intraclass correlation coefficients (ICC) in the concentrations of urinary metals for selected school-children across areas.

Metals		σ_W^2	σ_B^2	σ_T^2	σ_W^2 (%)	σ_B^2 (%)	ICC
As	μg/g-creatinine	0.339	0.139	0.478	71.0	29.0	0.290
	μg/L	0.581	0.150	0.731	79.5	20.5	0.205
Cr	μg/g-creatinine	2.53	0.121	2.65	95.5	4.55	0.046
	μg/L	1.29	0.151	1.44	89.5	10.5	0.105
Co	μg/g-creatinine	0.153	0.188	0.342	44.9	55.1	0.551
	μg/L	0.422	0.297	0.718	58.7	41.3	0.413
Cu	μg/g-creatinine	0.090	0.051	0.142	63.8	36.2	0.362
	μg/L	0.392	0.087	0.479	81.8	18.2	0.182
Mo	μg/g-creatinine	0.266	0.053	0.319	83.4	16.6	0.166
	μg/L	0.492	0.151	0.644	76.5	23.5	0.235
Ni	μg/g-creatinine	0.384	0.106	0.490	78.3	21.7	0.217
	μg/L	0.492	0.137	0.629	78.3	21.7	0.217
Cd	μg/g-creatinine	0.174	0.058	0.232	74.8	25.2	0.252
	μg/L	0.488	0.138	0.626	78.0	22.0	0.220
Pb	μg/g-creatinine	0.223	0.101	0.324	68.9	31.1	0.311
	μg/L	2.99	0.371	3.36	89.0	11.0	0.110
V	μg/g-creatinine	0.253	0.030	0.283	89.3	10.7	0.107
	μg/L	0.283	0.060	0.343	82.5	17.5	0.175

σ_W^2: within-subject variability, σ_B^2: between-subject variability.
σ_T^2: total variance ($\sigma_W^2 + \sigma_B^2$), ICC: σ_B^2 / σ_T^2

Table 4
The probability of overexposure (θ) for urinary metals overall and by area.

Metals	Reference value, RV_{95}	Overall	Urban	Suburban	Rural
As	17.7[a]	>99.9%	>99.9%	>99.9%	>99.9%
Cr	0.77[b]	>99.9%	>99.9%	>99.9%	>99.9%
Co	1.70[a]	0.21%	0.12%	0.43%	0.21%
Cu	25[c]	20.9%	27.1%	4.75%	54.4%
Mo	200[a]	0.51%	0.16%	0.66%	0.87%
Ni	4.4[c]	84.5%	87.7%	88.7%	59.1%
Cd	0.157[a]	>99.9%	>99.9%	>99.9%	>99.9%
Pb	1.14[a]	87.1%	91.8%	89.1%	>99.9%
V	-[d]	–	–	–	–

RV_{95} is a reference value in the 95th percentile of the measured urinary metal concentration.
[a] US National Health and Nutrition Examination Survey (NHANES) in 2015–2016 for 6–11 years old children (unit: μg/g-creatinine).
[b] German Environmental Survey 2014–2017 (GerES V) 2014–2017 for 3–17 years old children, (unit: μg/g-creatinine).
[c] Canadian Health Measures Survey (CHMS) in 2009–2011 for 6–19 years old children, (unit: μg/L).
[d] Not available.

(not shown). The selected variables could only partially (<5%) explain the total exposure variability in the urinary levels of Mo, Cu, As, Ni, V, and Co.

We also stratified sex to evaluate the effects of location, season, and time in the urinary metal concentrations (μg/g creatinine) if those variables were still significant in the models for boy or girl populations. As shown in Table S4, there were sex-specific differences in location and time variables; for instance, significantly higher levels of urine As, Co, and Ni in the urban area and urine As in the morning time for girls were found. However, there was no sex-specific difference in the seasonal variation.

4. Discussions

Tables S5–S13 show the comparisons of urinary metals (GM and arithmetic mean) for children of similar ages and survey years in different countries. We found that the urinary As (GM = ~60 μg/g creatinine) in schoolchildren in the current study was relatively higher than that in other studies. The middle-range levels of urinary Co, Cu, Ni, and V were observed in children of this study. The concentrations of urinary Mo, Cr, and Cd were also higher than those in other countries but lower than those in one study for children who reside close to a large coking plant in China. By contrast, the urinary Ni, V, and Pb concentrations in the current study were similar to those in Italy, Mexico, and Spain but were lower than those in Brazil (coal-mining region) and China (agricultural area). Overall, the average levels of urinary As, Cd, and Pb in schoolchildren in Taiwan were 2–10 times higher than those obtained from the US NHANES.

The higher value of urinary As in children, particularly in urban areas, was obtained and compared with previous studies (>99.9% of the θ, RV_{95} = 17.7 μg/g creatinine). Urinary As can be attributed to exposure to drinking water, food, and air particles contaminated with As. Reports indicated that the southwest coast of Taiwan has high As in the groundwater and soil (Liang et al., 2016), and As-contaminated groundwater is usually used for fish farming and food crops. Studies reported that Taiwan has a high potential risk from dietary exposure to As because of the consumption of aquacultured fish, shrimp, and rice (Chen et al., 2016; Kar et al., 2011). Although As-contaminated groundwater is a potential exposure route, the use of tap water

Table 5
Model coefficients and variance components for spatial, temporal, and biological factors associated with urinary metals (μg/g-creatinine).

Variables	As		Cr		Co		Cu		Mo		Ni		Cd		Pb		V	
	Effect%	SE	Effect%	SE	Effect%	SE	Effect%	SE	Effect%	SE	Effect%	SE	Effect%	SE	Effect%	SE	Effect%	SE
Location (Ref.: rural)																		
Urban	26.0*	0.089	48.0*	0.187	20.0*	0.081	7.00	0.051	1.00	0.067	33.0*	0.085	−25.3*	0.058	−41.8*	0.065	11.0	0.062
Suburban	7.00	0.085	3.00	0.178	21.0*	0.077	1.00	0.049	−9.00	0.065	9.00	0.081	−32.6*	0.056	−35.0*	0.062	3.00	0.060
Season (Ref.: cold)																		
warm	−15.1*	0.037	−67.8*	0.100	−10.1*	0.025	12.0*	0.019	−15.3*	0.032	−12.2*	0.040	−8.80*	0.027	16.0*	0.029	−17.5*	0.033
Time of sampling (Ref.: Afternoon)																		
Morning	18.0*	0.036	13.0	0.097	5.00	0.024	7.00*	0.018	−16.0*	0.031	16.0*	0.039	−4.00	0.026	11.0*	0.028	3.00	0.033
Sex (Ref.: girls)																		
Boys	−7.20	0.057	−28.4*	0.114	−22.6*	0.053	−9.90*	0.033	7.00	0.042	−21.5*	0.053	8.00*	0.036	−12.3*	0.041	−6.90	0.039
BMI (Ref.: 18.5≤ and <24)																		
<18.5	11.0	0.058	−6.40	0.124	8.00	0.050	5.00	0.033	16.0*	0.044	2.00	0.056	11.0*	0.038	10.0*	0.043	6.00	0.043
≥24	12.0	0.092	39.0	0.196	−1.80	0.079	8.00	0.051	6.00	0.070	6.00	0.088	2.00	0.060	0.00	0.067	0.00	0.067
σ_W^2	0.324	0.017	2.02	0.121	0.149	0.008	0.082	0.004	0.248	0.013	0.377	0.020	0.169	0.009	0.202	0.011	0.214	0.013
σ_B^2	0.136	0.020	0.227	0.091	0.158	0.017	0.053	0.007	0.053	0.011	0.090	0.017	0.045	0.008	0.061	0.010	0.029	0.009

*p-value ≦0.05, Effect% = (exp(β)-1) × 100; SE: standard error, σ_W^2: within-subject variability, σ_B^2: between-subject variability.

containing extremely low As has been popular in Taiwan since the 1980s. Therefore, exposure to As via drinking water in the population of the current study is unlikely to be a major concern. During biomonitoring, ambient metals in fine and coarse particles for the same schools were investigated (Hsu et al., 2016, 2019). We found that there was no significant difference in $PM_{2.5}$-bound As concentrations in urban, suburban, and rural areas (Table S14). The annual mean concentration of $PM_{2.5}$-bound As (2–3 ng/m^3, with an elevated level of ~4 ng/m^3 in the winter; Table S15) was not significantly higher than those in previous studies (Yadav and Turner, 2014; Xie et al., 2019). In addition, an insignificant correlation was obtained between daily air concentrations and integrated urine levels (by school) for As, although we were not able to conduct personal monitoring to capture individual exposure to airborne As. In the current study, inhalation exposure to ambient As might not be a significant route for urinary As in schoolchildren. We presumed that schoolchildren may have additional exposure to As via the ingestion of contaminated food, soil, and dust. Tobacco smoke from adult family members is also a source of inorganic As.

Urinary Cr, Ni, Cd, and Pb with the $\theta > 90\%$ observed in our study population should also be considered. Those toxic metals at relatively low levels associated with adverse health outcomes on children's neuro, kidney, brain, and bone organs have been documented (Sanders et al., 2019; Gundacker et al., 2021; Shen et al., 2021). For instance, children with a less developed blood–brain barrier are highly susceptible to Pb exposure (Massaro 2002). Rural schoolchildren with higher urinary Pb levels (2.54 μg/g creatinine; Table S12) may be exposed to Pb from the air and food, and an insignificant difference in ambient $PM_{2.5}$-bound Pb was found among the three areas (Table S14). Ambient Pb from traffic emissions has decreased markedly in recent decades owing to the introduction of unleaded gasoline (Wu et al., 2011). As a result, Pb emissions from stationary sources (such as coal combustion) to ambient air are predominant (Hsu et al., 2016). Airborne Pb can be deposited on soil and water and can reach the general population via the food chain. Although we recognized several high metal exposures in the urinary samples, no conclusive explanation could be established without sufficient information on external exposure to food, drinking water, or air for our study population.

The RV_{95}s have been used in public health, which provides a basic identification for individuals or populations of interest with an increased exposure compared to a background level of the general population. The exceed RV_{95} in individuals or populations indicates the requirement for the follow-up to explore key exposure sources and factors associated with elevated exposure in study populations compared to the background. This approach is able to aid in developing statistical associations between increased exposure and health outcome in the populations. Unlike health-based guidance values, such as permissible exposure limit (PEL), the RV_{95} does not consider the toxicological effects of biomarkers (Ewers et al., 1999; Saravanabhavan et al., 2017).

Substantial within-subject variability in the concentration of urinary metals (As, Cr, Cu, Mo, Ni, Cd, Pb, and V) measured across a year was obtained in our study population. High within-subject variability in urinary metal concentrations over short and long periods of time from measurements of consecutive days has been reported. Wang et al. (2016) presented poor reproducibility (ICCs = 0.01–0.29) in the serial measurements of As, Co, Cu, Pb, Mo, and Ni in spot urinary samples collected during a 3-month period in 11 adult men but indicated fair to good reproducibility for Cd (ICC = 0.53). Chen et al. (2019) presented high within-subject variability (creatinine-corrected ICC = 0.01–0.12) in urinary Cr, Mn, Fe, and Se over a period of days to months for healthy adult men. In general, within-subject variations in the concentrations of urinary metals are likely affected by the elimination of half-life (Aylward et al., 2014). A longer half-life of urine metals leads to a larger between-subject variation. Cd (half-life of 10–30 years) in the liver and kidneys that sufficiently reaches stability in the body from chronic exposure can result in good reproducibility in urinary levels in adults (Chen et al., 2019; Gunier et al., 2013; Smolders et al., 2014; Wang et al.,

2016). Blood Cd with a half-life of 3–4 months reflecting short-term exposure is an appropriate matrix to measure short-term variability (days), while urine Cd reflecting cumulative exposure is more suitable for measuring long-term variability (e.g., season). Urinary Cr has a very short half-life (<2 days) mainly determined by the temporal factor. However, the concentrations of Cd and Zn in spot urine samples with lower ICCs were obtained in the current study probably owing to the differences in study design (such as study population, sample types, and sampling period). Smolders et al. (2014) concluded that sex, age, underlying physiology of the participants, and geography that interacted with sources of exposure can partly explain the variation. However, there was no sex-specific difference from the between-subject variation (i.e., ICC) in urinary metal concentrations in our study. One measurement of urinary metals provides only a brief snapshot of the exposure levels in an individual and can result in exposure misclassification and the attenuation of risk estimations (Chen et al., 2019; Wang et al., 2016). In the population of the current study, four-spot urine samples in the individual collected at different times and locations to estimate subject-specific means of metal exposure and determine the variability is valuable; however, we are not able to tackle how many repeated measurements in the individual are representative to characterize exposure variability in the current study design.

To determine the significant factors associated with urinary metals in schoolchildren, we included temporal (season and time of sampling) and spatial (location) variables and biological factors (sex and BMI) when adjusting the model. We found that urban schoolchildren had higher urinary As, Cr, Co, and Ni levels and rural children had higher urinary Cd and Pb. Spatial and temporal variations that play a key role in determining urinary metal concentrations have been increasingly documented. Kafaei et al. (2017) reported that children living near industrial areas had higher urinary levels of As, V, Mn, and Ni than the reference population. Roca et al. (2016) reported significant differences between rural and urban areas for As, Co, Cs, Se, U, and Hg, with higher levels in children living in urban areas than in rural areas. Wang et al. (2019) indicated that children living in industrial areas had higher concentrations of urinary Cr, Cu, Cd, and Pb than those living in non-polluted areas. However, Aguilera et al. (2010) reported an insignificant difference between the industrial area and reference for urinary metals (Cr, Cu, and Ni) in children, except Cd levels, which were significantly higher in the reference group. Pérez et al. (2018) also indicated that there was no significant differences between urinary metal levels (i.e., As, Cd, Cu, Ni, Pb, Mn, and Zn) in children living in urban or rural areas (except for Co and Hg). Xu et al. (2020) reported that children living near a municipal waste incinerator did not suffer considerable long-term accumulation of Cd and Cr in urine samples compared with a control group. The comparison of the differences in metal concentrations in the spot urine samples between locations (urban vs. rural or industrial area vs. reference) is usually based on the assumption that inhalation exposure is the predominant route of exposure. However, inconsistent results might be caused by differences in design because covariant variables or confounders, such as seasonal variations in diet, sampling time/-frequency, individual activity patterns, lifestyle, and biological factors, were not included in the analysis. For instance, Kafaei et al. (2017) reported significantly higher levels of urinary As, Cd, V, and Ni in schoolchildren in fall than in spring. Paglia et al. (2017) demonstrated that the concentrations of 12 urinary metals among 7 healthy adults were higher in summer than in spring, fall, and winter because of the increased intake of water during warmer days. Our study revealed that higher levels of urinary metals were observed in schoolchildren during the cold season, except for Cu and Pb. When the models were stratified by sex, the seasonal variation in urinary metal concentrations was still consistent for the girl and boy populations (Table S4), indicating the season variable as a strong exposure determinant.

Furthermore, we evaluated the associations with ambient airborne metal concentrations on the basis of the measurements taken on the same day and in the same school. The negative or insignificant correlations between air and urine in metal concentrations allowed us to presume that inhalation is likely not a primary exposure route for metals in our study population (Table S16). However, other important factors, such as food consumption, lifestyle, and socioeconomic status may distort the associations and effects on urinary metals. Although the suburban schoolchildren living near a large-fired power plant were recruited, the school area is not a hot spot of air pollution based on the height of the target emission source and prevailing wind direction. But we cannot rule out its potential influence of emitted dust on the ecosystem, food chain, water, and soil. Pérez et al. (2018) indicated that children with higher consumption of vegetables had higher levels of urinary Cd, Cu, Mo, Sb, Tl, V, and Zn, and higher urinary As correlated with a higher consumption of cereals and fish. The use of first morning urine samples to evaluate personal exposure has been proposed because it is more concentrated and correlated with the 24 h urine collection (Scher et al., 2007). Our data showed higher urinary As, Cr, Cu, Ni, and Pb in children collected in the morning compared with samples collected in the afternoon. Following the circadian rhythm of urine excreted per day, higher concentrations of urinary metals observed in the morning are likely due to increased glomerular filtration (Akerstrom et al., 2014). However, the concentrations of urinary As, Cu, Pb, Mo, and Ni in a single first morning or 24 h urine sample may not provide accurate estimates of individual exposures over weeks or months (Wang et al., 2016). Sex in adults is also a significant factor that affects urinary metal levels. We found that girl schoolchildren had significantly higher levels of urinary As, Cr, Co, Cu, and Ni, and this finding is comparable with that of Berglund et al. (2011). Significantly higher levels of urinary As, Co, and Ni in the urban area were likely attributable to sex-specific difference (Table S4). The sex-specific difference in urinary metal exposure may be associated with their environmental exposure and sexual dimorphisms in anatomy, gray matter distribution, hormones, epigenetics, or metabolism (Mergler 2012). Our study also revealed that low (<18.5) BMIs are associated with an increase in urinary metals (Mo, Cd, Pb, and Cu) in schoolchildren. A similar result was reported in a previous study (Lewis et al., 2018), but there is no reasonable explanation. Therefore, further investigations are needed. Our findings also support the use of creatinine-corrected metal concentrations to control urinary dilution (Table S3). The urine dilution explained some variations in the concentrations of urinary metals and improved model fitting, and creatinine correction can reflect true variations in metal excretions (Wang et al., 2016). The previous study suggested that biomarker measures in creatinine-corrected concentrations, including creatinine as a covariate in statistical models, can improve the estimation of the effects of environmental exposures on human health. It is likely to control confounding and measurement error more effectively in the association of chemical exposure between the health effect (O'Brien et al., 2016). Our study also used the same approach to evaluate the effects of urinary metal exposure in creatinine-corrected concentrations (not shown). However, the effects had no significant changes across metals compared with the no-creatinine covariate model. The urine sample with a value below 0.3 g/L or above 3 g/L creatinine was suggested to exclude from the survey (Cocker et al., 2011). Only 5% of urine samples with creatinine concentrations out of the range were observed in this study, not distorting the results.

The most important limitation of this study is that data on food and water consumption are not available, which are the most important factors for metal concentrations in urine. This study also has several limitations. We did not distinguish inorganic and organic forms and the valence of As in the urinary samples by chemical analyses as an example. Inorganic As with trivalent compounds demonstrated higher acute toxicity than pentavalent inorganic As. Inorganic As is usually present in groundwater used for drinking, and organic As compounds are primarily found in fish for human exposure. However, there were no sufficient reports of the questionnaire associated with metal exposure, which captures information on the consumption of food and drinking water, family smoking, skin contact with soil, and lifestyle. As a result, we were

not able to explore the potential contributions of these most important sources to urinary metals. Although 24 h urine samples have been recommended as a "gold standard" for estimating the internal exposure of toxic elements excreted mainly from urine, it is nearly impossible to follow this ideal guideline, particularly in a community-based study with a large population. The use of four repeated measurements for urinary samples in evaluating the significant factors and metal exposure with within-subject variability may result in potential bias. The variability of metal levels in urine samples may be influenced by the kinetics of absorption, distribution, metabolism, and elimination, which could not be taken into account. Using Spearman's correlation measure, a significantly positive correlation ($r = 0.60$) between Cd and Mo in urine samples was obtained. The effect of molybdenum oxide (MoO) interference on urinary Cd analyses has been reported by Akerstrom et al. (2013), which may introduce bias in this study's urinary measurements. In our study population, four spot urine samples in the individual were collected at different times and locations to estimate subject-specific means of metal exposure and determine the variability, which is valuable. The results may be generalized to other schoolchildren within the same location. However, we cannot tackle how many repeated measurements in the individual represent long-term exposure variability in the current study design. The assumption of common variance components by location and season is also limited to our study.

5. Conclusions

High levels of urinary As were observed in schoolchildren in Taiwan, which may result in potential risk with long-term exposure. The overexposure (θ) values with relatively high levels in urinary As, Cr, Ni, Cd, and Pb for schoolchildren should be paid attention to, and further investigations on these toxic metal exposures and health effects are needed. Substantial within-subject variability in the concentrations of urinary metals (As, Cr, Cu, Mo, Ni, Cd, Pb, and V) was obtained. Spatial and temporal variables and biological factors could explain the exposure variability for Pb, Cr, and Cd in urine samples among schoolchildren. The sex-specific difference for some metals of urine samples in the exposure assessment of the population study plays an important role. We suggest that the repeated measurements of spot urine samples should be conducted across seasons for a long-term exposure estimate of metals.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113976.

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Home gardens and distances to nature associated with behavior problems in alpine schoolchildren: Role of secondhand smoke exposure and biomarkers

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ABSTRACT

Background: Behavior problems in children are shaped by a complex intertwining of environmental, social, and biological factors. This study investigated whether more nature exposure was associated with less behavior problems and whether these associations were mediated by differences in secondhand smoke in the home, sleep problems, adiposity, and inflammation.

Methods: We used cross-sectional data collected from 1251 schoolchildren (8–12 years old) in the Tyrol region of Austria and Italy. Information on sociodemographics, lifestyle, perinatal data, and housing conditions was obtained by questionnaire. Behavior problems in the past school year were rated by teachers using the Needleman questionnaire for classroom performance. We estimated nature exposure using a comprehensive naturalness index called "distance to nature" (*D2N*). This was calculated in several buffer sizes around the child's home and school. Presence of a home garden was assessed by self-report. Data on potential mediators including parental smoking in the home, child's urinary cotinine concentrations, sleep problems, body mass index, and urinary neopterin concentrations were also collected. Structural equation modeling was used to examine the multiple pathways between nature exposure and behavior problems.

Results: Children who lived in a home with a garden and those whose school was closer to nature exhibited less school behavior problems. The effect of proximity to nature from the school was directly associated with less behavior problems, and the effect of a home garden on behavior problems acted indirectly through a lower likelihood of secondhand smoke exposure. Associations were in unexpected directions between behavior problems and residential proximity to nature and depended on the outcome and context.

Conclusions: Natural environments in school and home surroundings might be beneficial for school conduct and performance. Lower secondhand smoke exposure at home might be a pathway between home gardens and children's behavioral problems. Associations with residential proximity to nature in this study were in unexpected directions and warrant further investigation. These findings serve as a point of departure for investigating how proximal nature might activate health supportive pathways in children and simultaneously confer childhood health benefits via positive behavioral changes in their parents.

1. Introduction

Behavior problems are commonly diagnosed in children (aged 5 to 17), affecting between 5 and 15% of them (Ghandour et al., 2019). In the long-term, manifestations such as irritability, impulsiveness, aggressiveness, hyperactivity, and distractibility during childhood can have profound negative impacts on quality of life, academic achievement in

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List of abbreviations used in the text

BMI	body mass index
CFI	comparative fit index
D2N	distance to nature
DWLS	diagonally weighted least squares
L_{den}	day-evening-night noise level
LULC	land use/land cover
NO_2	nitrogen dioxide
RMSEA	root mean square error of approximation
SEM	structural equation modeling
SRMR	standardized root mean square residual

school, professional status, social and relationship problems, and mental health later in life (Erskine et al., 2016; Rivenbark et al., 2018; Usami, 2016).

A large body of evidence supports the idea that behavior problems cannot be explained by the child's genetic background alone but are instead shaped by a complex intertwining of environmental, social, and biological factors (Shaw, 2013). The role of the landscape around the home and school has attracted particular interest (Zare Sakhvidi et al., 2022; Browning and Rigolon, 2019). Proximity and access to outdoor nature have been associated with improved neurocognitive development (de Keijzer et al., 2016; Luque-García et al., 2021; Yuchi et al., 2022), emotion regulation (Vanaken and Danckaerts, 2018), and emotional intelligence (Browning et al., 2022) among children. Private natural space can also support child development, as children with home gardens have been found to be more physically active, play more outdoors (Armstrong et al., 2019), and have better social, emotional, and behavioral functioning (Richardson et al., 2017). According to the stress reduction theory, even brief encounters with natural elements through various sensory modalities can dampen psychological and physiological stress (Ulrich, 1984; Ulrich et al., 1991). The complementary attention restoration theory holds that natural settings can effortlessly engage attention, providing physical and cognitive distances from stressors and demands such as those experienced in the school and family environments (Kaplan, 1995; Kaplan and Kaplan, 1989; Hartig, 2021).

Children's immediate family surroundings and individual actions of their parents can also impact behavior problems. The harmful effect of secondhand smoke on behavior and cognition have been particularly well-documented (Julvez et al., 2007; Chen et al., 2013; Martin et al., 2020). Recently, Maitre et al. (2021) reported that early-life environmental exposures in 6–11-year-old children affected behavior problems. In addition, behavioral and lifestyle risk factors are of growing interest, including sleep problems. Sleep impairment may contribute to both internalizing and externalizing symptoms (Maitre et al., 2021) and childhood obesity (Felső et al., 2017), and undermine school achievement and cognitive abilities (Martin et al., 2018; Spruyt, 2019). Systemic inflammation has been suggested as a biological pathway between these exposures. Behavior problems in children/adolescents (Jiang et al., 2018; Adelantado-Renau et al., 2020; Swan and Lessov-Schlaggar, 2007) may relate to children's inflammation levels though influences at different environmental scales and contexts (i.e., home and school).

To understand the effects of multiple exposures and individual-level factors for childhood behavior, mechanistic studies disentangling the contribution of different pathways are critically needed. The novel finding of Martin et al. (2020) on lower smoking prevalence in greener neighborhoods serves as a basis to suggest that nature exposure may change children's inflammation levels through modification of parents' smoking behavior. This pathway might act along with better studied pathways between nature and health, like reduced adiposity (Luo et al., 2020; Bao et al., 2021) and improved sleep quality (Astell-Burt and Feng, 2021; Feng et al., 2022), which have also been linked to lower inflammation levels (Irwin et al., 2016; Miller and Spencer, 2014). However, the extant evidence of associations between nature exposure, smoking, sleep quality, adiposity, inflammation, and child behavior problems remains fragmented and sparse due to the limited number of studies that have considered their interweaving roles (cf. Dzhambov et al., 2020; Luque-García et al., 2021), sufficient confounding by family socioeconomic status (cf. Russell et al., 2016), and general living conditions (Evans et al., 2013).

1.1. Current study

We sought to understand the complex interplays between nature exposure, mediating pathways, and school behavior problems in children. We hypothesized that home gardens and shorter distances to nature from the home and school would be associated with less behavior problems at school. Moreover, we expected that these relationships would be mediated by reduced exposure to secondhand smoke in the home, less sleep problems, and lower adiposity, which would lead to less inflammation and behavior problems. To examine these relationships, we selected alpine valleys in Austria where high shares of students (16.5%) are at risk of emotional and behavioral problems (Philipp et al., 2018). In doing so, we expand the understanding of this unique geographic context where distinctive patterns of association between environmental exposures and child health outcomes have been previously observed (Dzhambov et al., 2019, 2021, 2022).

2. Methods

2.1. Study design

We leveraged 2004–2005 questionnaire, biomarker, and residence data from schoolchildren in the Tyrol region of Austria and Italy. Data in the Brenner Base Tunnel Study were obtained from 1251 children (response rate = 85.5%; Dzhambov et al., 2022) from 3rd and 4th grade classrooms (8–12 years old) across all 49 public schools in the study area. Mothers completed questionnaires on sociodemographics, lifestyle, perinatal data, and housing conditions. Children answered questions on sleep problems. The legal guardians/mothers provided written informed consent for the children. Ethical approval was obtained from the Ethics committee of the Medical University Innsbruck (Ethics commission number 2105/2004).

2.2. Study area

Children lived in the Lower Inn, Wipp, and side valleys. The Lower Inn valley (Unterinntal) extends east of Innsbruck towards the Austrian-German border. The Wipp valley (Wipptal) extends along the river Sill southward from Innsbruck up to the Brenner Pass and down to Fortezza. Most lived in densely populated small communities that relied on tourism, industry, agriculture, and small businesses for their livelihoods (Dzhambov et al., 2019, 2021, 2022). Highways and railway lines ran through these valleys and communities, transporting heavy goods from northern to southern Europe and vice versa. Levels of traffic emissions varied across valleys based on road and railroad proximity (Lercher et al., 2008; Lercher and Botteldooren, 2006; von Lindern et al., 2016) as well as topographic and meteorologic differences (Heimann, 2007).

2.3. Measures

2.3.1. Behavior problems

Behavior problems in the past school year were rated by children's teachers using a questionnaire originally developed by Herbert Needleman to study classroom performance in relation to lead neurotoxicity (Needleman et al., 1979; Yule et al., 1984). The items of this scale are given in Section S1. Teachers answered "yes/no" to 11 items tapping three broad domains: Distractibility (4 items), Hyperactivity (3 items),

and Performance (4 items). Example items included: "Is this child easily distracted during his/her work?" (Distractibility), "Do you consider this child hyperactive?" (Hyperactivity), and "In general, is this child functioning as well in the classroom as other children his/her own age?" (Performance). A total score ("Needleman score") was calculated as the sum of all items (0–11), after reverse-coding five items where higher scores represented less (rather than more) behavior problems. The internal consistency of the scale was high (McDonald's $\omega = 0.82$; 95% CI: 0.80, 0.83). The three-factor theoretical structure was supported in a confirmatory factor analysis under the diagonally weighted least squares (DWLS) estimator ($\chi^2_{(41)} = 113.03$, $p < 0.001$; comparative fit index (CFI) = 0.98; root mean square error of approximation (RMSEA) = 0.04; standardized root mean square residual (SRMR) = 0.05).

2.3.2. Nature exposure

We estimated nature exposure with three measures. First, we used an index called "distance to nature" (*D2N*, Rüdisser et al., 2012) to capture the manifold effects of naturalness as well as the competing effects of natural and artificial land use/land cover (LULC) around the home. D2N comprehensively quantifies the anthropogenic influence on a given landscape by considering the degree of naturalness of a location as well as the distance to other natural habitat patches (Rüdisser et al., 2012). D2N has been successfully used as a comprehensive measure for anthropogenic influence on the landscape in biodiversity and ecosystem service modeling (Kirchner et al., 2015; Rüdisser et al., 2015), soundscape modeling (Dein and Rüdisser 2020), and environmental health research (Dzhambov et al., 2021, 2022). We calculated D2N by multiplying two indicators: degree of naturalness and distance to natural habitat. The degree of naturalness indicator portrays biodiversity-relevant anthropogenic interferences on plants, animals, and ecosystems by classifying LULC categories across seven levels (e.g., natural, near-natural, semi-natural, altered, cultural, artificial with natural elements, and artificial). The seven levels exist within a theoretically continuous interval scale but are defined by thresholds with proportional stretches. The distance to natural habitat indicator is defined as the Euclidean distance to the nearest natural or near-natural patch, as defined by the degree of naturalness. For calculating D2N, the two indicators are normalized to range from 0 (natural/no distance) to 1 (artificial/far from natural habitat). A cutoff of 1000 m is applied to the distance to natural habitat indicator where all values above 1000 m are counted equally. The resulting D2N variable is continuous and ranges from 0 to 1. Indicator estimations for the D2n are based on various LULC information sources, including CORINE, agricultural land use data, forest maps, soil sealing data, and transportation infrastructure (Rüdisser et al., 2012).

Five landscape types associated with D2N ranges have been proposed. Natural or near-natural ($0 < D2N \leq 0.06$) are landscapes where natural land cover (e.g. natural grasslands, moors and heathland, water bodies; natural and sustainably managed forests) is dominant. Extensively cultivated with natural elements ($0.06 < D2N \leq 0.25$) are agricultural landscapes with a high degree (>50%) of natural or near-natural landscape elements such as natural forests. Cultivated ($0.25 < D2N \leq 0.35$) are intensively used landscapes with a substantial extension of natural habitats. Intensively cultivated ($0.35 < D2N \leq 0.65$) are agricultural landscapes with no or very little natural habitats. Last, urbanized ($0.65 < D2N \leq 1$) are landscapes with a high amount of impervious soils (>50%) (Rüdisser et al. 2012, 2015).

We calculated D2N within Euclidean buffers for 100, 500, and 1000-m radii around the child's home (**D2N home**). These distances were derived from past research on independent mobility in children aged 10–12 years old that found children reported traveling approximately 500 m to access greenspace and 1000 m to access other common activity spaces (Hand et al., 2018; Loebach and Gilliland, 2014; Villanueva et al., 2012). 500-m home buffers were selected as the primary size based on their relevance in the broader literature on greenspace and health (Browning and Lee, 2017; Labib et al., 2020a,b).

Our second measure of nature exposure was the D2N in a 100-m buffer around the children's school (**D2N school**). This buffer size corresponds to children's expected mobility while at school (Carlson et al., 2017). All geographical information system (GIS) preparations and calculations were conducted in ArcMap 10.6 and QGIS v3.8.

Mothers were also asked whether there was a **garden** at the home (yes/no). Garden denoted private green space, green yards, orchards, floral gardens, or vegetable gardens.

2.3.3. Potential mediators

2.3.3.1. Smoking in the home. Mothers provided information on the **number of cigarettes smoked in the home daily (smoking in the home)** to measure reported secondhand smoke exposure. We also measured children's 12-h urine concentrations of **cotinine** as a biomarker of secondhand smoke exposure (Haufroid and Lison, 1998; Boyaci et al., 2006). For these urine samples, a complete set (plastic box including syrings, monovettes, cooling pads, urine cups) was delivered and prefrozen in the freezer compartment at home. The mother was instructed how to collect 12-h overnight urine samples in the morning with 10 ml monovettes. The sample was immediately put back in the box and into the freezer compartment. It was kept frozen at −20 °C in a portable freezer box for immediate analysis. Samples not analyzed within 2 h were frozen at −80 °C. Analysis was conducted with Cotinine Direct ELISA Kits (BQ086D, LOT: EK2072, ED: 11/06) from BioQuant. Samples were defrosted at +4 °C and brought to room temperature. Sample absorbencies were measured at a dual wavelength of 450 nm and 650 nm, using a Microplate Scanning Spectro-photometer from Bio-Tek Instruments. This method of cotinine measurement provided a limit of detection of 0.7 ng/dl. Concentrations are expressed in micromole per mol creatinine to compensate for variations in urine density (Boeniger et al., 1993; Muscat et al., 2011).

2.3.3.2. Sleep problems and body mass index (BMI). **Body mass index** (BMI) was calculated from measured height and weight following standard guidelines. Trained and supervised team members recorded standing height without shoes to the nearest 0.1 mm using a portable stadiometer (Seca Model 214, Hamburg, Germany). Weight was measured in minimal clothing and barefoot, to the nearest 0.1 kg using a digital scale (Tanita BWB 600, Tanita Corporation, Tokyo, Japan). While BMI was modeled as a continuous variable, for descriptive purposes, children were also categorized based on BMI, age, and sex, according to the cutoff points recommended by the Childhood Obesity Working Group of the International Obesity Taskforce (Cole et al., 2000). For the categorization of children's BMI, we used the function zbmicat (Vidmar et al., 2004, 2013).

Child's sleep problems were measured with three questions that the child answered on a 5-point scale (never, seldom, sometimes, often, or very often). These items included recent time problems falling asleep, uneasy sleep, and feeling tired in the morning.

2.3.3.3. Systemic inflammation. We collected urine **neopterin** concentrations to record cellular immune activation (Murr et al., 2002; Wachter et al., 1989). Neopterin is produced by monocytes/macrophages and microglia and may serve as a marker of cellular immune system activation (Daubner and Lanzas, 2018), where elevated neopterin has been found in children with neurodevelopmental disorders, such as autism (Zhao et al., 2015). Collection and storage paralleled the process reported for cotinine. Concentrations were calculated with High Pressure Liquid Chromatography (Model LC 550; VarianAssociates, Palo Alto, CA) as described elsewhere (Wachter et al., 1989).

2.3.4. Potential confounders and effect modifiers

We retrieved data for covariates at the individual and area-levels. We

collected **socio-demographic** data on the child's age and sex. Maternal education was categorized as basic for ≤9 years of school, skilled labor, vocational, or higher/A-level as well (Lercher and Schmitzberger, 1997). **House type** information was collected as an additional proxy for socioeconomic status (single family detached house, row house, and multiple dwelling).

Mothers were asked about **time spent outdoors** by their children in the summer (<2, 2–4, 4–6, or > 6 h/d). Information on **preterm births** (delivery <37 gestational weeks) was obtained from the Mother and Child Passport (for Austrian children) and Mother Passport (for Italian children), which every pregnant woman in the study area completed for her medical records (Bancher-Todesca, 2014).

We accounted for the **geographic region** (valley) where the child lived, as each region displayed differences either in topography/meteorology or the amount of traffic volume and emissions (Dzhambov et al., 2019). Geographic regions were Inn valley, Wipp valley north (side), Wipp valley south (side), Wipp valley north (main), and Wipp valley south (main).

Nitrogen dioxide (NO_2) and **day-evening-night noise level (L_{den})** exposures were not included in the main analyses due to their high correlations with D2N and concerns about overfitting the models and biasing the D2N effect estimates, since traffic infrastructure information was used in D2N calculations (Rüdisser et al., 2012). Briefly, annual means for NO_2 were calculated with the meteorological model Graz Mesoscale Model at a horizontal resolution of 10-m^2 with a vertical resolution of 2-m (Öttl et al., 2007). Total L_{den} from road traffic and railway noise (for those living in the Wipp valley) was calculated at the most exposed façade of the home and at the school coordinates using an early version of the Harmonoise source model and calibration against field measurements (Jonasson, 2007).

2.4. Statistical analyses

2.4.1. Data inspection and patterns of association tests

Inspection of the dataset revealed that few children had missing data in the variables of interest. For example, only 75 (6%) children had no information on the Needleman score. Therefore, we conducted complete-case analyses without imputing missing values. As a result, the sample size varied slightly across analyses.

Descriptive analysis of the outcome variables indicated that the Needleman score, smoking in the home, cotinine, and neopterin had right-skewed distributions. To address this, the Needleman score was treated as an ordered-categorical variable with a logit link, smoking in the home was dichotomized due to severe zero-inflation, and cotinine and neopterin were natural log-transformed as in earlier studies (Gatzke-Kopp et al., 2020).

Before the structural equation modeling (SEM), we used Pearson (phi, point-biserial) and Spearman correlation coefficients and Mood's median test to describe general patterns of association in the data. We also assessed multivariate associations between the outcomes and nature exposures in main effect models.

Bivariate and regression analyses were conducted in Stata/MP (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC).

2.4.2. Main effect models

We fitted ordinal (with Needleman score), logistic (with smoking in the home), and linear regressions (with cotinine, neopterin, BMI, and sleep problems) with robust standard errors to assess associations between these outcomes and nature exposures (D2N home, D2N school, and garden). All models were adjusted for child's sex and age, maternal education, house type, and geographic region. The shape of relationships between each exposure and outcome variable was tested with restricted cubic splines with five knots.

Models did not suffer from multicollinearity according to tolerance (>0.2) and Variance Inflation Factor (VIF, < 5.0) values. The intraclass correlation coefficient (ICC, < 0.05) did not support spatial clustering in our study regions (cf. Bliese, 2000). However, the small number of clusters in our dataset (<50) precluded more robust checks of clustering and multilevel modeling (Hox and Maas, 2001). Therefore, we included dummy variables for region (valley) that accounted for mean-level differences between the valleys into the regressions to correct the otherwise underestimated standard errors.

2.4.3. Structural equation modeling (SEM)

To examine the multiple pathways between nature exposure and behavior problems simultaneously, we employed SEM as one of the more flexible modeling approaches increasingly used in environmental epidemiology (Dzhambov et al., 2020). We specifically tested whether associations between residential and school D2N, presence of a garden, and the Needleman score were mediated by smoking in the home, sleep problems, BMI, and systemic inflammation (Fig. 1).

Covariances between nature exposure variables were specified a priori. We treated presence of a garden and smoking in the home as categorical endogenous variables and employed weighted least squares mean and variance adjusted estimation methods with robust standard errors. Because this estimator could handle non-normally distributed and categorical data (Flora and Curran, 2004; DiStefano and Morgan, 2014), we used cotinine and neopterin in their original form without log-transformations. Coefficients reported in the SEM were probit regression estimates (i.e., changes in units of standard deviation for the transformed outcome variable, where the probit model transforms probabilities into *z*-scores from a standard normal distribution). Indirect effects were defined as the product of the regression weights associated with their constituent paths, and standard errors for these defined parameters were computed using the Delta method.

Goodness-of-fit was evaluated using robust indices of acceptable model fit provided in Hu and Bentler (1999): non-significant χ^2 ($p > 0.05$); CFI ≥0.95; RMSEA ≤0.06 with a 90% CI ≤ 0.06; and SRMSR ≤0.08. Modification indices and standardized residuals were used as additional criteria to improve model fit by model re-specification. Standardized residuals ≤ |2.58| were expected from a good-fitting model (Byrne, 2014; Brown, 2015).

Structural equation modeling was conducted with the lavaan v. 0.6–9 package (Rosseel, 2012) in R v. 4.0.3. (R Core Team, 2020). A p-value of <0.05 was considered statistically significant.

2.4.4. Sensitivity analyses

We fitted the main effect models with residential D2N $_{100\text{-m}}$ and D2N $_{1000\text{-m}}$ to check whether the strength of association with our primary measure (D2N $_{500\text{-m}}$ home) varied by buffer size. We further tested whether relationships varied among children who had not moved since birth, as determined by length of residency in questionnaires with mothers. In another sensitivity analysis, we regressed the subscale scores of the Needleman questionnaire (Distractibility, Hyperactivity, and Performance) on each of the nature exposure measures to inspect whether behavior problem domains would be affected differentially.

2.4.5. Effect modifiers and stratified analyses

We conducted stratified analyses to test for differential effects in the nature exposure and behavior problems relationship. We presented effect estimates across levels (subgroups) of all pre-specified putative modifiers, regardless of the presence of a significant interaction term.

Our putative moderators included sex, school grade (3rd vs. 4th), maternal education, preterm birth, time spent outdoors (dichotomized as ≤ 6 vs. > 6 h/d), geographic region, residential NO_2, and L_{den}. Continuous modifiers were split at the median value.

In effect modification tests, the criterion for statistical consideration of interactions was relaxed to $p < 0.1$ (i.e., Type I error rate of 10%) (Selvin, 1996; Greenland and Rothman, 1998; Marshall, 2007).

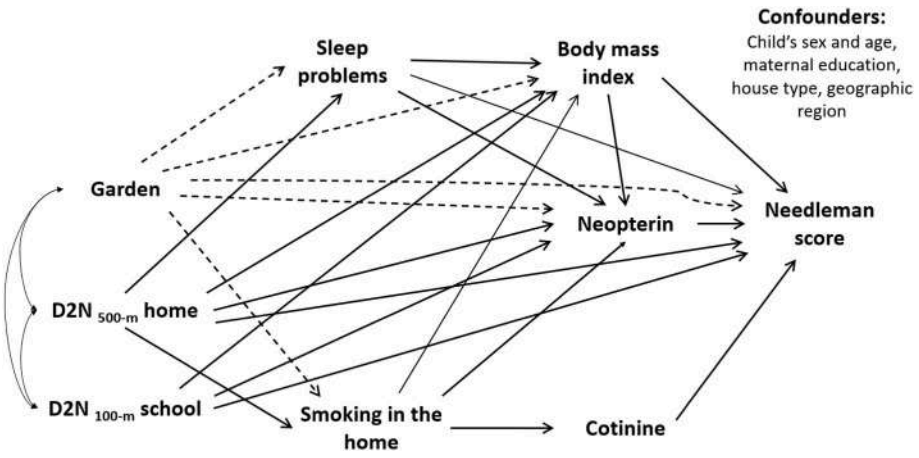

Fig. 1. Hypothesized pathways between nature exposure and behavior problems. Note: Solid lines represent hypothesized associations with a positive sign and dashed lines represent hypothesized inverse associations. Abbreviations: D2N – distance to nature.

3. Results

3.1. Sample characteristics and patterns of association

The sample was balanced in terms of boys and girls and maternal education levels (Table 1). Normative scores for the Needleman score were not available. However, our sample mean (8.84) was consistent with those in the original study by Needleman et al. (1979): 8.2 in the high lead exposure group, 9.5 in the low lead exposure groups.[2]

Most children had normal weight. About one third of children lived in a home where the parents smoked. As in our previous studies in this area (Dzhambov et al., 2019, 2021, 2022), we note that the level of nature exposure was high both in terms of D2N and presence of garden at home.

Bivariate correlations are presented in Table S1. Higher Needleman scores were observed in boys as well as in children exposed to smoking in the home, children with higher BMIs, and children reporting more sleep problems. Conversely, higher maternal education and a home garden were associated with lower Needleman scores. A home garden was inversely associated with smoking in the home, cotinine, BMI, and sleep problems. Opposite trends were observed for D2N. Single family houses were associated with a lower Needleman score, less smoking in the home and cotinine, lower neopterin and BMI, and less sleep problems (Table S2). Across geographic regions there were differences in Needleman scores and potential mediators without clear patterns. The multivariate models regressing behavior problems and potential mediators on nature exposure and corresponding sensitivity analyses supported this pattern of associations (Table S3). Greater distances to nature from school (D2N $_{100\text{-m}}$) were associated with more behavior problems.

3.2. Structural equation modeling

According to the robust model fit indices, the initial model poorly fit the data: $\chi^2_{(63)} = 468.39$, $p < 0.001$; CFI = 0.77; RMSEA = 0.08 (90% CI: 0.07, 0.09); SRMR = 0.06. Following an inspection of modification indices, model fit was improved by relaxing the constraints on covariances between exogenous variables in the model to be equal to zero (i.e., we allowed exogenous variables to be correlated). The revised model converged in 598 iterations. Robust goodness-of-fit statistics associated with this path model indicated good consistency with the data: $\chi^2_{(27)} = 39.13$, $p = 0.062$; CFI = 0.98; RMSEA = 0.02 (90% CI: 0.00, 0.04); SRMR = 0.01. The model explained 9.7% of the variance in the Needleman score and a small portion of the variance in most mediators. However, the explained variance was high for cotinine, garden, and D2N.

Fig. 2 and Table 2 present the final SEM results (full output in Table S4). Residential D2N $_{500\text{-m}}$ and school D2N $_{100\text{-m}}$ were differentially associated with behavior problems. No indirect associations with behavior problems were found for these nature exposures. Specifically, farther distances to nature from the home were associated with lower Needleman scores ($\beta = -1.97$; 95% CI: −3.69, −0.25) and farther distances to nature from school were associated with higher Needleman scores ($\beta = 1.92$; 95% CI: 0.43, 3.41). A home garden was only indirectly associated with lower Needleman scores ($\beta = -0.05$; 95% CI: −0.09, −0.02) and this relationship was driven by lower likelihood of smoking in the home, which in turn led to lower cotinine, and then to lower Needleman scores ($\beta = -0.03$; 95% CI: −0.05, −0.01). Interestingly, BMI was positively associated with neopterin, which in turn was unexpectedly associated with lower Needleman scores. While a home garden was associated with less sleep problems and lower BMI, and those were in turn associated with Needleman scores (Fig. 2), these indirect paths between a home garden and the Needleman score did not reach formal significance (Table 2).

