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The association of prenatal and childhood pyrethroid pesticide exposure with school-age ADHD traits

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ABSTRACT

Background: Pyrethroid insecticides are commonly used in residential settings, and their use has increased rapidly. Although research has been scarce, they have been reported to be associated with impaired neurodevelopment. Moreover, susceptible exposure windows and the long-term effects of pyrethroids have not been investigated. We examined the association between pyrethroid exposure and attention-deficit/hyperactivity disorder (ADHD) symptoms over time, with exposure windows spanning from the prenatal period to school-age.
Methods: Using 524 mother–child pairs, we measured urinary concentrations of 3-phenoxybenzoic acid (3-PBA), a major pyrethroid metabolite, and asked parents to fill-out the ADHD Rating Scale IV (ARS). We used Poisson regression to identify the susceptible periods of pyrethroid exposure, by correlating various 3-PBA exposure windows (prenatal, ages 2, 4, 6 and 8) with ADHD symptoms at ages 6 and 8.
Results: Doubling of prenatal and age 2 3-PBA concentrations was associated with increased ADHD symptoms at age 6 (2.7% change, 95% confidence interval [CI]: 0.3, 5.2; 5.2% change [95% CI: 0.5, 10.2], respectively). The 3-PBA concentrations at age 4 and age 6 were linked with ADHD symptoms at age 8 (2.7% change [95% CI: 0.3, 5.3]; 3.3% change [95% CI: 0.2, 6.4], respectively). There were no clear sex-specific patterns in association.
Discussion: Both prenatal and early-childhood exposure to 3-PBA were found to be associated with ADHD symptoms. Exposure during pregnancy, and at ages 2 to 6 were found to be susceptible periods for pyrethroid neurotoxicity at ages 6 and 8.

1. Introduction

Pyrethroid insecticides are the most widely used insecticides for residential purposes, and their use has increased dramatically following the ban of two organophosphate pesticides in 2000–2001 (Williams et al. 2008). They are also used in agriculture, particularly on crops such as cotton, vegetables and nuts (Barkoski et al. 2021). Residues in foods are the main source of exposure to pyrethroids, but inhalation or ingestion of contaminated household dust may be another important source of exposure, and dermal absorption, to a smaller degree, is a significant route in homes with frequent insecticide application (Agency for Toxic Substances and Disease Registry, 2003; Bao et al., 2020; Morgan 2012). Exposure to pyrethroids in the general population is widespread, as 3-phenoxybenzoic acid (3-PBA), a major metabolite of up to 20 synthetic pyrethroid insecticides, was detected in over 70% of urine samples in a US study (Barr et al. 2010). Children are at a higher

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risk of exposure because they eat more food per kilogram of body weight than adults (Hauptman et al., 2017), and also because of their proximity to the floor and surfaces where insecticides may be applied, and higher urinary concentrations have been reported compared to adolescents and adults (Viel et al. 2015).

Although pyrethroids have low volatility and mammalian toxicity compared to organophosphates, they have been documented to cross the blood–brain barrier during both the prenatal and postnatal period (Sinha et al. 2004), exhibiting neurotoxicity in the developing brain. Animal studies have found alterations in the dopaminergic system and attention-deficit/hyperactivity disorder (ADHD)-like behavior in mice exposed to pyrethroids (Burns et al. 2013; Elwan et al. 2006; Gillette and Bloomquist 2003). However, there is limited research on the association between pyrethroids and ADHD symptoms in humans. Prenatal exposure to pyrethroid has been reported to be associated with ADHD-related traits at ages 2–4 years (Dalsager et al. 2019). Cross-sectional studies in children have yielded inconsistent results, with some reporting positive associations between pyrethroid exposure and ADHD symptoms (Lee et al. 2020; Wagner-Schuman et al. 2015), while another study showed no association (Quiros-Alcala et al. 2014). Although previous studies only targeted preschool ages, ADHD symptoms can also be detected in the school-age period after age 6 (Kessler et al. 2005), but no study has investigated whether prenatal pyrethroid exposure has an effect on ADHD at this age. Moreover, no studies to date have simultaneously examined the neurotoxic effect of prenatal and postnatal 3-PBA exposure, and therefore, the most susceptible exposure windows are unknown.

The purpose of this study was to examine the relationship between pyrethroid exposure at multiple time periods and ADHD symptoms in school-age children. We aimed to identify the susceptible periods of pyrethroid exposure that are associated with increased ADHD symptoms by correlating urinary 3-PBA exposure at multiple time points (prenatal and ages 2, 4, 6, and 8) with ADHD symptoms at ages 6 and 8.

2. Methods

2.1. Study design and participants

Our research was based on data from the Environment and Development of Children (EDC) study, an ongoing prospective cohort study designed to evaluate the association between prenatal and postnatal environmental exposures and physical/cognitive development. Detailed information of the study design has been described elsewhere (Kim et al. 2018). We randomly contacted women that had participated in a larger study called the Congenital Anomaly Study (CAS). The CAS recruited 13,484 pregnant women during the second trimester from eight local hospitals (5 primary hospitals and 3 tertiary hospitals) in the Seoul and Gyeonngi provinces of South Korea from August 2008 to July 2010. The inclusion criteria for GAS were 1) pregnant women in the second trimester of pregnancy, 2) pregnant women who provided informed consent for follow-up and the exclusion criteria were 1) pregnant women with congenital abnormalities, including genetic disorders, or immunodeficiency disorders and 2) individuals unable to communicate in Korean. Data on their individual characteristics (sociodemographic and lifestyle factors, past medical history), and spot urine/blood samples at enrollment to estimate the exposure of environmental factors during the second trimester of their pregnancy (between 14 and 27 weeks of gestation; median 17 weeks of gestation) were collected. After the CAS ended in 2011, participants for the EDC study were recruited by randomly contacting the mothers after birth. Children who had a congenital deformity at birth (n = 115) or invalid contact information (n = 218) were excluded from the potential contact list. A total of 726 mother–child pairs were enrolled in the EDC study and participated in follow-up. The children were followed up every two years, from 2012 to 2019. A total of 425 children at age 2, 645 children at age 4, 574 children at age 6, and 525 children at age 8 received follow-up. Neurodevelopment was assessed using parent-report questionnaires and formal testing (including tests for intelligence, attention and executive function) by professional psychologists. As the intelligence quotient (IQ) level is considered to be stable (Schalke et al. 2013), children's IQ scores were assessed once at age 6 using the Korean Educational Developmental Institute's Wechsler Intelligence Scale for Children (Park et al. 1996). Additionally, mothers' IQ scores were evaluated using the Korean Wechsler Adult Intelligence Scale (Lim et al. 2000). After excluding 151 children who were missing ARS scores at age 6 or 8 and children from multiple births (twin or triplets, n = 51), a total of 524 mother–child pairs were included in this study. All children had data on 3-PBA concentrations for more than one exposure period (prenatal, age 2, 4, 6 or 8). Fig. 1 depicts the flow of the participants throughout the study.

Informed consent was obtained from the parents (since pregnancy) and children (aged ≥ 8 years) after sufficient explanation of the study. The study protocol was reviewed and approved by the Institutional Review Board of Seoul National University Hospital. This study was conducted in accordance with the principles of the Declaration of Helsinki.