3.3. Sensitivity analyses

Fitting the main effects model with D2N in alternative buffers revealed a significantly higher likelihood of smoking in the home with increasing D2N $_{100\text{-m}}$ (Table S5). The three subscales of the Needleman questionnaire performed differently, and Performance was the only subscale to be associated with garden and school D2N in the expected direction (Table S6). No significant associations were observed between the Needleman score and having a garden or D2N $_{500\text{-m}}$ in children who had not changed residence since birth (Table S7).

3.4. Stratified and effect modification tests

Stratified results testing for effect modification are shown in Table S8. Needleman scores were differentially associated with residential D2N $_{500\text{-m}}$ across geographic regions. NO_2 was also an effect modifier for D2N $_{500\text{-m}}$, with the direction of the association depending on NO_2 levels. The positive association between school D2N $_{100\text{-m}}$ and Needleman score was largely consistent but showed larger effects in girls. A home garden was associated with lower Needleman scores in single-family households and 3rd graders. The effects of a home garden on Needleman scores were also larger in children born prematurely.

[2] Comparison required reversing our Needleman score to match the scaling in Needleman et al. (1979), where higher values indicated more positive outcomes.

Table 1
Study population characteristics ($N = 1251$).

Characteristics	
Socio-demographics	
Age [years] (Mean ± SD)	9.36 ± 0.65
Boy (N, %)	623 (49.8)
School grade (N, %)	
3rd	618 (49.40)
4th	633 (50.60)
Maternal education (N, %)	
Basic	279 (22.3)
Skilled labor	396 (31.7)
Vocational	287 (22.9)
A-level	247 (19.7)
Behavior problems	
Needleman score (Median, 25th – 75th)	1.00 (0.00–4.00)
Nature exposure	
D2N $_{100\text{-m}}$ home (Median, 25th – 75th)	0.27 (0.18–0.39)
D2N $_{500\text{-m}}$ home (Median, 25th – 75th)	0.21 (0.14–0.27)
D2N $_{1000\text{-m}}$ home (Median, 25th – 75th)	0.16 (0.12–0.22)
D2N $_{100\text{-m}}$ school (Median, 25th – 75th)	0.42 (0.28–0.48)
Presence of garden (N, %)	927 (74.1)
Potential mediators	
Smoking in the home (N, %)	365 (32.07)
Cotinine [μmol/mol] (Median, 25th – 75th)	0.47 (0.12–1.75)
Neopterin [μmol/mol] (Median, 25th – 75th)	200.00 (166.00–245.00)
Sleep problems (Median, 25th – 75th)	7.00 (4.00–9.00)
BMI [kg/m^2] (Mean ± SD)	17.00 (2.65)
Underweight (N, %)	155 (12.39)
Normal weight (N, %)	883 (70.58)
Overweight (N, %)	170 (13.59)
Obese (N, %)	43 (3.44)
Other confounders and modifiers	
NO_2 [μg/m^3] (Median, 25th – 75th)	12.68 (9.73–18.53)
L_{den} home [dBA] (Median, 25th – 75th)	53.00 (44.23–59.43)
L_{den} school [dBA] (Median, 25th – 75th)	47.15 (38.32–56.12)
Geographic region (N, %)	
Inn valley	251 (20.06)
Wipp valley north (side)	326 (26.06)
Wipp valley south (side)	133 (10.63)
Wipp valley north (main)	326 (26.06)
Wipp valley south (main)	215 (17.19)
Preterm birth (N, %)	106 (9.08)
Time spent outdoors >6 h/d (N, %)	530 (43.69)
House type (N, %)	
Single family attached	780 (62.35)
Row house	176 (14.07)
Multiple dwelling	295 (23.58)

Notes: Depending on their distribution, we report mean and standard deviation (SD) for normally-distributed variables, median and percentiles for non-normality distributed variables, and number of cases and percentage across categories for categorical and ordinal variables. Abbreviations: BMI – Body mass index, D2N – Distance to nature, L_{den} – total day-evening-night noise level; NO_2 – nitrogen dioxide.

Having a home garden was associated with lower Needleman scores only at lower levels of NO_2 and L_{den}.

4. Discussion

4.1. Main findings

This cross-sectional study investigated associations between gardens and distances to nature and school behavior problems, and the role of biomarkers of secondhand smoke and systemic inflammation in schoolchildren residing in alpine valleys. We found that children who lived in a home with a garden and those whose school was closer to nature exhibited less behavior problems at school. The effects of proximity to nature from the school was directly associated with less behavior problems, while the presence of a home garden worked indirectly through a lower likelihood of secondhand smoke exposure. Associations were in unexpected directions between behavior problems and residential proximity to nature, and dependent on the outcome and context.

The salutary effects of home gardens and school nature proximity may be explained by nature conferring restorative experiences that support cognitive functioning (Collado and Staats, 2016). Multiple studies have suggested that such experiences can improve mood, self-discipline, impulse control, coping with stressors, and emotion regulation (Taylor et al., 2002; Wells and Evans, 2003; Wells, 2000; Barger et al., 2021). Several reviews have also found positive overall associations of nature with self-regulation (Weeland et al., 2019), prosocial behavior (Putra et al., 2020), and, in general, neuropsychological development and children's mental health (Luque-García et al., 2021). In the present study, we were able to corroborate that greater proximity to nature from the school was associated with less behavior problems, even though children in 3rd and 4th grades in Austria spend only about five hours/day at school. Unexpectedly, this pattern was not observed in the residential context beyond the home garden. Previously, in a study of 2593 schoolchildren in the 2nd to 4th grades in Barcelona, Spain, Dadvand et al. (2015) also reported more pronounced benefits from school greenness than residential or commuting greenness. Further, Maitre et al. (2021) found that the presence of greenspace within 300 m of the home during pregnancy was associated with increased externalizing symptoms in the child. We posit that our observed protective effects of nature proximity from schools and not homes (beyond the garden) may be attributable to the data on behavior problems being collected from teachers and that research on problem behavior at home may have shown different relationships with residential nature proximity.

There is no clear-cut explanation of these divergent results, as the reasons could be manifold. Differential associations are not uncommon across topographically or geographically diverse study areas. In our previous studies in these valleys, we have seen such differential associations between indicators of nature exposure and systolic blood pressure (Dzhambov et al., 2022) and allergic symptoms (Dzhambov et al., 2021). In addition, others have reported null associations in less urbanized areas compared with urban areas. For example, Bijnens et al. (2020) found no associations between green space and behavioral problems in children living in suburban and rural residents compared with urban children. It has been suggested that in less urbanized areas, private gardens may play a more important role for children's health than greenspace in the more distant neighborhood environment (McCrorie et al., 2021). Moreover, children cannot travel by themselves far from home and are more likely to engage in outdoor activities in a home garden (Gundersen et al., 2016). This may be the case here, as the effect of garden was more robust in sensitivity analyses than that of residential D2N.

Residential exposures may also reflect unmeasured area-level effects that extend beyond contact with nature. Greater school D2N (in other words, a less natural school setting) was consistently associated with more behavior problems, with no evidence of effect modification by geographic factors or co-exposures. This finding is not surprising, given that higher degree of naturalness of the immediate school surroundings may directly improve attention and decrease stress levels, and thereby impact on the child's school behavior and academic performance (Browning and Rigolon, 2019; Putra et al., 2020). Setting aside unmeasured confounding, another explanation could be that we did not account for theoretical factors driving relational dynamics between children and their residential surroundings, such as parental supervision, social interaction with others, and attitudes towards these settings.

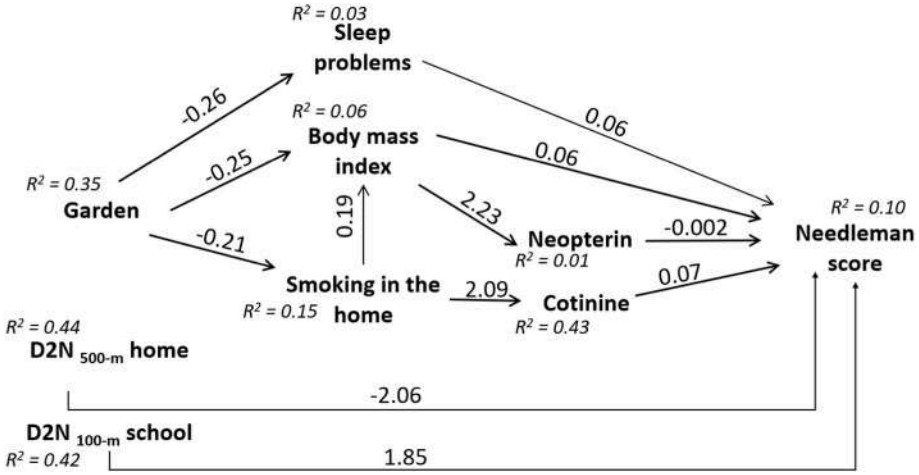

Fig. 2. Structural equation model showing estimated paths linking nature exposure to behavior problems, as measured by the Needleman score (N = 1014). Notes: Statistically significant regression weights are shown. R^2 shows proportion of variance explained in endogenous variables. Control variables (child's sex, age, maternal education, house type, and geographic region), covariances, and errors terms are not displayed to enhance readability. Abbreviations: D2N – Distance to nature.

Table 2
Adjusted associations between nature exposures and behavior problems, as measured by the Needleman score, in the structural equation model (N = 1014).

	Estimate (95% CI)
Total associations	
Garden	−0.10 (−0.30, 0.09)
D2N $_{500\text{-m}}$ home	**−1.97 (-3.69, -0.25)***
D2N $_{100\text{-m}}$ school	**1.92 (0.43, 3.41)***
Direct associations	
Garden	−0.05 (−0.25, 0.15)
D2N $_{500\text{-m}}$ home	**−2.06 (-3.77, -0.35)***
D2N $_{100\text{-m}}$ school	**1.85 (0.38, 3.33)***
Total indirect associations	
Garden	**−0.05 (-0.09, -0.02)***
D2N $_{500\text{-m}}$ home	0.09 (−0.15, 0.32)
D2N $_{100\text{-m}}$ school	0.07 (−0.07, 0.21)
Specific indirect associations	
Garden → Sleep problems	−0.02 (−0.03, 0.004)
Garden → BMI	−0.01 (−0.03, 0.004)
Garden → Neopterin	−0.01 (−0.03, 0.01)
Garden → Sleep problems → BMI	−0.00 (−0.001, 0.001)
Garden → Sleep problems → Neopterin	0.00 (−0.001, 0.001)
Garden → BMI → Neopterin	0.001 (−0.00, 0.002)
Garden → Smoking in the home → BMI	−0.002 (−0.01, 0.001)
Garden → Smoking in the home → Cotinine	**−0.03 (-0.05, -0.01)***
Garden → Smoking in the home → Neopterin	0.00 (−0.003, 0.003)
Garden → Smoking in the home → BMI → Neopterin	0.00 (−0.00, 0.00)
D2N $_{500\text{-m}}$ home → Sleep problems	0.09 (−0.05, 0.23)
D2N $_{500\text{-m}}$ home → BMI	−0.04 (−0.14, 0.07)
D2N $_{500\text{-m}}$ home → Neopterin	−0.004 (−0.12, 0.11)
D2N $_{500\text{-m}}$ home → Sleep problems → BMI	0.00 (−0.01, 0.01)
D2N $_{500\text{-m}}$ home → Sleep problems → Neopterin	−0.001 (−0.01, 0.00)
D2N $_{500\text{-m}}$ home → BMI → Neopterin	0.002 (−0.01, 0.01)
D2N $_{500\text{-m}}$ home → Smoking in the home → BMI	0.002 (−0.01, 0.01)
D2N $_{500\text{-m}}$ home → Smoking in the home → Cotinine	0.03 (−0.08, 0.15)
D2N $_{500\text{-m}}$ home → Smoking in the home → Neopterin	−0.00 (−0.003, 0.003)
D2N $_{500\text{-m}}$ home → Smoking in the home → BMI → Neopterin	−0.00 (−0.001, 0.00)
D2N $_{100\text{-m}}$ school → BMI	0.04 (−0.06, 0.14)
D2N $_{100\text{-m}}$ school → Neopterin	0.03 (−0.08, 0.14)
D2N $_{100\text{-m}}$ school → BMI → Neopterin	−0.003 (−0.01, 0.004)

Notes: Unstandardized probit regression estimates are shown. Abbreviations: BMI – Body mass index, D2N – Distance to nature. *$p < 0.05$.

4.2. Pathways between nature exposure and behavior problems

Reviews on nature exposure and child behavior problems have noted a general lack of mechanistic studies, which would enable understanding the contribution and relevance of different mediators (Putra et al., 2020; Luque-García et al., 2021). Our conceptual model was informed by Martin et al. (2020) who found that living in a greener area could reduce smoking prevalence in adults, possibly through improved mental well-being. We further extended this idea, considering that secondhand smoking is itself a risk factor for poor neurodevelopment (Julvez et al., 2007; Chen et al., 2013), by showing that children living in a home with a garden were less likely to be exposed to secondhand smoke in the home, which in turn benefited their school behavior and performance. Other potentially relevant pathways have been explored largely in relation to adult mental health (Dzhambov et al., 2020), including better sleep quality and lower adiposity, both of which support neurodevelopment (Li et al., 2018; Kamara and Beauchaine, 2020). We are unaware of past research that suggests parental smoking could explain the pathway between nature and child behavior problems.

We unexpectedly found that cellular immune activation, as measured by neopterin, was generally not associated with other variables except for a positive relationship with BMI. We hypothesized that neopterin would be related with behavior problems and act as a central mediator bringing together other indirect pathways starting from nature exposure (cf. Irwin et al., 2016; Miller and Spencer, 2014). Despite our model explaining 1% of the variance in neopterin, neopterin was still associated with less behavior problems. It appears this biomarker may not have captured neuroinflammation in a way consistent with our study objectives. Urinary neopterin is increased in a number of inflammatory states, such as viral and bacterial infections, autoimmune diseases, and even following routine vaccination (Hamerlinck, 1999). Urinary neopterin measured at one point in time may also not reflect long-term patterns of inflammation, which would be relevant for behavior problems. Additionally, there is evidence to suggest that brain neopterin may actually have a protective role in response to inflammation (Daubner and Lanzas, 2018) and even enhance cognitive performance in test animals (Ghisoni et al., 2016). Thus, our seemingly counterintuitive findings are not entirely unexpected in light of the multiple determinants and physiological properties of neopterin as well as alternative measures of neopterin not utilized in this study.

4.3. Strengths and limitations

Our study is not without limitations. First, its cross-sectional design precludes formal claims of causality between environmental exposures and outcomes. Longitudinal analyses have failed to replicate the cross-

sectional associations between environmental exposures and behavior problems (Naya et al., 2021; Tangermann et al., 2022). Moreover, cross-sectional tests of mediation can yield evidence of mediation even in the absence of such in a follow-up with the same subjects (Maxwell and Cole, 2007; Maxwell et al., 2011). In this vein, one-point-in-time measures, as captured here with urinary metabolites, may not represent long-term systemic inflammation. Still, it is reassuring that the pattern of associations was preserved in the subgroup of children who had not changed residence since birth and D2N and presence of garden reflected stable long-term patterns in LULC. Moreover, conceptual time-ordering of the constructs in the mediation model was supported by theory (cf. Tate, 2015).

Second, some of our findings could have been caused by uncontrolled residual confounding by area-level factors, daily activity patterns, home and school building characteristics, and social environment. Static exposure assessment is a common issue in environmental epidemiology and entails exposure misclassification (Helbich, 2018), as it ignores commuting exposure and leisure mobility of children. Furthermore, we did not examine the role of personality traits, which may be relevant for restoration in nature (Feng et al., 2022).

Third, it is possible that socioeconomic status, as measured by parental education and house type, might explain the effects of home garden on smoking in the home and behavior problems. Families that obtained higher levels of education may be less likely to smoke (Saito et al., 2018). Moreover, garden was associated with less behavior problems only in single family houses. We acknowledge that maternal education and house type largely accounted for having garden and smoking, but the associations of interest persisted nonetheless. The presence of a home garden and living close to nature is a common feature and not unequivocally related to higher socioeconomic status (Evans et al., 2002) in the study region, so this potential for residual confounding by socioeconomic status was difficult to determine.

Fourth, the Needleman scale has mostly been used in studies on cognitive and behavioral effects of lead exposure, which precludes direct comparison of our findings with other nature exposure studies. Still, the Needleman scale has shown similar results as other behavior problems scales (Yule et al., 1984).

Finally, although air pollution and noise reduction are considered important pathways underpinning health benefits of nature, we did not investigate them here. This choice was driven by the fact that the correlation between D2N and NO_2 and L_{den} was artificially inflated because traffic infrastructure information was used in the calculation of D2N (Rüdisser et al., 2012). Moreover, the complex and unexplained patterns of association in the dataset would render interpretation of these results untenable.

Relatedly, we did not use the commonly used NDVI indicator as it is too generic and not informative enough about LULC (Gascon et al., 2016; Astell-Burt and Feng, 2021). Future studies may consider including such indicators to ease with comparison across studies, however.

Despite these limitations, our study has several strengths. Unlike earlier studies (Luque-García et al., 2021), we used a comprehensive measure of nature/greenspace exposure. We investigated both residential and school exposures across different buffer sizes and geographically diverse alpine valleys. We tested complex underlying pathways beyond what would have been available in simple mediation analyses. And our model included, for the first time, parental smoking as a pathway in the relationship between nature exposure and children's behavioral problems using biomarkers of actual secondhand smoke exposure and systemic inflammation. These features are unique to our investigation and can trigger an expansion of more narrowly defined conventional models in this area of environmental epidemiology.

5. Conclusions

Natural environments in school and home surroundings might be beneficial for school conduct and performance. Lower secondhand smoke exposure at home might be a pathway between home gardens and children's behavioral problems. Associations with residential proximity to nature are in unexpected directions and warrant further investigation. These findings serve as a point of departure for investigating how proximal nature might activate health supportive pathways in children and simultaneously confer childhood health benefits via positive behavioral changes in their parents.

CRediT authorship contribution statement

Angel M. Dzhambov: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Peter Lercher:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Project administration. **Johannes Rüdisser:** Methodology, Investigation, Writing – original draft, Writing – review & editing. **Matthew H.E.M. Browning:** Methodology, Investigation, Writing – original draft, Writing – review & editing. **Iana Markevych:** Methodology, Investigation, Writing – original draft, Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113975.

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Longitudinal measures of phthalate exposure and asthma exacerbation in a rural agricultural cohort of Latino children in Yakima Valley, Washington

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ABSTRACT

Phthalates are a class of widely used synthetic chemicals found in commonly used materials and products. Epidemiological studies suggest phthalate exposure is associated with asthma outcomes, though most studies have not investigated phthalates as triggers of exacerbations in children diagnosed with asthma. This study used data from the Home Air in Agriculture Pediatric Intervention Trial (HAPI) to examine relationships between phthalate exposure and outcomes related to childhood asthma exacerbation. We used measures of phthalate metabolites and respiratory health measures including fractional exhaled nitric oxide (FENO), the Asthma Control Test (ACT), caregiver report of symptoms, and urinary leukotriene E_4 ($uLTE_4$) to estimate longitudinal associations using mixed effects models, adjusted for covariates. For 100% (i.e., doubling) increases in mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-2-ethylhexyl phthalate (MEHP), and mono-ethyl phthalate (MEP), concentrations of FENO increased by 8.7% (95% CI: 0.7–17.3), 7.2% (95% CI: 0.0–14.9), and 6.4% (95% CI: 0.0–13.3), respectively. All phthalate metabolites demonstrated associations with $uLTE_4$, effect sizes ranging from an 8.7% increase in $uLTE_4$ (95% CI: 4.3–12.5) for a 100% increase in MEHP to an 18.1% increase in $uLTE_4$ (95% CI: 13.3–23.1) for a 100% increase in MNBP. In models of caregiver report of symptoms, no phthalate metabolites were significantly associated in primary models. No phthalate metabolites were associated with standardized ACT score. Our results suggest urinary phthalate metabolites are significant predictors of inflammatory biomarkers related to asthma exacerbation in children but not child and caregiver report of airway symptomatology.

1. Introduction

Phthalates are a class of synthetic chemicals primarily used as plasticizers in manufactured materials, most prominently in polyvinyl chloride (PVC), with a multitude of additional applications in building materials, medical devices, food and beverage packaging, personal care products, and other consumer products (Schettler, 2006). The widespread use of phthalates and their ability to migrate from polymers contributes to their proliferation as indoor environmental and dietary contaminants (Erythropel et al., 2014; Mitro et al., 2016; Serrano et al., 2014). Sources and routes of exposure in humans vary by individual phthalate esters, but exposure is common and occurs through ingestion, inhalation, and dermal routes, with children at risk for increased exposure compared to adults due to increased surface area to volume

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ratio, increased respiratory rate, and increased hand to mouth behaviors compared to adults (Sathyanarayana, 2008; Wang et al., 2019).

Numerous studies indicate phthalate exposure is linked to allergic disease and asthma, although underlying mechanisms are unclear (Bølling et al., 2020; Robinson and Miller, 2015; Wu et al., 2020). *In vitro* and *ex vivo* studies suggest phthalates may induce inflammatory mediators (e.g., interleukin-4 (IL-4), IL-6, IL-8, and tumor necrosis factor alpha (TNF-α)) (Jepsen et al., 2004; Lee et al., 2011; Oh and Lim, 2009) and modulate other immune cells (e.g., macrophages and dendritic cells) involved in respiratory outcomes related to asthma, while mouse models demonstrate a role for phthalates as adjuvants in allergic airway reactions (Bølling et al., 2020). Cross-sectional and longitudinal studies in children have demonstrated positive associations between urinary phthalate metabolites and increased asthma risk (Wu et al., 2020), airway inflammation (Just et al., 2012; Kim et al., 2018), and reduced lung function (Kim et al., 2018). Several knowledge gaps persist, especially with respect to potential for phthalate-induced exacerbation of symptoms among children with preexisting asthma diagnoses. Airway inflammation is a key characteristic of asthma, and biomarkers of inflammation in exhaled breath can function as informative measures of asthma pathophysiology in children (Tenero et al., 2018). Fractional exhaled nitric oxide (FENO) is a biomarker of eosinophilic airway inflammation that offers utility as an objective measure in child asthma assessment with predictive potential for the risk of asthma exacerbation (Dweik et al., 2011; Rao and Phipatanakul, 2016). Urinary biomarkers also inform assessment of airway and systemic inflammation (James and Hedlin, 2016). Cysteinyl leukotrienes (CysLTs) are a class of inflammatory lipid mediators leading to the metabolic end product urinary leukotriene E_4 ($uLTE_4$), a biomarker correlated with airway inflammation and asthma exacerbation that has shown promise in pediatric asthma research (Hoffman and Rabinovitch, 2018). Longitudinal studies are needed to investigate phthalates as triggers of airway inflammation and asthma symptoms among children with asthma, especially within understudied racial, geographic, and socioeconomic groups. Studies have reported elevated urinary phthalate metabolite concentrations among Latino children (Galvez et al., 2018; Hoffman et al., 2018), and such populations in rural, low-income settings may experience unique combinations of factors, including socioeconomic status and health literacy, related to asthma morbidity and corresponding stressors (Estrada and Ownby, 2017; Rodríguez et al., 2020).

In this longitudinal study of Latino children with asthma in a primarily rural agricultural community in lower Yakima Valley, WA, we use repeated measures to examine the relationship between eleven individual urinary phthalate metabolites (mono-benzyl phthalate (MBZP), mono-(carboxy-isononyl) phthalate (MCINP), mono-(carboxy-isooctyl) phthalate (MCIOP), mono-(3-carboxypropyl) phthalate (MCPP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-ethyl phthalate (MEP), mono-isobutyl phthalate (MIBP), and mono-n-butyl phthalate (MNBP)) and repeated respiratory health assessment. We examined established clinical tools for assessing asthma status (FENO (Dweik et al., 2011) and the Asthma Control Test (ACT) (Nathan et al., 2004)), as well as caregiver report of symptoms and $uLTE_4$.

2. Material and methods

2.1. Study participants and setting

This study accessed participant samples and data from the Home Air in Agriculture Pediatric Intervention Trial (HAPI), a randomized trial of high-efficiency particulate air (HEPA) air cleaners with asthma education vs. education only (Masterson et al., 2020). The HAPI intervention was designed to examine the effectiveness of the HEPA air cleaners in rural homes in reducing particulate matter (PM) and ammonia concentrations as well as to examine health outcomes among participants. The original study was not designed to address phthalates specifically. Air cleaners would not be expected to reduce phthalate exposure assessed via urinary phthalate metabolites in this study, as diet is likely the dominant source of phthalate exposure in children aged 3–11 years (Wang et al., 2019), and children's urinary phthalate metabolite levels have not been affected by air purifiers in another setting (Lee et al., 2020). Enrollment occurred on a rolling basis beginning in 2015 and data collection concluded in 2019. The present study uses data from the total sample of 79 HAPI participants who provided at least a single urine specimen. Details regarding recruitment and follow up are provided elsewhere (Masterson et al., 2020). Briefly, participants were recruited through the Yakima Valley Farmworker Clinic (YVFWC), and eligibility criteria included: (i) child's age six to twelve years, (ii) poorly controlled asthma identified through a screening questionnaire, (iii) no smokers in the home, and (iv) residential proximity (i.e., $<$ ½ mile) to crop and/or dairy production. Human subjects research approval was granted by the University of Washington Institutional Review Board and the YVFWC research review committee.

The HAPI study followed each participant for one year, with data and sample collection at four separate time points. Same-day exposure and outcome assessments occurred at each time point. The first time point took place at enrollment, while the second, third, and fourth occurred at follow up visits approximately two, six, and 12 months later, respectively. After the second time point, participant households were randomized to one of two study arms: (i) asthma education, or (ii) asthma education plus two portable HEPA air cleaners, one placed in the child's sleeping area and the other in the main living area.

2.2. Urine sample collection and analysis

Spot urine samples were collected at each time point in phthalate-free polyethylene containers and transported on dry ice to a field laboratory where specific gravity was measured using a handheld refractometer and samples were stored at −20 °C. At the field laboratory, 24 trip blanks were prepared using high performance liquid chromatography (HPLC) water (VWR, Radnor, Pennsylvania, USA). Urine samples along with the investigator-initiated blinded quality control (QC) blanks and 5 aliquots of National Institute of Standards and Technology (NIST) standard reference material (SRM) 3673 (Organic Contaminants in Non-Smokers' Urine) were randomized and shipped to the Icahn School of Medicine at Mount Sinai laboratory hub of the National Institutes of Health (NIH) Children's Health Exposure Analysis Resource (CHEAR) (Balshaw et al., 2017), where they were analyzed for the target metabolites using isotope-dilution liquid chromatography with tandem mass spectrometry following a Centers for Disease Control and Prevention (CDC) method with modifications (Dewalque et al., 2014; Silva et al., 2004; Stroustrup et al., 2018). CHEAR laboratory hubs include their own internal quality assurance and QC protocols, including analysis of pooled samples to assess analytical precision. Quality assurance measures included the insertion of CHEAR QC pools A and B (three each per 100 samples), NIST SRM 3672 and 3673 (two each per 100 samples), and bi-annual participation in the German External Quality Assessment Scheme (G-EQUAS) (http://www.g-equas.de/) (Göen et al., 2012). Trip and laboratory blanks were all $<$ limit of detection (LOD). Mean ($N = 5$) recoveries of the analyte-specific NIST 3673 reference values ranged from 88% (coefficient of variation (CV) 4%) for MIBP to 102% (CV 12%) for MNBP, indicating good analytical accuracy, while % CVs calculated using the CHEAR QC pools ranged from 3 to 8%, indicating good analytical precision.

2.3. Respiratory health assessment

The respiratory health assessment included measurement of FENO, caregiver report of symptom days in the prior 2 weeks, age-appropriate administration of the ACT (child ACT for age 4–11 years, adult ACT for age $\geq$ 12 years), and a urinary biomarker of inflammation, $uLTE_4$. The

assessments are described briefly here but are described in detail elsewhere (Masterson et al., 2020).

FENO was measured using the NIOX Vero (Aerocrine Inc., Stockholm, Sweden) following standardized procedures recommended by the American Thoracic Society (ATS) and European Respiratory Society (ERS) (American Thoracic Society and European Respiratory Society, 2005). FENO values < 20 parts per billion (ppb) in children < 12 years are interpreted to be low and indicate that pulmonary eosinophilic inflammation is less likely, while values > 35 ppb in children are interpreted to be high and indicative of pulmonary eosinophilic inflammation (Dweik et al., 2011).

An aliquot of the same urine sample used for the urinary phthalate metabolite analyses was sent to the Icahn School of Medicine at Mount Sinai CHEAR laboratory hub for $uLTE_4$ analysis via enzyme-linked immunosorbent assay (No. 501060; Cayman Chemical, Ann Arbor, Michigan, USA). Urinary creatinine was measured using a G-EQUAS proficiency test validated colorimetric method based on Taussky (1954) (Taussky and Kurzmann, 1954), so that creatinine-corrected $uLTE_4$ and urinary phthalate metabolite concentrations could be reported, to allow comparison with prior research (Hoffman and Rabinovitch, 2018).

As a part of a broader questionnaire, the primary caregiver was asked at each urine sample collection time point: "During the past two weeks, how many days did the child have any asthma symptoms, such as wheezing, coughing, tightness in the chest, shortness of breath, waking up at night because of asthma symptoms, or slowing down of usual activities because of asthma?"

Field staff also deployed the ACT, a validated composite measure of asthma control recommended for the assessment of asthma control in both children and adults (Cloutier et al., 2012; Nathan et al., 2004). Children up to eleven years of age were given the Childhood Asthma Control Test (C-ACT), a 7-item questionnaire which contains 4 questions for the child soliciting perceptions of asthma severity, limitation of physical activity, cough, and sleep disturbances, and three questions for the caregiver (with a 4-week recall period) on daytime symptoms, daytime wheeze, and sleep disturbances (Liu et al., 2007). Responses to the first four questions are scored 0–3 and the latter three questions 0–5, for a summed score ranging from 0 to 27. Higher scores represent responses indicating less frequent signs and symptoms (better asthma control). Children ≥ 12 years were given the 5-item ACT, which includes four questions for the child soliciting perceptions on their asthma's impact on schoolwork, shortness of breath, sleep disturbances, and rescue medication use, and a question on overall perception of asthma control, all for the prior 4-week recall period. Each response is scored 1–5 for a total summed score range of 5–25. ACT and C-ACT scores of ≤ 19 are commonly used to identify poorly controlled asthma (Reddel et al., 2009). 70 participants completed the C-ACT (252 observations) and 9 completed the ACT (27 observations). We computed a percentage-scale score to standardize the ACT and C-ACT results by dividing the score observed by the total possible score. The ACT and C-ACT independently correlate at similarly high levels with the Global Initiative for Asthma (GINA) guidelines (Koolen et al., 2011), a gold standard for asthma control assessment, and a standardized ACT/C-ACT score has been used in other analyses within the HAPI study (Drieling et al., 2022).

2.3.1. Covariate assessment

Various participant-specific sociodemographic characteristics and factors related to asthma health were also collected in the HAPI study (Masterson et al., 2020). These included skin prick testing to determine atopy based on at least one positive among 6 common aeroallergens, determination of use of asthma controller medications, and assessment of additional home environment and demographic factors (e.g., annual household income) potentially affecting asthma morbidity. Height and weight measurements were collected in clinic at enrollment.

2.4. Statistical analysis

Variables of interest were evaluated for normality by visualizing distributions, and urinary phthalate metabolite, FENO, and $uLTE_4$ concentrations were natural log transformed. Pearson correlation coefficients were calculated between pairs of the 11 individual urinary phthalate metabolites. The four metabolites of di-2-ethylhexyl phthalate (DEHP), MECPP, MEHHP, MEHP, and MEOHP, were grouped by summing their individual molar concentrations to produce a single summed DEHP metabolite variable, $\sum$DEHP (Zota et al., 2014). For urinary phthalate metabolite concentrations below the analytical LOD, the instrumental values were used. In descriptive analyses, we addressed urine dilution by calculating uncorrected and specific gravity-corrected urinary phthalate metabolite concentrations. In descriptive analyses of $uLTE_4$, creatinine-corrected values were calculated to allow comparison with other published data. In regression models, specific gravity was included as a covariate to correct for urine dilution (Barr et al., 2005; Ferguson et al., 2019), and values of urinary phthalate metabolites and $uLTE_4$ were not corrected.

To estimate associations between urinary phthalate metabolite concentrations and respiratory health measures, and account for the longitudinal study design, we used linear mixed effects models (LMMs) for continuous outcomes and generalized linear mixed effects models (GLMMs) for dichotomized outcomes. Model specifications included fixed effects for the predictor of interest and corresponding covariates, and a by-participant random intercept. The overall model equation is as follows:

$$Y_{ij} = \beta_0 + \beta_1 * ph_{ij} + \sum \beta_k * X_{ijk} + \gamma_i + \varepsilon \quad (1)$$

In equation (eq.) 1, Y_{ij} denotes the j-th observation on the i-th participant, ph_{ij} denotes the phthalate metabolite, X_{ijk} denotes the covariates, and γ_I denotes the random intercept. We fit LMMs for natural log-transformed FENO, natural log-transformed $uLTE_4$, and a transformed percentage-scale ACT score outcome. The ACT transformation addresses the heterogeneity of variance associated with the fixed upper and lower limits of the percentage scale. This ACT outcome was calculated using a logit-like transformation:

$$log\left(\frac{Y-5}{(100-(Y-5))}\right) \quad (2)$$

In eq. (2), Y denotes the percentage-scale score on the ACT or C-ACT. We also fit GLMMs with a binary outcome indicative of an ACT or C-ACT score ≤ 19 (poorly controlled) versus a score > 19 (controlled, set as the reference group). In the analysis of our symptom questionnaire, we fit GLMMs for this dichotomized outcome variable indicating whether the caregiver provided an affirmative response (any symptom days) to any of four questions regarding asthma symptoms in the prior 2 weeks. In all outcome models, we specified fixed effects for a single predictor of interest (a given urinary phthalate metabolite or $\sum$DEHP) and all model covariates, and we specified a random intercept for participants.

Three separate models were fit for each of the twelve separate exposure variables (eleven individual natural log-transformed urinary phthalate metabolites and natural log-transformed $\sum$DEHP) and the respiratory outcomes: (1) a crude model, (2) a minimally adjusted model, and (3) a fully adjusted model (primary). Participant and demographic factors were selected *a priori* as potential confounder or precision variables with respect to the relationship being modeled (a general hypothesized directed acyclic graph (DAG) is presented in Figure S1).

The crude models consisted of only the natural log-transformed urinary phthalate metabolite (or natural log-transformed $\sum$DEHP) as the independent variable and the respiratory outcome as the dependent variable, with specific gravity as a covariate. The minimally adjusted models included sociodemographic covariates potentially associated with asthma outcomes, namely age (Ko et al., 2014), sex (Fu et al.,

Table 1
Study population and household characteristics at baseline.

Characteristic	N (%)		
	All	Atopy (N = 77)[d]	
	(N = 79)	No (N = 29)	Yes (N = 48)
Sex (female)	29 (36.7%)	12 (41.4%)	15 (31.2%)
Age at enrollment[a]	9.0 (2.1)	8.40 (2.0)	9.41 (2.2)
Ethnicity[b]			
Hispanic/Latino	77 (100%)	28 (100%)	47 (100%)
BMI (kg/m^2)[a,c]	20.9 (5.5)	19.9 (5.5)	21.6 (5.5)
BMI-for-age percentile at enrollment[a,c]	79 (26)	79 (22)	78 (28)
Baseline controller medication[b]	69 (89.6%)	25 (89.3%)	42 (89.4%)
HAPI intervention group[c]	38 (50%)	14 (50%)	22 (47.8%)
Primary household language			
English	18 (22.8%)	4 (13.8%)	13 (27.1%)
Spanish	61 (77.2%)	25 (86.2%)	35 (72.9%)
Annual household income[b]			
<$14,999	12 (15.6%)	4 (14.3%)	8 (17.0%)
$15,000-$29,999	33 (42.9%)	15 (53.6%)	16 (34.0%)
$30,000-$60,000	27 (35.1%)	9 (32.1%)	18 (38.3%)
>$60,000	5 (6.5%)	0 (0%)	5 (10.6%)

[a] Mean (SD).
[b] (N = 77).
[c] (N = 76).
[d] Missing values may preclude 100% summation.

2014), and HAPI intervention status. HAPI intervention status was coded as a binary variable to indicate whether or not a given observation had received an air cleaner at that time point. This study design included measures for the intervention group participants at two time points prior to receiving the air cleaner and two time points after, while the control participants had measures at all time points without an air cleaner present. The fully adjusted models added additional asthma-related factors: baseline clinic visit body mass index (BMI)-for-age percentile (Ahmadizar et al., 2016) (continuous percentile generated from CDC growth charts (Ogden et al., 2002)), atopy (Forno and Celedón, 2012), meteorological season as a nominal variable with four levels: (i) March, April, May, (ii) June, July, and August, (iii) September, October, November, and (iv) December, January, and February (Forno and Celedón, 2012), annual household income (as an ordinal variable with four levels: (i) < $14,999, (ii) $15,000-$29,999, (iii) $30,000-$60,000, and (iv) > $60,000) (Louisias and Phipatanakul, 2017), baseline assessment of controller medication utilization (yes/no), visit number (i.e., of the 4 time points possible), and in the primary models estimating effects on FENO only, we also included measurement time of day as a covariate. To examine potential effect modification of atopy in FENO models, we conducted an additional analysis including an interaction term. LMM β-estimates were interpreted as percent change in outcomes corresponding to a 100% increase in predictors of interest via the equation: $(((2^{\beta\text{-estimate}})-1)*100)$.

In supplementary analyses, we assessed the effect of the HAPI intervention (HEPA air cleaners) on urinary phthalate metabolite concentrations via (i) tabulating urinary phthalate metabolite distributions among households with and without air cleaners and (ii) estimating the association between air cleaner status and phthalate exposures. We carried out the latter by fitting LMMs with natural log-transformed urinary phthalate metabolites as dependent variables (12 separate models), a binary indicator variable for air cleaner status as the predictor of interest, and specific gravity as a covariate to correct for urine dilution. As in primary analyses, models included a by-participant varying random intercept.

All statistical analyses were conducted in R version 3.6.2 (The R Foundation for Statistical Computing), with package "lme4" for LMMs and GLMMs. Analyses were interpreted as statistically significant at p-value < 0.05.

3. Results

A total of 79 HAPI participants were included in the present study (Table 1). The mean (±SD) age at enrollment was 9 years ± 2.1 years, and 29 (36.7%) were female. All were Hispanic/Latino, and 61 (77.2%) came from households where the primary language spoken was Spanish. The mean BMI-for-age percentile at enrollment was 79% ± 26%. Use of controller medication was documented at baseline for 69 participants (89.6%). The number of atopic children was 48 (60.8%). Thirty-eight participants (50.0%) were in the HAPI intervention group, resulting in 65 urine samples collected where/when HEPA air cleaners were present and 216 urine samples collected with no air cleaners. An annual household income level of < $14,999 was reported by 12 households (15.6%), with another 33 (42.9%) at the $15,000-$29,999 level, 27 (35.1%) at the $30,000-$60,000 level, and 5 (6.5%) > $60,000.

A total of 281 urine samples were collected and analyzed for target phthalate metabolites: 54 participants provided 4 urine samples each, 19 provided three samples each, two provided two samples each, and 4 provided one sample each. A total of 77 urine samples were collected at time point 1, 75 at time point 2, 73 at time point 3, and 56 at time point 4. The number of observations of each exposure and outcome variable, including urinary phthalate metabolites, are listed in Table S1 both by visit and in total. Analyte-specific LODs ranged from 0.05 ng/mL for MCINP, MCIOP, and MCPP, to 0.2 ng/mL for MBZP, MECPP, and MEP.

The target phthalate metabolites were detected in 100% of samples, except for MEHP which was detected in 99%. Table 2 presents descriptive statistics of the unadjusted and creatinine-corrected concentrations, as well as comparison data from the National Health and Nutrition Examination Survey (NHANES) for similarly aged children in the U.S. for the years 2015–2016. The unadjusted geometric mean (GM) (geometric SD (GSD)) concentrations ranged from 1.4 ng/mL (2.6 ng/mL) for MEHP to 24.7 ng/mL (2.8 ng/mL) for MEP, while the creatinine-corrected concentrations ranged from 1.4 μg/g creatinine (2.5 μg/g creatinine) for MEHP to 25.4 μg/g creatinine (2.5 μg/g creatinine) for MEP. Overall, creatinine-corrected GMs were slightly lower in our sample compared to NHANES data. Specific gravity-corrected concentrations are shown in Table S3. Pearson correlation coefficients were lowest for MCINP and MEP ($\rho = 0.22$) and generally higher among DEHP metabolites (e.g., MEHHP and MEOHP, $\rho = 0.99$) (Table S2). Table S10 presents overall concentrations by household air cleaner status (i.e., HAPI intervention). Table S11 presents beta estimates for the

Table 2
Distributions of unadjusted and adjusted urinary phthalate metabolite concentrations, with NHANES 2015–2016 comparisons.

Metabolite	Present study[a]		Unadjusted values (ng/mL)								Creatinine-corrected values (μg/g creatinine)							
	LOD (ng/mL)	%>LOD	Present study[a]				NHANES (6–11 years, 2015–2016)[b]				Present study[a]				NHANES (6–11 years, 2015–2016)[b]			
			GM	GSD	50th%	90th%	GM	(95% CI)	50th% (95% CI)	90th% (95% CI)	GM	GSD	50th%	90th%	GM	(95% CI)	50th% (95% CI)	90th% (95% CI)
MBZP	0.2	100%	12.4	3.3	13.0	56.4	10.7	(8.6–13.3)	10.9 (8.4–13.8)	59.2 (41.6–73.2)	12.7	2.8	12.5	47.3	13.8	(11.3–16.8)	12.0 (10.0–15.6)	57.9 (49.0–85.7)
MCINP[c]	0.05	100%	2.3	2.4	2.1	6.5	2.3	(2.0–2.6)	2.4 (2.0–2.8)	6.7 (5.9–8.0)	2.3	2.3	2.2	7.0	2.9	(2.7–3.2)	2.8 (2.5–3.0)	6.7 (5.9–8.3)
MCIOP[d]	0.05	100%	9.5	2.8	8.0	40.9	11.1	(9.2–13.4)	10.2 (8.5–12.0)	51.9 (34.3–71.5)	9.7	2.6	7.9	40.6	14.3	(12.0–16.9)	11.5 (9.6–14.3)	59.6 (39.6–90.4)
MCPP	0.05	100%	2.4	2.5	2.3	7.3	1.8	(1.6–2.0)	1.8 (1.5–2.0)	6.8 (4.7–7.9)	2.5	2.2	2.3	6.6	2.3	(2.1–2.6)	2.2 (1.8–2.6)	6.7 (5.5–7.1)
MECPP	0.2	100%	16.9	2.4	16.9	50.8	14.6	(12.9–16.6)	14.9 (13.3–16.7)	42.5 (35.3–54.8)	17.3	2.1	16.1	45.8	18.9	(17.5–20.4)	17.5 (16.6–19.1)	48.3 (35.6–57.5)
MEHHP	0.1	100%	10.3	2.6	11.0	34.4	8.8	(7.8–9.9)	9.0 (7.9–10.0)	27.5 (20.0–35.9)	10.6	2.1	10.2	26.9	11.3	(10.5–12.3)	10.5 (9.6–11.6)	28.8 (21.7–35.2)
MEHP	0.1	99%	1.4	2.7	1.3	4.9	1.4	(1.3–1.6)	1.3 (1.0–1.6)	4.2 (3.6–4.8)	1.4	2.5	1.4	4.3	1.8	(1.6–2.0)	1.8 (1.6–2.1)	4.7 (4.1–5.2)
MEOHP	0.15	100%	6.8	2.6	7.0	23.0	6.0	(5.4–6.7)	6.1 (5.3–6.8)	19.0 (14.7–21.5)	7.0	2.1	6.7	18.2	7.7	(7.1–8.3)	7.3 (6.6–8.0)	19.4 (14.6–22.9)
MEP	0.2	100%	24.7	2.8	24.3	92.2	24.5	(18.6–32.3)	22.1 (16.3–31.1)	115.0 (70.4–203.0)	25.4	2.5	22.8	75.8	31.6	(25.6–39.0)	26.5 (21.8–33.6)	120.0 (77.8–205.0)
MIBP	0.1	100%	13.5	2.6	14.3	43.1	11.2	(9.3–13.5)	11.6 (10.2–13.7)	39.2 (31.1–52.1)	13.8	2.2	12.8	38.6	14.5	(12.6–16.6)	13.5 (11.9–15.0)	42.5 (28.4–49.6)
MNBP	0.1	100%	15.7	2.5	16.6	44.4	14.4	(13.1–15.8)	15.4 (13.4–17.3)	40.6 (36.0–43.0)	16.1	1.9	16.2	35.7	18.5	(17.4–19.7)	18.6 (17.2–19.8)	40.1 (35.5–44.3)
∑DEHP[e]	NR	NR	35.3	2.5	35.9	108.7	NR	NR	NR	NR	36.2	2.1	33.4	90.1	NR	NR	NR	NR

NR = not reported.

[a] $N = 281$ urine specimens from 79 participants.

[b] Source: CDC. *National Health and Nutrition Examination Survey (NHANES) Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2019* (U.S. Centers for Disease Control and Prevention) (Centers for Disease Control and Prevention, 2019).

[c] MCINP: Mono-(7-carboxy-2-methyloctyl) phthalate (product # MP015, CanSyn Chem. Corp., Toronto, Ontario, Canada) and Mono-(4-methyl-7-carboxyoctyl) phthalate (ring-1,2–$^{13}C_2$, dicarboxyl-$^{13}C_2$,99%) (product # CLM- CLM-10196-1.2, Cambridge Isotope Laboratories, Inc., Andover, MA,USA) were used as the unlabeled and labeled standards in the calibration curve for the quantification of MCNP isomers as a summation. MCINP in this study is compared with MCNP in the NHANES studies.

[d] MCIOP: Mono-(6-carboxy-2-methylheptyl) phthalate (product # MP014, CanSyn Chem. Corp., Toronto, Ontario, Canada) and Mono-(4-methyl-7-carboxyheptyl) phthalate (ring-1,2–$^{13}C_2$, dicarboxyl-$^{13}C_2$,99%) (product # CLM-10192-1.2, Cambridge Isotope Laboratories, Inc., Andover, MA,USA) were used as the unlabeled and labeled standards in the calibration curve for the quantification of MCOP isomers as a summation. MCIOP in this study is compared with MCOP in the NHANES studies.

[e] The four metabolites of DEHP (MECPP, MEHHP, MEHP, and MEOHP) were grouped by summing their individual molar concentrations to produce a single summed DEHP metabolite variable, ∑DEHP. The four metabolite concentrations were each divided by their respective molecular weights (MW), summed, and the sum was multiplied by the average MW of the four metabolites (Zota et al., 2014). ∑DEHP = ((MECPP/308.33) + (MEHHP/294.34) + (MEHP/278.34) + (MEOHP/292.33)) X ((308.33 + 294.34 + 278.34 + 292.33)/4).

Table 3
Summary of respiratory outcome measures.

Respiratory outcome	N[a]	Participants	Summary measures
FENO (ppb)	258	76	22.9 (25.5)[c]
$uLTE_4$ (μg/g creatinine)	274	78	1.43 (0.56)[c]
Caregiver report of symptoms in prior 2 weeks	281	79	
No			119 (42.3%)[b]
Yes			162 (57.6%)[b]
ACT	279	79	
Controlled[d]			204 (73.1%)[b]
Poorly controlled[d]			75 (26.9%)[b]
Percent score (%)[e]			80.3 (12.8)[c]

[a] Number of observations with matched urinary phthalate metabolite concentrations.
[b] Expressed as N (%).
[c] Expressed as mean (SD).
[d] Scores ≤ 19 = poorly controlled.
[e] Score on age-appropriate ACT divided by total possible (27 on C-ACT and 25 on ACT).

association between metabolite concentrations and HAPI air cleaner status. No statistically significant associations were observed.

Table 3 summarizes the respiratory outcomes measures. A total of 258 FENO measurements were available from 76 participants. The mean FENO values were 22.9 ppb ± 25.5 ppb, falling between the "low" and "high" inflammation cut-offs of < 20 ppb and > 35 ppb, respectively (Dweik et al., 2011). A total of 274 $uLTE_4$ measures were available from 78 participants, with a mean of 1.43 μg/g creatinine ± 0.56 μg/g creatinine. Caregiver reports of symptom days in the prior 2 weeks were available from all 79 participants at all time points, with 162 instances (57.6%) of a caregiver reporting symptoms. Results were available from 279 age-appropriate administrations of the ACT from all 79 participants, with 75 (26.9%) resulting in a score indicating poorly controlled asthma. The mean percent score across all ACT administrations was 80.3% ± 12.8%. We tabulated time point-specific summary statistics of covariates and outcomes in Table S13 and exposures in Table S14 and observed no meaningful differential missingness across study time points.

Tables 4a–c present the estimated regression coefficients of urinary phthalate metabolites and respiratory outcomes for the minimally

Table 4b
Estimated regression coefficients from mixed models of urinary phthalate metabolites and standardized ACT score.

Metabolite (ng/mL)[b]	ACT Score[a]			
	Minimally adjusted		Fully adjusted (primary)	
	β	95% CI	β	95% CI
MBZP	0.04	-0.05 – 0.13	0	-0.09 – 0.09
MCINP	0	-0.11 – 0.11	0	-0.11 – 0.11
MCIOP	-0.02	-0.11 – 0.08	0.01	-0.08 – 0.10
MCPP	-0.04	-0.15 – 0.07	-0.04	-0.15 – 0.07
MECPP	0.02	-0.10 – 0.14	-0.03	-0.15 – 0.08
MEHHP	0.03	-0.07 – 0.14	-0.01	-0.11 – 0.10
MEHP	0.02	-0.08 – 0.12	0.01	-0.09 – 0.11
MEOHP	0.04	-0.07 – 0.15	0	-0.11 – 0.11
MEP	0.04	-0.06 – 0.14	0.02	-0.08 – 0.11
MIBP	0.07	-0.04 – 0.17	-0.01	-0.13 – 0.10
MNBP	0.01	-0.10 – 0.13	-0.06	-0.18 – 0.06
∑DEHP[c]	0.03	-0.09 – 0.14	-0.02	-0.14 – 0.09

LMM β-estimates were interpreted as percent change in outcomes corresponding to a 100% increase in predictors of interest via the equation: $((2^{\beta\text{-estimate}})-1)*100$).
[a] Minimally adjusted model covariates: specific gravity, age, sex, intervention; fully adjusted: minimal model plus BMI, atopy, season, income, baseline medication, visit.
[b] Values were natural log-transformed.
[c] The four metabolites of DEHP (MECPP, MEHHP, MEHP, and MEOHP) were grouped by summing their individual molar concentrations to produce a single summed DEHP metabolite variable, ∑DEHP. The four metabolite concentrations were each divided by their respective molecular weights (MW), summed, and the sum was multiplied by the average MW of the four metabolites (Zota et al., 2014). ∑DEHP = ((MECPP / 308.33) + (MEHHP / 294.34) + (MEHP / 278.34) + (MEOHP / 292.33)) X ((308.33 + 294.34 + 278.34 + 292.33) / 4).

Table 4a
Estimated regression coefficients from mixed models of urinary phthalate metabolites and biomarkers of inflammation.

Metabolite (ng/mL)[c]	Biomarker of inflammation							
	FENO (ppb)[a,c]				$uLTE_4$ (ng/mL)[b,c]			
	Minimally adjusted		Fully adjusted (primary)		Minimally adjusted		Fully adjusted (primary)	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
MBZP	0.06	-0.03 – 0.16	0.05	-0.05 – 0.15	**0.15**	**0.11 – 0.20**	**0.15**	**0.10 – 0.20**
MCINP	0.01	-0.09 – 0.11	0.01	-0.09 – 0.11	**0.13**	**0.07 – 0.19**	**0.13**	**0.08 – 0.19**
MCIOP	0.06	-0.02 – 0.14	0.06	-0.02 – 0.15	**0.09**	**0.04 – 0.14**	**0.12**	**0.07 – 0.17**
MCPP	0.08	-0.01 – 0.18	0.09	-0.02 – 0.19	**0.10**	**0.05 – 0.16**	**0.12**	**0.06 – 0.18**
MECPP	**0.11**	**0.00 – 0.21**	**0.12**	**0.01 – 0.23**	**0.16**	**0.10 – 0.22**	**0.18**	**0.12 – 0.25**
MEHHP	0.08	-0.02 – 0.17	0.08	-0.02 – 0.18	**0.15**	**0.10 – 0.20**	**0.17**	**0.12 – 0.23**
MEHP	**0.11**	**0.02 – 0.21**	**0.10**	**0.00 – 0.20**	**0.08**	**0.03 – 0.13**	**0.12**	**0.06 – 0.17**
MEOHP	0.08	-0.02 – 0.18	0.08	-0.02 – 0.18	**0.17**	**0.11 – 0.22**	**0.19**	**0.13 – 0.24**
MEP	0.08	-0.01 – 0.16	**0.09**	**0.00 – 0.18**	**0.13**	**0.09 – 0.18**	**0.13**	**0.08 – 0.18**
MIBP	**0.10**	**0.01 – 0.20**	0.06	-0.05 – 0.17	**0.14**	**0.08 – 0.19**	**0.15**	**0.09 – 0.21**
MNBP	**0.13**	**0.02 – 0.23**	0.11	-0.00 – 0.22	**0.22**	**0.16 – 0.28**	**0.24**	**0.18 – 0.30**
∑DEHP[d]	0.09	-0.01 – 0.19	0.1	-0.01 – 0.21	**0.16**	**0.10 – 0.22**	**0.19**	**0.13 – 0.25**

Bolded values denote statistical significance: $p<0.05$.
LMM β-estimates were interpreted as percent change in outcomes corresponding to a 100% increase in predictors of interest via the equation: $((2^{\beta\text{-estimate}})-1)*100$).
[a] FENO minimally adjusted model covariates: specific gravity, age, sex, intervention; fully adjusted: minimal model plus BMI, atopy, season, income, time of day, baseline medication, visit.
[b] $uLTE_4$ minimally adjusted model covariates: specific gravity, age, sex, intervention; fully adjusted: minimal model plus BMI, atopy, season, income, baseline medication, visit.
[c] Values were natural log-transformed.
[d] The four metabolites of DEHP (MECPP, MEHHP, MEHP, and MEOHP) were grouped by summing their individual molar concentrations to produce a single summed DEHP metabolite variable, ∑DEHP. The four metabolite concentrations were each divided by their respective molecular weights (MW), summed, and the sum was multiplied by the average MW of the four metabolites (Zota et al., 2014), as follows: ∑DEHP = ((MECPP / 308.33) + (MEHHP / 294.34) + (MEHP / 278.34) + (MEOHP / 292.33)) X ((308.33 + 294.34 + 278.34 + 292.33) / 4).

Table 4c
Odds ratios (OR) between urinary phthalate metabolites and caregiver symptom report in prior 2 weeks.

Metabolite (ng/mL)[b]	Caregiver report of symptoms in prior 2 weeks[a]			
	Minimally adjusted		Fully adjusted (primary)	
	OR	95% CI	OR	95% CI
MBZP	0.91	0.68 – 1.21	0.98	0.70 – 1.37
MCINP	1.26	0.88 – 1.82	1.34	0.89 – 1.99
MCIOP	**1.37**	**1.00 – 1.86**	1.23	0.86 – 1.74
MCPP	1.05	0.74 – 1.51	1.00	0.66 – 1.50
MECPP	1.20	0.83 – 1.75	1.13	0.76 – 1.69
MEHHP	1.03	0.73 – 1.45	0.96	0.65 – 1.43
MEHP	1.12	0.81 – 1.55	1.02	0.72 – 1.45
MEOHP	1.04	0.74 – 1.47	0.98	0.66 – 1.46
MEP	0.82	0.60 – 1.11	0.91	0.64 – 1.29
MIBP	0.82	0.58 – 1.17	1.00	0.64 – 1.55
MNBP	1.01	0.70 – 1.48	1.15	0.76 – 1.73
∑DEHP[c]	1.13	0.78 – 1.63	1.07	0.70 – 1.63

Bolded values denote statistical significance: $p<0.05$.

[a] Minimally adjusted model covariates: specific gravity, age, sex, intervention; fully adjusted: minimal model plus BMI, atopy, season, income, baseline medication, visit.

[b] Values were natural log-transformed.

[c] The four metabolites of DEHP (MECPP, MEHHP, MEHP, and MEOHP) were grouped by summing their individual molar concentrations to produce a single summed DEHP metabolite variable, ∑DEHP. The four metabolite concentrations were each divided by their respective molecular weights (MW), summed, and the sum was multiplied by the average MW of the four metabolites (Zota et al., 2014). ∑DEHP = ((MECPP / 308.33) + (MEHHP / 294.34) + (MEHP / 278.34) + (MEOHP / 292.33)) X ((308.33 + 294.34 + 278.34 + 292.33) / 4).

adjusted and fully adjusted primary models (Tables S4 – S7 include results from the crude models). Log FENO concentrations generally increased as log urinary phthalate metabolite concentrations increased, although not all associations were statistically significant (Table 4a). For a 100% (i.e., a doubling) increase in MECPP, MEHP, and MEP, estimated concentrations of FENO increased by 8.7% (95% CI: 0.7–17.3), 7.2% (95% CI: 0.0–14.9), and 6.4% (95% CI: 0.0–13.3), respectively, in the primary models. We did not observe statistically significant interactions by atopy (Table S8).

In the $uLTE_4$ models, all phthalate metabolites (including ∑DEHP) had statistically significant, positive associations (Table 4a). In the primary models, effect sizes ranged from an 8.7% increase in $uLTE_4$ (95% CI: 4.3–12.5) for a 100% increase in MEHP to an 18.1% increase in $uLTE_4$ (95% CI: 13.3–23.1) for a 100% increase in MNBP.

None of the phthalate metabolites were significantly associated with caregiver report of any symptom days in the prior two weeks in the primary models. MCIOP was marginally significant in the minimally adjusted model (odds ratio (OR) 1.37 (95% CI: 1.00–1.86)) (Table 4c). No urinary phthalate metabolites were significantly associated with standardized ACT score (Table 4b) or dichotomized ACT outcome (Table S12).

4. Discussion

In this longitudinal study of repeat measures of phthalate exposure and respiratory outcomes in a population of immigrant Latino children with asthma in a rural, agricultural community, we found that urinary phthalate metabolite concentrations were associated with objective biomarkers of inflammation (FENO, $uLTE_4$) that have been linked with asthma exacerbation in children (Dweik et al., 2011; Hoffman and Rabinovitch, 2018). In contrast, we did not observe associations with caregiver report of symptoms in the prior 2 weeks, or with standardized ACT scores, a composite measure based on child and/or caregiver report of asthma related symptoms and disruptions in the prior month.

The cohort represents a relatively understudied group (rural Latino children with asthma). Exposures assessed based on urinary phthalate metabolite concentrations in this cohort were comparable or lower to those reported for a representative sample of U.S. children of similar age in the NHANES populations from 2015 to 2016 (Table 2) (Centers for Disease Control and Prevention, 2019). Unadjusted phthalate metabolite GMs for the study cohort were just slightly above the 95% confidence interval for NHANES GMs for four metabolites (MCPP, MECPP, MEHPP, MEOHP), whereas most of the phthalates assessed as adjusted for creatinine had GMs that were just below the 95% confidence interval for NHANES GMs. Median phthalate levels in our study were lower across all 11 metabolites compared to those of younger participants in the Toddlers Exposure to SVOCs in Indoor Environments (TESIE) Study in North Carolina (N = 180) (Hoffman et al., 2018), and we observed lower concentrations of MEP and MBP but slightly elevated MIBP concentrations compared to older female participants in The Health and Environmental Research on Makeup of Salinas Adolescents (HERMOSA) Study (Berger et al., 2019), a cohort of Mexican American adolescents in California's Salinas Valley (N = 100).

Few studies to date have evaluated associations between urinary phthalate metabolites and FENO. The first was a cross-sectional analysis of 244 children (mean age 6.5 years, SD: 1.3) of Dominican and African American mothers living in the highly urban environment of New York City, U.S. (Just et al., 2012). This study, not conducted exclusively among children with asthma, reported positive associations between MEP, MNBP, and MBZP and FENO, similar to our observation of MEP and MNBP as significant predictors of FENO. A more recent longitudinal study of 56 children (mean age 8.6 years ± 2.5 years) with asthma living near Seoul, South Korea, reported positive associations between MEHHP and MEOHP and FENO (Kim et al., 2018). Although these specific DEHP metabolites were not significantly associated with FENO in our study, we did observe statistically significant associations across FENO models with DEHP's primary metabolite, MEHP. The Korean study did not measure MEHP.

DEHP and its metabolite MEHP are among the most well-studied phthalates with respect to adverse allergy and asthma outcomes, and animal studies primarily in BALB/c mice suggest DEHP can induce eosinophilic immune responses and act as an adjuvant in allergic airway reactions (Bølling et al., 2020; Robinson and Miller, 2015). Eosinophilic airway inflammation is a characteristic of atopic asthma (Comberiati et al., 2017), motivating our assessment of interaction between concentrations of phthalate metabolites and atopy in the association with FENO vs. our other outcome measures. We did not observe a significant interaction, although our sample size limited power for assessment across strata. The study in New York City children also reported no interaction between atopy (as assessed by serum specific immunoglobulin E (IgE)) and phthalate metabolites with FENO. Phthalates are associated with increased release of immune mediators related to eosinophilic airway inflammation (Bølling et al., 2020), but potential mechanisms linking exposure to elevated FENO are not well understood and eosinophilic airway responses may occur in non-allergic individuals with asthma (Brusselle et al., 2013). Phthalates increase production of TNF-α (Lee et al., 2011; Rakkestad et al., 2010), a proinflammatory cytokine known to upregulate inducible nitric oxide (NO) synthase (iNOS), the enzyme responsible for NO synthesis from L-arginine in the respiratory tract (Hoyte et al., 2018). Phthalates also increase expression of IL-4 and act as adjuvants in expression of IL-13 (He et al., 2013; Lee et al., 2011), both T helper 2 (Th2) inflammatory cytokines thought to drive increases in FENO (Hoyte et al., 2018).

Although $uLTE_4$ has been utilized as an informative asthma outcome measure (Hoffman and Rabinovitch, 2018), we are not aware of any epidemiologic studies examining its relationship with phthalate exposure. Our consistent findings of significant associations between all urinary phthalate metabolites tested and $uLTE_4$ support the relevance of this biomarker of effect in studies of phthalate exposure and childhood outcomes related to inflammatory processes. Animal models demonstrate MEHP's influence on the lipoxygenase pathway and leukotriene release in rat alveolar macrophages, however further study is needed to

explicate this relationship in pediatric asthma (Rakkestad et al., 2010). LTE_4 synthesis occurs through arachidonic acid metabolism via the 5-lipoxygenase enzymatic pathway (Hoffman and Rabinovitch, 2018), and *in vitro* and *ex vivo* studies suggest phthalates may be involved in this process as demonstrated by their ability to increase release of arachidonic acid (Oh and Lim, 2011) and their interplay with the lipoxygenase pathway (Rakkestad et al., 2010). Multi-pronged assessments of airway and systemic inflammation via diverse biomarkers may assist in understanding the underlying pathophysiological relationships between phthalates and asthma outcomes. While FENO is interpreted as an indicator of eosinophilic airway inflammation related to allergic asthma, $uLTE_4$ is a more generalized biomarker of inflammation.

The significant associations we found between urinary phthalate metabolites and the inflammatory biomarkers FENO and $uLTE_4$ contrasted with the null associations we found with caregiver- and self-reported symptom reports. This could be due to several factors. Questionnaire data are vulnerable to potential errors in recall and individual differences in recognition of symptoms and disruption, which may vary by respondent and by time of assessment. In addition, the recall periods of symptoms comprise experience over two weeks (symptom day reports) and four weeks (ACT) prior to urinary biomarker concentrations derived from a single urine sample. Urinary phthalate metabolites generally reflect exposures in the past 24 h (Wang et al., 2019), and temporal reliability is moderate, with intraclass correlation coefficients (ICCs) for spot urine samples varying widely between individual metabolites (Johns et al., 2015). Our symptom-related outcomes that summarize asthma experience in the previous 2–4 weeks may be less sensitive to the influence of phthalate exposures assessed as a single measure at the end of that time period.

There are additional limitations to our study. In using urinary biomarkers to represent total phthalate exposures, we cannot provide insight on exposure routes or biologically active doses. The significance of individual routes of phthalate exposure (e.g., inhalation vs. ingestion) with respect to asthma pathogenesis and exacerbation is not understood (Bølling et al., 2020), and there could be links between routes due to the potential for inhaled phthalates to be ultimately ingested after mucociliary clearance from the lung. Measures of parent phthalate concentrations in indoor media such as air, house dust, and building materials (Hammel et al., 2019), and detailed dietary data and information on personal care product use are needed to elucidate the influences of specific exposure routes on asthma-related health impacts. Furthermore, as noted, our exposure assessment may have been temporally mismatched to the questionnaire-based outcome assessments.

Our statistical approach analyzed each phthalate metabolite as a predictor in separate regression models. Thus, we report on multiple statistical comparisons tested, and do not consider exposure to phthalate mixtures (e.g., through aggregate analyses such as weighted quantile sums (Carrico et al., 2015)). However, metabolite-specific models are widely used, provide important phthalate-specific data, and do not rely on assumptions of similar biological activity across phthalates and their metabolites (Johns et al., 2015), including directional homogeneity or linear and/or additive effects.

We attempted to control for unmeasured confounding due to indoor air contaminants such as PM, based on whether an outcome was assessed when a HEPA air cleaner was in the home or not. HEPA air cleaners were shown in the HAPI study to effectively reduce PM with aerodynamic diameter < 2.5 μm ($PM_{2.5}$) in the children's bedrooms by approximately 50%, using baseline and one year follow-up 14-day integrated samples (Riederer et al., 2021). We did not have repeated measures of indoor $PM_{2.5}$ concurrent with each urinary specimen, however, we evaluated the potential for confounding using the 14-day measurements. We observed largely negative correlations between $PM_{2.5}$ and urinary phthalate metabolite concentrations in this study (Table S9), and overall the correlations were low (-0.2 – 0.16). We also found weak correlations between 14-day integrated PM_{10} and $PM_{10\text{-}2.5}$ (assessed contemporaneously with $PM_{2.5}$) and urinary phthalates, reducing our concern about meaningful residual confounding after controlling for the intervention.

There are several strengths of our study. Few studies of phthalate exposure and asthma have been conducted in rural settings where asthma morbidity may be comparable or worse than for urban counterparts and for which exposure to phthalates has not been well characterized (Karr, 2012; Loftus et al., 2015; Rosser et al., 2014; Valet et al., 2009). Yakima County, Washington is a rural area of industrial scale agriculture with both elevated child poverty and asthma hospitalization rates compared to the rest of Washington and the U.S. (CDC, n.d.; Loftus et al., 2015; WA DOH, 2013). This is also the first study to our knowledge to use repeated measures of both phthalate exposure and asthma morbidity in a rural population, and measures of $uLTE_4$, a relatively novel and promising biomarker of airway inflammation that is sensitive to subclinical airway changes (Hoffman and Rabinovitch, 2018). Last, our repeated measures design conducted in a cohort of children with asthma provides insight on the relationship between phthalate exposure and asthma exacerbation – an area of inquiry with scant human data (Just et al., 2012; Kim et al., 2018). To date, research on phthalates and asthma has almost exclusively focused on the occurrence of disease (e.g. asthma/no asthma) as the primary outcome.

5. Conclusions

We provide novel evidence to understand associations between urinary phthalate metabolites and asthma exacerbation with a strong repeated measures design, utilizing a suite of measures commonly used to assess asthma status among children with asthma. The goal of childhood asthma treatment is control of symptoms and reduction in disruptions in sleep, school, and activities, as well as control of the underlying pathophysiological processes, namely inflammation (Reddel et al., 2009). Short term changes in environmental conditions may trigger the inflammatory response, subsequent symptoms, and perceptions of disease. Our study adds to limited data on the role of phthalates in asthma exacerbations (Kim et al., 2018). Additional studies characterizing phthalate exposure and its association with respiratory outcomes in children with asthma are needed. Future studies that incorporate well-characterized co-exposures to common allergens in order to investigate potential adjuvant effects (Strassle et al., 2018) would further advance this field, as would studies well-powered to assess effect modification in subgroups (e.g., atopy, sex). Additional biomarkers of effect may assist in elucidating potential mechanisms, such as oxidative stress, a well-characterized factor in asthma responses (Sahiner et al., 2018), which may play a role in the adjuvant pathway of phthalate-induced asthma responses (You et al., 2014), and more generally in the toxic effects of phthalate exposures that are related to asthma (North et al., 2014).

Institutional Review Board statement

This study was approved by the Institutional Review Board of the University of Washington (UW) Human Subjects Division and the YVFWC Research Review Committee approved the HAPI study protocol, and consenting participants signed Health Insurance Portability and Accountability Act (HIPAA) forms.

Data availability statement

Partial datasets generated and analyzed during the current study are available in the National Institute of Environmental Health Science's (NIEHS) Human Health Exposure Analysis Resource (HHEAR) public data repository, https://hhearprogram.org/

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Supplementary data

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Polybrominated diphenyl ethers in early pregnancy and preterm birth: Findings from the NICHD Fetal Growth Studies

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ABSTRACT

Background: Studies suggest associations between exposure to individual polybrominated diphenyl ethers (PBDEs) with preterm birth (PTB) and shorter gestational age. Little is known about exposure to PBDE mixtures and these outcomes. We evaluated associations of multiple PBDEs in early pregnancy with gestational age at delivery and PTB.

Methods: Data were collected from 2046 women without obesity and 396 women with obesity from the NICHD Fetal Growth Studies, who had early pregnancy plasma PBDEs concentrations and gestational age at delivery. PTB was defined as < 37 weeks of gestation at delivery and further categorized into subtypes (late or very early/moderate; spontaneous or medically indicated). We applied (1) generalized linear models (GLM); (2) principal component analysis (PCA); and (3) Bayesian Kernel Machine Regression (BKMR) to evaluate the individual and joint associations of log-transformed PBDE concentrations with gestational age at delivery and PTB, adjusting for potential confounders and evaluating effect modifiers.

Results: In GLM analyses, a 1-standard deviation (SD) increase in log-PBDE 153 was associated with shorter gestational age at delivery [adjusted β (95% CI) = −0.19 (−0.31, −0.06) weeks] among women without obesity. In PCA analyses, 1-SD increase in the principal component summarizing most of PBDE 153 variability was associated with shorter gestational age at delivery [adjusted β (95% CI) = −0.18 (−0.30, −0.06) weeks], very early/moderate PTB [adjusted OR (95% CI) = 1.91 (1.19, 3.07)], and spontaneous PTB [adjusted OR (95% CI) = 1.34 (1.00, 1.80)] among women without obesity. Associations were stronger among non-Hispanic Black women, women with BMI ranging between 25 and 30 kg/m^2, and women who were ≥35 years old among those without obesity. In BKMR analyses, a suggestive inverse association between PBDE 153 and gestational age at delivery, and an inverse U-shaped association between PBDE 154 and gestational age at delivery were observed in women without obesity. No statistically significant association of PBDEs and gestational age or PTB was observed among women with obesity.

Conclusions: PBDEs, specifically PBDE 153, were associated with shorter gestation and higher risk of certain PTB subtypes among pregnant women without obesity.

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1. Introduction

Preterm birth (PTB), defined as delivery prior to 37 weeks of gestation, affects 9–10% of pregnancies in the U.S. (Martin and Osterman, 2018), and is a strong predictor of neonatal mortality and morbidity (Behrman and Butler, 2007). PTB is also associated with an increased risk of maternal morbidity and mortality, as well as adverse health outcomes later in life in both mothers (such as increased risk of type 2 diabetes and cardiovascular disease) (Henderson et al., 2016; Wu et al., 2018) and their offspring (such as developmental disabilities, asthma, and metabolic syndromes) (Parkinson et al., 2013; Saigal and Doyle, 2008; Sonnenschein-van der Voort et al., 2014). In addition, recent studies suggest a gradient of increasing risk of poorer outcomes with decreasing gestational age at delivery instead of a fixed threshold for PTB on perinatal mortality and morbidity (Boyle et al., 2012; Zhang and Kramer, 2009). Although certain factors, such as prior PTB, multiple pregnancy, age, maternal comorbidities (e.g. hypertension, diabetes), psychosocial stress, and tobacco smoking, are established risk factors for PTB (Behrman and Butler, 2007), there remains a large proportion of cases with unknown etiology (Ferrero et al., 2016). Thus, further identification of contributors to shortened gestational age at delivery is needed, such as exposure to environmental chemicals with endocrine disrupting properties (Ferguson et al., 2013).

Polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs) are environmentally persistent polyhalogenated organic compounds that are ubiquitously distributed due to their use as flame retardants in a variety of consumer products, including electronic equipment, furniture, textiles, and small appliances (Eskenazi et al., 2013). Since PBDEs are semi-volatile and not chemically bound to a substrate, they can leach from products and become environmental contaminants (Darnerud et al., 2001). Exposure to PBDEs in the general population occurs through inhalation of contaminated air, ingestion of contaminated water and food, indoor dust, as well as dermal contact (Johnson-Restrepo and Kannan, 2009; Stapleton et al., 2005). PBDEs can bioaccumulate in the body, with long half-lives ranging from 2 to 12 years (Chevrier et al., 2010). Exposure to PBDEs have been associated with a range of adverse perinatal outcomes such as altered thyroid hormone levels in pregnant women and infants (Herbstman et al., 2008) and low birth weight (Harley et al., 2011; Zhao et al., 2017). Potential mechanisms linking PBDEs to PTB are supported by the results of *in vitro* studies, where PBDEs were linked with biomarkers indicating altered placental development during mid-gestation (Varshavsky et al., 2020a), with oxidative stress and pro-inflammatory mediators (Park et al., 2014; Peltier et al., 2012), and with altered placental DNA methylation (Zhao et al., 2016), which are on pathophysiological pathways leading to both spontaneous and medically-indicated PTB (Farina and Winkelman, 2005; McElrath et al., 2008; Morgan, 2016).

Previous findings from population-based studies on the associations between PBDEs and PTB or gestational age at delivery have been inconsistent. One study found no association between 4 PBDE congeners (PBDE 47, 99, 100, 153) and gestational age at delivery (Harley et al., 2011), while two other studies found higher maternal serum concentrations of PBDE 47 associated with increased odds of PTB (Peltier et al., 2015) and shorter gestational age at delivery (Eick et al., 2020). However, most studies have focused on a few specific PBDE congeners, failing to account for the complex nature of these exposures as a joint exposure to a PBDEs mixture with potential synergistic/antagonistic effects. In addition, previous studies had relatively small sample sizes and under-represent certain racial/ethnic groups, and thus may have inadequate power to detect associations or ascertain racial/ethnic differences, especially given the pronounced racial/ethnic disparities in both PTB (14% in non-Hispanic black compared to 9% in non-Hispanic white women) (Burris et al., 2011, 2019) and exposure levels to PBDEs (e.g. higher median levels of PBDE 47, 99, 100, 153 and 154 found in non-Hispanic black women compared to non-Hispanic white women in a healthy obstetric cohort) (Buck Louis et al., 2018; Nguyen et al., 2020; Varshavsky et al., 2020b) that are disproportionally affecting non-Hispanic black women in the U.S.

In this study, we aimed to prospectively evaluate the associations of exposures to multiple PBDE congeners during early pregnancy with both gestational age at delivery and PTB within a large, multi-center, multiracial/ethnic cohort of U.S. pregnant women. We used three different statistical approaches that accommodated individual effects and mixtures effects. As secondary aims, we evaluated whether these associations differed across race/ethnicity, age, or pre-pregnancy BMI, or by categorizing PTB subtypes based on the length of gestation at delivery and indication of delivery.

2. Methods

2.1. Study population

This study was conducted among women from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies – Singleton Cohort, a multi-center, multiracial prospective study of pregnant women recruited between July 2009 and January 2013 from 12 U.S. clinical sites (Grewal et al., 2018). Women aged 18–40 years with a viable singleton pregnancy were enrolled between 8 and 13 weeks of gestation and followed through delivery. Specifically, two cohorts of singleton pregnancies were included: (1) a cohort of 2334 women with a low-risk pregnancy and without obesity (i.e. pre-pregnancy BMI <30 kg/m^2) across four self-reported race/ethnicity groups (non-Hispanic white, non-Hispanic Black, Hispanic, or Asian/Pacific Islander); and (2) a cohort of 468 women with a low-risk pregnancy and with obesity (i.e. pre-pregnancy BMI ≥ 30 kg/m^2) unselected by race/ethnicity. Both cohorts excluded women with major chronic conditions before pregnancy (autoimmune disease, cancer, chronic hypertension, chronic renal disease, diabetes, HIV/AIDS, or psychiatric disorders). In addition, the non-obese cohort further excluded several other conditions or comorbidities (shown in Supplemental Fig. S1). Further details of the study can be found elsewhere (Grewal et al., 2018; Zhang et al., 2018).

For this analysis, we further restricted the study population to women who: (1) remained eligible for study participation after enrollment and consented to having their biospecimen used for research; (2) provided first trimester blood samples with available measurement of 10 brominated chemicals (PBB 153; PBDE 28, 47, 85, 99, 100, 153, 154, 183, and 209); (3) had data on gestational age at delivery; and (4) had a live birth. The final study population consisted of 2046 women without obesity and 396 women with obesity. All analyses were conducted separately in the non-obese and obese cohorts because chemical assays were performed at separate times and locations for each of these cohorts, and the cohorts had different eligibility/exclusion criteria that may reflect different demographic, lifestyle, and health conditions. For all statistical analyses evaluating the associations of PBDEs with gestational age at delivery and PTB, we further excluded observations with PBDEs concentrations greater than 5 standard deviations from the mean values to avoid potential distortion from extreme values ($n = 38$ in women without obesity; $n = 6$ in women with obesity). A detailed flow diagram of the inclusion and exclusion criteria is provided in Supplemental Fig. S1.

2.2. Blood collection and PBDE quantification

Concentrations of 1 polybrominated biphenyl (PBB 153) and 9 PBDEs (PBDE 28, 47, 85, 99, 100, 153, 154, 183, and 209) were measured from blood specimens, which were collected at enrollment (median: 11 weeks of gestation) for both cohorts. Samples were processed immediately after collection and stored at -80 °C until analysis. Analysis was performed at the Wadsworth Center, New York State Department of Health (for samples from the non-obese cohort) and NYU Langone (for samples from the obese cohort) based on an isotope

dilution method described previously using a gas chromatograph (GC 7890A, Agilent Technologies, Atlanta, GA) coupled with a high resolution mass spectrometer (MSD 5975 as well as JEOL800D) as previously discussed in detail (Ma et al., 2014). All chemical concentrations were reported as ng/mL plasma. The limits of quantification (LOQs) varied by congener, ranging between 0.0025 and 0.01 ng/mL for both the obese and non-obese cohorts. Machine-observed values were used for all chemicals in the analysis without substitution, including concentrations below the LOQ, as this has been shown to yield the least biased estimates compared to simple imputations (Schisterman et al., 2006). In addition, plasma lipids were measured from non-fasting blood samples collected at enrollment (Bao et al., 2018). Lipids were quantified using commercially available enzymatic methods as described in previous studies (Akins et al., 1989).

2.3. Outcome measurement

The two primary study outcomes of interest were: (1) gestational age at delivery (weeks), calculated as the difference between date of delivery and ultrasound validated self-reported date of first day of last menstrual period (LMP); and (2) a binary outcome of PTB, defined as delivery prior to 37 weeks of gestation. Delivery dates were abstracted by trained staff from the medical records. Only those with consistent self-reported LMP dates and ultrasound-estimated dates (i.e. within 5 days for those from 8^{+0} to 10^{+6} weeks, within 6 days for those from 11^{+0} to 12^{+6} weeks, and within 7 days for those from 13^{+0} to 13^{+6} weeks) were enrolled (Skupski et al., 2017).

Secondary study outcomes of interest included: (1) PTB further categorized as "spontaneous/inflammatory" or "medically indicated/placental dysfunction" based on prior classifications (McElrath et al., 2008) and (2) PTB further categorized as very early (<32 weeks), moderate (32 to < 34 weeks), or late preterm (34 to < 37 weeks) (Ananth et al., 2013) based on gestational age at delivery. Spontaneous/inflammatory PTB was defined by the absence of a maternal or fetal indication/intrauterine growth restriction (IUGR) and included preterm labor, preterm premature rupture of membranes, cervical insufficiency, or placental abruption. Medically indicated/placental dysfunction PTB was characterized by infarcts and increased placental syncytial knots with the relative absence of inflammation and included maternal indications (such as preeclampsia, hypertension, or diabetes), or fetal indications (such as IUGR, non-reassuring fetal testing, or anomalies) (James-Todd et al., 2018).

2.4. Covariates

Based on *a priori* knowledge, the following variables were selected as potential confounders: maternal age, self-reported race/ethnicity, pre-pregnancy BMI, parity, education level, marital status, family income during last year, plasma cotinine level, plasma total lipids, total activity, sedentary activity, and acculturation status. Information about socio-demographic, lifestyle and reproductive factors were collected from a standardized questionnaire at baseline. Acculturation status in this analysis was classified as U.S.-born, recent immigrant (defined as lived in the U.S. for less than 10 years), or long-term immigrant (defined as lived in the U.S. for ≥ 10 years) based on previous publications (Mitro et al., 2019). Maternal weight and height were measured by trained research staff at enrollment using standardized methods (Grewal et al., 2018). Pre-pregnancy BMI was calculated from self-recalled pre-pregnancy weight divided by measured height squared (Pugh et al., 2017). Total plasma lipids (in ng/mL) were calculated using the following equation described elsewhere: total lipids = (total cholesterol × 2.27) + triglycerides + 62.3 (Bernert et al., 2007; Phillips et al., 1989). Plasma cotinine levels (in ng/mL) were measured in specimens collected at enrollment using an ultraperformance liquid chromatography coupled with an electrospray triple quadrupole tandem mass spectrometry (Buck Louis et al., 2018).

2.5. Statistical analysis

Baseline characteristics of the non-obese and obese cohorts were reported in the form of mean ± standard deviation (SD) for continuous variables or number (percentage) for categorical variables, in the overall cohorts as well as stratified by PTB (yes/no). Distributions of each of the 10 chemicals were summarized with number (percentage) above the LOQ and lipid-standardized geometric means (95% confidence intervals) and lipid-standardized medians (interquartile ranges). Molar sum of the congeners was calculated by dividing each chemical concentration by its molecular weight and summing all detectable concentrations. Total PBDEs was calculated using the sum of the detectable concentrations of all PBDE congeners. Chemicals that had quantification rates >30% in each cohort were included in the main analyses. Correlations between the chemical concentrations were assessed using Spearman correlation coefficients.

For all analytical models measuring the associations between PBDEs and gestational age at delivery (weeks) or PTB (yes/no), we natural log-transformed the machine-observed values of the chemical concentrations to account for skewedness of their distributions, using ln (1 + concentration) transformation for PBDE congeners 28, 100, 153, 154 and 183, and ln (5 + concentration) for PBDE congeners 47 and 99 to ensure positive minimum values. Log-transformed chemicals were then standardized (subtracted the mean and divided by the SD) to generate comparable scales. We did not include lipid-standardized chemical concentrations in the models when analyzing the associations between PBDEs and gestational age at delivery or PTB. Instead, we adjusted for plasma total lipids as a covariate in the model, as a simulation study showed this to provide the less biased results (Schisterman et al., 2005). All models were adjusted for the following covariates: maternal age (years), race/ethnicity (non-Hispanic white, non-Hispanic Black, Hispanic, Asian/Pacific Islander), pre-pregnancy BMI (kg/m^2), parity (0, 1, 2+), education level (< college degree, some college/undergraduate, graduate/post-graduate), marital status (married or living with partner, not married), family income during last year (<$30,000, $30,000-$49, 999, $50,000-$99,999, ≥ $100,000, not reported), plasma cotinine level (ng/mL), plasma total lipids (ng/mL), total activity (MET hours/week), sedentary activity (MET hours/week), and acculturation (U.S.-born, recent immigrant, long-term immigrant). We utilized three approaches to evaluate the associations between PBDEs and gestational age at delivery (weeks) or PTB (yes/no): (1) generalized linear model regression (GLM) approaches; (2) GLM combined with principal component analysis (PCA) to identify a reduced set of exposure factors; and (3) Bayesian Kernel Machine Regression (BKMR), as described below.

First, we used multivariable linear regression models to identify the association between each individual PBDE congener and gestational age (weeks) at delivery. Beta coefficients (95% CI) for one standard deviation (SD) increase in each log-transformed PBDE congener concentration were calculated in three models: (1) crude; (2) adjusted for the above-mentioned covariates; and (3) mutually adjusted (i.e. further adjusted for all the other PBDE congeners). Likewise, we used multivariable logistic regression models to evaluate the individual associations between PBDEs and odds of PTB. Odds ratios (OR) and 95% CIs of PTB for a one-SD increase in each log-transformed PBDE congener concentration were calculated in the crude, adjusted, and mutually adjusted models.

Second, we used PCA as a dimension reduction approach, an unsupervised method to reduce the number of correlated variables to a smaller number of uncorrelated principal components (PCs) that explain most of the variability of the data (Chiu et al., 2018). In this study, we used PCA with varimax rotation and identified four PCs that cumulatively explained >90% of variability of the PBDE congeners. We then fitted a multivariable linear regression and a multivariable logistic regression model of gestational age at delivery and PTB as outcomes, respectively, using the selected PCs as exposure measures, adjusted for the above-mentioned covariates. As secondary analyses, we also evaluated the associations between the PCs and PTB further categorized as

spontaneous or medically indicated (compared to non-preterm), as well as further categorized as very/moderate preterm or late preterm (compared to non-preterm), using multinomial logistic regression models in the non-obese cohort only (due to insufficient numbers in the obese cohort). In addition, we assessed potential effect measure modification via stratifying the multivariate linear regression models by race/ethnicity (non-Hispanic white, non-Hispanic Black, Hispanic, Asian/Pacific Islander) or maternal age (<35 vs. ≥ 35 years) in the non-obese and obese cohorts, and by pre-pregnancy BMI (<25 vs. ≥ 25 kg/m^2) in the non-obese cohort only. P-for-interactions were obtained via including interactions terms of the principal components and the proposed modifier(s), and p-for-interactions < 0.10 were conventionally considered as statistically significant.

Third, we applied BKMR to evaluate the associations between PBDE mixtures and gestational age at delivery. BKMR is a flexible approach that utilizes a kernel function that allows for non-linear estimation of individual exposure-outcome relationships for each chemical while taking into account the correlation structure of the multiple chemicals; it also provides intuitive graphical visualization of potential interactions between chemicals (Bobb et al., 2015). We applied the Gaussian kernel and the default tuning parameters for Markov Chain Monte Carlo (MCMC) processes with 50,000 iterations to assess the relative importance of each standardized log-transformed PBDE congener on standardized gestational weeks at delivery based on their posterior inclusion probabilities (PIPs), and qualitatively evaluated potential interactions between the chemicals.

To determine the robustness of the results, we additionally performed several sensitivity/secondary analyses. First, based on *a priori* knowledge of the potential correlations of certain dietary factors with PBDEs concentrations and PTB (Gete et al., 2020; Schecter et al., 2010), we evaluated the correlations between PBDEs and several dietary factors [including dairy intake, meat from beef/pork/lamb/organ, processed meats, fish/seafood, and a summarized Alternate Healthy Eating Index 2010 (AHEI-2010)] in a subset of the study population who completed a food frequency questionnaire (FFQ) at baseline, and further adjusted for these dietary factors to account for any potential confounding. Second, in the generalized linear models, we examined the associations based on quantiles of the chemical concentrations as predictors of gestational age at delivery to relax the linearity assumptions. Third, given PBDEs were shown to disturb lipid metabolism (Cowens et al., 2015), we conducted a sensitivity analysis without adjusting for plasma lipids to avoid any potential blockage of the causal pathway between PBDEs and gestational age or PTB. Fourth, we conducted survival analyses modelling gestational age at delivery as a time-to-event outcome to evaluate the associations between PBDEs and time to delivery using Cox proportional hazards models. Lastly, we conducted a sensitivity analysis where we

Table 1
Main characteristics of the study population among the non-obese and obese cohorts, total and stratified by gestational age at delivery.

	Non-obese cohort (n = 2046)			**Obese cohort** (n = 396)		
	Total (n = 2046)	**Preterm (GA < 37 weeks)** (n = 122)	**Non-preterm (GA ≥ 37 weeks)** (n = 1924)	**Total** (n = 396)	**Preterm (GA < 37 weeks)** (n = 26)	**Non-preterm (GA ≥ 37 weeks)** (n = 370)
Age (years)	28.3 ± 5.5	28.2 ± 6.0	28.3 ± 5.4	27.9 ± 5.6	29.3 ± 5.3	27.8 ± 5.6
Race/ethnicity						
Non-Hispanic White	563 (27.5)	29 (23.8)	534 (27.8)	122 (30.8)	12 (46.2)	110 (29.7)
Non-Hispanic Black	517 (25.3)	48 (39.3)	469 (24.4)	142 (35.9)	7 (26.9)	135 (36.5)
Hispanic	576 (28.2)	27 (22.1)	549 (28.5)	127 (32.1)	6 (23.1)	121 (32.7)
Asian/Pacific Islander	390 (19.1)	18 (14.8)	372 (19.3)	5 (1.3)	1 (3.9)	4 (1.1)
Pre-pregnancy BMI (kg/m^2)	23.6 ± 3.0	24.2 ± 3.1	23.6 ± 3.0	34.5 ± 3.9	35.9 ± 4.1	34.4 ± 3.8
Parity						
0	1001 (48.9)	68 (55.7)	933 (48.5)	136 (34.3)	8 (30.8)	128 (34.6)
1	703 (34.4)	32 (26.2)	671 (34.9)	133 (33.6)	8 (30.8)	125 (33.8)
2+	342 (16.7)	22 (18.0)	320 (16.6)	127 (32.1)	10 (38.5)	117 (31.6)
Education level						
Less than college degree	561 (27.4)	32 (26.2)	529 (27.5)	151 (38.1)	6 (23.1)	145 (39.2)
Some college or undergraduate	1107 (54.1)	71 (58.2)	1036 (53.9)	213 (53.8)	18 (69.2)	195 (52.7)
Graduate or post-graduate	378 (18.5)	19(15.6)	359 (18.7)	32 (8.1)	2 (7.7)	30 (8.1)
Marital status[a]						
Married or living with partner	1565 (76.6)	86 (70.5)	1479 (77.0)	266 (67.2)	23 (88.5)	243 (65.7)
Not married	479 (23.4)	36 (29.5)	443 (23.1)	130 (32.8)	3 (11.5)	127 (34.3)
Family income during last year						
Less than $30,000	479 (23.4)	28 (23.0)	451 (23.4)	129 (32.6)	6 (23.1)	123 (33.2)
$30,000-$49,999	294 (14.4)	23 (18.9)	271 (14.1)	88 (22.2)	7 (26.9)	81 (21.9)
$50,000-$99,999	464 (22.7)	30 (24.6)	434 (22.6)	93 (23.5)	5 (19.2)	88 (23.8)
$100,000 or more	530 (25.9)	24 (29.7)	506 (26.3)	52 (13.1)	5 (19.2)	47 (12.7)
Unknown	279 (13.6)	17 (13.9)	262 (13.6)	34 (8.6)	3 (11.5)	31 (8.4)
Plasma cotinine (ng/mL)[a]	1.1 ± 12.6	0.6 ± 3.6	1.1 ± 13.0	3.4 ± 19.3	3.3 ± 14.4	3.4 ± 19.6
Plasma total lipids (mg/dL)[a]	609.9 ± 99.2	613.4 ± 104.8	609.7 ± 98.9	607.5 ± 101.7	636.8 ± 97.3	605.5 ± 101.8
Alternative Healthy Eating Index (AHEI-2010) score[a]	51.4 ± 9.7	50.0 ± 9.5	51.5 ± 9.7	49.2 ± 8.7	47.6 ± 8.3	49.3 ± 8.7
Total activity (MET hours per week)[a]	323.1 ± 167.9	351.9 ± 207.7	321.3 ± 165.0	330.8 ± 156.3	268.1 ± 127.4	335.2 ± 157.3
Sedentary activity (MET hours per week)[a]	26.1 ± 18.3	30.4 ± 19.5	25.8 ± 18.2	27.2 ± 18.7	29.0 ± 16.3	27.1 ± 18.9
Acculturation[a]						
U.S.-born	1353 (66.2)	89 (73.0)	1264 (65.8)	327 (82.6)	20 (76.9)	307 (83.0)
Recent immigrant (<10 years)	305 (14.9)	12 (9.8)	293 (15.3)	24 (6.1)	2 (7.7)	22 (6.0)
Long-term immigrant (≥10 years)	385 (18.8)	21 (17.2)	364 (19.0)	45 (11.4)	4 (15.4)	41 (11.1)

Means ± SD for continuous variables. N (%) for categorical variables.
GA = gestational age.
[a] Numbers may not add up to total numbers due to missing values. Variables with missing values (missing rate) included: marital status (0.1%), plasma cotinine (1.6%), plasma total lipids (1.1%), AHEI 2010 score (37.4%), total activity (0.2%), sedentary activity (0.2%), acculturation (0.1%).

included data from the individuals with extreme values of PBDEs (which was excluded in our main analyses).