2.2. Measurement of 3-PBA concentrations

We measured 3-PBA concentrations using maternal urine samples collected between 14 and 27 weeks of gestation, at a mean of 20 weeks, and from children at ages 2, 4, 6 and 8. The first urine after fasting overnight was collected with a 10 ml conical tube, stored on ice at the site, and transported on the same day to SMARTIVE Co. a commercial lab, for storage at −70 °C until analysis. The laboratory methods and processes for 3-PBA analysis followed a previously reported study (Schettgen et al. 2002). Our detailed method of 3-PBA concentration assessment was described in a previous article (Lee et al. 2019). To explain briefly, after 1 ml of concentrated hydrochloric acid (37%) was added for hydrolysis of the samples, the samples were heated at 90 °C for 1 h with the standard solution, n-hexane was added, shaken for 20 min, and the mixture was centrifuged at 2500 rpm for 5 min. n-Hexane was further added to the supernatant. The collected mixture was combined with 0.1 M NaOH, shaken for 10 min, and centrifuged at 2500 rpm for 5 min. The separated upper layer was removed and concentrated hydrochloric acid and n-hexane were added. The mixture was shaken for 10 min and then centrifuged at 1500 rpm for 5 min. After removing the upper layer solution, the solvent was dried with a nitrogen concentrator, and then mixed with toluene and N-Methyl-N-(*tert*-butyldimethylsilyl) trifluoroacetamide. The mixture was allowed to react at 70 °C for 60 min and urinary 3-PBA concentration was determined by a gas chromatograph-mass spectrometer (Clarus 600 T, Perkin Elmer (GC–MS/MS)).

Strict internal quality control was performed prior to analysis of the whole samples and included tests of linearity, accuracy and precision and detection limit. In the linearity test, R^2 was 0.999 in the calibration curve in pooled urine. The accuracy test was performed using ClinChek I and II (catalog no. 8928, ClinChek® Urine Controls, Recipe® Chemicals + Instrument GmbH, Germany) and showed 99–116% accuracy for ClinCheck I and 87–98% recovery. Coefficient of variations (CV%) were 3.0%, 2.6% and 1.5% in low, medium and high concentrations, respectively. For values below the limit of detection (LOD), we assigned the LOD value divided by the square root of 2 (Hornung et al. 1990). In this study, the LOD was 0.015 μg/L. Our laboratory was involved in the German External Quality Assessment Scheme and has passed the 57th to 60th (2017–2018: urinary 3-PBA) programs. Creatinine values (g/L) were included as covariates in subsequent analyses to correct for urine dilution (Barr et al., 2005; O'Brien et al., 2016). For measuring creatinine, CREA reagent (Roche, Indianapolis, IN, USA) was used with a Hitachi 7600 machine (Hitachi, Tokyo, Japan) for a kinetic colorimetric assay (rate-blanked and compensated).