Data analyses were conducted in SAS version 9.4 (SAS Institute Inc) and R version 4.0.2 (R Foundation for Statistical Computing). P-values < 0.05 were conventionally considered statistically significant unless otherwise specified. R packages used for main analyses included *stats* (for GLMs and PCA) and *bkmr* (for BKMR) (Bobb et al., 2018).

3. Results

Table 1 presents baseline characteristics of the study population in the non-obese (n = 2046) and obese cohorts (n = 396). Study participants in both cohorts predominantly had some college degree or higher, were married or living with a partner, and were U.S.-born. The non-obese cohort consisted of 28% non-Hispanic white, 25% non-Hispanic Black, 28% Hispanic and 19% Asian/Pacific Islander women, while the obese cohort consisted of 31% non-Hispanic white, 36% non-Hispanic Black, 32% Hispanic, and only 1% Asian/Pacific Islander women. The non-obese cohort also had higher percentages of nulliparous women, lower percentages of family income < $30,000, lower mean plasma cotinine levels, as well as lower total and sedentary activities compared to the obese cohort. The percentages of PTB were 6.0% in the non-obese and 6.6% in the obese cohort. The mean gestational ages at delivery and further categorizations of PTB are summarized in Supplemental Table S1.

Table 2 shows the distributions of lipid-adjusted PBDEs concentrations in the non-obese and obese cohorts. Chemicals that had quantification rates >30% in each cohort (PBDE 28, 47, 99, 100, 153, and 154 among those without obesity; PBDE 47, 99, 100, 153, 154, and 183 among those with obesity) were included in the main analyses. In the non-obese cohort, PBDE congeners were detected in plasma samples in the decreasing order of PBDE 47, 100, 99, 153, 154, and 28. In the obese cohort, PBDE congeners were detected in plasma samples in the decreasing order of PBDE 153, 100, 154, 47, 99, and 183. Among the five congeners that had quantification rates >30% in both cohorts, women with obesity had higher geometric means and higher quantification rates compared to women without obesity. The mean molar sum of PBDEs and total PBDEs were also higher in women with obesity than women without obesity. PBDEs concentrations were weakly to highly correlated with each other (Supplemental Fig. S2). Concentrations for most of the PBDEs were lower than the concentrations from a representative sample of women from the U.S. National Health and Nutrition Examination Survey (NHANES) 2005–2014 (geometric means in NHANES for PBDE 28, 47, 99, 100, and 154 were: 1.2, 23.6, 4.7, 7.9 and 0.4 ng/g lipid, respectively), except for higher geometric mean of PBDE 154 in those with obesity (5.7 ng/g lipid, compared to 0.4 ng/g lipid in NHANES) (Sjödin et al., 2019).

3.1. Associations of individual PBDEs with gestational age at delivery and PTB

Table 3 displays the crude, covariate adjusted, and mutually adjusted results from linear regression models for the associations between individual PBDEs and gestational age at delivery. We found that a 1-SD increase in log-PBDE 153 concentration was associated with 0.19-week (~1.3 day) shorter gestational age at delivery in the mutually adjusted model among those without obesity [β (95% CI) = −0.19 (−0.31, −0.06) weeks], while there was no significant association between PBDE 153 and gestational age among those with obesity [β (95% CI) = 0.26 (−0.16, 0.68) weeks]. We did not observe any statistically significant associations of other PBDE congeners or the sum of PBDEs with gestational age at delivery in either cohort.

Results from sensitivity analyses of associations of PBDE concentrations divided into quartiles above the LOQ with gestational age at delivery are presented in Supplemental Table S2. Among those without obesity, we found that the adjusted average gestational age at delivery in the highest quartile of PBDE 153 was 0.34-week shorter than that in the category below the LOQ (p-trend = 0.01). In addition, among those with obesity, we found that the adjusted average gestational age at delivery in the highest quartile of PBDE 183 was 0.91-week shorter than that in the category below the LOQ (p-trend = 0.02).

Table 4 shows the results from logistic regression models for the association between individual PBDEs and the odds of PTB. We did not observe any statistically significant associations of any PBDEs with the odds of PTB in either cohort.

3.2. Principal component analysis of PBDEs with gestational age at delivery and PTB

In each cohort, application of varimax-rotated PCA yielded four principal components (PCs) that cumulatively accounted for >90% of the variability in PBDE concentrations. Loading factors for each chemical in each of the PCs are presented in Supplemental Table S3. The associations of the PCs as predictors of gestational age at delivery (from

Table 2
Distributions of lipid-standardized PBDEs concentrations of the study population among the non-obese and obese cohorts.

Chemicals, ng/g lipid	Non-obese cohort (n = 2046)			Obese cohort (n = 396)		
	> LOQ n (%)	Geometric mean (95% CI)[a]	Median (IQR)[b]	> LOQ n (%)	Geometric mean (95% CI)[a]	Median (IQR)[b]
PBB 153	25 (1.2)	1.5 (1.5, 1.5)	0 (0, 0)	78 (19.7)	0.2 (0.2, 0.2)	0 (0, 0.6)
PBDE 28	749 (36.6)	0.3 (0.2, 0.3)	0 (0, 1.0)	51 (12.9)	0.4 (0.4, 0.5)	0 (0, 0)
PBDE 47	1895 (92.6)	5.6 (5.1, 6.1)	8.7 (3.9, 17.4)	370 (93.4)	6.2 (5.4, 7.3)	7.8 (3.1, 16.1)
PBDE 85	52 (2.5)	0.4 (0.4, 0.4)	0 (0, 0)	0 (0.0)	0 (0, 0)	0 (0, 0)
PBDE 99	1267 (61.9)	0.3 (0.3, 0.4)	2.2 (0, 5.4)	341 (86.1)	2.3 (2.0, 2.5)	2.3 (1.4, 4.5)
PBDE 100	1466 (71.7)	0.9 (0.8, 0.9)	2.2 (0, 4.3)	392 (99.0)	4.4 (4.1, 4.7)	4.7 (3.0, 6.3)
PBDE 153	846 (41.4)	1.2 (1.1, 1.3)	0 (0, 7.0)	393 (99.2)	5.3 (4.8, 5.7)	5.5 (3.0, 8.8)
PBDE 154	781 (38.2)	0.1 (0.1, 0.1)	0.4 (0, 2.8)	378 (95.5)	5.7 (5.3, 6.1)	6.7 (5.0, 8.4)
PBDE 183	4 (0.2)	3.6 (3.6, 3.6)	0 (0, 0)	319 (80.6)	2.2 (1.9, 2.6)	3.3 (0.8, 7.1)
PBDE 209	2 (0.1)	947.2 (940.6, 953.9)	0 (0, 0)	0 (0.0)	0 (0, 0)	0 (0, 0)
Molar sum of PBDEs, pmol/g lipid[c]	/	37.1 (35.3, 39.0)	39.4 (18.5, 79.9)	/	61.0 (57.2, 65.0)	57.7 (39.8, 85.7)
Total PBDEs, ng/g lipid[d]	/	19.8 (18.8, 20.8)	21.2 (9.9, 43.3)	/	35.6 (33.5, 37.9)	34.6 (24.2, 49.3)

Chemicals with >30% detection rate in each cohort (i.e. PBDE 28, 47, 99, 100, 153, and 154 in the non-obese cohort; PBDE 47, 99, 100, 153, 154 and 183 in the obese cohort) were selected for the main analyses, respectively.

[a] Geometric means (95% CI) calculated using all machine-observed values including those below the LOQs, where zero and negative values were assigned the value of (lowest positive value)/2.

[b] Percentiles and median calculated among all machine-observed values including those below the LOQs.

[c] Molar sum of PBDEs (in pmol/g lipid) were calculated by dividing each lipid-standardized chemical concentration by its molecular weight and summing all detectable concentrations.

[d] Total PBDEs (in ng/g lipid) were the sum of the detectable concentrations of all PBDE congeners.

Table 3
Association between each standard deviation increment in log transformed plasma PBDE concentrations and gestational age at delivery (weeks) among the non-obese and obese cohorts.

Plasma PBDEs	Non-obese cohort[a]			Obese cohort[a]		
	Crude β (95% CI) (n = 2008)	Adjusted[b] β (95% CI) (n = 1975)	Mutually adjusted[b,c] β (95% CI) (n = 1975)	Crude β (95% CI) (n = 390)	Adjusted[b] β (95% CI) (n = 356)	Mutually adjusted[b,c] β (95% CI) (n = 356)
PBDE 28	−0.15 (−0.35, 0.05)	−0.12 (−0.33, 0.09)	0.00 (−0.27, 0.28)	/[d]	/[d]	/[d]
PBDE 47	−0.11 (−0.21, −0.01)	−0.07 (−0.18, 0.03)	−0.05 (−0.22, 0.12)	0.06 (−0.33, 0.45)	0.04 (−0.38, 0.45)	0.07 (−0.80, 0.95)
PBDE 99	−0.09 (−0.26, 0.08)	−0.05 (−0.23, 0.12)	−0.02 (−0.22, 0.17)	0.03 (−0.59, 0.65)	0.05 (−0.60, 0.70)	0.20 (−1.20, 1.60)
PBDE 100	−0.14 (−0.26, −0.02)	−0.11 (−0.23, 0.01)	0.01 (−0.19, 0.22)	−0.27 (−0.80, 0.26)	0.01 (−0.55, 0.57)	−0.34 (−1.30, 0.62)
PBDE 153	−0.17 (−0.27, −0.06)	−0.17 (−0.28, −0.06)	−0.19 (−0.31, −0.06)	−0.01 (−0.33, 0.31)	0.19 (−0.14, 0.52)	0.26 (−0.16, 0.68)
PBDE 154	0.05 (−0.04, 0.13)	0.04 (−0.05, 0.13)	0.08 (−0.01, 0.17)	−0.12 (−0.33, 0.09)	−0.21 (−0.43, 0.02)	−0.20 (−0.44, 0.03)
PBDE 183	/[d]	/[d]	/[d]	−0.04 (−0.25, 0.19)	0.01 (−0.22, 0.23)	−0.09 (−0.35, 0.16)
Molar sum of PBDEs	−0.11 (−0.27, 0.04)	−0.06 (−0.22, 0.10)	/	−0.01 (−0.69, 0.68)	0.07 (−0.64, 0.79)	/
Total PBDEs	−0.13 (−0.35, 0.08)	−0.06 (−0.28, 0.15)	/	0.03 (−1.92, 1.87)	0.38 (−1.63, 2.38)	/

SD = standard deviation; CI = confidence interval; β estimates represent the mean differences in gestational age at delivery per 1-SD increase in log transformed plasma concentrations of each of the PBDEs.

[a] For all statistical analyses on the associations of PBDEs with gestational age at delivery and preterm birth, we excluded observations with PBDEs concentrations greater than 5 SDs from the mean values to avoid potential distortion of extreme values.

[b] Adjusted for maternal age (years), race/ethnicity (Non-Hispanic white, Non-Hispanic Black, Hispanic, Asian/Pacific Islander), pre-pregnancy BMI (kg/m^2), parity (0, 1, 2+), education level (<college degree, some college/undergraduate, graduate/post-graduate), marital status (married or living with partner, not married), family income during last year (<$30,000, $30,000-$49,999, $50,000-$99,999, $100,000 or more, not reported), plasma cotinine level (ng/mL), plasma total lipids (ng/mL), total activity (MET hours per week), sedentary activity (MET hours per week), and acculturation (US-born, recent immigrant, long-term immigrant). Observations with missing covariates were excluded from the adjusted models.

[c] Additionally adjusted for all the other PBDEs.

[d] Results not available, as only congeners with >30% detection rate in each cohort (i.e. PBDE 28, 47, 99, 100, 153, and 154 in the non-obese cohort; PBDE 47, 99, 100, 153, 154 and 183 in the obese cohort) were selected for the main analyses, respectively.

Table 4
Association between each standard deviation increment in log transformed plasma PBDEs concentrations and odds of PTB among the non-obese and obese cohorts.

Plasma PBDEs	Non-obese cohort[a]			Obese cohort[a]		
	Crude OR (95% CI) (n = 2008)	Adjusted[b] OR (95% CI) (n = 1975)	Mutually adjusted[b,c] OR (95% CI) (n = 1975)	Crude OR (95% CI) (n = 390)	Adjusted[b] OR (95% CI) (n = 356)	Mutually adjusted[b,c] OR (95% CI) (n = 356)
PBDE 28	1.20 (0.72, 1.90)	1.02 (0.58, 1.69)	0.87 (0.42, 1.73)	/[d]	/[d]	/[d]
PBDE 47	1.17 (0.92, 1.44)	1.04 (0.79, 1.32)	1.01 (0.66, 1.49)	0.54 (0.14, 1.35)	0.61 (0.13, 1.79)	1.05 (0.11, 9.59)
PBDE 99	1.10 (0.70, 1.64)	0.98 (0.64, 1.47)	0.95 (0.59, 1.51)	0.20 (0.01, 1.27)	0.16 (0.01, 1.54)	0.02 (0.00, 1.66)
PBDE 100	1.23 (0.93, 1.58)	1.12 (0.83, 1.46)	1.10 (0.68, 1.74)	1.49 (0.57, 3.26)	1.59 (0.42, 4.41)	7.23 (0.64, 91.1)
PBDE 153	1.21 (0.95, 1.50)	1.19 (0.92, 1.50)	1.20 (0.89, 1.59)	1.23 (0.68, 1.97)	1.21 (0.57, 2.24)	1.05 (0.40, 2.41)
PBDE 154	0.91 (0.71, 1.14)	0.89 (0.69, 1.12)	0.85 (0.66, 1.09)	1.26 (0.83, 2.02)	1.34 (0.79, 2.40)	1.38 (0.78, 2.55)
PBDE 183	/[d]	/[d]	/[d]	0.85 (0.50, 1.29)	0.77 (0.40, 1.30)	1.11 (0.51, 1.17)
Molar sum of PBDEs	1.13 (0.79, 1.46)	0.98 (0.64, 1.29)	/	0.49 (0.06, 2.02)	0.50 (0.03, 2.98)	/
Total PBDEs	1.14 (0.66, 1.58)	0.97 (0.49, 1.38)	/	0.24 (0.01, 9.29)	0.24 (0.00, 25.4)	/

SD = standard deviation; OR = odds ratio; CI = confidence interval; OR estimates represent the odds ratio of PTB per 1-SD increase in log transformed plasma concentrations of each of the PBDEs.

[a] For all statistical analyses on the associations of PBDEs with gestational age at delivery and preterm birth, we excluded observations with PBDEs concentrations greater than 5 SDs from the mean values to avoid potential distortion of extreme values.

[b] Adjusted for maternal age (years), race/ethnicity (Non-Hispanic white, Non-Hispanic Black, Hispanic, Asian/Pacific Islander), pre-pregnancy BMI (kg/m^2), parity (0, 1, 2+), education level (<college degree, some college/undergraduate, graduate/post-graduate), marital status (married or living with partner, not married), family income during last year (<$30,000, $30,000-$49,999, $50,000-$99,999, $100,000 or more, not reported), plasma cotinine level (ng/mL), plasma total lipids (ng/mL), total activity (MET hours per week), sedentary activity (MET hours per week), and acculturation (US-born, recent immigrant, long-term immigrant). Observations with missing covariates were excluded from the adjusted models.

[c] Additionally adjusted for all the other PBDEs.

[d] Results not available (/) for this congener in this cohort, as only congeners with >30% detection rate in each cohort (i.e. PBDE 28, 47, 99, 100, 153, and 154 in the non-obese cohort; PBDE 47, 99, 100, 153, 154 and 183 in the obese cohort) were selected for the main analyses, respectively.

linear regression models) and the odds of PTB (from logistic regression models) are presented in Fig. 1 and Supplemental Table S4. Among those without obesity, we found that a 1-SD increase in PC2 scores (with high loadings for PBDE 153 and 100) were associated with shorter gestational age at delivery [adjusted β (95% CI) = −0.18 (−0.30, −0.06) weeks]. However, none of the four PCs was significantly associated with odds of

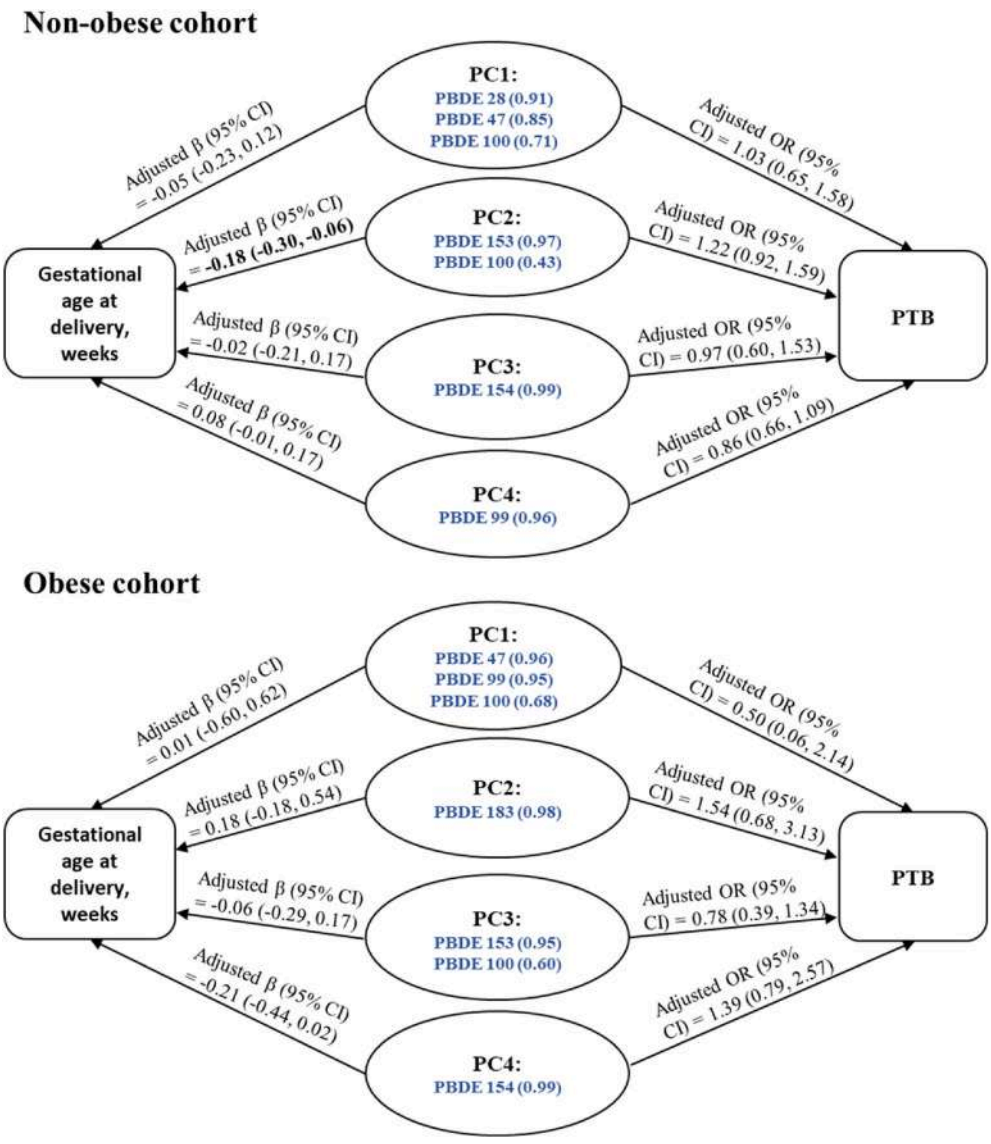

Fig. 1. Adjusted associations between each standard deviation increment in principle components scores of PBDEs and gestational age at delivery (weeks) or odds of preterm birth among the non-obese and obese cohorts.
PC = principal component; SD = standard deviation; PC = principle component; PTB = preterm birth; CI = confidence interval; OR = odds ratio; β estimates represent the mean differences in gestational age at delivery per 1-SD increase in each of the PC scores. ORs represent the odds ratio of PTB per 1-SD increase in each of the PC scores. For each PC, blue, bolded PBDE congeners (loadings) with varimax-rotated |loadings| > 0.4 are listed. All estimates (95% CIs) adjusted for maternal age (years), race/ethnicity (Non-Hispanic white, Non-Hispanic Black, Hispanic, Asian/Pacific Islander), pre-pregnancy BMI (kg/m2), parity (0, 1, 2+), education level (<college degree, some college/undergraduate, graduate/post-graduate), marital status (married or living with partner, not married), family income during last year (<$30,000, $30,000-$49,999, $50,000-$99,999, $100,000 or more, not reported), plasma cotinine level (ng/mL), plasma total lipids (ng/mL), total activity (MET hours per week), sedentary activity (MET hours per week), and acculturation (US-born, recent immigrant, long-term immigrant). Observations with missing covariates were excluded from the adjusted models. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

PTB. Among those with obesity, we did not observe statistically significant association between the PCs with gestational age at delivery or PTB.

3.3. PBDE mixtures and gestational age at delivery from BKMR

Based on the estimated PIPs from the BKMR analyses, PBDE 153 was considered as the most important contributor to the overall association between the PBDE mixtures and gestational age at delivery among those without obesity (PIP = 0.90), while PBDE 154 was considered the most important contributor among those with obesity (PIP = 0.44). Fig. 2 shows the results from BKMR modelling PBDEs as mixtures, where the covariate-adjusted univariate exposure-response functions and 95% credible intervals for each chemical and standardized gestational age at delivery are presented separately for each cohort, while holding all other PBDEs at their median concentrations. We found that: (1) Among women without obesity, there was a suggestive relationship of higher PBDE 153 concentrations with shorter gestational age at delivery. (2) Among women without obesity, there was a suggestive non-linear relationship between PBDE 154 and gestational age, with a positive association at lower concentrations and an inverse association at higher concentrations. (3) Among women with obesity, there was a suggestive relationship of higher PBDE 154 and 183 concentrations with shorter gestational age at delivery. However, in both cohorts, the 95% credible intervals of the differences in gestational age included zero, when comparing each chemical's 90th percentile to the 10th percentile while holding all other chemicals at the median (Supplemental Fig. S3). In addition, no apparent trend was observed in either cohort when evaluating the overall joint effect of the PBDE mixture on gestational age at delivery (Supplemental Fig. S4).

To explore interactions between chemicals, we plotted the exposure-response functions of each chemical with a second chemical fixed at various percentiles (25th, 50th, 75th), holding all other chemicals at the median. We observed a potential interaction between PBDEs 100 and 154 among those without obesity (Fig. 3A), where there was a stronger relationship of higher PBDE 100 concentrations with shorter gestational age at delivery when PBDE 154 concentrations were fixed at lower levels. We also observed a potential interaction between PBDEs 154 and 183 among those with obesity (Fig. 3B), where the suggestive relationship of higher PBDE 154 concentrations with shorter gestational age at delivery was stronger when the PBDE 183 concentrations were fixed at higher levels.

3.4. Secondary and sensitivity analyses

The associations between the PCs from PCA and categories of PTB are

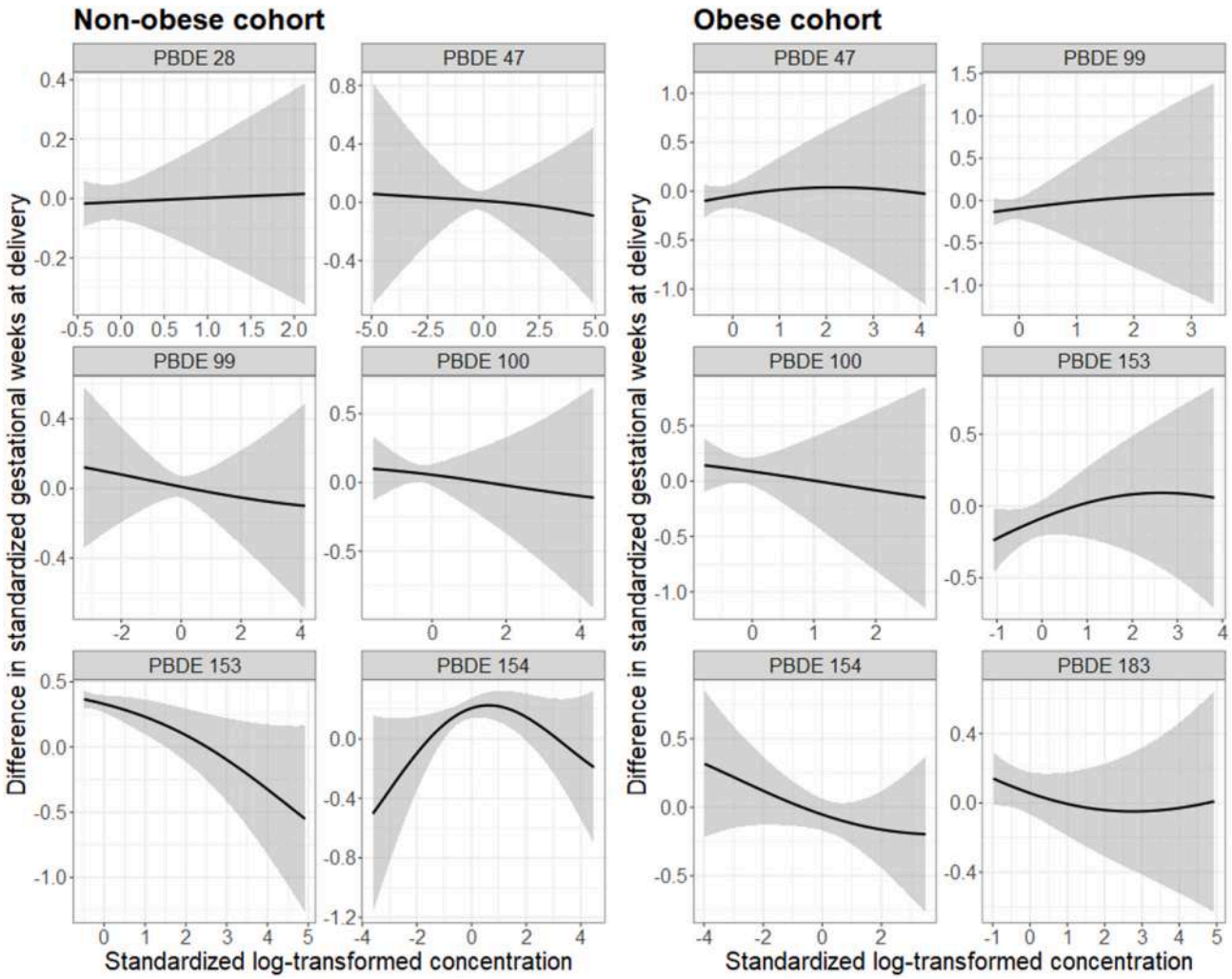

Fig. 2. Univariate exposure-response functions (95% credible intervals) between PBDEs concentrations and standardized gestational age at delivery estimated by BKMR, holding all other congeners at the median levels, among the non-obese cohort (left, n = 1975) and the obese cohort (right, n = 356).
All estimates (95% CIs) adjusted for maternal age (years), race/ethnicity (Non-Hispanic white, Non-Hispanic Black, Hispanic, Asian/Pacific Islander), pre-pregnancy BMI (kg/m2), parity (0, 1, 2+), education level (<college degree, some college/undergraduate, graduate/post-graduate), marital status (married or living with partner, not married), family income during last year (<$30,000, $30,000-$49,999, $50,000-$99,999, $100,000 or more, not reported), plasma cotinine level (ng/mL), plasma total lipids (ng/mL), total activity (MET hours per week), sedentary activity (MET hours per week), and acculturation (US-born, recent immigrant, long-term immigrant). Observations with missing covariates were excluded from the adjusted models.

presented in Supplemental Tables S4 and S5. We found that among those without obesity, the PC2 (predominantly contributed by PBDEs 153 and 100) scores were associated with higher odds of very early/moderate PTB [OR (95% CI) = 1.91 (1.19, 3.07)], but not significantly associated with odds of late PTB (Supplemental Table S5). We also found that the PC2 scores were marginally associated with higher odds of spontaneous PTB [OR (95% CI) = 1.34 (1.00, 1.80)], but not significantly associated with the odds of medically indicated PTB among those without obesity (Supplemental Table S6).

Supplemental Table S7 presents the associations between the PCs and gestational age at delivery, stratified by race/ethnicity. There was statistically significant effect modification (p-for-interaction = 0.01) by race/ethnicity in the non-obese cohort for the relationship between PC4 (predominantly contributed by PBDE 99) and gestational age, where there was a positive association between PC4 and gestational age only among non-Hispanic Black women [β (95% CI) = 0.33 (0.09, 0.58)]. We also observed a significant inverse association between the PC2 scores and gestational age only among non-Hispanic Black women in the non-obese cohort [β (95% CI) = −0.40 (−0.67, −0.12)]. There was statistically significant effect modification by maternal age (p-for-interaction < 0.01) for the associations between higher PC2 scores and shorter gestational age in the non-obese cohort, where the associations appeared stronger among those aged ≥ 35 years compared to those aged < 35 years (Supplemental Table S8). In addition, the associations between the PC2 scores and gestational age were also modified by BMI (p-for-interaction = 0.04) in the non-obese cohort, where the associations appeared stronger among those with BMI ≥ 25 kg/m^2 compared to those with BMI < 25 kg/m^2 (Supplemental Table S9).

In sensitivity analyses, we identified a few dietary factors that were weakly correlated with PBDE concentrations among a subset of women who completed a baseline FFQ (Supplemental Fig. S5). Further adjusting for the Alternative Healthy Eating Index (AHEI-2010) score and these dietary factors resulted in stronger inverse associations of PBDE 153 with gestational age among those without obesity (Supplemental Table S10). In addition, not adjusting for plasma total lipids had very little impact on the effect estimates in either cohort (data not shown). In the survival analysis evaluating gestational age at delivery as a time-to-event outcome, we found similar associations between PBDE 153 and time-to-delivery [adjusted hazard ratio (95% CI) = 1.10 (1.02, 1.19)] in the non-obese cohort. Lastly, including data from individuals with

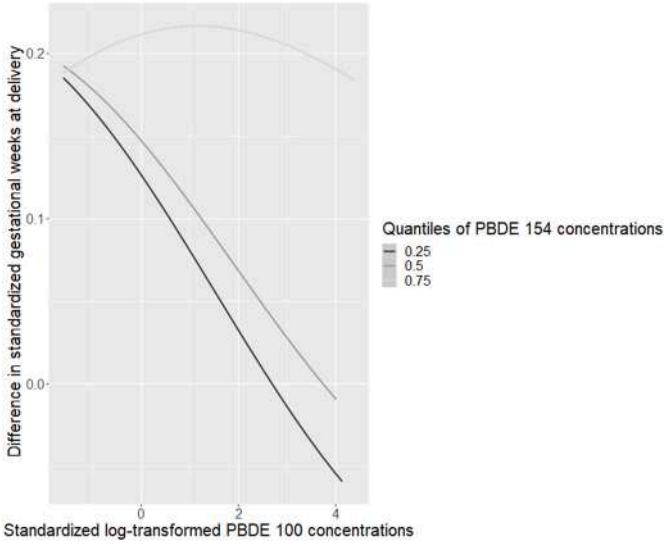

Fig. 3A. Exposure-response functions between PBDE 100 and gestational age at delivery, when fixing PBDE 154 concentrations at the 25th, 50th, and 75th percentiles, and all other congeners at the median, estimated with BKMR in the non-obese cohort.

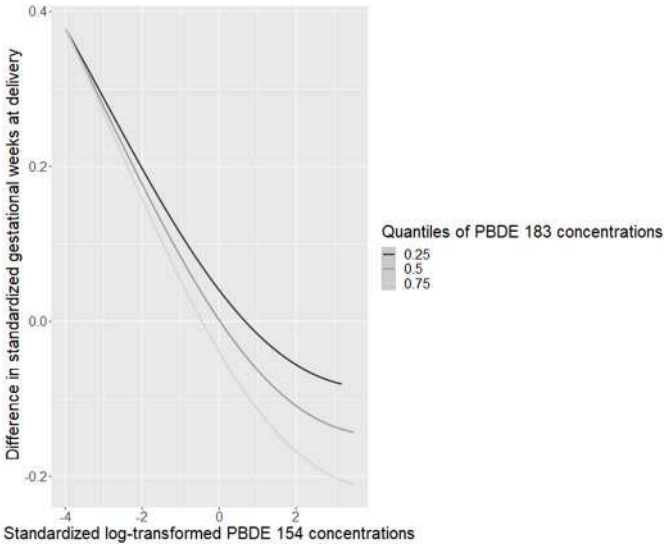

Fig. 3B. Exposure-response functions between PBDE 154 and gestational age at delivery, when fixing PBDE 183 concentrations at the 25th, 50th, and 75th percentiles, and all other congeners at the median, estimated with BKMR in the obese cohort.

extreme values of PBDEs yielded results in similar directions and magnitudes (data not shown).

4. Discussion

In this study of pregnant U.S. women from a large, multi-center, multi-racial/ethnic cohort, we observed associations of higher concentrations of plasma PBDE 153 in early pregnancy with shorter gestational age at delivery among women without obesity, via different statistical approaches that evaluated individual and mixture effects of PBDEs. We also observed associations of higher levels PBDE 153 and 100 (represented by PC2 from PCA) with higher odds of very early/moderate PTB and spontaneous PTB among women without obesity. There was also a suggestive non-linear dose-response relationship between PBDE 154 concentrations and gestational age at delivery among women without obesity. On the other hand, in the smaller sized obese cohort, there was no statistically significant association among women with obesity, although suggestive inverse dose-response relationships of PBDE 154, 183 and their interactions with gestational age at delivery were observed in BKMR analyses.

Previous epidemiological studies that evaluated certain PBDEs and PTB or gestational age at delivery have reported inconsistent results. One longitudinal cohort study of low income and predominantly Hispanic women recruited during 1999–2000 (n = 286) evaluated four serum PBDE congeners (PBDE 47, 99, 100, 153, and their molar sum) in the second trimester (mean: 26.1 weeks), and found no association with gestational age at delivery (Harley et al., 2011). In contrast, another cohort study during 2014–2018 (n = 506) that evaluated serum PBDE 47 and 99 in the second trimester (range: 12–28 weeks) found an inverse association between PBDE 47 and gestational age at delivery (Eick et al., 2020). A smaller sized case-control study (n = 138) during 2008–2011 that specifically measured PBDE 47 (collected at delivery) found higher concentrations associated with a higher odds of PTB (Peltier et al., 2015). In addition, a study conducted at a brominated flame retardant production area in China during 2010–2012 (n = 207) showed a positive association between PTB and serum PBDE 153 (collected at delivery), but not with other PBDE congeners (Gao et al., 2016). However, these studies were limited to evaluating the individual effects of certain PBDE congeners, or only used an additive summary measure of multiple chemicals, without taking into account the potential correlations and interactions between these chemicals. Moreover, most of the existing studies had relatively small sample sizes and might not have sufficient power to detect any racial/ethnic disparities. Our study addresses these limitations via evaluating the effect of PBDEs, both individually and as a mixture, on gestational age and PTB in a larger, multi-center, multi--racial/ethnic cohort of U.S. pregnant women.

In our study, we utilized three different statistical approaches to evaluate the individual and joint associations of PBDEs with gestational age and PTB. We found statistically significant or suggestive evidence of associations of plasma PBDE 153 concentrations with shorter gestational age at delivery among women without obesity from all three methods. Despite the fact that a 0.19-week reduction in gestational age at delivery might be of less clinical relevance, we also found significant evidence of PBDE 153 and 100 (represented by PC2) associated with higher odds of very early/moderate PTB and spontaneous PTB, which carries important clinical implications. These findings suggest a potential adverse role of PBDE 153 on gestation length/PTB. PTB is a complex phenotype that involves multiple etiologic factors acting through multiple pathophysiological pathways. One pathway is through inflammation, where higher levels of cytokines promotes labor through stimulating the production of prostaglandin that leads to cervical dilation, stimulating the expression of oxytocin receptors, as well as inhibiting adhesions in fibroblasts that lead to premature membrane rupture (Farina and Winkelman, 2005). Another pathway involves placental dysfunction, where abnormal uterine spiral artery remodeling contributes to several adverse pregnancy outcomes, including PTB (Morgan, 2016). PBDE 153 has been shown to play a role in both pathways. For example, PBDE 153 has been shown to increase the production of reactive oxygen species (Huang et al., 2010), contributing to the inflammatory pathway that may result in spontaneous PTB, which is consistent with our findings. In addition, PBDE 153 has been found to alter DNA methylation of the IGF2 gene that plays a role in placental development and fetal growth (Zhao et al., 2016), potentially contributing to the progression of medically indicated PTB. However, we did not any observe significant association between PBDE 153 and medically indicated PTB. Additional mechanistic studies are needed to further explore and validate these findings.

We also found a suggestive inverse U-shaped dose-response relationship between PBDE 154 and gestational age among women without obesity from BKMR analyses, with a similar non-linear pattern observed in the sensitivity analysis categorizing PBDE 154 into quantiles. The scientific literature on the toxicity of PBDE 154 is lacking, with only one

study that showed PBDE 154 was less toxic than PBDE 47 in inducing apoptotic cell death in liver cells (Souza et al., 2016). To date, no studies have evaluated the toxicity of PBDE 154 in the placenta or other tissues. On the other hand, we did not find any significant association between PBDE 47 and gestational age or PTB, despite PBDE 47 having the highest detection rate among those without obesity. A case-control study found PBDE 47 associated with higher odds of PTB (Peltier et al., 2015). However, this study had a small sample size (n = 138) and collection of PBDE 47 was cross-sectional (at delivery). One study conducted in rats demonstrated that for CYP1A1, an oxidative stress-related gene that was found to be associated with PTB risk (Sheikh et al., 2016), its expression level was positively correlated with increased bromine content of the three PBDE congeners (PBDE 47, 99, or 153) (Sanders et al., 2005), pointing to potentially stronger adverse effects on PTB comparing PBDE 153 to PBDE 47. However, further research in comparing the toxicity of PBDE congeners in human cell lines or tissues relevant to pregnancy outcomes is warranted to complement epidemiological findings.

Unlike the associations among women without obesity, we did not observe statistically significant associations of PBDE 153 with gestational age at delivery or PTB among women with obesity. The analyses in the obese cohort were limited by lower power due to a much smaller sample size. However, we did find effect modification by BMI in the non-obese cohort, suggesting some modification potentially related with adiposity. In addition, a suggestive yet consistent dose-response relationship of PBDE 154 and gestational age at delivery were observed in the obese cohort, across all three statistical approaches. Within women with obesity, each 1 SD increase in log PBDE 154 corresponded to a 0.20-week decrease in gestational age at delivery; this finding was similar to the 0.21-week decrease in gestational age at delivery observed for PC4 (which consisted almost entirely of PBDE 154) from the PCA, and similar linear patterns were suggested by the BKMR analyses. One possible explanation for the differences observed between the obese and non-obese cohorts might be that the obese cohort had higher detections and mean concentrations of PBDEs (particularly PBDE 153 and 154) than the non-obese cohort, and therefore might be capturing different patterns of the dose-response relationships between these chemicals and gestational age at delivery. In addition, the two cohorts had different eligibility criteria where the non-obese cohort excluded more medical conditions, some of which might be risk factors for adverse pregnancy outcomes including PTB. Furthermore, PBDEs have been shown to alter adipocyte metabolism, a hallmark feature of metabolic obesity (Hoppe and Carey, 2007). Therefore, it is possible that PBDEs interact with unmeasured metabolic features related to adiposity or lifestyle factors that lead to different effects on gestational length in individuals with and without obesity. Studies in rodents also have shown that different PBDE congeners may have different disposition patterns (e.g. more PBDE 47 distributed to adipose tissue, while more PBDE 153 accumulated in the liver) (U.S. EPA, 2008). Thus, future studies beyond plasma measurements of PBDEs, such as measurements in adipose tissue, might be helpful to further investigate their potential effects in women with obesity.

We observed effect modification by race/ethnicity for the association of PBDEs with gestational age at delivery among women without obesity. A positive association between PBDE 99 (presented by PC4) and gestational age at delivery was found only among non-Hispanic Black women. We also observed a significant inverse association between the PC2 (predominantly contributed by PBDEs 153 and 100) scores and gestational age only among non-Hispanic Black women in the non-obese cohort. Potential explanations may include higher exposure levels to these chemicals (Nguyen et al., 2020) among non-Hispanic black women, as well as potential interactions of higher PBDE concentrations with factors such as diet/micronutrients (e.g. vitamin D deficiency was shown to be associated with PTB in some observational studies, and vitamin D deficiency is more common among black women) (Burris et al., 2019), higher psychosocial stress levels (Forrester et al., 2019; Gump et al., 2014) and higher exposures to other pollutants (Muller et al., 2018; Stieb et al., 2012) that might jointly impact non-Hispanic Black women to disproportionately increase the risk of adverse pregnancy outcomes (Grobman et al., 2018). We also observed significant effect modification by age, suggesting that women with older age at pregnancy might be more susceptible to the potential adverse effect of PBDE 153 on shorter gestation length.

Our study had some limitations. First, chemical concentrations were measured only once during the first trimester, which may have misclassified exposure for some women at biologically relevant windows. Nevertheless, PBDEs are persistent chemicals that are expected to be stable over time with long half-lives (Chevrier et al., 2010). Second, this study population constitutes low-risk pregnancies only; thus, the results may not be generalizable to higher-risk populations. Third, potential batch effects may exist for comparing chemical concentrations in the non-obese and obese cohorts given that chemical assays were performed at different locations multiple years apart. However, inter-lab variability is less likely given the assay was performed by the same laboratory scientist with the same QA/QC standards. Fourth, type 1 errors might occur from multiple testing, but our findings were consistent across three different statistical approaches. Fifth, the arbitrary cutoff of 30% above the LOQ for selection of PBDE congeners to be included in the main analyses was lower compared to other studies. Furthermore, the obese cohort consisted of a much smaller sample size compared to the non-obese cohort. We therefore have less power to detect any potential associations in the obese cohort even if they did exist (e.g. the suggestive yet non-significant relations between PBDE 154 and gestational age at delivery among those with obesity could be due to insufficient power). Lastly, selection bias may occur from restricting to live births, since PBDEs were shown to increase the risk of pregnancy loss (Choi et al., 2019), and unmeasured or residual confounding may exist.

Despite these limitations, our study has several strengths. First, the relatively large sample size of pregnant U.S. women allowed us to apply mixtures analyses to estimate the association of multiple PBDE congeners with gestational age at delivery and PTB in addition to evaluating individual effects. Second, this study population consists of four relatively evenly distributed racial/ethnic groups, which allowed for sufficient power to detect effect measure modification by race/ethnicity in the non-obese cohort. The large sample size also allowed us to evaluate potential effect modification by other factors, such as age and BMI. Third, we were able to evaluate clinically confirmed endpoints classified by PTB subtypes as secondary outcomes. Lastly, compared to other studies that measured PBDEs concentrations during the second trimester or upon delivery, the prospective design of this cohort minimized the possibility of reverse causation, and the comprehensive questionnaire and biomarker data allowed for proper control of confounding.

5. Conclusions

In conclusion, using different statistical approaches that evaluated individual and mixture effects of PBDEs, we found evidence of associations of higher early pregnancy plasma PBDE 153 concentrations with shorter gestational age at delivery and higher odds of very early/moderate PTB and spontaneous PTB among women without obesity in a cohort of low-risk U.S. pregnant women. These associations were stronger among non-Hispanic Black women, women with BMI ranging between 25 and 30 kg/m^2, and women aged ≥35 years. Suggestive inverse relationships of PBDE 154 with gestational age at delivery were observed among women with obesity. Given the ubiquitous presence of PBDEs and the increasing disparities in PTB, further research is needed to understand the effects of exposures to PBDEs on gestation length, especially in high exposure and high outcome risk subgroups.

Declaration of competing interest

None.

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Appendix A. Supplementary data

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Public health performance of sanitation technologies in Tamil Nadu, India: Initial perspectives based on *E. coli* release

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ABSTRACT

Sanitation is intended to reduce the spread and burden of diseases transmitted from excreta. Pathogen reduction from excreta before sludge or effluent discharge to the environment would seem a logical and useful performance indicator for sanitation systems. However, the relative magnitudes of pathogen release from common sanitation technologies are not well understood. We, therefore, investigated the feasibility of performance measurement of different sanitation technologies in Tamil Nadu, India in reducing the release of the pathogen indicator *Escherichia coli* (*E. coli*). After conducting users' surveys and technical assessments of the locally prevalent sanitation systems, we classified them into 7 distinct categories (based on both observed physical characteristic and usage) within a widely-accepted physical typology. Faecal sludge and wastewater samples were collected and analysed for *E. coli* and total solids from 136 household systems, 24 community systems, and 23 sanitary sewer oveflows. We estimated the average volumetric release rates of wastewater and faecal sludge from the different sanitation technologies. Average daily per capita *E. coli* release was computed, and used as one indicator of the public health performance of technologies. We found that on-site installations described by owners as "septic systems" included diverse forms of tanks and pits of uncertain performance. We observed a statistically significant difference in the average daily per capita *E. coli* release from different sanitation technologies ($p = 0.00001$). Pathogen release from the studied on-site sanitation technologies varied by as much as 5 orders of magnitude from "lined pits" (5.4 Log10 *E. coli* per person per day) to "overflowing sanitary sewers" and "direct discharge pipes" (10.3–10.5 Log10 *E. coli* per person per day). Other technologies lay between these extremes, and their performances in *E. coli* removal also varied significantly, in both statistical and practical terms. Our results suggest that although faecal sludge management along the sanitation service chain is important, sanitation planners of the observed systems (and probably elsewhere) should direct higher priority to proper management of the liquid effluents from these systems to minimize public health hazards. We conclude that (i) the work demonstrates a new and promising approach for estimating the public health performance of differing sanitation technologies, (ii) if *E.coli* is accepted as an indicator of the public health hazard of releases from sanitation systems, our results strongly suggest that safe containment of excreta for an extended period substantially reduces pathogen numbers and the risk of pathogen release into the environment; and (iii) there are some simple but little-used technical improvements to design and construction of on-site sanitation systems which could significantly reduce the release of pathogens to the environment.

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1. Introduction

Sanitation-related diseases caused by exposure to faecal pathogens (including various bacteria, viruses, helminths, and protozoa) cause a substantial global burden of disease including 1.7 billion episodes of diarrhoea every year in children under 5 years (Walker et al., 2013). Access to adequate sanitation alone would eliminate about 0.5 million deaths and 26 million DALYs of diarrhoea every year, especially in the Low and Middle Income Countries (Prüss-Ustün et al., 2019).

Communities everywhere include households with diverse individual and collective ways of managing excreta. It has been estimated that over 3.1 billion people globally rely on household on-site sanitation facilities (pit latrines, cesspits and diverse "septic systems") (UNICEF and WHO, 2019), and this population is anticipated to increase to 5 billion by 2030 (Strande and Brdjanovic, 2014). The on-site systems function by containing excreta, either in a pit latrine (which receives excreta with minimal water until it is filled, when its contents are emptied as faecal sludge) or in some form of "septic system" (which allows for the management of large amounts of wastewater without necessarily spilling directly into the local environment). In this paper the term "septic system" in quotation marks refers to any of a wide variety of poorly designed and operated on-site sanitation systems which receives wastewater, stores septage, and discharges liquid effluent to the environment. In contrast, the term septic system *without* quotation marks refers to a much narrower, and rarer, subset of well-designed and operated systems which meet common widely accepted engineering design criteria for septic systems to improve performance. The term pit refers to an on-site sanitation system which receives excreta or wastewater into a hole, and stores faecal sludge as the liquid fraction ex-filtrates into the surrounding soils.

In India, about 45% of the urban households (approximately 600 million people (Plecher, 2020)) are served by on-site sanitation systems –mainly "septic systems" (Census of India, 2011; Rohilla et al., 2016b). In Urban Tamil Nadu, India, around 38% of households use "septic systems" for their sanitation needs, 27% are connected to sanitary sewers, and 35% use others (such as pit latrines (6.0%), shared facilities (9.9%), direct discharge pipes (1.2%), open defecation (16.5%), etc.) (Census of India, 2011; IIPS and ICF, 2017). This range of sanitation technologies and service chains poses practical and important questions for sanitation managers. Which is a greater public health priority in a given city: reduction of covert faecal sludge dumping from "septic systems" and pit latrines, or better wastewater treatment? Reduction of the immediate direct discharge of black-water to the environment or open defecation by a small fraction of the population, or better treatment or control of "septic system" effluent discharged by many? Such decisions should reflect the relative benefits, costs, reliability, and operation and maintenance requirements of different technologies, which all vary with local conditions.

In principle, a septic system consists of both (i) a well-designed watertight chamber (i.e. fully-lined tank) that receives domestic wastewater for basic treatment through sedimentation and anaerobic processes to reduce organics and total solids; and (ii) the effluent receiver (such as a drain field, etc.) for further treatment and disposal of the tank effluent (Missouri DHSS., 2018; Georgia Department of Public Health, 2019; Feachem et al., 1981; Wang et al., 2021; Koottatep et al., 2014). However, the design, construction, operation, and maintenance of septic systems are not well understood by users, policy makers, and utility authorities especially in the global south. This confusion has resulted in a chaotic mixture of poorly designed and constructed tanks/on-site sanitation systems for management of excreta, with widely varying effluent quality and disposal practice, with little or no concern for public health (Strande et al., 2018).

How can we begin to estimate the effectiveness (or public health threats) from diverse sanitation technologies in a community without understanding the pathogen inactivation and releases of these systems? Without measurement and analysis, the effects of different design, construction, and operational features of these systems on pathogen inactivation and release will remain poorly understood. While the types and typical concentrations of pathogens present in excreta have been documented (e.g. Feachem et al. (1981); Harwood et al. (2017); Penn et al. (2018)), the pathogen releases in liquid effluent or emptied faecal sludge from on-site sanitation systems, remain scarcely characterised (Williams and Overbo, 2015; Wang et al., 2021; Foster et al., 2021; Amin et al., 2020; Manga et al., 2016, 2019, 2021).

The problem is complex because faecal sludge and effluent from on-site sanitation systems vary substantively, depending upon factors including type of containment, detention time, desludging practice, quality of construction, household usage, and operation of the system. Previous studies on the performance of on-site sanitation technologies (especially "septic systems") focus on removal of physical-chemical pollutant indicators (e.g. pH, conductivity, total suspended solids, biochemical oxygen demand, algal nutrients, etc.) from liquid effluent or faecal sludge (Abbassi et al., 2018; Bounds, 1997; Burubai et al., 2007; Levett et al., 2010; Nasr and Mikhaeil, 2013; Philippi et al., 1999; Rich et al., 2004; Strande et al., 2018; Prasad et al., 2021; Englund et al., 2020). However, studies of pathogen reduction in septic systems are few. Some studies demonstrate that the fully-lined tanks of the septic systems act as primary treatment units for solids removal from wastewater, reducing *E. coli* concentrations by 1–2 Log_{10} – mainly through sedimentation (Abbassi et al., 2018; Pfluger et al., 2009; Stenström et al., 2011; Brandes, 1978; Wang et al., 2021); however these studies do not account for the release of *E. coli* in emptied faecal sludge and/or liquid discharge.

We set out to investigate (i) the characteristics and key design features of local on-site sanitation systems, (ii) relative *E. coli* concentrations and average daily volumetric discharges of excreta from different sanitation technologies, (iii) relative *E. coli* releases from different sanitation technologies in liquid discharges and/or faecal sludge removal through periodic desludging, (iv) the effectiveness of local sanitation technologies in reducing *E. coli* release to the environment and/or the next stage of the sanitation service chain, and (v) the effect of key design, construction, and operational features of these systems on their performance in terms of *E. coli* release. To meet these objectives, we collected, synthesised and analysed field data from community transect walks, household interviews, key informant interviews, and observational surveys, technical assessments, and environmental sampling of locally prevalent sanitation technologies from two study sites within Tamil Nadu, India.

2. Methods

2.1. Study areas

Our study was conducted in two urban communities in the south Indian state of Tamil Nadu: (i) the Town Panchayat of Narasimhanaicken-Palayam (NNP) in Coimbatore district at an altitude of 473 m above mean sea level (geographical coordinates 11°7′31.44″ N latitude, 76°55′33.24″ E longitude), and (ii) Tiruchirappalli (Trichy) City Corporation (TCC) at altitude of 88 m above mean sea level (geographical coordinates 10°48′18″ N latitude, 78°41′8.16″ E longitude). Both communities have been supported by the TNUSSP - Tamil Nadu Urban Sanitation Support Programme (TNUSSP is a Technical Support Unit set up to support the Govt. of Tamil Nadu in scaling up urban sanitation across the state. It is a consortium, in which the Indian Institute for Human Settlements is the lead partner). These sites reflect a spectrum of excreta return pathways from common sanitation technologies in Tamil Nadu (Tiruchirappalli City Corporation, 2018; TNUSSP, 2018a; TNUSSP, 2017; TNUSSP, 2016). These communities use similar sanitation technologies, including various on-site sanitation systems discharging liquid effluent, although NNP lacks the municipal sewerage and wastewater treatment found in Trichy. The communities we selected differ in scale—a major city (Trichy) versus a smaller urban

administrative unit (NNP) on the outskirts of the major city of Coimbatore. The sanitation service chains for these sites also differ, with, for example, municipal faecal sludge decanting stations in Trichy, in contrast to the widespread direct agricultural reuse of untreated faecal sludge just outside NNP.

Sanitation challenges common to both study communities include: households without access to individual toilets, and a high proportion of households dependant on poorly designed and constructed on-site sanitation systems (pits and tanks) with no proper faecal sludge management services (TNUSSP, 2018a; TNUSSP, 2017; TNUSSP, 2016). The small sewerage system of Trichy is inadequately operated and managed and has insufficient wastewater and faecal sludge treatment capacity.

2.2. Community transect walks and key informant interviews

Field work for this cross-sectional study was conducted between March 2018 and October 2019. Initially, we conducted key informant interviews with the stakeholders along the sanitation service chain (such as pit emptiers, managers of community toilets, operators of sanitary sewers and FS treatment facilities, etc.). In addition to these interviews, we conducted 20 transect walks in Trichy and 10 transect walks in NNP to identify the different sanitation technologies used in the study area.[1] All community transect walks were conducted using well-tested and documented methods (World Bank Group (2016). The route for each transect walk was identified, discussed, and agreed on by all the participants at least a day in advance. Such routes crossed the study communities following a winding path to include a variety of areas that represent the study area. The route taken for each transect walk was planned and recorded using GPS data.

2.3. Sanitation Technology Typology in the study communities

Fig. 1 illustrates the Sanitation Technology Typology by the structural characteristics of the different systems identified in the study communities, based on the work of the SFD Promotion Initiative (2017). Table 1 describes the different routes through which these sanitation technologies release *E. coli* (and other pathogens) to the environment and/or next stage of the sanitation service chain. The typology used thus classifies sanitation technologies by physical measurements and observation to identify relevant pathogen release mechanisms for each system.

We used a "mass balance" approach to explore the release of *E. coli* from household sanitation systems: In this approach, *E. coli* enter the system with excreta, and are removed either by (1) liquid discharge (in releases of effluent, or overflows) or exfiltration, (2) die-off during treatment or storage in the system, or (3) faecal sludge or septage removal through periodic desludging. Effluent refers to the liquid discharge from on-site sanitation systems (for example, a tank with discharge pipes) to open ground, surface water, or open drain. By contrast, overflow refers to (i) sewage overflows at parts of the sanitary sewer associated with frequent blockages and overflows, or (ii) direct discharge without treatment (i.e. direct discharge pipes) to the open drains or environment. Exfiltration refers to seepage through the floor or side walls of the tank, pit and/or discharge to a soakaway. These technologies are illustrated in Fig. 1.

As indicated in Table 1 and shown in Fig. 1, a **lined pit** is constructed with an open bottom and permeable linings (e.g. honeycombed lined walls or perforated pre-cast concrete rings) through which exfiltration can occur into the surrounding soils. A **lined tank** refers to a tank constructed with impermeable sidewalls and permeable bottom/base, while a **fully-lined tank** is constructed with both impermeable sidewalls and base (See Fig. 1). In this paper the term **decanting station** refers to a designated facility through which faecal sludge is discharged into the sanitary sewer that conveys it with sewage to the wastewater treatment plant (TNUSSP, 2018b).

2.4. Selection of sanitation systems for study

An inventory of all the sanitation systems identified in the study communities based on community visits and key informant interviews was prepared, and used as a sampling frame. The final selection of the sanitation systems for detailed technical review and effluent, septage, and faecal sludge sampling was purposive and reflected a balance of criteria: (i) logistical or resource constraints; (ii) accessibility of the containment systems and sampling points for on-site visual assessment and sample collection; and (iii) the willingness of the system owners and users to have their systems included in the study. The results are shown in Table 2.

2.5. Field worker training and piloting of data collection tools

Experienced local research assistants and enumerators with knowledge of WaSH, expertise of using mWater (New York, USA) mobile platform and fluent in English and the local language (Tamil), were recruited and trained in data collection for this study in a five-day facilitated workshop.

The surveys were independently checked, pretested during surveyor training, and piloted in non-study villages. Rigorous testing and retesting of the surveys were carried out during programming of the electronic survey in mWater.

2.6. Initial site surveys

Prior to detailed surveys and environmental sampling, the initial site data were collected through user surveys and technical surveys at each of the selected sanitation systems. The user and observation survey questions were communicated by the enumerators in either English or the local language (Tamil), as preferred by the respondent. Data collected through these surveys were captured electronically using the mWater data collection platform, using Android-enabled smartphones or tablets.

2.6.1. User (household) surveys

A total of 203 user surveys were conducted with household owners or users of the 178 containment systems and 25 direct discharge pipes in the study. Users were asked about (i) the number of users of the containment systems, (ii) the emptying frequency over the past 5 years, (iii) the date when the containment system was last emptied and (iv) primary means of greywater disposal (where greywater is discharged to open drain, open ground, containment system, soakaways or others). A complete list of user survey questions is provided in Appendix C (Table S10) of the Supplementary Material. These data were used to (i) guide the technical assessment of the containment system; (ii) estimate storage periods between desludging; and (iii) permit analysis of the field measurements and samples collected from the studied systems.

2.6.2. Technical surveys

Data were collected by on-site visual inspections of the containment systems, taking measurements as appropriate. Each of the 178 containment systems was surveyed to determine (i) the shape and key design features of the system (e.g. plastic sanitary inlet or outlet tee-pipes, effluent pipes, the number of chambers, and lastly, the inlet and outlet pipe configurations), (ii) the structural integrity and permeability of both sidewalls and bottom of the containment systems and (iii) blackwater or effluent receivers. During the last round of sampling, the containment systems were fully emptied and re-inspected for features

[1] A 'transect walk' is a systematic walk by researchers and community members along a defined path (transect) across the community/project area to explore the sanitation conditions by observing, asking, listening, looking and producing a transect diagram (World Bank, 2016; Keller, 2020).

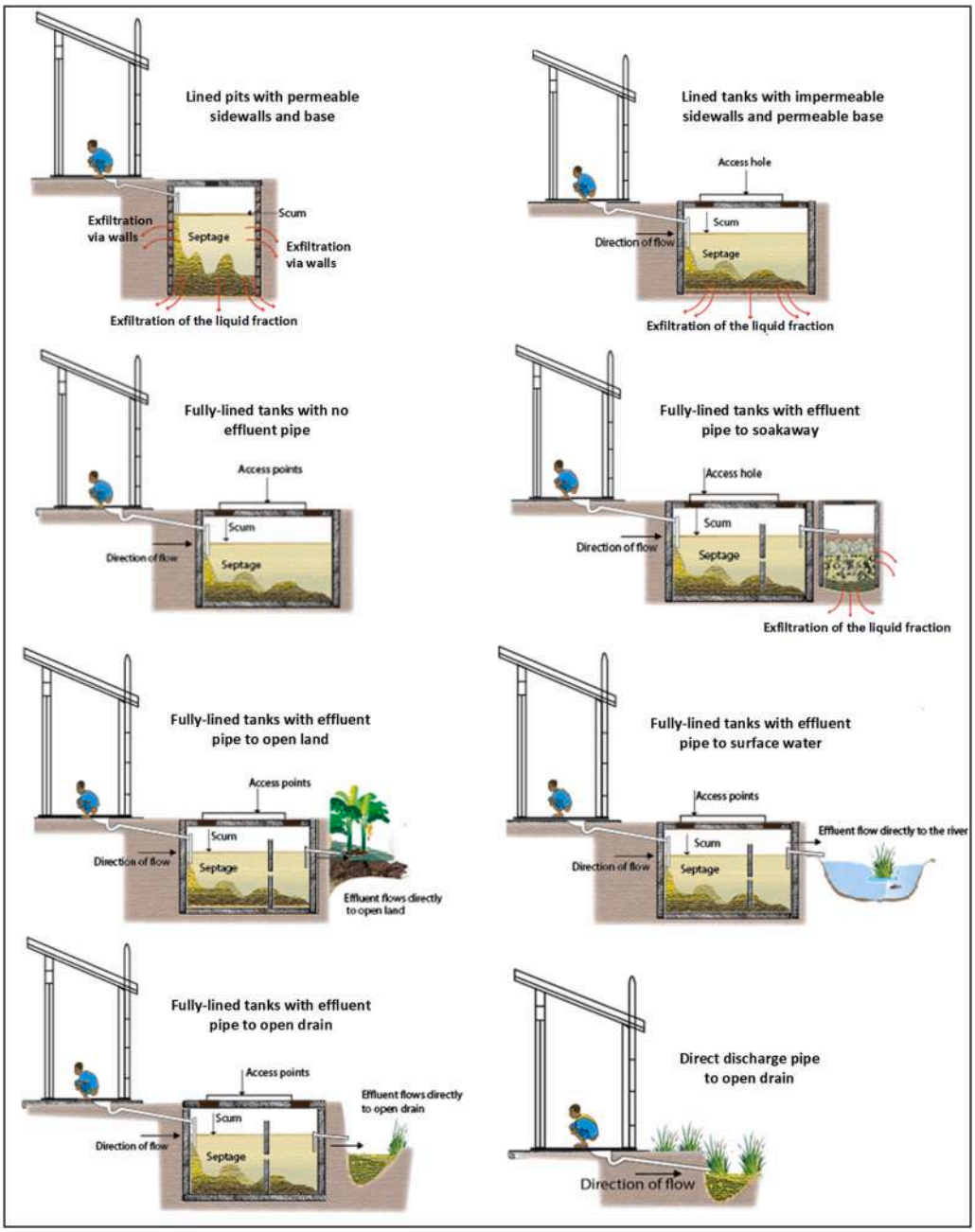

Fig. 1. Common sanitation technologies in the study area.

that may have been missed or not visible in previous inspections. The collected observational data were used to guide the subsequent physical design, construction, structural assessment and classification of containment systems encountered in the field. The complete observation survey checklist is provided in Appendix C (Table S11) of the Supplementary Material.

The length, width, or diameter of the 178 containment systems in the study were measured using a Bosch Blaze GLM50C Bluetooth Enabled 165-Foot Laser distance measure with color backlit display (Malaysia) and/or an Ironton 44045 steel tape measure 1-inch by 25-Foot (USA). The laser measure tool was calibrated for accuracy using the standardized steel tape measure. The depth of the tank, freeboard, and faecal sludge level were measured using a 12 mm MS dipping stick fabricated locally, and an Ironton 44045 steel tape measure. From these, the effective depth, effective volume of the tank, and actual volume of faecal sludge/septage in the tank were computed. All the key design features observed in the containment system were inspected and their measurements taken.

2.6.3. *Quality control of field surveys*

Enumerators were audited by the Water Institute staff by resurveying a selection of households and re-inspecting/re-assessing the on-site sanitation systems. Data quality comparison and quality assurance checks and procedures were carried out at multiple stages of data collection.

2.7. *Detailed surveys and environmental sampling*

Detailed surveys and environmental sampling were conducted in two phases for a period of 6 months, the first phase in April–June 2018 and the second phase in August–October 2019 with the intent of capturing seasonal differences (Weather Spark, 2022). Despite the deliberate

Table 1
Typology of sanitation used in this study. (adapted from SFD Promotion Initiative (2017)).

Technology Type	Structural characteristics of tank or system	Release of *E. coli* (and other pathogens) from sanitation systems		
		Discharge		Septage/ sludge removal by tank emptiers
		Effluent to open ground, surface water, or open-drain	Overflows to environment	
Lined Pit	Permeable sidewall and base	No	No	Yes
Lined Tank	Impermeable sidewalls and permeable base	No	No	Yes
Fully-Lined Tank *without* effluent pipe	Impermeable sidewalls and base	No	No	Yes
Fully-Lined Tank *with* effluent pipe		Yes	No	Yes
Community Toilet Fully-lined Tank (Fully-Lined Tank with effluent pipe, shared by multiple households)		Yes	No	Yes
Direct Discharge Pipes (also known as "straight pipes", "black-water pipes")	Short pipe discharging directly to the environment	No	Yes, constantly	No
Sanitary Sewers	Pipes carrying wastewater to the municipal network	No	Yes, at locations where sewers are broken or often blocked	No

timing of our field activities for both the usual "rainy" and "dry" seasons, there was no rainfall during our sample collection period, including the six weeks that overlapped with the expected rainy season for 2019. All the environmental sampling activities were conducted in the dry season with average temperatures of about 24–31 °C and humidity of 54%–78% (Weather Spark, 2022). Data collected through physical measurements and environmental sampling of the different sanitation systems were used in computation of *E. coli* releases from the different sanitation systems.

2.7.1. Containment systems

Data were collected from the selected 135 containment systems (75 household fully-lined tanks, 15 lined pits, 21 lined tanks, and 24 community toilet fully-lined tanks) (See Table 2 and Fig. 1). Data collection from each sampling site included a user interview, observational survey, physical measurement, and technical assessment of the containment system.

Effluent and faecal sludge/septage samples were collected from each studied containment system at least 2 times during the study. On each such visit, two types of samples were collected: (i) a single composite sample from the containment system liquid discharge (for systems with effluent pipes to the environment), and (ii) a composite sample of faecal sludge/septage from the containment system (a mixture of samples from the bottom, middle and top of the tank). All effluent and faecal sludge/septage samples were collected from the different containments systems according to sampling procedures in Koottatep et al. (2021), Bassan et al. (2016) and Koottatep et al. (2014).

For the liquid discharge (i.e. effluent) composite sample, 10 grab samples of each 2-L (at an interval of 5 min) were collected from the effluent pipe of the containment system (Koottatep et al., 2014; Nam et al., 2006; Amin et al., 2020). These were then mixed in a sterile container to form a composite sample from which a 1-L aliquot was collected, labelled, and taken to the lab for analysis.

For faecal sludge/septage samples; during the first and/or second round of sampling, composite samples of containment system faecal sludge/septage were collected from each of three depths below the faecal sludge/septage surface: top (between 0.0 and 0.15m); middle (about 0.5–1.0 m); and bottom (about 1.0–1.5 m). At each depth, 10–15 samples of 1-L were collected randomly using an adjustable handle metallic deep-sludge sampler, making a total of between 30 and 45 samples from each containment system. These were then transferred into a sterile container and the samples from each depth were thoroughly mixed to form a composite sample from which a 1-L aliquot was collected and transported to the laboratory for analysis. The metallic deep-sludge sampler was disinfected and sterilized with either bleach and/or 96% alcohol and flamed to sterilize between uses (before and after use). The deep-sludge sampler used in this study was designed and fabricated locally according to Nabateesa et al. (2017) device specifications, but with some modifications to suit the sanitation technologies within the study area. The modifications were aimed at limiting chances of cross-contamination of the collected sample when drawing the equipment from a deeper depth.

During the final round of sampling, faecal sludge samples were collected during a complete emptying of the tank. About 10 grab samples of each 2-L were collected at each of three stages of the emptying

Table 2
Distribution of observed and studied sanitation systems by technology in Trichy, and NNP.

Study Areas	Row Total of Sites	Household Fully-lined Tanks	Lined Pits	Lined Tanks	Community Fully-lined Tanks	Direct Discharge Pipes	Sanitary Sewer Discharges
Trichy Inventory	465	260	26	15	37	80	47
NNP Inventory	88	12	43	20	13	N/A	N/A
Selection for Initial Site Surveys							
In Trichy	183	105	2	10	18	25	23
In NNP	43	1	17	15	10	N/A	N/A
Total in both Trichy and NNP	**226**	**106**	**19**	**25**	**28**	**25**	**23**
Final Selection for Detailed Surveys and Environmental Sampling							
In Trichy	148	**74**	2	10	14	25	23
In NNP	35	1	13	11	10	N/A	N/A
Total in both Trichy and NNP	**183**	**75**	**15**	**21**	**24**	**25**	**23**

N/A – Not applicable as that sanitation system was not found in the study area.

process using grab sampling beaker device: (the start, the middle, and the end). The collected grab samples at each stage were mixed to form a composite sample of the faecal sludge being emptied, from which a 1-L aliquot was collected and taken to the laboratory for analysis.

All collected samples were stored in a portable ice chest/cooler box with ice packs and transported to the PSG Institute of Medical Sciences and Research laboratory, where they were stored at below 4 °C until analysis. The average daily discharge as liquid effluent release (q_D) from the containment systems (in litres/day) was estimated based on one of two ways: (1) the hydrostatic/volume balance and/or (2) a collected volume of liquid discharge (i.e. effluent) in a given time period. The minimum liquid detention time of each containment system was computed, based on the effective tank volume and the estimated inflow and/or discharge rate of liquid effluent into the immediate environment. Details of these procedures for estimating or computing the flow rate and average daily volumetric discharges are provided in Appendix A-1 of the Supplementary Material.

2.7.2. Sanitary sewer overflows

Sewage samples were collected from the Trichy sanitary sewers at 23 locations associated with frequent overflows. Each location was sampled at least three times during the study; once at each time interval of the day (i.e. morning between 6 and 9 a.m., afternoon between 12:00 to 3:00 p.m., evening between 4:30 to 6:00 p.m., and/or night between 7:00 p.m. and 10:00 p.m.). Samples at each site were collected through the nearest manholes. About 10 grab sub-samples of each 1-L (at an interval of 5 min) were collected from each location using either a wastewater sampler or sterilized 1-L plastic container. These sub-samples were transferred in a sterile 20-L plastic container and mixed to form a composite sample from which a 1-L aliquot was collected and taken to the laboratory for analysis. The collected samples were used to determine the pathogen load in the sewage that gets, or would get, to the surrounding environment whenever the blockages or overflows occur at the sampled locations.

According to TNUSSP (2017) and Rohilla et al. (2016a), 30% of the sewage collected in Trichy returns to the environment unsafely before reaching the treatment plant. The unsafe return per household connected to the sanitary sewer was therefore computed based on: (i) the reported daily water usage per capita in Trichy, (135 L/day), (TNUSSP, 2017); (ii) the average household size; (iii) the assumption that 80% of water used returns as wastewater; and (iv) the assumption that 30% of sewage returns to the environment unsafely. The computed volume was considered as the household average daily discharge as sewage overflow release (q_D) in litres/day to the environment.

2.7.3. Direct discharge pipes

Samples were collected from 25 household direct discharge pipes of black-water to the environment (i.e. open drains and open ground) in Trichy; none were found in NNP. Each location was sampled at least two times during the study. Samples were collected using 1000 gauge PVC plastic bags placed at the end of the selected household direct discharge pipes for about 24 h. The plastic bags were exchanged every 12 h or when full. When exchanging the plastic bags, the volume of black-water trapped in the plastic bag was measured in a sterile graduated container, and a sample collected after mixing the collected black-water. The volume of black-water collected from each direct discharge pipe was measured, and a composite sample formed using an equal volume of each of the exchanged bags (i.e. in most cases, the sub-sample volume was proportional to the collected bag volume). A 1-L sample was collected from this composite sample and taken to the laboratory for analysis. The average daily discharge as overflow (q_D) from each direct discharge pipe (litres/day) was computed based on the collected volume of black-water discharge in a 24-h period.

2.8. Analytical laboratory methods

All samples were stored at below 4 °C until analysis, and these were processed within 24 h of sampling. Total solids of faecal sludge and septage samples were analysed following section 2540 B of APHA-AWWA-WEF (2017). During laboratory analysis, about 10% of the samples were analysed in duplicate and a maximum relative error of 9% was observed between the duplicates.

Samples were analysed for *E. coli* using dilution spread plate counting technique with *E. coli*-Coliforms Chromogenic Agar (Oxoid Ltd, UK), according to section 9215C of APHA-AWWA-WEF (2017). Counts were Log_{10}-transformed and expressed as Log_{10} CFU per g of sample dry weight (for faecal sludge samples from lined tanks and pits) or Log_{10} CFU per ml (for liquid samples). Thereafter, *E. coli* concentrations in emptied faecal sludge or septage (C_d) and overflows and/or effluent (C_e) in Log_{10} *E. coli* per litre were computed.

2.9. Computations of E. coli release

Pathogens, including *E. coli,* are released from containment systems through periodic desludging as well as in liquid effluent or overflow. "Release" in this study refers to "removal from the containment system" either as "release to the environment" or "release to the next stage of the sanitation management chain"; the fate of emptied faecal sludge is often unclear, with widespread reports of clandestine dumping or use as agricultural fertilizer.

2.9.1. Daily release from discharge (effluent and overflow)

The average daily *E. coli* release to the environment due to discharges (effluent and overflow) ($\overline{R}_D$) was computed as a product of the estimated average daily overflows or effluent release (q_D) in litres/day and *E. coli* concentrations in overflow/effluent discharge (C_D) in *E. coli*/litre (See Eq. (2) for each system). The average daily per capita *E. coli* release ($\overline{R}_{D,pc}$) was computed by dividing $\overline{R}_D$ by the number of users of the sanitation technology (Eq. (3)).

2.9.2. Daily release from desludging

The periodic *E. coli* release of desludging operations to either the environment or the "next stage of the sanitation service chain" was estimated for each containment system. During desludging of containment systems, the faecal sludge volume emptied (V_S in litres) from each system was estimated based on the capacity of the emptying cesspool truck (gauge scale) and/or the faecal sludge volume in the tank or pit before emptying. The total *E. coli* release from the system from a single desludging operation (R_S) was computed as a product of the estimated volume desludged (V_S) and the *E. coli* concentration of this faecal sludge (C_S) (Eq. (4)). The average daily *E. coli* release from this desludging operation ($\overline{R}_S$) was then computed by dividing R_S by the time T in days since the last desludging (Eq. (5)). Finally, the average daily per capita *E. coli* release due to periodic desludging ($\overline{R}_{S,pc}$) was calculated by dividing R_S by the number of users of the system (Eq. (6)).

The average combined daily per capita *E. coli* release from sanitation technologies since last emptying ($\overline{R}_{c,pc}$) was computed as a sum of estimated average daily per capita *E. coli* releases due to (a) discharge as effluent and/or overflow release ($\overline{R}_{D,pc}$), and (b) periodic desludging operations ($\overline{R}_{S,pc}$) (See Eq. (7)).

2.9.3. Summary of all E. coli release equations

$$\overline{R}_D = q_D \times C_D \quad \text{Eq. (2)}$$

$$\overline{R}_{D,pc} = \frac{(q_D \times C_D)}{number\ of\ users} \quad \text{Eq. (3)}$$

$$R_S = V_S \times C_S \quad \text{Eq. (4)}$$

$$\overline{R}_S = \frac{V_S\ X\ C_S}{T} \quad \text{Eq. (5)}$$

$$\overline{R}_{S,pc} = \left(\frac{V_S\ X\ C_S}{T}\right) \Big/ number\ of\ users \quad \text{Eq. (6)}$$

$$\overline{R}_c = (q_D \times C_D) + \left(\frac{V_S\ \times\ C_S}{T}\right) \quad \text{Eq. (7)}$$

$$\overline{R}_{C,pc} = \overline{R}_{D,pc} + \overline{R}_{S,pc} = \left(\frac{(q_D \times C_D)}{number\ of\ users}\right) + \left[\left(\frac{V_S\ X\ C_S}{T}\right) \Big/ number\ of\ users\ )\right] \quad \text{Eq. (8)}$$

Where $\overline{R}_D$ = Average daily *E. coli* release per system due to effluent and/or overflows
$\overline{R}_{D,pc}$ = Average daily per capita *E. coli* release due to effluent and/or overflows
R_S = Average accumulated *E. coli* release per system at desludging
$\overline{R}_S$ = Average daily *E. coli* release due to periodic desludging
$\overline{R}_{S,pc}$ = Average daily per capita *E. coli* release due to periodic desludging
$\overline{R}_c$ = Average combined daily *E. coli* release (effluent + overflow + desludging)
$\overline{R}_{C,pc}$ = Average combined daily per capita *E. coli* release
q_D = Average daily discharge as overflows/effluent release in litres
C_D = *E. coli* concentrations in overflow/effluent discharge in *E. coli*/litre
V_S = Volume desludged from the containment systems in litres
C_S = *E. coli* concentrations in emptied faecal sludge
T = Time in days since the previous desludging operation

2.10. Statistical analysis

Household survey data were exported from mWater into Stata/SE 13.0 (College Station, Texas, USA) for cleaning and analysis. Physical measurements and laboratory data were also exported into Stata/SE 13.0. Taking the average combined daily per capita *E. coli* release of direct discharge pipes as the input or baseline, the performance of the different sanitation technologies was assessed. Data were analysed using a one-way Analysis of Variance (ANOVA) parametric test at a significance level of $p = 0.05$ to assess (a) differences amongst average daily *E. coli* release from different sanitation technologies; (b) differences between the mean values of the physical measurements (effective tank volume per capita, liquid detention time, storage periods of excreta, and emptying frequency) of the different containment system types. Average daily per capita *E. coli* release due to periodic desludging and effluent liquid discharge were compared. All significant one-way ANOVA test results were followed with a pairwise Post-hoc Tukey test, to check for the difference in means between each pair of sanitation technologies. Linear regression was used to examine the association between average daily *E. coli* release and the key design, construction, and operation features of the different sanitation technologies, with "statistical significance" determined by a >95% confidence level. Standard multiple regression analysis was conducted to identify the key design, construction, and operational features that influence performance of containment systems especially "septic tanks" (fully-lined tanks) in terms of *E. coli* release.

2.11. Ethical approvals

Prior to conducting user surveys, site surveys, and some key informant interviews, we obtained written informed consent from the respondents. Written consent was also obtained from the owners or operators of the sanitation systems before conducting physical measurements and environmental sampling at least 24 h in advance of the detailed survey. If owners refused or later retracted their consent, another sanitation system was randomly selected from the inventory, and the owner contacted for consent. Prior to the detailed technical study, the top slabs of the selected sanitation systems were broken to gain full access to the tank or pit and sample collection and physical measurements. However, these systems were re-sealed, and restored to their original condition or better at the end of the study. The study protocols were reviewed and approved by the Institutional Review Board (IRB) of the University of North Carolina at Chapel Hill (Reference #249163).

3. Results

3.1. Structural and physical characteristics of containment systems

The majority of the household and community containment systems in our study receive only excreta and flushing water (black-water) from the toilet facilities; only 3% of the surveyed households used containment systems as their primary means of greywater disposal; with the majority (71%) discharging greywater directly to open drains (Table S1).

All observed fully-lined tanks (household and community toilets) were rectangular. The majority (64%) of lined tanks and all lined pits were circular, with the former constructed of pre-cast concrete rings, and the latter with open-jointed pre-cast concrete rings to permit exfiltration of the liquid fraction into the surrounding soils (Table S1). Interviewees were not always aware of whether their containment were fully-lined or not; some indicated fully-lined tanks without effluent pipes where the physical survey revealed they were in fact lined.

Sixty-one percent of the observed containment systems were constructed with discharge (effluent/overflow) pipes; these included 77% of the household fully-lined tanks and 96% of the community toilet fully-lined tanks. All effluent/overflow pipes discharged to open drains (72%); stream, pond, or river (5%); open ground (12%), and soakaways or drain fields (11%) (Fig. 1 and Table S1). We frequently observed fresh faeces in the effluent from community toilet fully-lined tanks to the environment. All lined tanks and lined pits had no discharge pipes, and their pathogen release is entirely through exfiltration and desludging. Note that we never observed any exfiltrated excreta/liquid on the surface within the household or community environment during field investigation. Containment system characteristics are shown in Table S1.

3.2. Design and operational characteristics of the containment systems

The main design and operational characteristics of the studied containment systems were computed and compared, to permit study of their effect upon performance in *E. coli* removal (Table 3). The effective tank volume per capita varied significantly by technology. Community toilet fully-lined tanks had the lowest effective tank volume per capita, less than 10% of that for the fully-lined household tanks; these differences were statistically significant at $p < 0.0001$ (Table S4 which includes the pairwise Post-hoc Tukey test results). The mean liquid detention time of household fully-lined tanks (15.6 h) was significantly different to that of community toilet fully-lined tanks (5.0 h) ($p = 0.0001$).

Lined pits were associated with the longest storage periods of excreta between desludging of about 57 months; followed by household fully-lined tanks with effluent pipes (24 months), lined tanks (21 months), fully-lined tanks without effluent pipes (11 months), and community toilet fully-lined tanks (about 1 month).

The emptying frequency in the past 5 years differed significantly across the observed containment system ($p < 0.0001$, See Table S4 - for details). The majority of the household fully-lined tanks with effluent pipes, fully-lined tanks without effluent pipes and lined tanks were emptied about 2–10 times in the past 5 years. In contrast, community

Table 3
Key design and performance characteristics of studied containment systems.

Type of the containment systems	178 Containment Systems studied				
	Household Systems				Community Toilets
	Fully-Lined Tank with effluent pipes (n = 81)	Fully-Lined Tank without effluent pipes (n = 24)	Lined Tanks (n = 24)	Lined Pits (n = 19)	Fully-Lined Tank (n = 28)
Characteristics and Design Features	Median or Mean ± SD; Range (Min - Max)	Median or Mean ± SD; Range (Min - Max)	Median or Mean ± SD; Range (Min - Max)	Median or Mean ± SD; Range (Min - Max)	Median or Mean ± SD; Range (Min - Max)
Number of users	8 ± 6 (2–35)	8 ± 5 (3–20)	6 ± 3 (2–15)	5 ± 2 (3–8)	204 ± 129 (30–500)
Effective Tank Volume per capita (litres/user)	880[a] (126–3,689)	952[a](214–3,964)	245[a] (40–2,170)	191[a] (40–640)	84[a] (14–510)
Liquid Detention time (hours)	15.6 ± 11.8 (3.1–68.6)	N/A	N/A	N/A	5.0 ± 3.1 (1.3–15.1)
Storage periods between emptying (months)	24.3[a] (2.9–61.6)	11.1[a] (0.8–29.4)	21.2[a] (0.03–313)	57.1[a] (12.3–178.8)	0.9[a] (0.03–313.4)
Emptying Frequency in the past 5 year	3[a] (1–15)	7[a] (2–120)	5[a] (1–30)	1[a] (1–5)	60[a] (1–1800)

[a] Median, Min = Minimum, Max = Maximum, and SD=Standard deviation. Data collected on some variables were unevenly distributed and very highly variable, therefore, median instead of mean values are reported, denoted with *.

toilet fully-lined tanks were emptied >60 times, and lined pits <2 times in the same period (Table S3).

3.3. *E. coli* release estimation from sanitation systems

3.3.1. *E. coli* release due to liquid discharge

Fig. 2 (A) presents the arithmetic means of the average daily per capita *E. coli* release to the environment due to liquid discharge from the different sanitation technologies with effluent or overflows. Overflows from sanitary sewer points and direct discharge pipes exhibited the highest per capita daily *E. coli* release, with the arithmetic means of 10.3 and 10.5 Log_{10} *E. coli* per person per day, respectively. Household fully-lined tanks with effluent pipes to the environment exhibited the lowest average liquid daily *E. coli* release per capita (8.8) followed by community toilet fully-lined tank, (9.1) Log_{10} *E. coli* per person per day. There are significant differences in the daily per capita *E. coli* releases by discharges from different sanitation technologies, with $p = 0.0001$. Similarly, the pairwise Post-hoc test with a Tukey adjustment revealed significant differences in *E. coli* release between each pair of sanitation technologies (with $p = 0.0001$), with exceptions of (i) direct discharge pipes and sanitary sewers ($p = 0.9$), and (ii) household fully-lined tanks with effluent pipes to environment and community fully-lined tanks ($p = 0.4$) (Table S5).

3.3.2. *E. coli* release due to periodic desludging

Fig. 2 (B) presents the arithmetic means of the average daily per capita *E. coli* releases due to periodic desludging of the different technologies. Lined pits recorded the lowest average daily per capita *E. coli* release to the environment (or next stage of the sanitation service chain) due to periodic desludging, with the arithmetic mean of 5.4 Log_{10} *E. coli* per person per day; followed by lined tanks (6.7), household fully-lined tanks with effluent pipes (7.1), community toilet fully-lined tanks (8.7) and finally household fully-lined tanks without effluent pipes (9.0). Based on the pairwise post-hoc tests with a Tukey adjustment, the difference in average daily *E. coli* release due to periodic desludging between each pair of sanitation technologies was highly significant with $p < 0.05$, with the exceptions of (i) household fully-lined tanks with effluent pipes to the environment and those with soakaways, (ii) lined tanks and household fully-lined tanks with effluent pipes to environment, (iii) lined tanks and household fully-lined tanks with effluent pipes to soakaways, (iv) lined pits and household fully-lined tanks with effluent pipes to soakaways, (v) household fully-lined tanks without effluent pipes and community toilet fully-lined tanks, (vi) lined pits and lined tanks (Table S5).

3.3.3. *Combined E. coli release from liquid discharge and desludging*

For each individual system, the average daily per capita *E. coli* release from liquid discharge was arithmetically summed with the average daily per capita *E. coli* release from periodic desludging to compute the average combined daily per capita *E. coli* release. Fig. 2 (C) thus shows the arithmetic means of the average combined daily per capita *E. coli* release to the environment and/or the next stage of the sanitation service chain. This statistic differed significantly across the sampled sanitation technologies, with $p = 0.00001$ (See Fig. 3). The lined pits, lined tanks and household fully-lined tanks with effluent pipes to soakaways exhibited the lowest arithmetic average combined daily per capita *E. coli* release of about 5.4, 6.7 and 7.0 Log_{10} *E. coli* per person per day, respectively, as shown in Fig. 2 (C). Unsurprisingly, direct discharge pipes recorded the highest average combined daily *E. coli* release, with 10.5 Log_{10} *E. coli* per person per day; as shown in Fig. 2(C), these were followed by sanitary sewers (10.3), community toilet fully-lined tanks (9.5), household fully-lined tanks without effluent pipes (9.0), household fully-lined tanks with effluent pipes to the environment (8.8). The pairwise post-hoc tests with a Tukey adjustment further confirmed that the difference in average combined daily per capita *E. coli* release between each pair of sanitation technologies was significant with $p < 0.05$ but with the following exceptions: (i) household fully-lined tanks without effluent pipes and those with effluent pipes to the environment, (ii) household fully-lined tanks without effluent pipes and community toilet fully-lined tanks, (iii) lined tanks and household fully-lined tanks with effluent pipes to soakaways, (iv) lined pits and lined tanks, (v) sanitary sewer overflows and community toilet fully-lined tanks, and (vi) sanitary sewers and direct discharge pipes (Table S5).

3.4. *Containment system performance in reducing E. coli release*

We have termed household systems which discharge the deposited excreta directly to the environment with no storage or treatment as "direct discharge pipe systems". Taking the average combined daily per capita *E. coli* release of these systems as the baseline or input, we can compare the relative effectiveness of other technologies in reducing *E. coli* before their release. Lined tanks, lined pits and household fully-lined tanks with effluent pipes to soakaways exhibited excellent performance with approximately 3.5–5.1 Log_{10} *E. coli* per capita reduction, followed by household fully-lined tanks with effluent pipes to the environment (1.7), household fully-lined tanks without effluent pipes (1.5), community toilet fully-lined tanks (1.0), and sanitary sewer overflows (0.2) (See Fig. 2 (C)).

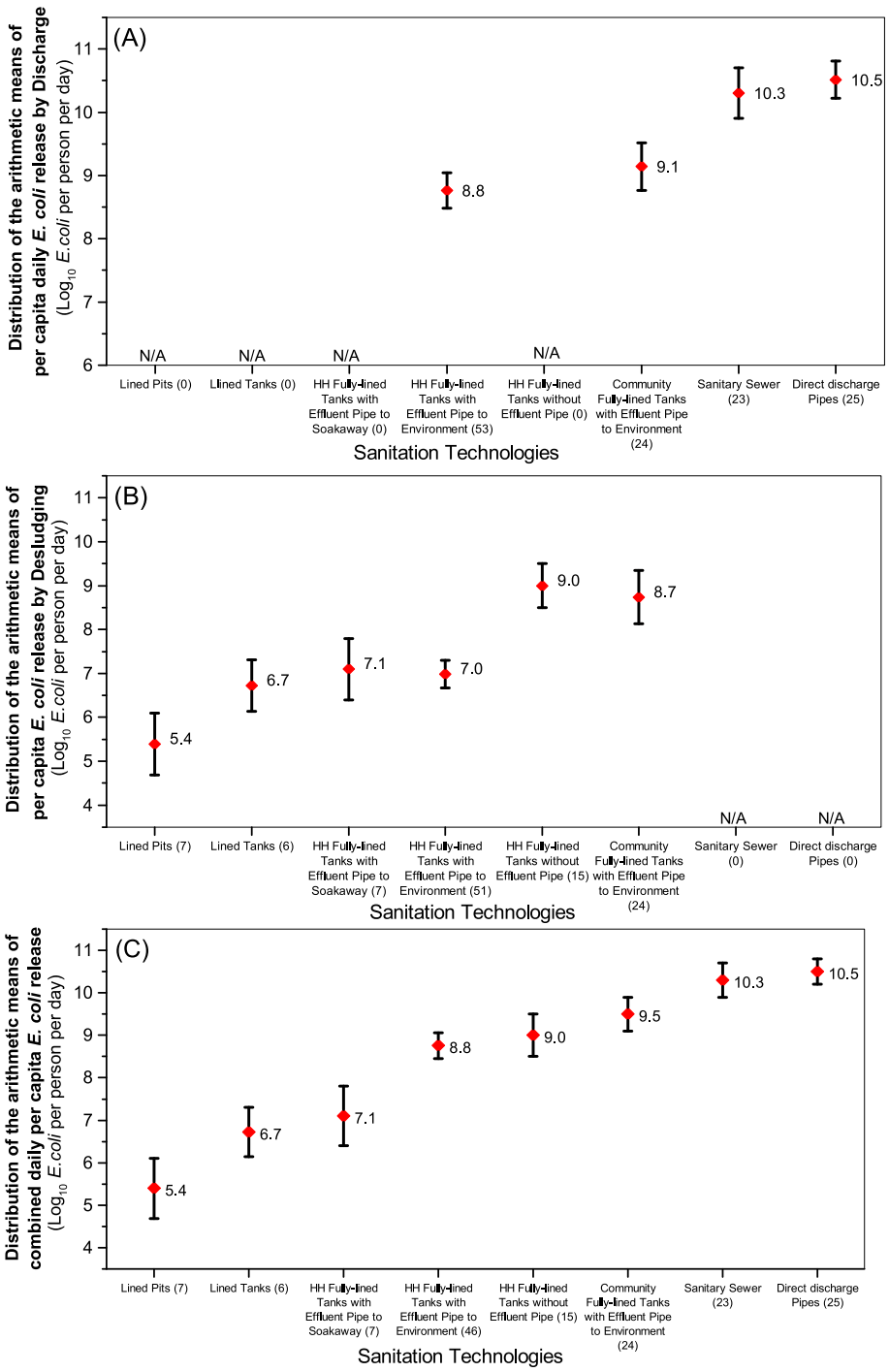

Fig. 2. (A) Arithmetic mean of the average daily per capita *E. coli* release from sanitation technologies to the environment due to discharge (i.e. effluent and/or overflows); (B) Arithmetic mean of the average daily per capita *E. coli* release to the next stage of the sanitation service chain and/or environment due to period desludging; (C) Arithmetic Mean of the average combined daily per capita *E. coli* release to the next stage of the sanitation service chain and/or the environment. *Error bars represent 95% confidence limits of the geometric means.*

4. Discussion

4.1. Design and operational characteristics of on-site sanitation technologies

The design, structural and operational characteristics of containment systems such as effective tank volume, effective tank volume per capita, liquid detention time, storage periods of excreta prior to desludging, effluent disposal, and emptying frequency varied significantly between the different sanitation technologies (Table S4).

4.1.1. Community fully-lined tanks vs household fully-lined tanks

The storage volume/user in the community toilet fully-lined tanks is less than a tenth of that for the household units (See Table 3). This greater storage volume also implies that, depending on the daily wastewater generation rate per capita in the study communities, the household fully-lined tanks would be associated with longer hydraulic detention times (of about 15 h) than community toilet fully-lined tanks (of about 5 h). This is the reason for considering "household fully-lined tanks" and "community fully-lined tanks" as distinct technologies. The average dentition time of household fully-lined tanks observed in our study is within the threshold detention time range (of 12–24 h) that is acceptable for removal of total solids in "septic systems" (Nnaji and Agunwamba, 2012).

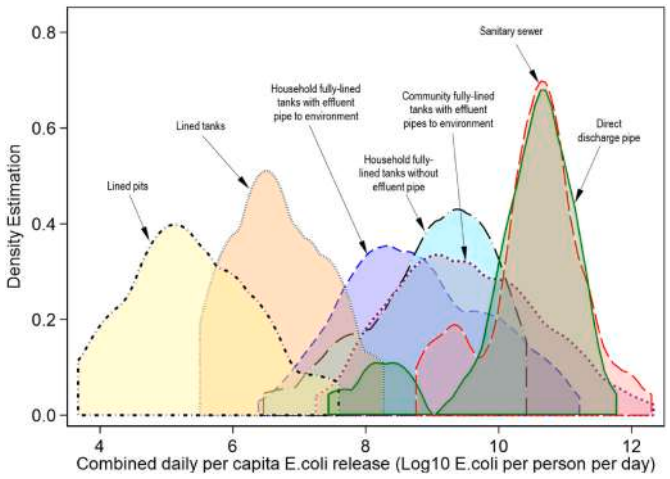

Fig. 3. Distribution of the average combined daily per capita *E. coli* release from the different sanitation technologies to the environment and/or next stage of the sanitation service chain. Density estimation represents the probability density function of the normalised variable (i.e. average combined daily per capita *E. coli* release across the population of each sanitation technology). This density plot displays the distribution of data over a continuous interval of combined daily per capita *E. coli* release. The peaks of a Density Plot for each sanitation technology shows where values are concentrated over the interval of average combined daily per capita *E. coli* release.

4.1.2. Lined pits vs lined tanks

Our study revealed that some of the key design and operational features (such as effective tank volume per capita, storage periods of excreta before emptying, etc.) of the lined pit and lined tanks were not significantly different (Tables S1–S4), suggesting that these containment systems are similar in their design and functionality. However, the lined tanks had to be emptied nearly three times as often as the lined pits. This may be because the lined tanks are associated with limited liquid exfiltration through the tank bottom only, while the lined pits are designed and constructed to allow exfiltration of the liquid fraction both through the bottom and semi-permeable sidewalls of the pit into the surrounding soils. We also observed a difference in the characteristics of faecal sludge collected from lined pits and lined tanks – as the faecal sludge from the former was more viscous and contained more solids content (with about 11.2% total solids) than that from the latter (with about 6.9% total solids). This may be attributable to (a) possible differences in influent material, with less water being flushed into pits than tanks, and (b) the corresponding difference in the storage periods of faecal sludge between desludging - as previous research has shown the total solids of faecal sludge from the containment systems (especially lined pits) to increase with the increase in storage times between emptying (Englund et al., 2020).

4.1.3. Number of users, detention time, and emptying frequency

Community toilet fully-lined tanks were associated with both the shortest storage periods between emptying and the highest emptying frequency over the previous 5 years (Table 2). This is logically due to the high user rate (about 170 people per community toilet fully-lined tank compared to 6–7 people for other lined tanks), combined with smaller effective tank volume per capita of the community toilet fully-lined tanks – all resulting from the under-design of the community toilet containment systems, with reference to recommended hydraulic detention time (Bounds, 1997; Bureau of Indian Standards, 1993; USEPA, 2002; Brandes, 1978). A similar observation was reported in Englund et al. (2020) study that found the emptying frequency of "septic tanks" to increase with increase in the number of users and decrease in the volume of the containment system. The short storage periods between desludging of community toilet fully-lined tanks observed in this study are comparable to those reported in literature, for example, Strande et al. (2018) similarly found community toilet "septic tanks" to be associated with high emptying frequency (of about 1825 in 5 years) and very short storage periods between emptying of as short as one day.

Lined tanks and pits have longer solids storage and lower emptying frequency than other technologies, perhaps because some of the liquid fraction of the biodegrading excreta exfiltrates into the surrounding soils, while the solids accumulate in containment more slowly. In the same vein, household fully-lined tanks without effluent pipes were associated with shorter storage periods between emptying and thus higher emptying frequency, than those fully-lined tanks with effluent pipes (See Table 2); this reflects the fact that fully-lined tanks without effluent pipes are constructed to store both the liquid and solid fraction of excreta, while those with effluent pipes are designed and built to release the liquid fraction to either the environment or soakaways while the solid fraction slowly accumulates in the tank.

4.2. E. coli concentrations and average daily volumetric discharges

The arithmetic mean of *E. coli* concentrations we found in the liquid discharges and faecal sludge/septage from the different sanitation technologies were in the range of 7.0–9.0 Log_{10} and 5.5–8.2 Log_{10} *E. coli*/L, respectively, and 9.0 Log_{10} *E. coli*/L for direct discharge black-water. (Table S8). Our findings broadly align with the published *E. coli* concentrations in faecal sludge/septage (Bassan et al., 2013; Manga et al., 2016; Manga, 2017) or liquid effluent (Abbassi et al., 2018; Amin et al., 2020; Pang et al., 2004; Richards et al., 2016; Humphrey et al., 2011; Harwood et al., 2017) from sanitation technologies, and direct discharge black-water (Mawioo et al., 2016; Eregno et al., 2018). However, no study to our knowledge has systematically studied concentrations of *E. coli* in both the liquid discharges and faecal sludge/-septage from on-site sanitation technologies.

Table S8 presents the arithmetic means of the average daily per capita liquid discharges and faecal sludge/septage volumes from the different sanitation technologies, and these were in the range of 20–69 L per capita per day. Currently, we are unaware of any published studies which reports volume estimates of liquid and faecal sludge discharges from sanitation technologies.

4.3. E. coli release by liquid discharge (overflows and/or effluents)

Our study found that direct discharge pipes and sanitary sewer overflows released a higher average daily per capita *E. coli* load in liquid discharge to the environment than on-site containment systems (e.g. household and community toilet fully-lined tanks); recall that lined pits and tanks in our study areas have no effluent or overflows. The results indicate that shifting from direct discharge pipes to on-site sanitation technologies with containment systems (especially those with the added advantage of liquid fraction exfiltration) may result in a 1–2 Log_{10} reduction in the average daily per capita *E. coli* release to the environment due to liquid discharge. This is in alignment with the well-established fact that some partial treatment of excreta and inactivation or removal of *E. coli* occurs during safe containment of excreta (Feachem et al., 1981; Franceys et al., 1992; Mara, 1996). Other studies (Odagiri et al., 2021; Maxcy-Brown et al., 2021) have also found that the direct discharge of black-water to the environment is a common practice in India and elsewhere, which we believe results in the substantial release of faecal pathogens to the environment, and a substantive public health hazard. We observe from our data in Fig. 2 that direct discharge pipes and sanitary sewer overflows (without treatment) may be viewed as "hypodermic needles" of pathogens, injecting up to 1000 times more *E. coli*/person/day than the other on-site sanitation systems we observed.

The volumetric liquid discharges (litres per capita per day) from household fully-lined tanks with effluent pipes to the environment were about 3 times *higher* than those from community toilet fully-lined tanks

(Table S8). However, the daily per capita *E. coli* discharge from these household systems were about 3 times (0.4 Log_{10} *E. coli*) *lower* than these community toilets. This may be due to the longer liquid detention times in household tanks (of about 15.6 h), relative to community systems (5.0 h). Previous studies have similarly shown that septic tanks with longer hydraulic detention time perform better in terms of both solids and pathogen removal from tank effluent (Bounds, 1997; Nasr and Mikhaeil, 2013; Koottatep et al., 2004; Nnaji and Agunwamba, 2012). In this study, we observed evidence that an increase in liquid detention time of the containment system by 1 day was associated with 0.7 Log_{10} reduction in the average daily per capita *E. coli* release due to liquid discharge ($R^2 = 0.11$, $p = 0.003$, see Table S7). In the same vein, the high *E. coli* release associated with the community toilet fully-lined tanks can be attributed to hydraulic overload due to the high usage rate of the community toilet fully-lined tanks and small effective tank size per capita, which all result in shorter and inadequate liquid detention times.

Observed discharge concentrations from fully-lined tanks with longer hydraulic detention times were lower than those with shorter hydraulic detention times. This is consistent with previous studies that found longer liquid detention times permit better removal of settleable solids and associated pathogens from the liquid discharge through sedimentation (Brandes, 1978; Nnaji and Agunwamba, 2012).

4.4. Release of E. coli from periodic desludging

Community toilet fully-lined tanks and household fully-lined tanks without effluent pipes showed the highest average daily per capita *E. coli* release from desludging (See Fig. 2 (B)) than other containment systems. This may be attributed to the short storage of excreta before desludging associated with community toilets and household fully-lined tanks without effluent pipes. This observation was supported by a meaningful association between the storage periods of excreta and average daily per capita *E. coli* release due to periodic desludging ($R^2 = 0.21$, $p = 0.00001$). Further, we observed evidence that an increase in the emptying frequency of the containment system was significantly associated with a higher average daily per capita *E. coli* release due to periodic desludging ($R^2 = 0.16$, $p = 0.00001$).

Our study results suggest that excreta safely contained for an extended storage period in lined tanks and pits before desludging, contains less pathogen indicator organisms and thus presents less of a public health hazard than fresher excreta from community toilet fully-lined tanks. A similar observation was reported by Mills et al. (2018) who indicated that reducing the emptying frequency and extending the storage periods of human excreta has a potential of reducing the public health risks - as no exposures are assumed to be associated with safely contained and unemptied faecal sludge. In the same vein, previous researchers have similarly found safe containment of excreta for longer periods to be responsible for pathogen reduction (Feachem et al., 1983). The difference in the average daily *E. coli* release due to periodic desludging between the lined tanks and household fully-lined tanks with effluent pipes was not significant ($p = 0.994$) implying similar performance of these two types of tanks when it comes to the containment of the solid fraction of excreta or septage. This evidence was also confirmed by the similarity in storage periods of both types which were in the range of 21–24 months (see Table 3).

4.5. Effectiveness of sanitation technologies in reducing E. coli release

In this study, we have focused on pathogen release routes that were accessible to us: liquid discharge, and desludging. We cannot, from our study, know what fraction of desludging is safely managed, as we were not able to trace the fate of such faecal sludge from emptying to its final return to the environment. Our work, however, enables one to assess the relative benefits of improved management of liquid discharges, versus better offsite treatment of faecal sludge and its safe disposal. In the following discussion, we have tentatively assumed, for demonstrative purposes, that all the pathogens still present in faecal sludge at emptying are unsafely returned to the environment, to assess the degree of indicator organism *E. coli* removed by on-site containment and treatment, by the different technologies; the implications of this assumption are explored later.

Overall, the performance of the different sanitation technologies varied significantly (Table S5). This can be attributed to the principal routes through which the different sanitation technologies release *E. coli* to the environment or the next stage of the sanitation service chain. For example, in our study, the lined tanks, lined pits, and household fully-lined tanks with effluent pipes to soakaways, primarily released *E. coli* through a single route of periodic desludging after safe containment of excreta for long periods of about 21–57 months (see Table 3) as the liquid fraction exfiltrates through the sidewalls and/or bottom of the tanks/pits. Similarly, the household fully-lined tanks without effluent pipes release *E. coli* through a single route of periodic desludging, but only after safe containment of both the liquid and solids fraction of excreta for a short time of about 11 months (see Table 3). However, community toilet fully-lined tanks and household fully-lined tanks with effluent pipe to the environment released *E. coli* through both periodic desludging after storage periods of about 1–24 months, and frequent release of effluents. Currently, we have found no study in the literature reporting comparable data.

4.5.1. Benefits of soakaways

Previous studies have reported discharge of containment effluent to the environment as a common practice in India, with about 72% of containment systems discharging effluent to open drains, especially in the urban environment (Dasgupta et al., 2019). This study and other studies in Dhaka, Bangladesh (Amin et al., 2019) and Indonesia (Odagiri et al., 2021) have found this practice to contribute to the release of faecal pathogens to the environment, constituting a clear public and environmental health hazard. However, our findings suggest that, where feasible, changing from the household fully-lined tanks with effluent pipes to the environment to effectively the same system discharging to soakaways would yield a 1.8 Log_{10} reduction in the average combined daily per capita *E. coli* release. Similarly, switching from household fully-lined tanks with no effluent pipes, (in which case septage is simply stored until emptying) to the same system with a soakaway, can reduce the per capita *E. coli* release by 2 orders of magnitude. Fully-lined tanks with effluent pipes connected to soakaways pose less risks of public exposure to faecal pathogens than equivalent systems discharging their liquid content directly to the environment. For this reason, our findings are consistent with previous analyses of sanitation technologies that concluded that all “septic systems” should comprise of a drainfield or soakaway for exfiltration of the liquid effluent into the surrounding soils for further pathogen inactivation or removal (Foster et al., 2021). In the same vein, connection of fully-lined tanks effluent to soakaways reduces the faecal pathogens in the liquid fraction in the environment, which subsequently reduce exposure risks at the household and community open drains (Mills et al., 2018).

4.5.2. Relative magnitudes of E. coli release from effluent and sludge

In the case of household fully-lined tanks with effluent pipes to the environment, we found the daily per capita *E. coli* release associated with the liquid fraction (effluent discharge) is approximately 63 times higher than from faecal sludge emptying. The ratio of liquid/solid per capita loadings of *E. coli* is reduced to 2.5 in the case of community toilet fully-lined tanks. Importantly, these ratios of liquid effluent/faecal sludge loadings of *E. coli* “discharge to the environment” would be *higher* if the collected faecal sludge is in fact properly managed; we draw the key conclusion that whether or not the faecal sludge management chain is effective, the *E. coli* loading to the environment from these systems is predominantly in the liquid effluent. This is a significant finding for sanitation planners and engineers as it may well aid the prioritizing of sanitation interventions that will minimize release of pathogen indicator

organism *E.coli* to the environment and minimize e public health hazard. Our study findings are in agreement with Peal et al. (2020) that concluded that safely managed sanitation cannot be achieve by managing only faecal sludge but also the liquid effluent – as this will help reduce the release of pathogen hazards to the environment.

4.5.3. Ranking of technologies by E. coli release

These study findings are consistent with the observation made by Kolsky et al. (2019) that from a perspective of pathogen hazards release, the widely-held public view of a sanitation technology hierarchy of "safety" from pit latrines → septic tanks→ sanitary sewers, is probably incorrect as such systems are currently managed in resource-poor settings. Arguably, our data suggest that in many settings it may be reversed, and the hierarchy should start with the sanitary sewers with inadequate sewage treatment before discharge as the worst performance, lined pits with the best performance, and the other technologies in-between may be ranked on the basis of the per capita *E. coli* loadings to the environment.

4.5.4. Effects of excreta storage, liquid discharge, and faecal sludge management

The excellent performance exhibited by the lined tanks and lined pits can be attributed to (a) the long excreta storage periods prior to desludging and low emptying frequency associated with such containment systems, and (b) the lack of liquid discharge to the environment. An increase in the storage periods of excreta and a decrease in the emptying frequency was significantly associated with a decrease in the average daily per capita *E. coli* release, thus performance (Tables S6–S7). Even though the lined tanks and lined pits exhibited excellent performance, these systems still release 5.4–6.7 Log_{10} *E. coli* per person per day to the environment or next stage of the sanitation service chain; safer management of excreta along the service chain is still needed to minimize the unsafe returns and the associated pathogen hazards. Our findings are consistent with previous analyses on pathogen flows associated with sanitation technologies that if on-site sanitation technologies are to adequately protect public health and contamination of the wider environment, they all require appropriate and robust management regardless of their performance in terms of inactivating or removing faecal pathogens (Foster et al., 2021).

Amongst the containment systems, community toilet fully-lined tanks exhibited the worst performance, with the highest average daily per capita *E. coli* release. This can be attributed to the short liquid detention time, short storage periods of the excreta prior to emptying, and high emptying frequency associated with community toilet fully-lined tanks from hydraulic overloading or higher user rate, and smaller effective tank volume per capita. This observation was supported by a meaningful correlation between average daily per capita *E. coli* release and storage periods of excreta before desludging (R^2 = 0.16, p = 0.0006), liquid detention time (R^2 = 0.13, p = 0.0025) and emptying frequency (R^2 = 0.21, p = 0.0001).

Our results confirm that safe containment of excreta for an extended period, with resultant pathogen inactivation, has great potential to reduce the release of pathogen hazards to the next stage of the sanitation service and/or environment as well as the spread of excreta-related diseases. However, both the rate of pathogen inactivation and the factors influencing pathogen reduction or inactivation in containment systems vary widely between pathogens, and are still not well understood.

Our observation of no statistically significant difference between the performance of community toilet fully-lined tanks and sanitary sewer overflows (with p = 0.110), suggests that such community toilet fully-lined tanks probably function more like direct discharge pipes. The poor performance exhibited by community toilet fully-lined tanks could reflect both the short liquid detention time and the poor operation and emptying practices of these systems. By visual observation of the emptying operations, most of the these tanks were partially emptied especially from the second or third chamber of the tank; this could be due to the large sizes of the containment system, where the desludging operators could not completely desludge the tank in a single trip. Failure to empty the first chamber naturally leads to continuous accumulation of faecal sludge in this tank over several cleanings, thus gradually reducing the effective tank volume. Similar observation was made by Koottatep et al. (2014) and Nnaji and Agunwamba (2012) that the accumulation of faecal sludge in the containment system reduces the mean hydraulic detention times, which results in more short-circuiting and an increase in the pathogens and pollutant release from the containment system.

4.6. Design and operational features influencing performance

Regression results revealed safe disposal of the liquid effluent to soakaways as the most important design feature that reduces *E. coli* release from "septic systems", as this effectively eliminates the liquid release route. We observed that construction of the fully-lined tank with liquid effluent disposal to soakaways significantly reduces average daily combined per capita *E. coli* release by about 30 times (Coef. = −0.903, p = 0.029), and this was the highest reduction observed in the study. This was followed by the inlet and outlet pipe configurations (Coef. = −0.892, p = 0.0001), liquid detention time of ≥24 h (Coef. = −0.563, p = 0.011) and faecal sludge storage times between desludging (Coef. = −0.180, p = 0.026). These findings suggest some of the important design features and operational practices that should be considered for any "septic system"/fully-lined tank, from the perspective of reducing pathogen releases. Soakaways, however, may not be technically or financially realistic in areas with rock or tight soils; in this case, both inlet and outlet pipe configurations and detention times take on greater importance. Previous studies, have also found proper configuration of inlet and outlet pipes (Koottatep et al., 2014) and hydraulic detention time (Brandes, 1978; Nnaji and Agunwamba, 2012) as important features to consider during the design and construction of fully-lined tanks so as to improve the performance of the systems to better safeguard public health and protect environmental contamination.

Further, although some studies and design manuals (Koottatep et al., 2014; Georgia Department of Public Health, 2019; Missouri DHSS., 2018) have recommend other key design features of "septic systems" or fully-lined tanks (such as Length: Breadth ratio of ≥1.5, number of chambers, effective tank depth of 0.9–2, etc.), in our study we did not, however, observe a meaningful association between them and the performance indicator of average daily *E. coli* release (See Supplementary information; Table S7). This may be because our sample size was insufficient to detect the effect of these design features on the *E. coli* release, as some of these design features were missing in the majority of the sampled fully-lined tanks. However, our study still encourages us that there are many plausible ways to improve performance, and that rigorous studies to test these design features and their relative impacts would be desirable.

Inlet and outlet pipe configuration had a statistically significant association with average daily *E. coli* release due to average daily combined per capita *E. coli* release. Our results suggest that use of correct inlet and outlet pipe configuration reduces the average daily combined per capita *E. coli* release by about 8 times. Liquid detention time had a statistically significant association with the average daily combined per capita *E. coli* release. An increase in liquid detention time by 1 day reduces average daily *E. coli* release due to the average daily combined per capita *E. coli* release by about 7 times (See Table S7). Therefore, the proper designing of septic tanks/fully-lined tanks with a minimum liquid detention time of ≥1 days should be emphasised.

There is a practically meaningful and statistically significant relationship between storage periods of excreta before desludging and average daily per capita *E. coli* release due to average daily combined per capita *E. coli* release. We observed that safe containment of excreta for prolonged periods significantly reduces *E. coli* release to the

environment or next stage of the sanitation service chain. This implies that the containment system should be designed with a sufficient effective tank volume per capita for storage of the excreta to promote adequate pathogen inactivation or removal during containment. Sanitation technologies such as lined tanks and lined pits with storage periods of more than 21 months were associated with considerably lower *E. coli* release due to periodic desludging. Further studies are needed to better understand the die-off kinetics of pathogens in the septic tank sludge and timing of the pseudo-steady state of pathogen load in the tanks.

4.7. Limitations of the study

We believe that both the results of this study, and the approach taken of tracking pathogen flow through both effluent and faecal sludge disposal routes may be of significant value to those working in this area. Like all studies, however, this one has some limitations.

Due to logistical challenges, the sanitation systems studied were limited to those we encountered during the community visits and transect walks; all the systems in the final study were purposively selected to represent a range of sanitation technologies observed in the study communities. A fully representative sample was not possible. This means we cannot, with scientific rigour, generalize some of the key findings and conclusions of this study to the study area population, let alone to all the communities in Tamil Nadu or other cities in India. However, the study findings should be helpful for policymakers and sanitation planners in considering similar study communities or contexts, and to promote better priority setting and decision-making in sanitation interventions.

In broad terms of public health importance, we are at best estimating *E. coli* release to the environment, not public exposure to these *E. coli*; details of where such releases went and their possible impact on human exposure were beyond the scope of the study. The authors nevertheless believe that one of the first steps for better sanitation management for public health is an understanding of the pathogen flow and "leakage" from the systems designed to control excreta.

In our study, periodic desludging and liquid discharge (overflow or effluent release) were considered as the principal routes for pathogen (represented in this study by *E. coli*) release from sanitation technologies to the environment. We did not consider other pathogen release routes such as exfiltration of the liquid fraction from the lined tanks and lined pits, or the soakaways for fully-lined tanks. During our observations, we did not observe and were unaware of any surfacing of the exfiltrated excreta/liquid, but this could be much more significant during rains. The associated risk from exfiltration into soil is, of course, heavily dependent upon the location of any groundwater sources used for drinking water.

Secondly, the unsafe returns and *E. coli* releases associated with emptying, transportation, treatment, and disposal were not considered in this study as we focused on the release from the sanitation technologies or containment systems. However, our related work in progress on pathogen flow addresses pathogen releases from other stages of the sanitation service chain.

Like many before us, we used *E. coli* as an indicator for pathogens released from the different sanitation systems because it is has been widely reported in literature as an important and very widely-used faecal indicator when assessing the microbial and public health hazards and/or risks associated with faecal waste/human excreta (Feachem et al., 1983; Odonkor and Ampofo, 2013; Manga et al., 2021). However, *E. coli* may not be a suitable organism indicator for some pathogens (such as helminth eggs, viruses, protozoa oocysts etc.) found in faecal sludge/human waste from different sanitation technologies (Amin et al., 2019).

5. Conclusions

While there is a wide range of on-site sanitation technologies used in much of the world, there has been little systematic study of their relative effectiveness in reducing pathogen release in either liquid or solid waste streams, or of the combined release of pathogens to the environment from these two streams. Field investigations in Tamil Nadu, India were undertaken to assess the performance of local sanitation technologies in reducing the release of the indicator organism *E. coli* to either the environment or the next downstream step of the sanitation service chain. -We hope these findings, (combined with future work by this team and others adopting a similar approach with different pathogens, and in other sites), will be useful in improving the design, construction and operation, and performance of on-site sanitation technologies (and especially septic systems) in terms of pathogen inactivation and release. Based on the study findings, we draw the following conclusions:

i. Pathogen release from the studied on-site sanitation technologies varied by as much as 5 orders of magnitude from "lined pits" (5.4 Log10 *E. coli* per person per day) to "overflowing sanitary sewers" and "direct discharge pipes" (10.3–10.5 Log10 *E. coli* per person per day). Other technologies ("lined tanks", "household fully-lined tanks", and "community toilet fully-lined tanks") lay between these extremes, and their performances in *E. coli* removal also varied significantly, in both statistical and practical terms. "Household fully-lined tanks", a widespread technology, offered nearly a 2 Log10 reduction in *E. coli* compared to "direct discharge pipes" in which no detention or treatment occurs.
ii. Human excreta safely contained for an extended storage period in lined tanks and lined pits contained significantly less *E. coli* than fresh excreta discharged from direct discharge pipes or sanitary sewers without treatment. This study indicated that the direct discharge pipes and sanitary sewer overflows (without treatment) release up to 1000 times more *E. coli*/person/day to the environment than the on-site sanitation systems we observed. Therefore, sanitary sewer authorities and funders should not just prioritize the extension of sanitary sewer services but should also stress (i) improved operations and maintenance to reduce sanitary sewer blockages and overflows to minimize highly concentrated pathogen release, and (ii) effective and reliable pathogen removal at treatment works.
iii. Community toilet fully-lined tanks exhibited higher *E. coli* release per capita than household fully-lined tanks, and this was significantly influenced by the mode of operation. Therefore, sanitation engineers and authorities should pay more attention to improvement to the operation, routine maintenance of similar systems to improve performance.
iv. Community toilets and household "septic tanks" (the most commonly used technology in the study area) at present discharge on average 3 and 65 times, respectively, as many *E. coli* per person per day through the daily liquid release than through periodic desludging. Our study findings suggest that although faecal sludge management along the sanitation service chain is important, the highest priority should be directed to proper management of the liquid effluents from these containment systems. The significance of liquid stream release from "septic tanks" and other on-site sanitation technologies is also highlighted by the 2–3 Log10 difference between systems without significant liquid release (lined pits, lined tanks and fully-lined tanks with effluent pipes to soakaways), and those with significant liquid release (fully-lined tanks with effluent pipes to environment). The former exfiltrate their liquid fraction through soakaways or the soil, without creating, in the sites we studied, any visible threat or nuisance, while the latter release most of their *E. coli* to the surface as effluent or overflow.
v. Four design parameters were found to influence performance of on-site sanitation systems in *E. coli* removal: (i) disposal of the liquid effluent to soakaways, (ii) inlet and outlet pipe design to reduce short-circuiting, (iv) liquid detention time and (iii) faecal

sludge storage time to reduce the frequency of emptying, as it affected the *E. coli* released. The study suggests that to minimize average daily *E. coli* release associated with the liquid fraction of excreta, the plumbing errors in the inlet and outlet pipe configurations need to be addressed as soon as possible, as they lead to serious "short-circuiting". Further, proper design of the containment system is important – to improve the performance in terms of reducing pathogen indicator organism *E. coli.* However, proper operation and maintenance practices of the containment systems are at least as important as technology selection, design, and construction.

Future work should focus on i) detailed analyses of technical and process factors in performance, including a rigorous comparison of what are known locally as "septic tanks" with the key criteria adopted nearly universally by engineers and boards of health in defining an acceptable septic tank; and ii) development of a "pathogen flow diagram" for the communities to highlight where in the sanitation service chain the greatest pathogen leaks to the environment occur.

Disclaimer

The findings and conclusions of this study are solely of the authors, and do not represent the views, decisions or policies of the institutions with which they are affiliated.

Author contribution

Conceptualization (MM, PK, JS, JB, & JR); Methodology (MM, PK, JS, & JR); Software (MM); Formal Analysis (MM, & PK); Investigation (MM, PK, & JS); Resources (PK, JB, & JS); Data curation (MM, PK, JS, JB, & JR); Writing - original draft preparation (MM); Writing – Review and Editing (MM, PK, JB, JS, JR, SR, & LS); Visualization (MM, PK); Supervision (JS, PK, & JB); Project Administration (MM, JS & PK); Funding Acquisition (PK, JS & JB). All authors read and approved the final manuscript.

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Appendix A. Supplementary data

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Urinary 3-phenoxybenzoic acid (3-PBA) levels and changes in hematological parameters in Korean adult population: A Korean National Environmental Health Survey (KoNEHS) 2012–2014 analysis

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ABSTRACT

Pyrethroid insecticides have been broadly used as pest control in agriculture and residential spaces, exerting high effectiveness of insecticidal property and relatively low toxicity to humans. Several animal studies suggested that exposure to pyrethroids may induce hematological abnormalities, thereby altering the number of blood cells and resulting in blood disorders. However, no epidemiologic study has reported on the effect of pyrethroid insecticide exposure on hematological changes, except for occupational exposure. This study aimed to investigate the effect of urinary 3-phenoxybenzoic acid (3-PBA) concentrations on hematological parameters in a representative South Korean adult population. We analyzed data from 6296 adults enrolled in the Korean National Environmental Health Survey (2012–2014). We employed multiple linear regression analysis to evaluate the association of urinary 3-PBA levels with eight hematological profiles: white blood cells (WBCs), red blood cells (RBCs), hemoglobin, hematocrit, platelets, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The urinary 3-PBA levels were negatively associated with WBC, RBC, and hemoglobin levels and positively associated with MCV levels. The direction and magnitude of the association between the 3-PBA and hematological parameters varied according to sex and age. The adverse effects of 3-PBA on hematological parameters were distinctive among males aged 60 years and older. In this age group, 3-PBA levels were negatively associated with the WBC, RBC, hemoglobin, hematocrit, and MCHC levels among males. This study is the first to verify that urinary 3-PBA concentrations at the levels found in a Korean population are associated with blood parameters. This finding merits further investigation to understand the impact of 3-PBA on human blood function and public health.

1. Introduction

Pyrethroids are a class of synthetic chemicals originating from pyrethrin, which is a natural insecticide found in the flowers of Chrysanthemum. Pyrethroids have become the most widely used insecticides replacing traditional ones (e.g., organophosphate insecticides), owing to their high and long-lasting effectiveness of insecticidal properties and low mammalian toxicity (Barr et al., 2010). Accounting for over 30% of the global insecticide market, pyrethroids are frequently used in agriculture and residential settings and public pest control in the general population (USEPA, 2017). In the general population, pyrethroids are exposed through the diet, inhaled airborne pesticides, skin contact, and

Abbreviations: 3-PBA, 3-phenoxybenzoic acid; 4-F-3PBA, 4-fluoro-3-phenoxybenzoic acid; AMs, arithmetic means; BMI, body mass index; CI, confidence interval; cis/trans-DCCA, cis/trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid; GMs, geometric means; DBCA, cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid; GSE, geometric standard error of the mean; KoNEHS, Korean National Environmental Health Survey; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBCs, red blood cells; SE, arithmetic standard error of the mean; trans-DCCA, trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid; WBCs, white blood cells.

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unintentional ingestion (Tang et al., 2018). 3-phenoxybenzoic acid (3-PBA) is a non-specific metabolite of pyrethroids and is a commonly used urine biomarker from several nationwide biomonitoring programs worldwide. According to several national studies, 3-PBA has been detected in over 70% of Americans and 100% of Canadians (Health Canada, 2013; Jain, 2016). Given the increasing use of pyrethroids, the adverse health effects of pyrethroids in the general population are becoming an important public health issue.

Although the nervous system is the primary target of pyrethroids, endocrine disruption, hearing loss, decreased pulmonary function, and blood function disorders are also reported as adverse health effects of pyrethroids (Hu et al., 2021; Hwang et al., 2019; Park et al., 2019; Xu et al., 2020; Alavanja et al., 2014). Pyrethroids are metabolized into several metabolites (e.g., 3-PBA and trans-3-(2,2-dichlorovinyl)-2, 2-dimethyl-cyclopropane-1-carboxylic acid (trans-DCCA)) in the human body and destroy hematopoietic progenitor cells, thereby inhibiting blood cell production in the bone marrow (Arif et al., 2020; Mandarapu and Prakhya, 2015). Several experimental studies found that pyrethroids inhibit erythropoiesis and heme production by affecting hematopoietic progenitors and destroying erythroid cells, leading to lower RBC and hemoglobin levels (Khan et al., 2012; Mandarapu and Prakhya, 2015; Manna et al., 2004). Thus, pyrethroids may damage hematopoietic stem and progenitor cells and alter hematological parameters, resulting in aplastic anemia, leukopenia, thrombocytopenia, leukemia, or other blood cancers (Alavanja et al., 2014; ATSDR, 2003; Hofmann et al., 2021; Rusiecki et al., 2009; Shearer et al., 2019).

Many prior studies on the association of pyrethroids exposure with altered hematological parameters and blood disorders were primarily based on animal experiments, and only a few studies have confirmed these effects in humans. In a recent study in the U.S., 27 pesticide applicators exposed to permethrin during their lifetime experienced reduced RBC, elevated MCV, and increased risks of multiple myeloma (Shearer et al., 2019). In addition, a study on 83 farmers in Pakistan found that pyrethroid-used workers showed reduced RBC, hematocrit, and WBC levels and had a high risk of anemia (Azmi et al., 2009), while increased WBC levels and reduced hemoglobin and platelet levels were observed from 40 farmers in Turkey (Varol et al., 2014). However, all these studies showed different associations between hematologic parameters and partially different direction of the associations. Also, they used relatively small sample sizes. Most importantly, they mainly focused on occupational exposure, which exposed high concentrations of pyrethroids. There is a possibility that similar effects could be observed at relatively low concentrations during environmental exposure; however, no epidemiological study of associations between pyrethroid exposure and hematological parameters has been verified in the general population thus far. Since over 70% of pesticides in the Korean market are pyrethroids, with a likely higher rate of exposure than in other countries (Choi et al., 2020), a study on a South Korean population is needed.

Therefore, this study assessed the association between urinary 3-PBA levels and hematological parameters in adults recruited in the Korean National Environmental Health Survey (KoNEHS) 2012–2014.

2. Methods

2.1. Study population

This study drew data from adult participants in the second round of KoNEHS (2012–2014). Conducted by the Korea Ministry of Environment since 2009, KoNEHS is a nationwide survey that represents the South Korean population. It constitutes an ongoing series of three-year cross-sectional studies designed to collect nationally representative exposure profiles for environmental contaminants and associates socio-demographic and behavioral characteristics in a representative South Korean population. The survey was comprised of three parts—health and behavior interviews, environmental chemical analysis, and clinical examinations. Trained personnel performed the questionnaire surveys through face-to-face interviews, and a medical technologist performed the environmental chemical analysis and clinical exams by collecting urine and blood samples of participants (NIER, 2017).

A total of 6478 participants at age 19 and older were enrolled in the KoNEHS cycle 2 (2012–2014) based on a proportionally stratified two-stage sampling design, considering their sex, age, and geographical characteristics. The information about strata, clusters, and sample weights for design-based analysis were employed to account for the differential probability of selection and non-response. Among the initially recruited participants (n = 6478), participants who did not perform urinary 3-PBA tests (n = 76), those who did not take blood tests (n = 23), and those missing data on their urinary cotinine levels (n = 34) were excluded. Participants who were pregnant (n = 28) or were undergoing anemia treatment (n = 21) were also dropped, as differences in hematological variables due to either condition could confound the results. In addition, five participants taking blood-related medications were excluded (Hill and Pickinpaugh, 2008). Ultimately, data from 6296 participants were used for analysis (Supplemental Fig. S1).

KoNEHS was approved by the Research Ethics Committee of the National Institute of Environmental Research in Korea, and all the participants provided written informed consent.

2.2. Measurement of urinary 3-PBA

Technicians assembled participants' spot urine samples at survey centers. Urine samples were immediately delivered at 0–4 °C to laboratories and frozen at −20 °C within 24 h of collection. Urinary 3-PBA was estimated by liquid-liquid extraction (LLE) and derivatized to volatile esters using N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) and gas chromatography mass spectrometry (GC–MS) (Clarus 600T; Perkin Elmer) separation. The analytical method for urinary 3-PBA was performed following the descriptions in a previous report (NIER, 2015). Briefly, 2-PBA was added as a standard internal material, and acidic hydrolysis was performed for the split of conjugates. The LLE procedure was then used to extract 3-PBA analytes from the matrix using n-hexane under acidic conditions. NaOH was summed to the organic phase, and the carboxylic acids were re-extracted to the aqueous phase for further purification. After repeated acidification and extraction into n-hexane, the metabolites were derivatized to volatile esters using MTBSTFA. Separation and detection were conducted using capillary gas chromatography–mass spectrometry.

Quality control procedures followed the protocols recommended by the Korea National Institute of Environmental Research (NIER, 2015). Analytical laboratories successfully certified the quality control program set by the Korean Association of Quality Assurance for Clinical Laboratory and the German External Quality Assessment Scheme for Analysis of Organic Chemicals in Biological Materials (G-EQUAS) twice a year. The method detection limit (MDL) for urinary 3-PBA was 0.015 μg/dL (NIER, 2015). Only 2 (0.003%) adult samples of urinary 3-PBA had below the MDL. Values for each urinary 3-PBA level below the MDL were determined by dividing the MDL by $\sqrt{2}$ (0.011 μg/dL) (Hwang et al., 2019).

2.3. Hematologic parameters

The hematologic parameters were measured on blood samples collected simultaneously with urine samples. These parameters were analyzed by laser flow cytometry (ADVIA 2120i; Siemens, USA). Detailed information on sample collection and analysis protocols for hematologic parameters has been described in a previous report (NIER, 2017). We used the following hematological variables for the analysis: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin concentration, hematocrit concentration, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean

corpuscular hemoglobin concentration (MCHC). The MCV, MCH, and MCHC values were derived from the following formula: MCV (fL) = hematocrit/RBC × 10; MCH (pg) = hemoglobin/RBC × 10; and MCHC (g/dL) = hemoglobin/hematocrit × 10 (Dacie and Lewis, 2001).

2.4. Covariates

Relevant covariates were identified through literature review and were included in the final models based on the Pearson correlation coefficients (Azmi et al., 2009; Hassanin et al., 2018; Kassahun et al., 2020; Kimata et al., 2009). The correlation coefficients between the potential covariates were low ($r < 0.4$, p-value$\leq$0.05), thereby eliminating the multicollinearity concern among them. The selected variables were age, sex, region, monthly household income, urinary cotinine level, body mass index (BMI), physical activity, drinking status, agricultural occupation, household pest control and urinary creatinine level. Age, urinary cotinine level, BMI, and urinary creatinine level were used as continuous variables. Next, sex (male or female), region (urban or rural), monthly household income (low, mid-low, mid-high, or high), physical activity (little, moderate, or vigorous), drinking status (never, former, or current), agricultural occupation (no or yes), and household pest control (no or yes) were used as categorical variables. Also, monthly household income was divided by the income criteria according to the definition of the Statistics Korea. The cut-off level of high urinary cotinine level was $\geq$50 μg/L based on previous studies, which reported that >50 μg/L was considered a smoker (Hong et al., 2018). BMI was calculated by dividing the measured weight (kg) by the square of the measured height (m). To account for occupational exposure to insecticide, information on agricultural occupation (yes or no) at the participant's longest job was considered as a potential confounder. The participants' agricultural occupation was identified according to the Korean Standard Classification of Occupations (Statistics Korea, 2017). Household pest control (yes or no) in the past year was also considered as a potential confounder to account for non-occupational and household insecticide exposure. Finally, urinary creatinine level was inputted in all models as a confounder to correct the dilution effect of spot urine samples (James-Todd et al., 2016).

2.5. Statistical analysis

The urinary 3-PBA and hematological measures of WBC, RBC, hemoglobin, hematocrit, and platelet counts had a right-skewed distribution. As such, the values were log-transformed. For descriptive statistics, geometric means (GMs) or arithmetic means (AMs) with percentiles of urinary 3-PBA levels and hematological parameters were assessed. The student's t-test was used to evaluate the differences in the geometric mean or arithmetic mean between the groups. Multiple linear regression analysis was used to evaluate the association between urinary 3-PBA levels and hematological parameters. The model included urinary 3-PBA levels as independent variables, and hematological measures (WBC, RBC, hemoglobin, hematocrit, platelet, MCV, MCH, and MCHC) as dependent variables, and the 3-PBA levels were set as continuous, and categorical variables (quartiles) in all models. P-values for a linear trend were computed by fitting the 3-PBA quartiles as ordinal categorical variables coded using integer values (0–4). Potential confounders were classified into three: sociodemographic factors, behavioral and metabolic factors, and insecticide-related exposure factors. As a result, three sequential models were developed to identify the effects of confounders: Model A was adjusted for sociodemographic factors (age, sex, region, monthly household income, and urinary creatinine level); Model B was additionally adjusted for behavioral and metabolic factors (urinary cotinine level, BMI, physical activity, and drinking status); and Model C was additionally adjusted for insecticide-related exposure factors (agricultural occupation and household pest control).

3-PBA potentially interacts with sex hormones, affecting blood function depending on the sex of an individual (Tyler et al., 2000), and it affects hematological changes due to differences in metabolism and excretion depending on the age (Rosita et al., 2016). Also, occupational or residential insecticide use can vary the effects of 3-PBA on hematological parameters. Thus, stratified analyses were conducted to evaluate whether sex (male or female), age (19–60 years or $\geq$60 years), agricultural occupation (yes or no), and household pest control (yes or no) was potential effect modifiers of these associations. The cut-off value for categorizing participants into two age groups were set based on the reports demonstrating that the association between age and hematological parameters dramatically changed in the 60s with rapid aging (Tefferi, 2011). Moreover, the interactions of 3-PBA with sex and age on hematological parameters were evaluated by including a multiplicative interaction term between 3-PBA levels as continuous variables and sex or age group in the models.

Pearson's correlation analysis was performed to test the correlation between urinary 3-PBA and urinary cadmium, urinary mercury, and blood lead. As urinary cadmium, urinary mercury, and blood lead had a right-skewed distribution, and the values were log-transformed.

All statistical analyses were performed using the complex sample module in SPSS version 25.0 and "survey" package in R version 3.4.3, and the statistical significance level was set as a two-sided p-value of $<$0.05.

2.6. Sensitivity analysis

Sensitivity analyses were designed to control other environmental chemical risk factors of hematological parameters among the study participants. Heavy metals (e.g., cadmium, mercury, and lead) and pyrethroids are prevalent chemicals in daily living spaces. They share common exposure routes: contaminated food, water, and insecticide use for cadmium, mercury, and 3-PBA; polluted indoor and outdoor air, and consumer goods for lead and 3-PBA (Nguyen et al., 2021; Wang et al., 2019). Therefore, several studies reported that the urinary or blood levels of heavy metals were moderately correlated with urinary 3-PBA in the general populations (Lee et al., 2021; Nguyen et al., 2021). Furthermore, prior studies showed that these chemicals were associated with hematological alterations (Rehman et al., 2017). Therefore, this study verified whether heavy metals and urinary 3-PBA were significantly correlated based on Pearson's correlation analysis results, and sequentially added urinary cadmium, mercury, and blood lead levels in the multiple regression models.

3. Results

The geometric means or arithmetic means of hematological parameters by participant characteristics are shown in Table 1. In the study population, the mean (standard error, SE) age was 46.41 (0.36) years, and 3577 (56.8%) were female. More than half of the participants had a BMI of 23 or more (62.7%), and were current drinkers (59.4%). The hematological measures were lower among females than males, decreased with age, and significantly differed by BMI, monthly income, urinary cotinine, physical activity, and drinking status.

The unadjusted and creatinine-adjusted 3-PBA concentrations by participant characteristics are shown in Fig. 1. Both unadjusted and creatinine-adjusted 3-PBA concentrations were significantly higher in older participants, participants who resided in rural areas with lower household income, lower cotinine levels, and non-drinkers.

The distribution of urinary 3-PBA concentrations and hematological parameters are summarized in Table 2. The detection frequency of urinary 3-PBA was 99.97%. The geometric means of urinary 3-PBA level was 1.42 μg/L. The geometric means for hematological parameters were 6.72×10^9/μL for WBC, 4.63×10^6/μL for RBC, 13.98 g/dL for hemoglobin, 42.13×10^2 L/L for hematocrit, 257.86×10^9/μL for platelet, 91.17 fL for MCV, 30.27 pg for MCH, and 33.20 g/dL for MCHC.

The multiple linear regression results representing the associations of urinary 3-PBA levels with the hematological parameters were presented

Table 1
Geometric means (standard errors) or arithmetic means (standard errors) of hematological parameters by participant characteristics (n = 6296).

Characteristic	n (%)[a]	WBC[b]	RBC[b]	Hemoglobin[b]	Hematocrit[b]	Platelet[b]	MCV[b]	MCH[b]	MCHC[b]
		GM (GSE)	GM (GSE)	GM (GSE)	GM (GSE)	GM (GSE)	AM (SE)	AM (SE)	AM (SE)
Total	6296 (100)	6.72 (1.01)	4.63 (1.00)	13.98 (1.00)	42.13 (1.00)	257.86 (1.00)	91.17 (0.10)	30.27 (0.05)	33.20 (0.04)
Age (years)									
19–39	1520 (24.1)	6.99 (1.01) **	4.79 (1.00) **	14.27 (1.00)**	43.04 (1.00)**	267.14 (1.01) **	89.99 (0.15) **	29.86 (0.06) **	33.17 (0.05) **
40–59	2617 (41.6)	6.62 (1.01)	4.63 (1.00)	14.03 (1.00)	42.24 (1.00)	256.66 (1.01)	91.39 (0.13)	30.38 (0.06)	33.24 (0.05)
≥60	2159 (34.3)	6.45 (1.01)	4.36 (1.00)	13.40 (1.00)	40.42 (1.00)	244.91 (1.01)	92.74 (0.13)	30.76 (0.06)	33.16 (0.05)
Sex									
Male	2719 (43.2)	6.97 (1.01) **	4.92 (1.00) **	15.06 (1.00)**	45.05 (1.00)**	252.46 (1.00) **	91.69 (0.14) **	30.65 (0.05) **	33.44 (0.04) **
Female	3577 (56.8)	6.48 (1.01)	4.36 (1.00)	12.99 (1.00)	39.43 (1.00)	263.30 (1.00)	90.65 (0.11)	29.89 (0.06)	32.96 (0.05)
Region									
Urban	4954 (78.7)	6.70 (1.01)	4.63 (1.00)	13.98 (1.00)	42.13 (1.00)	257.90 (1.00)	91.16 (0.11)	30.26 (0.05)	33.19 (0.04)
Rural	1342 (21.3)	6.87 (1.02)	4.62 (1.01)	13.99 (1.01)	42.09 (1.01)	257.27 (1.02)	91.28 (0.23)	30.37 (0.17)	33.26 (0.14)
BMI (kg/m^2)									
<23	2294 (36.4)	6.45 (1.01) **	4.53 (1.00) **	13.64 (1.00)**	41.35 (1.00)**	254.11 (1.01)	91.39 (0.14) **	30.18 (0.06)*	33.01 (0.05) **
23–25	1567 (24.9)	6.65 (1.01)	4.62 (1.00)	14.02 (1.00)	42.15 (1.00)	257.80 (1.01)	91.29 (0.16)	30.38 (0.07)	33.28 (0.05)
≥25	2435 (38.7)	7.06 (1.01)	4.74 (1.00)	14.32 (1.00)	42.97 (1.00)	261.97 (1.01)	90.85 (0.13)	30.30 (0.05)	33.35 (0.04)
Monthly income (million Won)									
Low (<1)	1771 (28.1)	6.51 (1.01) **	4.44 (1.00) **	13.51 (1.00)**	40.82 (1.00)**	251.73 (1.01) **	92.10 (0.18) **	30.49 (0.06) **	33.11 (0.05)
Mid-Low (1–2)	2900 (46.1)	6.73 (1.01)	4.63 (1.00)	13.95 (1.00)	42.08 (1.00)	260.17 (1.01)	91.04 (0.19)	30.21 (0.08)	33.17 (0.05)
Mid-High (2–3)	1567 (24.9)	6.91 (1.01)	4.71 (1.00)	14.20 (1.00)	42.84 (1.00)	259.45 (1.01)	91.11 (0.20)	30.22 (0.08)	33.16 (0.06)
High (≥3)	58 (0.9)	6.71 (1.01)	4.68 (1.00)	14.12 (1.00)	42.47 (1.00)	258.77 (1.01)	90.79 (0.14)	30.22 (0.06)	33.28 (0.06)
Urinary cotinine (μg/L)									
Low (<50)	4986 (79.2)	6.50 (1.01) **	4.55 (1.00) **	13.66 (1.00)**	41.21 (1.00)**	255.56 (1.01) **	90.73 (0.10) **	30.10 (0.05) **	33.17 (0.04) **
High (≥50)	1310 (20.8)	7.38 (1.01)	4.87 (1.00)	14.96 (1.00)	44.97 (1.00)	264.75 (1.01)	92.45 (0.19)	30.78 (0.08)	33.29 (0.05)
Physical activity									
Never	3302 (52.4)	6.82 (1.01)	4.62 (1.00)	13.94 (1.00)**	42.01 (1.00)*	260.90 (1.01) **	91.09 (0.12)	30.24 (0.05)	33.19 (0.04)
Moderate	721 (11.5)	6.67 (1.01)	4.58 (1.01)	13.75 (1.01)	41.58 (1.01)	260.62 (1.01)	90.98 (0.28)	30.13 (0.11)	33.10 (0.07)
Vigorous	2273 (36.1)	6.58 (1.01)	4.66 (1.00)	14.11 (1.00)	42.47 (1.00)	252.78 (1.01)	91.33 (0.14)	30.35 (0.06)	33.23 (0.06)
Drinking status									
Never	2160 (34.3)	6.46 (1.01) **	4.48 (1.00) **	13.38 (1.00)**	40.47 (1.00)**	258.94 (1.01)*	90.41 (0.14) **	29.91 (0.06) **	33.06 (0.05) **
Former	394 (6.3)	6.54 (1.02)	4.61 (1.01)	13.91 (1.01)	42.13 (1.01)	244.04 (1.02)	91.54 (0.36)	30.25 (0.11)	33.05 (0.08)
Current	3742 (59.4)	6.85 (1.01)	4.70 (1.00)	14.26 (1.00)	42.90 (1.00)	258.52 (1.01)	91.47 (0.12)	30.43 (0.05)	33.26 (0.05)
Agricultural occupation									
No	5743 (91.2)	6.73 (1.01)	4.64 (1.00) **	13.99 (1.00)**	42.18 (1.00)**	258.52 (1.00) **	91.10 (0.10) **	30.25 (0.05) **	33.20 (0.04)
Yes	553 (8.8)	6.52 (1.02)	4.47 (1.01)	13.68 (1.00)	41.29 (1.00)	245.84 (1.02)	92.41 (0.29)	30.65 (0.15)	33.16 (0.12)
Household pest control									
No	909 (14.4)	6.65 (1.01)	4.63 (1.01)	13.97 (1.01)	42.15 (1.00)	253.07 (1.01)	91.25 (0.19)	30.26 (0.08)	33.16 (0.07)
Yes	5387 (85.6)	6.73 (1.01)	4.63 (1.00)	13.98 (1.00)	42.12. (1.00)	258.74 (1.01)	91.15 (0.11)	30.27 (0.05)	33.20 (0.04)

GM, geometric mean; GSE, geometric standard error of the mean; AM, arithmetic mean; SE, arithmetic standard error of the mean; WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

[a] Weighted percentage from survey frequency.

[b] *P*-values based on survey-weighted *t*-test for binomial groups and survey-weighted Wald F-test for categorical groups. *$p < 0.05$, **$p < 0.01$.

in Table 3. In the fully adjusted model (Model C), the urinary 3-PBA levels were negatively associated with WBC (β = −0.010; 95% confidence interval [CI]: −0.020, −0.001), RBC (β = −0.004; 95% CI: −0.007, −0.001), and hemoglobin (β = −0.003; 95% CI: −0.006, −0.000), whereas they were positively associated with MCV (β = 0.197; 95% CI: 0.018, 0.375). When the urinary 3-PBA levels were treated as categorical variables, the results were similar to those for continuous variables. The negative trends of WBC, RBC, and positive trends of MCV remained significant ($p < 0.05$), while negative trends of hemoglobin became marginal (Supplemental Table S1). A significant difference in magnitude of the associations was observed after adding more covariates from model A to model B. Whereas after adding more covariates from model B to model C, the difference in the magnitude of the associations became marginal.

Next, we evaluated whether sex or age modified the associations between the 3-PBA levels and hematological parameters. After

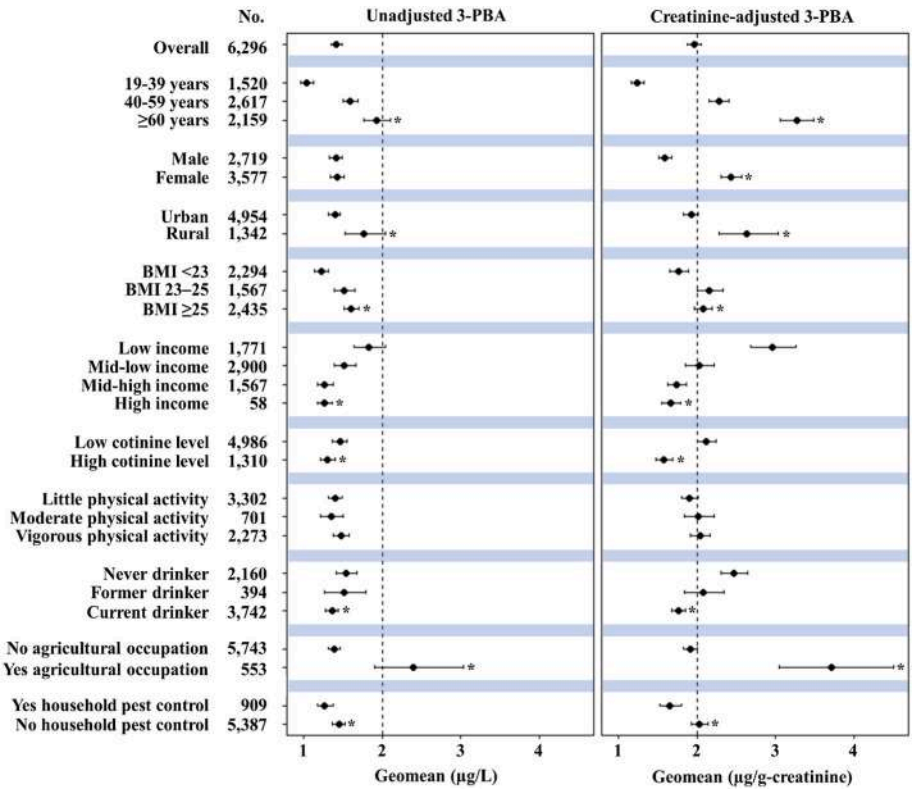

Fig. 1. Geometric means (95% CI) of unadjusted urinary 3-PBA concentrations (μg/L) and creatinine-adjusted urinary 3-PBA concentrations (μg/g-creatinine) by participant's characteristics. Survey-weighted Student's *t*-test was used for binomial groups and Wald F-test for categorical groups. *$p < 0.05$.

Table 2
Distributions of urinary 3-PBA levels and hematological parameters among total participants (n = 6296).

Variables	Detection frequency (%)	GM (or AM)	Percentile			
			25th	50th	75th	95th
Urinary 3-PBA levels						
3-PBA levels (μg/L)	99.97	1.42	0.73	1.47	2.87	8.48
Hematological parameters						
WBC (10^9/μL)	100	6.72	5.70	6.70	8.00	10.10
RBC (10^6/dL)	100	4.63	4.30	4.62	5.00	5.47
Hemoglobin (g/dL)	100	13.98	13.00	14.00	15.20	16.50
Hematocrit (10^2L/L)	100	42.13	39.30	42.20	45.50	49.40
Platelet (10^9/μL)	100	257.86	225.00	261.00	302.00	369.00
MCV (fL)	100	91.17[a]	88.56	91.33	93.86	98.40
MCH (pg)	100	30.27[a]	29.40	30.41	31.31	32.83
MCHC (g/dL)	100	33.20[a]	32.56	33.26	33.89	34.79

GM, geometric mean; AM, arithmetic mean; WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.
[a] Values are survey-weighted arithmetic mean.

Table 3
Regression coefficients (β, 95% CI) for hematological parameters by urinary 3-PBA among total participants (n = 6296)[1].

Outcomes	Model A[a]	Model B[b]	Model C[c]
WBC	−0.008 (−0.018, 0.002)	**−0.010 (-0.019, -0.001)**	**−0.010 (-0.020, -0.001)**
RBC	*−0.003 (-0.006, 0.000)*	**−0.004 (-0.007, -0.001)**	**−0.004 (-0.007, -0.001)**
Hemoglobin	−0.002 (−0.005, 0.001)	**−0.003 (-0.006, -0.000)**	**−0.003 (-0.006, -0.000)**
Hematocrit	−0.001 (−0.004, 0.002)	−0.002 (−0.004, 0.001)	−0.002 (−0.004, 0.001)
Platelet	−0.003 (−0.012, 0.006)	−0.005 (−0.013, 0.004)	−0.005 (−0.014, 0.004)
MCV	0.176 (−0.012, 0.365)	**0.192 (0.013, 0.371)**	**0.197 (0.018, 0.375)**
MCH	0.016 (−0.060, 0.092)	0.018 (−0.058, 0.094)	0.019 (−0.058, 0.096)
MCHC	*−0.043 (-0.096, 0.010)*	*−0.047 (-0.098, 0.004)*	*−0.047 (-0.099, 0.004)*

CI, confidence interval; WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.
Bold: $p < 0.05$; *Italics*: $p < 0.10$.
[a] Model A was adjusted for age, sex, region, monthly household income, and urinary creatinine.
[b] Model B was adjusted additionally for urinary cotinine, BMI, physical activity, and drinking status.
[c] Model C was adjusted additionally for agricultural occupation, and household pest control.

stratifying according to sex, we observed a sex difference in the association of the urinary 3-PBA levels and WBC, RBC, hemoglobin, hematocrit, MCH, and MCHC ($p < 0.05$, Fig. 2). Males showed significant negative associations for 3-PBA with RBC (β = −0.006; 95% CI: −0.010, −0.002), hemoglobin (β = −0.008; 95% CI: −0.012, −0.004), hematocrit (β = −0.005; 95% CI: −0.009, −0.001), and MCHC (β = −0.096; 95% CI: −0.165, −0.028). However, no significant associations between 3-PBA and these parameters were found in females. Meanwhile, females showed significant negative associations for 3-PBA with WBC (β = −0.014; 95% CI: −0.027, −0.001) and positive associations for 3-PBA with MCV (β = 0.281; 95% CI: 0.030, 0.533).

After stratifying by age, we found that the association trends were similar between the different age groups. However, the effect estimates

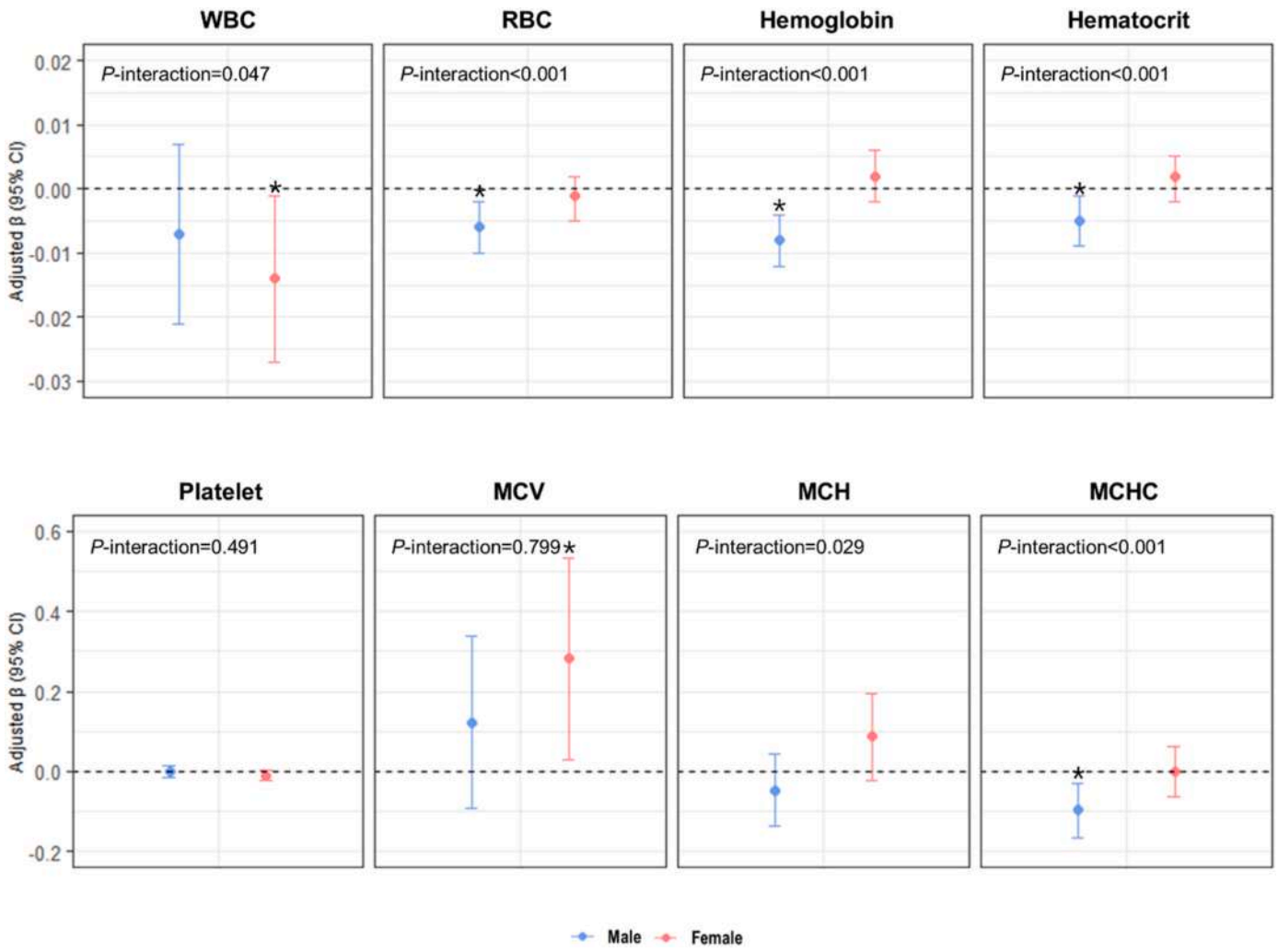

Fig. 2. Adjusted regression coefficients (β, 95% CI) for the association between the 3-PBA levels and hematological parameters stratified by sex. All models were adjusted for age, region, monthly household income, urinary cotinine, BMI, physical activity, drinking status, agricultural occupation, household pest control, and urinary creatinine. *$p < 0.05$.

of RBC, hematocrit, platelet, MCH, and MCHC were significantly more robust in adults aged ≥60 years than those aged 19–59 years ($p < 0.05$, Fig. 3). Adults aged ≥60 years showed significant negative associations between 3-PBA and WBC (β = −0.017; 95% CI: −0.031, −0.003), RBC (β = −0.007; 95% CI: −0.012, −0.001), hemoglobin (β = −0.007; 95% CI: −0.013, −0.001), and MCHC (β = −0.143; 95% CI: −0.212, −0.074), and showed significant positive associations between 3-PBA and MCV (β = 0.388; 95% CI: 0.077, 0.699). However, no significant associations were observed in adults aged 19–59 years for 3-PBA with any hematological measures.

Since the urinary 3-PBA levels were associated with hematological measures only in adults aged ≥60 years, next, we explored associations in adults aged ≥60 years in different sexes. We found that the adverse effect of 3-PBA on the hematological parameters was more substantial among males aged ≥60 years (Fig. 4). In this age group, the urinary 3-PBA levels were negatively associated with WBC (β = −0.037; 95% CI: −0.057, −0.017), RBC (β = −0.014; 95% CI: −0.023, −0.005), hemoglobin (β = −0.016; 95% CI: −0.026, −0.007), hematocrit (β = −0.010; 95% CI: −0.019, −0.002), and MCHC (β = −0.188; 95% CI: −0.284, −0.092) in males, while being positively associated with MCV (β = 0.363; 95% CI: 0.022, 0.704) and negatively associated with MCHC (β = −0.086; 95% CI: −0.183, −0.000) in females.

Stratified analysis by agricultural occupation showed that urinary 3-PBA levels were negatively associated with RBC (β = −0.011; 95% CI: −0.022, −0.001), hemoglobin (β = −0.012; 95% CI: −0.021, −0.003), and MCHC (β = −0.162; 95% CI: −0.310, −0.014) in participants who engaged in agricultural occupations. In participants who did not engage in agricultural occupations, urinary 3-PBA levels were negatively associated with RBC (β = −0.004; 95% CI: −0.007, −0.000) and positively associated with MCV (β = 0.218; 95% CI: 0.031, 0.405) (Fig. 5).

In stratified analysis by household pest control, we found that urinary 3-PBA levels were negatively associated with WBC (β = −0.010; 95% CI: −0.020, −0.000), RBC (β = −0.004; 95% CI: −0.007, −0.000), hemoglobin (β = −0.003; 95% CI: −0.007, −0.000), and MCHC (β = −0.060; 95% CI: −0.115, −0.005) in participants who controlled household pests in the past year. In participants who did not control household pests in the past year, urinary 3-PBA levels were negatively associated with RBC (β = −0.008; 95% CI: −0.017, −0.000) and positively associated with MCV (β = 0.513; 95% CI: 0.050, 0.976) and MCH (β = 0.210; 95% CI: 0.008, 0.412) (Fig. 6).

Pearson's correlation test showed that urinary 3-PBA was significantly correlated with cadmium ($r = 0.48$), mercury ($r = 0.29$), and lead ($r = 0.10$) (Supplemental Table S2). On the sensitivity analysis after adjusting heavy metals, the negative associations of 3-PBA with WBC, RBC, and hemoglobin were still significant. Also, the positive association of 3-PBA with MCV remained significant. After adding urinary cadmium, mercury, and blood lead level from model A to model C, the magnitude of estimates of 3-PBA became larger while statistical

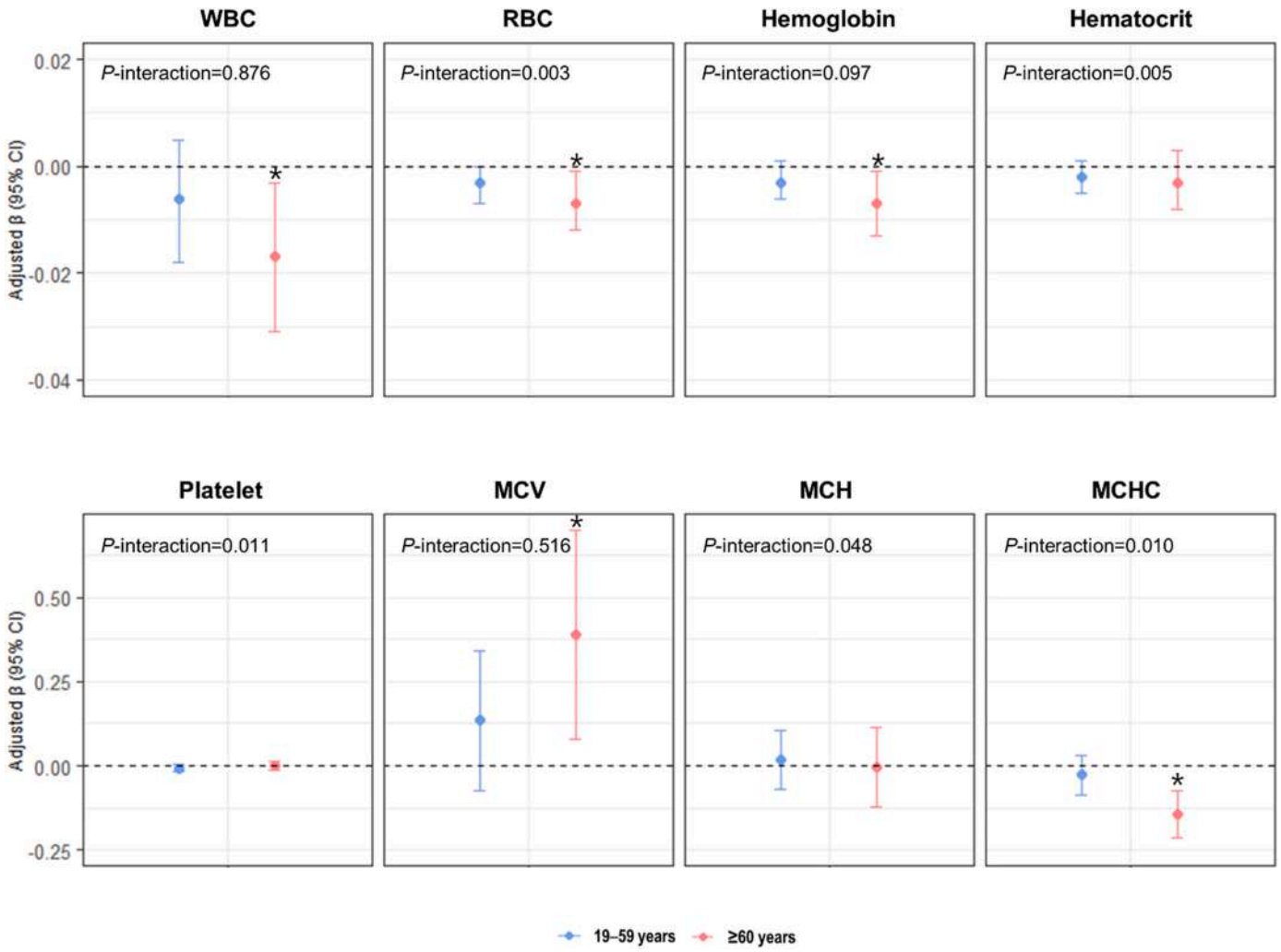

Fig. 3. Adjusted regression coefficients (β, 95% CI) for the association between the 3-PBA levels and hematological parameters stratified by age group. All models were adjusted for sex, region, monthly household income, urinary cotinine, BMI, physical activity, drinking status, agricultural occupation, household pest control, and urinary creatinine. *$p < 0.05$.

significance remained the same (Supplemental Table S3).

4. Discussion

This study found negative associations between the urinary 3-PBA levels and WBC, RBC, and hemoglobin levels but positive associations with MCV among Korean adults. Moreover, stronger associations between 3-PBA and hematological parameters were observed among males aged 60 years or older. To the best of our knowledge, this is the first epidemiological study that examined the association between urinary 3-PBA levels and hematological parameters in the general population.

4.1. Urinary 3-PBA levels of Korean adults

The 3-PBA levels observed in this study were compared with those observed in adults from other countries. The urinary 3-PBA levels observed in this study were similar to those in a previous study conducted on a similar population of the KoNEHS second round (GM 1.42 μg/g-creatinine in this study vs. GM 1.41 μg/g-creatinine in Hwang's study) (Hwang et al., 2019)). Meanwhile, the concentrations of 3-PBA differed by country (Supplemental Table S4). The Korean adults' urinary 3-PBA levels were three times higher than those in the American and Canadian adults (CDC, 2019; Health Canada, 2013; Lehmler et al., 2020) and were seven times higher than those in the Polish and French adults (Baudry et al., 2019; Wielgomas et al., 2013; Wielgomas and Piskunowicz, 2013). Moreover, the Korean adults' urinary 3-PBA levels were generally higher than those of other Asians. In China, the urinary 3-PBA level among adult populations had a GM of 0.7 μg/g-creatinine (Li and Kannan, 2018) and was3.07 μg/g-creatinine among young adults aged between 18 and 30 (Lin et al., 2021). In Japan, the GM of urinary 3-PBA level was 0.40 μg/g-creatinine among adults aged 39–85.

The difference between dietary habits and the patterns of indoor insecticide use can explain the higher concentrations of 3-PBA in Asia, especially in Korea, compared to Western countries. In the general population, pyrethroid exposure usually occurs through the intake of pyrethroid residues in food (e.g., fresh and cooked fruits or vegetables) (Saillenfait et al., 2015). Ye et al. (2015) announced that people who ate vegetables frequently had higher urinary 3-PBA levels than those who did not. Rice is the staple food in Korea, with an annual rice consumption per person (72.8 kg/year) higher than that of Japan (49.5 kg/year) and the U.S. (9.5 kg/year) (FAO, 2020). Thus, the higher consumption of kimchi and rice than in other countries could contribute to Koreans' higher pyrethroid metabolite levels. Also, residential insecticide use was a known contributor to pyrethroid metabolite exposure (Lu et al., 2013; Saillenfait et al., 2015). Koreans often use sprays and fumigants in their homes during the summer to eliminate mosquitoes and pests (Hwang et al., 2019). However, after household insecticide use was stratified in our study, the associations of urinary 3-PBA with RBC and MCV were

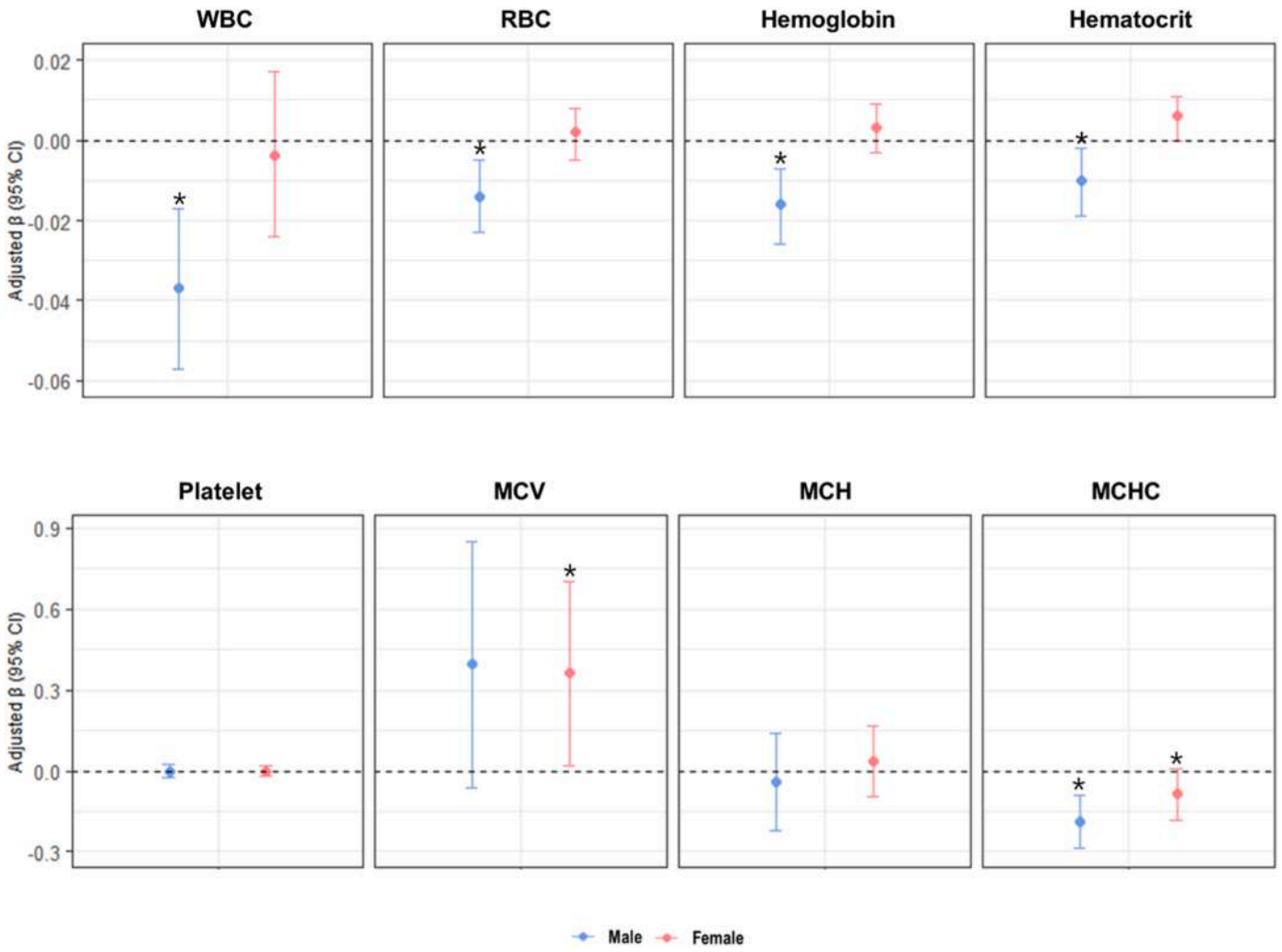

Fig. 4. Adjusted regression coefficients (β, 95% CI) for the association between the 3-PBA levels and hematological parameters in males and females ≥60 years. All models were adjusted for region, monthly household income, urinary cotinine, BMI, physical activity, drinking status, agricultural occupation, household pest control, and urinary creatinine. *$p < 0.05$.

observed between the participants who controlled household pests and who did not. This suggests that pyrethroids, independent of other pesticides (i.e., organophosphate insecticides) and herbicides in household insecticides, affect blood parameters. Hence, diverse pathways in environmental pyrethroids exposure in the general population lead to altered blood parameters. Since different populations are exposed to pyrethroids through varying sources, the study findings should be verified in other populations.

4.2. Associations between urinary 3-PBA and hematological parameters

The results of this study are generally consistent with animal experimental studies that found that pyrethroids could decrease the WBC, RBC, hemoglobin, and hematocrit levels, and increase MCV levels (ATSDR, 2003; Khan et al., 2012; Manna et al., 2004; Nair et al., 2010). However, only a few epidemiological studies based on occupational settings supported the study results. A longitudinal study in the U.S. found that 27 male pesticide applicators aged 50 years or older with self-reported high lifetime use of permethrin showed significantly lowered RBC and hemoglobin levels and elevated MCV levels, thereby leading to a higher risk of the destruction of hematopoiesis and multiple myeloma (Shearer et al., 2019). Next, 40 Turkish farmers (4 females and 36 males) with a mean age of 42.3 showed that self-reported exposure to pyrethroids increased the WBC counts and decreased hemoglobin and platelet counts (Varol et al., 2014). Also, 30 male greenhouse workers with a mean age of 35.6 reported that the GM of their urinary 3-PBA was 7.8 μg/g-creatinine and was associated with cytokine levels, though it showed a null association with WBC (Costa et al., 2013). This study had a GM level of 3-PBA is 1.94 μg/g-creatinine, with decreased RBC and hemoglobin levels and increased MCV levels similar to prior studies, as well as decreased WBC and hematocrit levels. However, all studies mentioned above focused on occupational settings, including workers exposed to high concentrations of pyrethroid pesticides, and had a small sample size. Using a large general population of over 6000 individuals, this study showed that urinary 3-PBA level was inversely associated with WBC, RBC, and hemoglobin and was positively associated with MCV, which is consistent with previous reports. Moreover, this study examined whether the effect of 3-PBA on hematological parameters varied according to sex and age.

Once pyrethroids enter the body, they are rapidly detoxified by carboxylesterase-mediated hydrolysis, produce several metabolites (e.g., 3-PBA, trans-DCCA, cis/trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis/trans-DCCA), 4-fluoro-3-phenoxybenzoic acid (4-F-3PBA), and cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DBCA)), and are eliminated in the urine (Barr et al., 2010). Therefore, these metabolites are widely used as biomarkers for exposure to pyrethroids. Pyrethroids and their metabolite 3-PBA penetrate various organs, including blood, causing

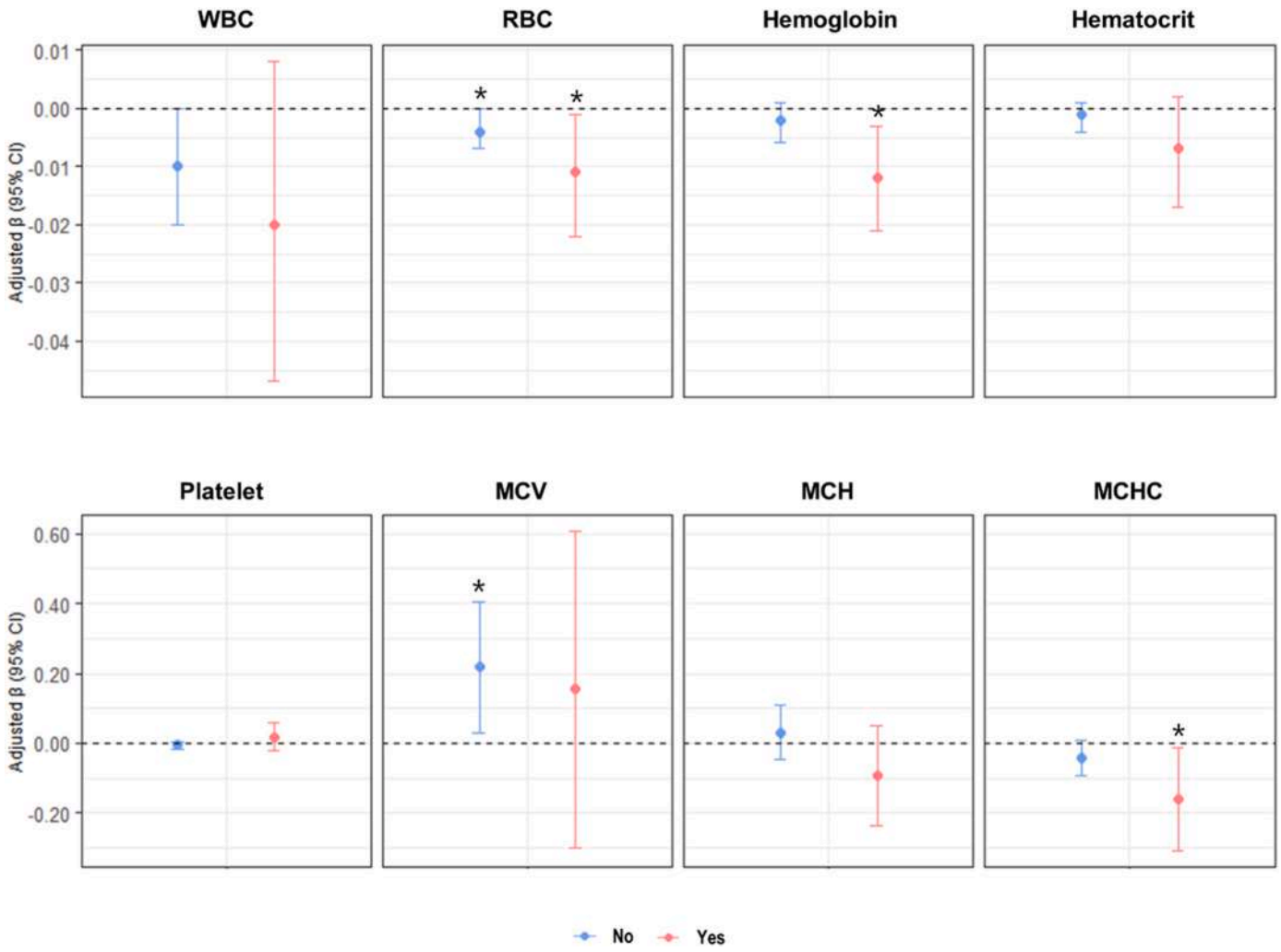

Fig. 5. Adjusted regression coefficients (β, 95% CI) for the association between the 3-PBA levels and hematological parameters in participants who did not engage in agriculture and those who engaged in agriculture. All models were adjusted for age, sex, region, monthly household income, urinary cotinine, BMI, physical activity, drinking status, household pest control, and urinary creatinine. *$p < 0.05$.

adverse effects on humans. Although the biological mechanisms by which pyrethroids affect blood function have yet to be fully clarified, multiple pathways may be involved. For example, cell destruction, decreased or delayed mitosis in hematopoietic progenitor cultures, production of immature granulocytes and RBCs due to the destruction of the cells of myeloid lineage, marrow cellular apoptosis, fewer leukocytes by a diminished function of the immune system, and oxidative stress induced by the pyrethroid chemicals are considered major mechanisms (Arif et al., 2020; Chatterjee et al., 2013; Mandarapu and Prakhya, 2015; Wang et al., 2016). Mandarapu and Prakhya (2015) demonstrated that bone marrow is one of the most sensitive tissues against pyrethroids, and that several pyrethroid metabolites destroy hematopoietic progenitor cells in the bone marrow, inhibit blood cell production, and finally cause degenerative diseases such as aplastic anemia. Several studies have suggested that pyrethroids destroy erythropoietin, inhibiting red blood cell and heme production, which decreases the number of RBCs, hemoglobin, and hematocrit in the blood (Khan et al., 2012; Manna et al., 2004). Other studies have reported that pyrethrin-induced oxidative stress leads to immune toxicity, resulting in leukopenia (ATSDR, 2003; Khan et al., 2012). Therefore, this study's observations that urinary 3-PBA decreased the WBC, RBC, hemoglobin, and hematocrit levels were supported by plausible biological explanations.

4.3. Sex- and age-dependent associations with hematological parameters

Different associations according to sex were found between the urinary 3-PBA levels and hematological parameters in this study. Our results showed that the urinary 3-PBA levels were negatively associated with RBC, hemoglobin, hematocrit, and MCHC among males; however, no significant association was found among females, and lower WBC and elevated MCV levels were observed only in females as well. Few epidemiological studies have observed sex-specific associations between 3-PBA and hematological parameters, making it difficult to compare the results of this study which are supported only by the results of studies on males (Hassanin et al., 2018; Shearer et al., 2019). Shearer et al. (2019) observed lower RBC counts and higher granulocytes in samples of male farmers exposed to permethrin, indicating an association with multiple myeloma development. Although clear mechanisms for the sex-dependent association are not yet available, the following mechanisms may partially explain such differences. First, 3-PBA directly interacts with the receptor of sex hormones (estrogen and androgen) and adversely affects blood function. Generally, androgen in men acts on the bone marrow to produce erythropoietin, generating red blood cells and hemoglobin or promoting erythropoietin production in the kidneys, whereas estrogen in women inhibits this action (Murphy, 2014). 3-phenoxybenzyl alcohol (3-PBA1c), which is degraded into 3-PBA, is known to induce an anti-androgenic activity (Tyler et al., 2000). Thus, it could

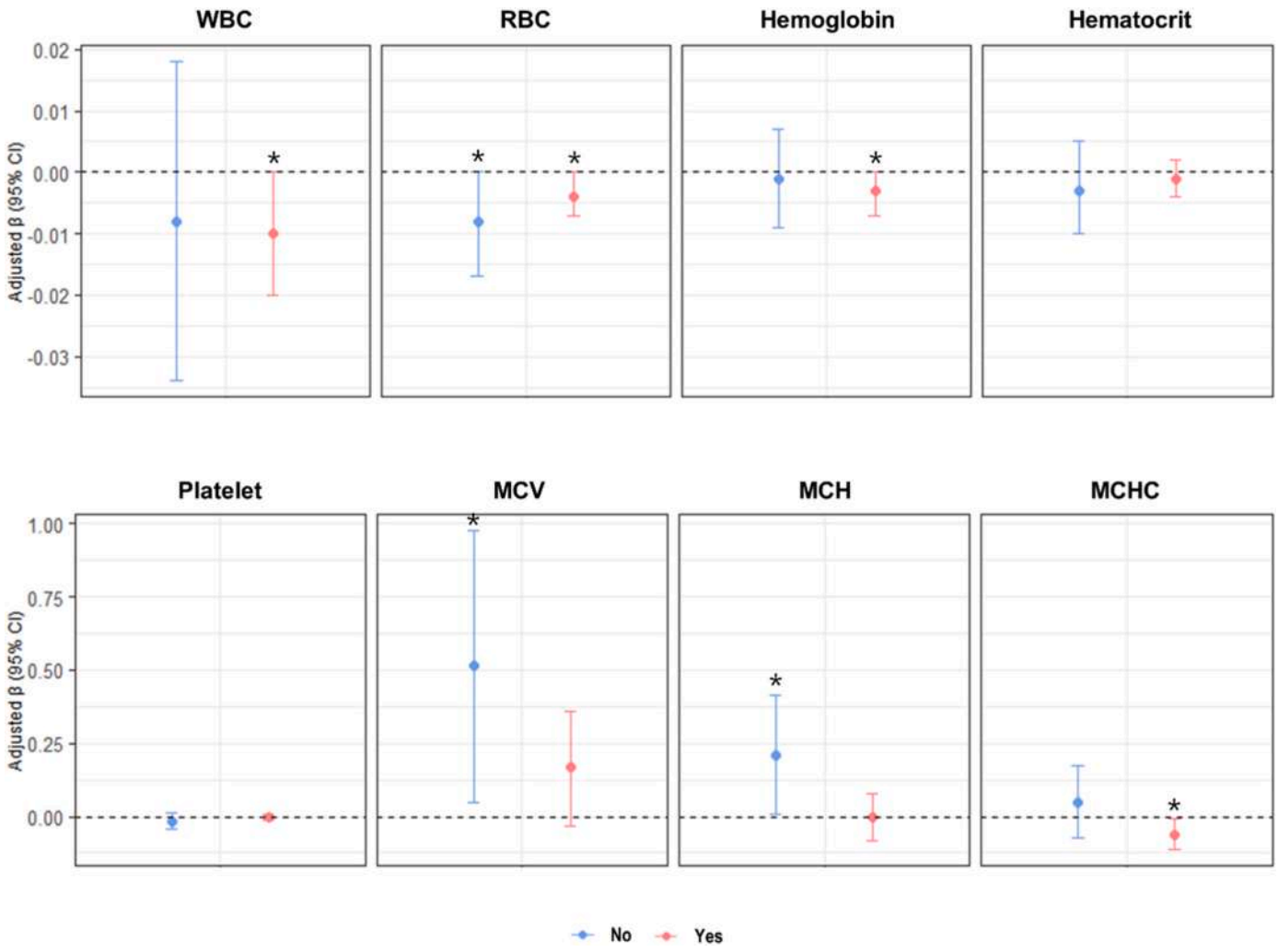

Fig. 6. Adjusted regression coefficients (β, 95% CI) for the association between the 3-PBA levels and hematological parameters in participants who did not control household pests in the past year and those who controlled household pests in the past year. All models were adjusted for age, sex, region, monthly household income, urinary cotinine, BMI, physical activity, drinking status, agricultural occupation, and urinary creatinine. *$p < 0.05$.

be inferred that 3-PBA inhibits blood cell production by disturbing the action of sex hormones in men. In addition, the endocrine-disrupting effects of pyrethroids may indirectly affect hematological parameters. 3-PBA interferes with the reproductive function related to the hypothalamic-pituitary-gonadal axis or transforms the metabolism and transport of sex steroid hormones (Ye and Liu, 2019). Also a neurotoxicant, pyrethroids target voltage-gated sodium channels and calcium channels and disrupt sex steroid hormone gene expression (Ye and Liu, 2019). This implies that abnormal sex hormone-related properties induced by pyrethroids or 3-PBA may impact the hematopoietic system. Finally, gender differences in the metabolism and excretion of 3-PBA can be explained. An *in vivo* study reported that the excretion rate of 3-PBA1c and 3-peoxybenzaldehyde in female mice was much faster than that in male mice (Ueyama et al., 2010). However, further studies are needed to verify these mechanisms due to insufficient evidence on the different vulnerabilities by sex.

Additionally, this study found that the 3-PBA levels were inversely associated with WBC, RBC, hemoglobin, and MCHC and were positively associated with MCV in participants aged 60 or older. Among participants aged 19–59, no associations were observed between 3-PBA concentrations and any of the hematological parameters (Fig. 4). Regarding the age-dependent association between the urinary 3-PBA levels and hematological parameters, several factors may explain these differences. Aging decreases the rate of blood flow and the size of the liver and kidneys, resulting in the reduced metabolism and excretion of pyrethroids (Ginsberg et al., 2005). Rosita et al. (2016) revealed that mice exposed to permethrin excreted less calcium and sodium ions from their kidneys and that permethrin accelerated aging.

4.4. Limitations and implications

First, this research was a cross-sectional study and did not establish a causal relationship. Therefore, we should interpret the biological implications from observed statistical associations cautiously. Second, it is difficult to collect 24-h urine samples in a large survey study (e.g., KoNEHS), so a spot urine measurement of 3-PBA was used. Since the half-life of 3-PBA in the human body is 7.5 h, using the urinary 3-PBA level as the long-term surrogate exposure is not certain, leading to measurement error. However, a previous study suggested that estimates of chemicals with short half-lives through a single urine sample may fairly represent long-term exposure (Li et al., 2019). Third, the lack of information on other pyrethroid metabolites was another limitation of this study, but 3-PBA is a generic metabolite of many pyrethroids (e.g., permethrin, cypermethrin, deltamethrin) used to assess pyrethroid exposure. Fourth, 3-PBA is a biomarker of exposure to several pyrethroids, which limits its ability to determine specific pyrethroids. Finally, missing information on the participants' nutritional statuses (e.g., calcium and iron concentrations) was a limitation, as those factors

may act as covariates for 3-PBA and blood parameters. Nonetheless, the study findings are based on a large sample representing the general Korean population, such that the resulting observations can be generalized. The results of this study provide reliable epidemiological evidence that pyrethroid at the levels currently observed in the general Korean adults are associated with hematological alterations.

5. Conclusion

Urinary 3-PBA levels are associated with the hematological parameters in Korean adults. Furthermore, this effect was more distinct among males aged 60 or older. This is the first epidemiological study that verified the association of urinary 3-PBA levels with hematological parameters based on a large size of the human population. Given the widespread use of pyrethroids worldwide, future longitudinal studies should be conducted to confirm the results of this study.

Author contributions

Yun-Hee Choi: Conceptualization, Methodology, Writing – Original draft preparation, Data curation; Ju-Yeon Lee: Writing – Original draft preparation, Writing – Review & Editing, Data curation; Da-An Huh: Writing – Review & Editing, Validation, Visualization; Kyong Whan Moon: Methodology, Writing – Review & Editing, Supervision.

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Ethics statement

The Institutional Review Board (IRB) of the National Institute of Environmental Research (NIER) approved the study (approval number: Environmental Epidemiology Division-1805) under NIER rule no. 569 (the NIER IRB Operating Procedure), and all participants provided written informed consent.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113988.

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Urinary phthalate metabolite concentrations during four windows spanning puberty (prepuberty through sexual maturity) and association with semen quality among young Russian men

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ABSTRACT

Aim: To prospectively investigate the associations of urinary phthalate metabolite concentrations measured at four time points spanning pubertal development with semen parameters in Russian men.
Design: 516 boys were enrolled at ages 8–9 years (2003–2005) and followed annually.
Methods: Urine samples were collected annually and pooled into four exposure windows [prepuberty, early puberty, late puberty and sexual maturity] based on physician assessed Tanner genitalia stages and testicular volume. Fifteen phthalate metabolites were quantified using isotope dilution HPLC-MS/MS at Moscow State University. We calculated molar sums (∑) of di-2-ethylhexyl phthalate (DEHP), di-isononyl phthalate (DiNP), di-isodecyl phthalate (DiDP) and anti-androgenic phthalate (AAP) metabolites. At sexual maturity (ages 18–19 years), the men provided 1–2 semen samples for analysis. We estimated the associations of quintiles of urinary ∑phthalate metabolites as well as mono-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), and mono-benzyl phthalate (MBzP) at each pubertal window, with semen parameters by fitting generalized linear mixed models with random intercepts and adjusting for confounders.
Results: A total of 223 men who provided semen samples had phthalates measured at one or more pubertal windows. Higher urinary concentrations of ∑DiNP metabolites during late puberty were related to poorer semen quality (men with the highest quintile of urinary ∑DiNP had 30% lower sperm concentration, 32% lower count and 30% lower progressive motile count, compared to men in the lowest quintile). Also, young men with higher urinary concentrations of MiBP metabolites in early puberty tended to have poorer semen quality. No associations were observed for ∑DEHP metabolites, ∑DiDP metabolites, ∑AAP, MBzP or MnBP metabolites with semen quality parameters.

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Conclusions: ∑DiNP metabolites measured during late puberty and MiBP metabolites at early puberty were related to poorer semen quality, highlighting the importance of considering specific windows of exposure when investigating chemical exposures in relation to measures of reproductive health in men.

Competing financial interests

None of the authors has any conflicts of interest to declare.

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Author contributions

Drs. Mínguez-Alarcón, Sergeyev, Burns, and Hauser had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Mínguez-Alarcón, Burns, Williams, Korrick, Lee, Sergeyev and Hauser. Acquisition of data (semen analysis): Sergeyev and Smigulina. Chemical analysis of urine samples: Kovalev, Sokolov, Lebedev and Koch (quality assurance). Analysis of data: Mínguez-Alarcón. Interpretation of data: All authors. Drafting of the manuscript: Mínguez-Alarcón. Critical revision of the manuscript for important intellectual content: All authors.

1. Introduction

Some phthalates are endocrine disrupting chemicals used in a multitude of consumer products, leading to widespread general population exposure (CDC 2018; Hauser and Calafat 2005; Koch et al., 2017; Silva et al., 2004; Zota et al., 2014). Exposure to phthalates occurs through ingestion, inhalation, and dermal absorption (Cirillo et al., 2013; Duty et al., 2005; Just et al., 2010; Langer et al., 2014; Rudel et al. 2003, 2011; Wilson et al., 2003; Wormuth et al., 2006). After entering the body, phthalates are quickly hydrolyzed to their respective biologically active monoester metabolites and excreted in urine. Although phthalates do not persist in the body and have short biological half-lives (<24 h), there is repeated, episodic, and chronic exposure to phthalates (Kao et al., 2012; Koch et al. 2006, 2012). Urine serves as a non-invasive and convenient medium for biological monitoring and has been shown to be the optimal matrix for measuring phthalates because of their short serum half-lives and non-persistent nature (Calafat et al., 2015). Di-2-ethylhexyl phthalate (DEHP) is commonly added to plastics to increase flexibility and can be found in consumer products, flooring and wall coverings, food contact applications, and medical devices. Metabolites of DEHP include mono(2-ethylhexyl) phthalate (MEHP), mono (2-ethyl-5-hydroxy-hexyl phthalate (MEHHP), mono (2-ethyl-5-oxo-hexyl) phthalate (MEOHP), and mono (2-ethyl-5-carboxy-pentyl) phthalate (MECPP). Other phthalate metabolites such as mono-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), and mono-benzyl phthalate (MBzP) can be found in personal care products and other consumer products. Di-isononyl phthalate (DiNP) is added to plastic consumer products including some polyvinyl chloride flooring, materials used in automobile interiors, wire and cable insulation, gloves, tubing, garden hoses, and shoes (Braun et al., 2014; Hauser and Calafat 2005).

As a result of their anti-androgenic activity, some phthalates have been associated with male reproductive tract abnormalities and lower circulating testosterone levels in experimental animal studies (Howdeshell et al., 2008; Johnson et al., 2012). In a recent systematic review and meta-analysis of epidemiology literature on phthalates and male reproductive health, the authors concluded that there was robust evidence of a negative association of DEHP and di-n-butyl phthalate (DnBP) with semen quality, and moderate evidence for associations of di-isononyl phthalate (DiNP), and butyl-benzyl phthalate (BBzP) with poorer semen quality (Radke et al., 2018). However, most of these epidemiologic studies of semen quality were cross-sectional with exposure biomarkers measured at the time of semen collection and thus precluded the ability to study specific windows of exposure during spermatogenesis or exposures earlier in life during pubertal development. In addition, urinary phthalate metabolites were quantified in a single urine sample leading to potential misclassification of the exposure.

Given the robust cross-sectional associations and the lack of studies on earlier exposure windows, such as during puberty, we leveraged our long-standing Russian Children's Study cohort to investigate the longitudinal associations of phthalate metabolite concentrations collected during four pubertal windows with semen parameters measured in young adulthood. Although fetal and neonatal periods involve biological processes which are highly sensitive to chemical exposures that can impact male reproductive tract development and subsequent pubertal timing, puberty is also considered a critical developmental stage with exposure sensitivity (Woodruff et al., 2010). During puberty, development and activation of the male reproductive system occurs, characterized by maturation of the supporting Sertoli cells, differentiation of the testosterone producing Leydig cells, and spermatogenesis. Therefore, with these critical developmental processes, the pubertal period may also be vulnerable to chemical exposures.

2. Methods

2.1. Study population

Our study population consists of a subset of 223 of the 516 boys residing in Chapaevsk, Russia, who were enrolled at 8 and 9 years old between 2003 and 2005 in the Russian Children's Study, and followed until time of semen collection approximately 10 years later (Supplemental Fig. 1). At enrollment, each boy underwent a complete physical exam and their adult guardian completed health, dietary, and lifestyle surveys. This initial assessment was followed by yearly physical exams and questionnaires as previously described (Burns et al., 2020; Sergeyev et al., 2017; Williams et al., 2019) as well as annual urine sample collections and biennial blood sample collections. Throughout the study follow-up, a standardized anthropometric examination was performed by a single trained research nurse and pubertal staging was performed by a single physician. Genitalia (G) and pubic hair (P) staging, graded as 1 (immature) to 5 (sexually mature) were assessed by visual inspection following standard procedures (Tanner and Whitehouse 1976). Testicular volume (TV) was measured using a Prader orchidometer.

Of the 516 boys initially enrolled in the cohort between 2003 and 2005, 139 (26%) were lost to follow up by the time they were eligible for semen collection either due to death (n = 6) or no longer residing in the study area (n = 129), and 4 were not invited to participate due to severe cognitive impairment (Supplemental Fig. 1). Of the 377 remaining boys, 152 declined to participate in the semen study. Thus, 225 (44%) young

men provided 1 or 2 semen samples between 2012 and 2018 and contributed urine samples during at least one pubertal window. Additionally, 1 participant who was diagnosed with severe chronic disease and 1 azoospermic young man were excluded. Thus, for this analysis, we included the remaining 223 men with semen samples. A total of 134 boys provided urine samples for phthalate metabolite quantification during the prepubertal period defined as TV = 1 or 2 cc and G = 1 or 2, or TV = 3 cc and G = 1; 219 boys provided urine samples during early puberty defined as TV = 3 cc and G = 2 or 3, or TV = 4–8 cc and G = 1 to 3, or TV = 10 cc and G = 1; 203 boys provided urine during the late pubertal period defined as TV = 10–15 cc and G = 2 to 5, or TV = 20 cc and G = 2 to 3; and 213 boys provided urine at sexual maturity defined as TV ≥ 20 cc and G = 4 or 5. Additionally, for those young men who contributed a semen sample, the sexual maturity pool was limited to urines collected 90 days or more before semen sample collection.

The study was approved by the Human Studies Institutional Review Boards of the Chapaevsk Medical Association (Chapaevsk, Russia), Harvard T.H. Chan School of Public Health, Brigham and Women's Hospital (Boston, MA, USA), and Nemours Children's Health (Wilmington, DE, USA). During the baseline assessment, the adult guardian/parent signed an informed consent, and each participant signed an assent before participation. When participants reached 18 years of age or older, they signed a consent form, including a separate consent for providing semen samples.

2.2. Urinary phthalate metabolite assessment

At enrollment and annually, spot urines collected in clean polypropylene containers were aliquoted into 15 ml sterile glass containers and stored at −35 °C. An aliquot of urine from each annual exam was defrosted, vortexed, and pooled for each boy into one of four pubertal categories (prepuberty, early puberty, late puberty, or sexual maturity) using pre-specified G and TV criteria described above. The pooled urine was thoroughly vortexed before measuring specific gravity (sg), then aliquoted into 1.8 ml polypropylene cryovials for storage at −35 °C. Urine samples collected during the first ten months of enrollment, n = 216, were stored at the Harvard T.H. Chan School of Public Health in Boston, Massachusetts, U.S.A. and unavailable for pooling because of Russian restrictions on shipping human biospecimens into the country.

The frozen pooled urine samples were transported in dry ice from Chapaevsk to Moscow State University (MSU) laboratory, Moscow, Russia, for analysis according to the methods of Koch et al., (2017). Phthalate metabolite concentrations were measured using online liquid chromatography tandem mass spectrometry (LC-MS/MS) (Koch et al., 2017). Urinary phthalate metabolites measured and included in analyses were: the metabolite of di-isobutyl phthalate (DiBP), MiBP; the metabolite of BBzP, MBzP; the metabolite of DnBP, MnBP; the metabolites of di-2-ethylhexyl phthalate (DEHP), MEHP, MEHHP, MEOHP, and MECPP; the metabolites of di-isononyl phthalate (DiNP), mono-hydroxy-iso-nonyl phthalate (MHiNP), mono-oxo-iso-nonyl phthalate (MOiNP), and mono-carboxy-iso-octyl phthalate (MCOP); and the metabolites of di-isodecyl phthalate (DiDP), mono-(-hydroxy-iso-decyl) phthalate (MHiDP), mono-(oxo-iso-decyl) phthalate (MOiDP), and mono-(carboxy-iso-nonyl) phthalate (MCNP); and mono-(3-carboxypropyl) phthalate (MCPP), which is a metabolite of multiple parent phthalates, e.g. DnBP, DiNP, DiDP, among others. Calibrations were performed using commercial reference standards from LGC (MiBP, MnBP, MBzP, MEHP; Teddington, UK), Biozol (MEHHP, MEOHP, MECPP; Eching, Germany), custom synthesized standards provided by Koch/IPA (MCPP, MHiNP, MOiNP, MCOP, MHiDP, MOiDP, MCNP; Bochum, Germany) and isotopically labelled internal standards such as LGC (MCPP, MEHP, MEOHP; Teddington, UK) and custom synthesized by Koch/IPA (MiBP, MnBP, MBzP, MEHHP, MECPP, MHiNP, MOiNP, MCOP, MHiDP, MOiDP, MCNP; Bochum, Germany). Analyses were performed in 34 batches of 50 samples each including two randomly selected participants' samples analyzed in duplicate, two quality control (QC) samples (from designated discard urines) with known low and high concentrations of each metabolite (1 QC_{low} and 1 QC_{high}), and 1 field blank. For each boy, pooled urines from each pubertal stage were analyzed in the same batch. When a peak was absent or indeterminate, a concentration of zero was assigned. Limits of detection (LOD) were defined as a signal-to-noise (S/N) ratio of 3 in a urine matrix assessed as part of method validation. External reference standards were not available for analysis, but the MSU laboratory's reproducibility measures were generally excellent. Specifically, the inter-assay relative SD percent (RSD%: (SD/mean)*100) across the 34 batches for QC_{high} ranged from 1.5 to 20.0%, except for MEHP (29.1%), whereas RSD% for QC_{low} were ≤20%, except for MEHP (50.8%) and DiDP metabolites (23.6–57.8%), one of which had 23% of values below the LOD. High RSD% are seen with both MEHP (it can be a contaminant in reagents) and DiDP metabolites (they are a complex mixture of isomers that do not have as well resolved analytic peaks as other phthalates).

2.3. Semen quality assessment

Each young man in the study was asked to contribute two semen samples after reaching 18 years of age. They were asked to abstain from ejaculation for 2–4 days prior to semen sample collection and each provided up to 2 semen samples (approximately one week apart) by masturbation inside a dedicated room next to the Andrology Lab. The physician recorded information regarding any viral/bacterial illness or fever in the months prior to the semen collection and date/time of last recalled ejaculation to calculate abstinence time. Three subjects reported their first ejaculation at the study visit so their values were included in the highest category of the abstinence time covariate. The samples were stored inside an incubator at 37 °C and analyzed within 1 h after sample collection. Most samples (99%), however, were analyzed within a half hour of the collection. All samples were assessed by a single andrology technician (LS) according to criteria updated by the Nordic Association for Andrology (NAFA) and European Society of Human Reproduction and Embryology–Special Interest Group in Andrology (ESHRE-SIGA) (Björndahl et al., 2010). All semen samples were analyzed by the technician prior to measurements of urinary phthalate metabolite concentrations, thus the technician and study staff were blinded to the exposure data.

Semen volume was measured using a one, five, or ten mL disposable pipette. Sperm motility was evaluated by microscopic examination of the semen sample in duplicate at 400 times magnification, using at least 200 spermatozoa and at least 5 microscope fields in each duplicate count. Results were reported according to the 1999 WHO manual for the examination and processing of human semen. Specifically, at least 200 sperm per duplicate were classified into one of 4 categories: Class A: rapidly progressive motile; Class B: slowly progressive motile; Class C: locally motile and Class D: immotile. The percent progressive motile sperm was calculated by summing the individual percentages of the WHO classes A and B of each sample (WHO 2010). Sperm concentration was quantified using standard dilutions (1:50, 1:20, or 1:10 by sperm immobilization solution), two aliquots and Improved Neubauer Chamber Hemacytometer (INCH) at 200 times magnification under a phase contrast microscope with typically at least 200 spermatozoa in each sample. Duplicates for sperm concentration and motility were assessed and compared for agreement. Differences between the duplicates did not exceed the corresponding acceptance limit in any of the 438 semen samples for both sperm concentration and motility and average values were used for statistical analysis. Within-observer mean coefficients of variation (CV) in duplicates were 6.4% for sperm concentration and 4.9% for progressive motility and Lin's concordance correlation coefficients (Lin 1989) reflected high agreement, rho = 0.97 for sperm concentration and rho = 0.92 for progressive motility.

2.4. Statistical analysis

We calculated medians and interquartile ranges (IQR) for participant demographics, dietary and parental characteristics that were continuous variables, and number and percentages for categorical variables. Semen parameters were reported as means (SD) and medians (IQR). We reported distribution of urinary phthalate metabolite concentrations as medians (IQR) for each pubertal period. Using their corresponding molar weights, we calculated the molar sum (μmol/L) of the ∑DEHP = (MEHP + MEHHP + MEOHP + MECPP), ∑DiNP = (MHiNP + MOiNP + MCOP), ∑DiDP = (MHiDP + MOiDP + MCNP), ∑ anti-androgenic (AAP) = (MEHP + MEHHP + MEOHP + MECPP + MnBP + MiBP + MBzP + MHiNP + MOiNP + MCOP) with DiNP metabolites weighted 0.43 because of its weaker relative impact on fetal testosterone production (Hannas et al., 2011). Within each pubertal window, molar sums of the different urinary phthalate metabolites as well as MnBP, MiBP, and MBzP, were divided into quintiles, and the first (lowest) quintile was used as the reference group. Total sperm count (volume x sperm concentration) and total progressive motile sperm count (total sperm count x % progressive motile sperm) were calculated. We calculated Spearman correlations between the first and the second semen sample for the measured semen parameters. Total sperm count, sperm concentration and total progressive motile sperm count were log-transformed to approximate a normal distribution. Multivariable linear regression models with random subject effects to account for repeated measurements within the same man were used to examine the relation between the urinary phthalate metabolites, in quintiles, and semen parameters. We compared semen parameters (semen volume, total sperm count, sperm concentration, % progressive motile sperm, and total progressive motile sperm count) among men with higher quintiles of urinary concentrations to those in the lowest quintile. Predicted marginal means for these semen parameters were estimated as least square means (Searle et al., 1980) (adjusted for confounders at the mean level for continuous variables and for categorical variables weighted according to their frequencies) and were back-transformed to allow presentation of results in the original scale. Tests for linear trends were conducted using quintiles of urinary phthalate metabolite concentrations as ordinal levels. Inverse probability of censoring weights (IPCW) were used to account for potential selection bias, based on fitting a logistic regression model to obtain predicted probabilities of having a semen sample available among men with urinary phthalate data. Covariates included in the IPCW model were urinary DEHP metabolite concentrations at early puberty (larger sample size for phthalate quantification), birthweight, breastfeeding weeks, body mass index (BMI), household income and intakes of total calories, fats and carbohydrates. We collected BMI data from the most recent physical examination. Diet intakes, neonatal and household characteristics were collected from the parent or guardian when the boys were age 8–9 years. Mean (range) of censoring weights were 2.02 (1.18, 3.91). Intakes of total calories at study entry as well as urinary DEHP metabolite concentrations were the most predictive variables in the IPCW model.

To supplement our primary analyses, we applied a multiple informant model (MIM) (Horton et al., 1999; Pepe et al., 1999) to compare associations of urinary phthalate metabolite concentrations across pubertal windows with semen quality parameters only for those phthalates in which our primary analysis demonstrated associations with semen quality. For these MIM analyses, we used the urinary phthalate metabolites as continuous exposure variables and we included one continuous outcome per man by calculating the geometric mean of each semen parameter for participants who contributed two semen samples. In each model, we included a pubertal variable with four levels, an interaction term for the urinary phthalate metabolites and the pubertal window variable as well as the confounders. Results from this model allowed evaluation of whether associations of semen quality with urinary phthalate metabolite concentrations differed across the four time periods (prepuberty, early puberty, late puberty, and sexual maturity). Results are presented as differences in β estimates (SE) with their corresponding p-value for each pairwise comparison of the three earlier time periods versus sexual maturity (as the reference group), along with the p-value for interaction for the overall comparison across the four pubertal periods. We also estimated specific associations for each semen parameter among samples collected in each pubertal window. IPCW was used in the MIM to account for potential selection bias, as described above. Lastly, we repeated our main models using log-transformed continuous exposures rather than quintiles, and tested for non-linearity by including a quadratic term of the log-transformed exposure concentration in the models.

Covariates which could be potential confounders were included in the primary models for semen parameters, and were selected based on *a priori* evidence from the literature and supported empirically by associations with one or more of the semen parameters or urinary phthalate metabolite concentrations. In addition, we included abstinence time (days) in the models regardless of statistical significance because this is a well-known predictor of most semen quality parameters, and thus can improve the precision of the exposure estimates in the model (Schisterman et al., 2009). Based on these criteria, statistical models were adjusted for urine dilution (SG), current BMI (kg/m^2), abstinence time (<2 days, 2–5 days, ≥ 5 days), and smoking (yes/no) as well as beer consumption (yes/no) both during the year of their most recent study questionnaire (up to 3 years prior to semen collection). For example, smoking status (yes/no), was collected using the question: "Have you smoked a cigarette, even a few puffs, within the past year?". We analyzed the data using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA).

3. Results

A total of 223 men had semen samples and contributed urines for phthalates for at least one pubertal window covering the four windows (Table 1). The study participants were all white and had a median (IQR) age at time of semen collection of 18.5 years (18.1, 19.1) and a median

Table 1
Demographic, dietary, reproductive and parental characteristics among 223 participants in the Russian Children's Study with semen samples and urine samples for at least one pubertal window for this analysis.

	Median (IQR) or N (%)
Demographics and dietary characteristics	
Age, years	18.5 (18.1, 19.1)
BMI, kg/m^2	20.9 (19.0, 23.1)
Smoking[a], n (%)	113 (51)
Beer intake[a], n (%)	139 (63)
Other alcohol intake[a], n (%)	70 (32)
At entry (8–9 years old)	
Total calorie intake[a] (kcal/day)	2777 (2139, 3385)
Carbohydrates[a] (% calories)	53.9 (49.6, 58.1)
Fat[a] (% calories)	34.5 (30.9, 37.6)
Protein[a] (% calories)	11.4 (10.5, 12.5)
Reproductive characteristics	
Cryptorchidism, n (%)	4 (2)
Varicocele, n (%)	7 (3)
Orchiditis, n (%)	1 (1)
Parental and residential characteristics	
Any household smoking during pregnancy[a],n (%)	31 (14)
Parental education[a], n (%)	
High school or less	11 (5)
College degree	131 (60)
Graduate degree or more	76 (35)

We collected BMI data from the most recent physical examination. All cases of cryptorchidism were verified after investigation of medical history and follow-up. Smoking status was based on the response to the question: "Have you smoked a cigarette, even a few puffs, within the past year?"). The questionnaire to collect smoking information was completed up to 3 years before the semen sample was collected. Diet and parental/residential characteristics were collected at age 8–9 years. [a]These variables had missing data.

(IQR) BMI of 20.9 (19.0–23.1) kg/m^2. More than half the men reported smoking (51%) or beer consumption (63%) during the year of their most recent study questionnaire. Four (2%), 7 (3%) and 1 (1%) of the participants had been previously diagnosed with cryptorchidism, severe varicocele (grade III or after surgery) and orchiditis, respectively. Twenty-eight (14%) of the young men's parents reported household smoking during pregnancy.

Overall, urinary concentrations of the DEHP metabolites were higher during the prepubertal and the early pubertal period, compared to those measured in urine samples collected in late puberty and at sexual maturity (Table 2). In contrast, urinary concentrations of the DiNP and the DiDP metabolites were higher at sexual maturity compared to the other three windows. Similar distributions of urinary phthalate metabolite concentrations were observed among a subset of 110 men who contributed to all four peripubertal windows (Supplemental Table 1). Participants in the Russian Children's Study had, overall, higher urinary phthalate metabolite concentrations compared to children in the U.S. NHANES (CDC 2019) and Germany (Kasper-Sonnenberg et al., 2012), except for MBzP which was comparable to a German cohort and lower than NHANES children, and MCNP which was higher in NHANES children. Mean (SD) and median (IQR) values for semen parameters are reported in Table 3. Median (IQR) abstinence time was 2.71 days (1.88, 3.96). Correlations between the first and the second semen sample for the measured semen parameters were moderate-to-high (r = 0.49 for total sperm count and 0.74 for sperm concentration) among 215 men who contributed 2 semen samples. Young men in the Russian Children's Study had similar median sperm concentrations compared to young men in Sweden (53.5 million/mL and 56 million/mL, respectively) (Axelsson et al., 2015), Finland (54 million/mL) and Estonia (57 million/mL) (Jørgensen et al., 2002). However, they had higher semen concentrations than men in Murcia, Spain (44 million/mL) (Mendiola et al., 2013), Australia (45 million/mL) (Hart et al., 2015), Norway and Denmark (41 million/mL) (Jørgensen et al., 2002), and worse than a young Spanish cohort in Almeria (62 million/mL) (Fernandez et al., 2012). Urinary concentrations of MBzP were moderately correlated in prepubertal and early pubertal samples (r = 0.63) as well as late pubertal and sexual maturity samples (r = 0.50) (Supplemental Table 2). We found weak-to-moderate correlations for the other phthalate metabolites across pubertal samples (r = 0.01–0.44).

When we investigated the associations of phthalate metabolite concentrations measured in pooled urine samples collected during the four pubertal windows with semen parameters, we found that young men with higher urinary concentrations of ∑DiNP metabolites in late puberty had poorer semen quality, on average, as measured by lower sperm concentration (p-trend = 0.09), count (p-trend = 0.07) and progressive motile count (p-trend = 0.12) (Table 4) than men with lower urinary concentrations. Specifically, men in the highest versus lowest quintile of urinary ∑DiNP had 30% lower sperm concentration, 32% lower count and 30% lower progressive motile count. Of note, ∑DiNP-associated differences in semen quality were most evident among men in the highest quintile of exposure supporting a potential non-linear relationship. Also, young men with higher urinary concentrations of MiBP metabolites in the early pubertal samples tended to have poorer semen quality, on average, as measured by lower ejaculated volume, sperm count, progressive motility and progressive motile count (Supplemental

Table 2
Distribution (median, IQR) of urinary phthalate metabolite concentrations (ng/mL) by pubertal window among participants in the Russian Children's Study.

	Prepuberty	Early Puberty	Late Puberty	Sexual Maturity
	134 men	219 men	203 men	213 men
	338 urines	578 urines	283 urines	891 urines
	Median = 2	Median = 2	Median = 1	Median = 4
	Range = 1-6	Range = 1-6	Range = 1-6	Range = 1-7
Mono-n-butyl phthalate (MnBP)	205 (118, 290)	183 (114, 281)	127 (68.3, 220)	107 (75.0, 161)
Mono-isobutyl phthalate (MiBP)	54.4 (33.7, 84.2)	68.9 (43.8, 115)	58.7 (36.4, 96.8)	66.7 (43.5, 112)
Monobenzyl phthalate (MBzP)	6.26 (3.01, 13.1)	7.69 (4.18, 20.8)	6.94 (3.63, 14.0)	7.27 (3.86, 15.6)
Di-2-ethylhexyl phthalate (DEHP) metabolites				
Mono-2-ethylhexyl phthalate (MEHP)	12.8 (6.60, 21.5)	15.4 (7.53, 26.2)	10.8 (6.40, 20.4)	11.1 (6.98, 19.8)
Mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP)	76.7 (46.4, 107)	77.6 (44.5, 136)	53.6 (30.8, 97.2)	50.4 (32.2, 80.7)
Mono-2-ethyl-5-oxohexyl phthalate (MEOHP)	61.5 (39.2, 94.2)	68.2 (37.2, 110)	46.2 (26.1, 82.3)	40.7 (26.6, 67.1)
Mono-2-ethyl-5-carboxypentyl phthalate (MECPP)	150 (98.2, 245)	165 (95.1, 259)	117 (69.0, 197)	105 (61.3, 160)
Di-isononyl phthalate (DiNP) metabolites				
Mono-hydroxy-iso-nonyl phthalate (MHiNP)	8.21 (4.93, 14.7)	10.0 (6.01, 18.3)	10.0 (5.39, 18.0)	19.5 (11.2, 41.5)
Mono-oxo-iso-nonyl phthalate (MOiNP)	2.88 (1.80, 5.77)	3.92 (2.04, 7.61)	3.97 (1.94, 7.27)	6.76 (3.96, 14.0)
Mono-carboxy-iso-octyl phthalate (MCOP)	5.93 (3.39, 12.7)	7.81 (4.59, 14.2)	7.45 (3.65, 15.0)	15.3 (8.24, 34.2)
Di-isodecyl phthalate (DiDP) metabolites				
Mono-(hydroxy-iso-decyl) phthalate (MHiDP)	3.79 (2.20, 7.07)	3.86 (2.18, 8.01)	3.73 (1.90, 7.55)	4.60 (2.57, 10.2)
Mono-(oxo-iso-decyl) phthalate (MOiDP)	0.41 (0.13, 0.68)	0.43 (0.17, 0.84)	0.41 (0.14, 0.93)	0.86 (0.42, 1.51)
Mono-(carboxy-iso-nonyl) phthalate (MCNP)	0.82 (0.49, 1.37)	0.94 (0.50, 1.53)	0.75 (0.47, 1.36)	1.18 (0.74, 1.78)

Table 3
Semen parameters and reproductive characteristics among 223 men contributing 438 semen samples in the Russian Children's Study who also contributed urine biomarkers of phthalate exposure to at least to one pubertal window for this analysis.

	Mean (SD)	Median (IQR)	Spearman correlation between the two semen samples (N = 215)	N (%) samples within normal NAFA-ESHRE ranges[a]
Ejaculated volume (mL)	2.76 (1.63)	2.40 (1.60, 3.50)	0.72	288 (66)
Sperm concentration (million/mL)	67.8 (58.1)	53.5 (28.3, 88.5)	0.74	378 (86)
Total sperm count (million/ejaculate)	174 (165)	128 (63.0, 230)	0.49	295 (67)
Progressive sperm motility (%)	53.1 (10.2)	55.0 (48.0, 60.0)	0.65	306 (70)
Total progressive motile count (million/ejaculate)	96.3 (92.4)	70.9 (31.4, 131)	0.50	–
Abstinence time (days)	4.92 (10.9)	2.71 (1.88, 3.96)	–	–

[a] Ejaculated volume ≥2 mL; sperm concentration ≥20million/mL; total sperm count ≥80 million; progressive sperm motility ≥50%.

Table 4
Adjusted semen parameters by quintiles of urinary phthalates (measured during the late pubertal window) among 203 men contributing 398 semen samples in the Russian Children's Study.

Urinary phthalates (range in μmol/L or ng/mL)	Ejaculate volume (mL)	Sperm concentration (million/mL)	Total sperm count (million/ejaculate)	Progressive motility (%)	Total progressive motile count (million/ejaculate)
∑DEHP					
Q1 (0.09–0.40)	2.34 (1.91, 2.76)	48.2 (33.3, 69.8)	95.1 (63.6, 142)	50.7 (47.2, 54.3)	46.5 (29.7, 73.0)
Q2 (0.41–0.64)	2.89 (2.41, 3.35)	46.2 (37.3, 57.1)	113 (86.6, 146)	53.6 (50.9, 56.3)	59.1 (44.3, 78.9)
Q3 (0.65–0.89)	2.86 (2.37, 3.34)	54.0 (43.1, 67.5)	124 (90.7, 171)	54.0 (51.6, 56.5)	66.2 (46.9, 93.4)
Q4 (0.90–1.43)	2.71 (2.27, 3.14)	48.2 (36.8, 63.1)	109 (81.8, 144)	53.2 (50.6, 55.9)	56.9 (41.7, 77.6)
Q5 (1.46–64.6)	2.66 (2.20, 3.11)	40.7 (29.5, 56.2)	89.3 (63.4, 126)	52.0 (49.2, 55.0)	45.3 (31.1, 65.9)
P-trend	0.75	0.60	0.70	0.79	0.78
∑DiNP					
Q1 (0.004–0.03)	2.60 (2.16, 3.05)	52.3 (38.5, 71.0)	112 (81.4, 155)	51.9 (48.9, 55.3)	56.5 (39.3, 81.2)
Q2 (0.04–0.05)	2.96 (2.51, 3.40)	48.9 (38.6, 62.1)	127 (94.6, 169)	54.1 (51.2, 57.0)	67.4 (48.6, 93.5)
Q3 (0.06–0.08)	2.62 (2.11, 3.11)	55.1 (42.3, 71.8)	109 (77.3, 154)	51.8 (49.0, 54.6)	55.5 (37.8, 81.5)
Q4 (0.09–0.16)	2.88 (2.40, 3.36)	46.2 (34.1, 62.6)	111 (79.6, 155)	52.7 (50.0, 55.4)	57.2 (39.9, 82.1)
Q5 (0.17–4.72)	2.39 (2.03, 2.76)	36.4 (26.6, 49.8)	75.7 (54.4, 105)	53.3 (50.5, 56.1)	39.4 (27.1, 57.2)
P-trend	0.41	0.09	0.07	0.76	0.12
∑DiDP					
Q1 (<LOD-0.007)	2.74 (2.28, 3.20)	42.0 (30.7, 57.4)	98.7 (69.5, 140)	52.5 (49.1, 56.0)	50.3 (33.7, 75.1)
Q2 (0.008–0.013)	2.88 (2.40, 3.36)	50.6 (39.8, 64.4)	117 (89.6, 153)	52.3 (49.5, 55.0)	59.9 (44.5, 80.5)
Q3 (0.014–0.018)	2.24 (1.94, 2.53)‡	61.4 (51.2, 73.7)	120 (91.4, 157)	53.7 (51.3, 56.1)	63.4 (47.5, 84.7)
Q4 (0.019–0.042)	2.71 (2.38, 3.04)	42.1 (28.7, 61.6)	102 (69.2, 150)	52.8 (49.9, 55.7)	52.6 (3.3, 80.6)
Q5 (0.044–1.20)	2.88 (2.32, 3.45)	42.6 (32.6, 55.7)	92.4 (66.1, 129)	52.3 (49.3, 55.3)	47.2 (32.4, 68.9)
P-trend	0.83	0.63	0.53	0.99	0.58
∑AA					
Q1 (0.23–1.04)	2.43 (1.99, 2.85)	50.8 (35.7, 72.3)	104 (70.4, 155)	53.1 (49.4, 56.7)	53.9 (34.7, 83.7)
Q2 (1.05–1.51)	2.92 (2.3, 3.47)	47.4 (37.3, 60.4)	113 (84.4, 152)	53.2 (50.3, 56.1)	59.1 (42.5, 82.1)
Q3 (1.52–2.25)	2.71 (2.31, 3.12)	52.1 (41.2, 66.0)	118 (89.0, 159)	51.9 (49.1, 54.7)	60.5 (43.9, 83.4)
Q4 (2.26–3.28)	2.62 (2.18, 3.06)	36.9 (28.4, 48.1)	81.7 (59.9, 111)	51.6 (48.9, 54.3)	41.4 (29.2, 58.6)
Q5 (3.29–69.9)	2.78 (2.25, 3.31)	51.0 (37.2, 69.9)	114 (81.4, 160)	53.9 (51.1, 56.8)	59.9 (41.5, 86.3)
P-trend	0.75	0.67	0.78	0.98	0.80
MBzP					
Q1 (0.10–2.81)	2.31 (1.88, 2.71)	55.7 (41.1, 75.4)	110 (77.7, 156)	54.3 (51.2, 57.5)	58.7 (39.6, 87.1)

(*continued on next page*)

Table 4 (*continued*)

Urinary phthalates (range in μmol/L or ng/mL)	Ejaculate volume (mL)	Sperm concentration (million/mL)	Total sperm count (million/ejaculate)	Progressive motility (%)	Total progressive motile count (million/ejaculate)
Q2 (2.82–5.49)	3.00 (2.54, 3.46)‡	47.9 (38.0, 60.5)	126 (97.9, 161)	53.7 (50.6, 56.8)	66.0 (50.0, 87.2)
Q3 (5.52–8.18)	2.85 (2.38, 3.32)‡	50.4 (39.2, 64.9)	117 (87.9, 156)	51.2 (48.1, 54.3)	58.6 (42.2, 81.3)
Q4 (8.31–18.4)	2.34 (1.92, 2.75)	44.3 (32.7, 60.0)	85.2 (59.0, 123)	50.9 (48.3, 53.6)	42.3 (28.3, 63.2)
Q5 (18.8–243)	2.89 (2.48, 3.46)‡	39.9 (28.9, 55.0)	95.6 (66.9, 137)	53.6 (51.0, 56.3)	50.1 (33.7, 74.6)
P-trend	0.53	0.14	0.19	0.44	0.20
MnBP					
Q1 (15.9–60.8)	2.43 (1.99, 2.87)	48.5 (34.4, 68.5)	96.8 (66.1, 142)	52.5 (49.1, 55.8)	49.5 (32.4, 75.7)
Q2 (61.1–106)	2.72 (2.31, 3.13)	53.4 (41.6, 68.6)	125 (90.2, 172)	52.8 (50.0, 55.7)	64.5 (44.9, 92.6)
Q3 (107–159)	2.81 (2.26, 3.37)	49.8 (39.2, 63.3)	113 (86.8, 146)	52.9 (50.0, 55.8)	58.3 (43.8, 77.7)
Q4 (159–242)	2.45 (2.10, 2.80)	40.4 (30.2, 54.1)	86.2 (62.0, 120)	52.1 (48.9, 55.30	43.8 (29.9, 64.1)
Q5 (244–1459)	3.06 (2.51, 3.61)	45.3 (35.5, 57.9)	112 (82.2, 153)	53.4 (50.8, 56.1)	58.8 (42.0, 82.4)
P-trend	0.29	0.37	0.88	0.84	0.89
MiBP					
Q1 (12.2–31.7)	2.83 (2.38, 3.28)	50.6 (39.7, 64.5)	123 (91.3, 166)	55.3 (52.4, 58.2)	66.7 (47.6, 93.5)
Q2 (32.9–50.8)	2.82 (2.31, 3.35)	43.7 (32.3, 59.0)	98.7 (70.7, 138)	52.4 (49.3, 55.6)	50.5 (34.6, 73.7)
Q3 (51.2–73.9)	2.82 (2.38, 3.260	50.5 (39.6, 64.4)	121 (91.5, 161)	52.2 (49.4, 55.0)	62.2 (45.8, 84.4)
Q4 (76.1–105)	2.48 (2.02, 2.94)	42.1 (31.8, 55.6)	85.2 (63.5, 114)‡	52.0 (49.5, 54.5) ‡	43.4 (31.6, 59.6)‡
Q5 (108–1019)	2.48 (2.02, 2.96)	50.4 (37.6, 67.6)	103 (71.4, 148)	51.8 (48.9, 54.7) ‡	51.8 (34.6, 77.6)
P-trend	0.25	0.98	0.43	0.11	0.31

Models adjusted for specific gravity, abstinence time, BMI, smoking, alcohol intake and household income. *p-value <0.05 when compared that quartile with the lowest quintile of exposure. ‡p-value <0.10 when compared that quartile with the lowest quintile of exposure. ∑DEHP = (MEHP + MEHHP + MEOHP + MECPP). ∑DiNP = (MHiNP + MOiNP + MCOP). ∑DiDP = (MHiDP + MOiDP + MCNP). ∑ anti-androgenic (AAP) = (MEHP + MEHHP + MEOHP + MECPP + MnBP + MiBP + MBzP + MHiNP + MOiNP + MCOP).

Table 4). We observed no evidence of consistent associations with semen quality parameters of urinary concentrations of ∑DiNP and MiBP measured in the other three pubertal windows (supplemental tables 3, 4 and 5) nor of ∑DEHP, ∑AA and ∑DiDP, MBzP and MnBP in the four periods (Table 4, supplemental tables 3, 4 and 5). However, isolated associations between higher urinary MBzP and MnBP at sexual maturity and decreased percent of progressive motility were found. The MIM models showed no meaningful associations between ∑DiNP and semen parameters for any of the windows. In addition, there were no differences in measures of association in relation to semen parameters when comparing prepuberty, early puberty and late puberty to sexual maturity (Supplemental Table 6). When repeating the primary analyses evaluating urinary ∑DiNP with respect to semen parameters using a continuous measure (log-transformed) rather than quintiles, no associations were observed for the late puberty period, consistent with the MIM model results (Supplemental Table 7). However, when a continuous measure of ∑DiNP was modeled, including a quadratic term, results supported potential non-linear associations between urinary ∑DiNP in late pubertal samples with sperm concentration, count and progressive motility consistent with findings in models of exposure quintiles (Supplemental Table 7). ”

4. Discussion

To our knowledge, this is the first study to prospectively investigate prepubertal and pubertal urinary phthalate metabolite concentrations and subsequent semen quality in young adults. Urine samples to quantify phthalate metabolite concentrations were collected annually and pooled for each boy from four windows spanning puberty: prepuberty, early puberty, late puberty and sexual maturity. Our primary finding was that, on average, men with higher urinary concentrations of ∑DiNP metabolites in late puberty had poorer semen quality than those with lower urinary ∑DiNP metabolite concentrations. We additionally found marginal associations with lower semen quality in men with higher, as

compared to lower, MiBP metabolite concentrations in early puberty. However, we did not find any evidence of associations between ∑DiNP or MiBP measured during the other three pubertal windows or of ∑DEHP, ∑DiDP or ∑AAP in any of the four exposure windows and semen quality. These results highlight the importance of considering different windows of exposure during pubertal development when investigating chemical exposures in relation to semen quality in men.

Despite the fact that urinary concentrations of DiNP metabolites were highest at sexual maturity in the Russian Children's Study participants, in our primary analysis we only found associations of ∑DiNP metabolites at late puberty with poorer semen quality. We defined "late" puberty in our cohort as TV = 10–15 cc and G = 2 to 5, or TV = 20 cc and G = 2 to 3. Potential explanations are that late puberty may be more sensitive to chemical exposures than other time windows or that the exposure impact on spermatogenesis during this late pubertal stage immediately preceding sexual maturity is critical for subsequent semen quality. During late puberty, serum concentrations of LH, FSH and testosterone are highest and AMH concentrations decline. Progression of spermatogenesis from spermatogonial differentiation to generation of mature spermatozoa occurs during late puberty with the full spermatogenic cycle taking 64 days (Rey 2021). Therefore disruption of this process may impair spermatogenesis and affect semen quality.

Another potential explanation is based on varying epigenetic susceptibility of the germ cells and the somatic Sertoli and Leydig cells involved in the regulation and progression of spermatogenesis across the different pubertal windows. Sperm epigenetic modifications may include clonal expansion of undifferentiated spermatogonia through mitosis starting in early puberty, and sperm-specific changes in DNA methylation, histone modifications, and histone to protamine exchanges in mature sperm during late puberty (Marcho et al., 2020; Pilsner et al., 2017). Marcho and colleagues suggested that mitotic divisions of undifferentiated spermatogonia occur before the blood-testis barrier is formed, and that the epigenome may be more susceptible to environmental conditions during spermatocytogenesis, the initial stage of spermatogenesis (Marcho et al., 2020).

Therefore, specific physiological changes in the reproductive organs, cells, hormone profiles, and epigenetic programming of both somatic and germ cells, together with the anti-androgenic effects of the DiNP may explain the negative associations found between ∑DiNP metabolites in the late pubertal samples with semen parameters. The MIM model findings suggested no association between urinary ∑DiNP metabolite concentrations in late puberty and semen parameters, and also identified no differences in associations of ∑DiNP with any semen parameter when comparing prepuberty, early puberty, and late puberty with sexual maturity. These findings were corroborated when using ∑DiNP metabolite concentrations as a log-transformed continuous exposure variables in the main models. However, the null findings observed when a continuous exposure measure was assessed in a linear regression model may be attributable to evidence of non-linear associations between ∑DiNP metabolite concentrations in late pubertal samples with sperm concentration, count and progressive motility – supporting the greater sensitivity of detecting associations in the main models based on exposure quintiles. It should be noted that some differences between the primary (time-specific) analyses and the MIMs remained, since we were able to include up to two semen parameters (outcomes) as repeated measures for each study participant in our primary analyses, whereas the MIM method is limited to a single outcome per subject, which we modeled as the geometric mean for men who contributed two semen samples. Yet another limitation of the MIM approach is that non-linear associations, which we observed in sensitivity analyses described above, are not easily incorporated.

Young men in our study with higher urinary MiBP metabolite concentrations showed a trend toward poorer semen quality, possibly because of the higher concentrations found in the Russian boys compared to boys in NHANES (CDC 2019) and in Germany (Kasper-Sonnenberg et al., 2012). However, urinary DEHP and other phthalate metabolites with anti-androgenic activity were not consistently associated with semen quality in this study despite previous cross-sectional epidemiologic evidence in adult men (Radke et al., 2018) and the high concentrations compared to the aforementioned studies. It is important to note that young men participating in this study (RCS) are generally healthy and have good semen quality as demonstrated by the high proportion of semen samples above the normal NAFA-EHSRE ranges for semen quality. Similar studies in other cohorts that assess urinary phthalate metabolite concentrations across different pubertal periods in relation to semen quality are needed to confirm these results.

Cross-sectional epidemiological studies in Chinese (Pan et al., 2015), Polish (Jurewicz et al., 2013), Swedish (Axelsson et al., 2015), and other European (Specht et al., 2014) adult men of reproductive age, have reported altered semen quality with increased urinary DiNP metabolite concentrations (MCiOP or MINP). Specifically, urinary DiNP metabolite concentrations were associated with poorer morphology (Axelsson et al., 2015; Jurewicz et al., 2013; Pan et al., 2015), lower motility (Axelsson et al., 2015; Pan et al., 2015), and lower sperm concentration (Pan et al., 2015; Specht et al., 2014). Since studies suggest that semen quality has generally declined during the past few decades in Western Countries (Levine et al., 2017; Minguez-Alarcon et al., 2018) and has been associated with higher risk of common chronic diseases and mortality (Choy and Eisenberg 2018; Eisenberg et al. 2014, 2016; Jensen et al., 2009; Latif et al., 2017), identifying potentially modifiable contributory factors, such as environmental exposures, is critical given the public health relevance beyond fertility and reproduction.

Limitations of this study include lack of information on parents' exposure to phthalates, including maternal prenatal exposure, which may have transgenerational epigenetic effects on spermatogenesis, and the father's reproductive health, including semen parameters (Soubry et al., 2014; Stuppia et al., 2015). Other potential limitations are that the urinary phthalate metabolite concentrations among participants in the Russian Children's Study were higher than other populations slightly reducing generalizability to other populations, the potential lack of power to detect associations in the prepubertal window due to a small sample size, and the nonconcurrent time frame (>1 year) between urines collected at sexual maturity and semen sample collection. Lastly, we did not have information regarding exposure to other environmental chemicals that might impact semen quality for each of our four exposure windows so, if other chemical measures are correlated with phthalate metabolites, unmeasured confounding could impact our results. The main strengths of this study are the collection of annual urine samples during the course of the study minimizing the risk of misclassification of the exposure for these chemicals with short half-lives, and its prospective design, limiting the possibility of reverse causation, specifically for the prepubertal, early and late pubertal analysis. Other strengths include the analysis of the semen samples by a single technician who was blinded to the urinary phthalate concentrations and the collection of two semen samples for most of the young men. We also used sophisticated statistical methods such as inverse probability weights to account for censoring, in order to reduce concerns regarding selection bias.

In conclusion, we found that young men with higher concentrations of ∑DiNP metabolites quantified in urine samples collected during late puberty had poorer semen quality at sexual maturity. Also, those with higher MiBP metabolites in early puberty tended to have poorer semen quality. We did not find any evidence of consistent associations with semen quality for ∑DiNP or MiBP measured at other pubertal windows or of urinary ∑DEHP, ∑DiDP and ∑AAP at any pubertal period. These results highlight the importance of considering different windows of exposure when investigating chemical exposures in relation to semen quality in men. Further studies are needed to confirm these results.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113977.

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