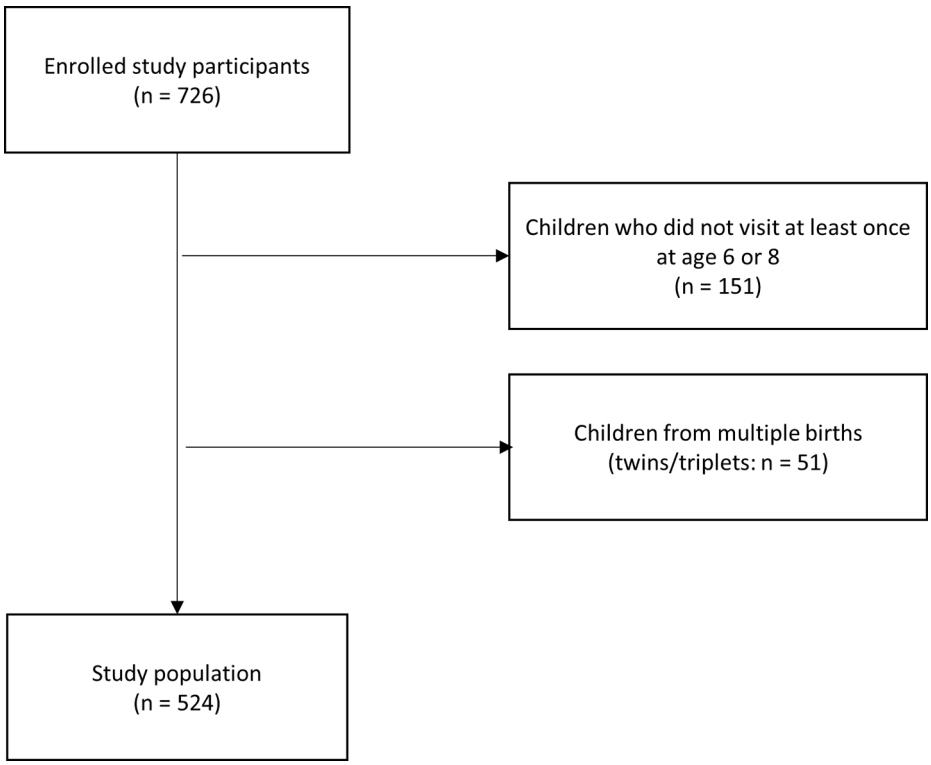

Fig. 1. Study flow of the participants Abbreviations: 3-PBA, 3-phenoxybenzoic acid.

2.3. Assessment of ADHD symptoms

ADHD symptoms were measured using a parent-report rating scale called the ADHD-Rating Scale IV (ARS) (Dupaul 1998). The ARS consists of 18 items rated on a scale from 0 to 3, with potential responses of "never or rarely", "sometimes", "often", or "very often". Each item corresponds with the diagnostic criteria of ADHD in the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) (American Psychiatry Association 2013). The total score ranges from 0 to 54, with nine items reflecting inattention symptoms (range from 0 to 27) and nine items addressing hyperactivity-impulsivity symptoms (range from 0 to 27). All higher scores indicate more ADHD traits. The internal consistency (Cronbach's α) of ARS in this study population was 0.88 at age 6 and age 8. The cut-off for possible clinical ADHD is $\geq$ 19.

2.4. Definition of covariates

We selected potential covariates based on previous literature (Dalsager et al. 2019; Lee et al. 2020; Wagner-Schuman et al. 2015). These included maternal age at pregnancy (years), maternal education (<or $\geq$ college education), family income status (monthly family income < or $\geq$ $3,500), maternal smoking during pregnancy (ex-smoker, current smoker, or non-smoker), diabetes mellitus (DM) during pregnancy (yes or no), child's age (in months), sex, body mass index (BMI) (kg/m^2), birth order (<or $\geq$ 2nd), delivery mode (vaginal delivery or cesarean section), prematurity (<or $\geq$ 37 weeks), low birth weight (<or $\geq$ 2.5 kg), breastfeeding status (exclusive breastfeeding, mixed feeding, or exclusive formula feeding), and season of 3-PBA exposure (spring, summer, autumn, or winter). We controlled for different covariates in prenatal and postnatal exposure models: DM during pregnancy, prematurity and low birth weight were only considered as covariates in postnatal models, as these are potential mediators lying on the pathway from prenatal 3-PBA exposure to ADHD symptoms. Breastfeeding was only considered in postnatal models as this occurred after prenatal 3-PBA exposure and couldn't have affected prenatal 3-PBA concentration. Based on exploratory analyses, we selected the final covariates that were associated with both ARS scores and 3-PBA concentrations ($p < 0.1$) (Hernán et al., 2002). Specifically, we first identified the covariates that were associated with ARS scores at age 6 or 8, and then selected the covariates that were associated with 3-PBA concentrations during pregnancy, age 2, 4, 6 or 8 (Table S1, S2). Urine creatinine concentrations were included as covariates in all analyses for correction of urine dilution. The final set of selected covariates were age, sex, family income, season of exposure, and urine creatinine concentration for the prenatal 3-PBA analyses and age, sex, family income, season of exposure, DM during pregnancy, breastfeeding, prematurity, and urine creatinine concentration for the postnatal 3-PBA analyses.

2.5. Statistical analysis

We used $\log_2$-transformed 3-PBA values in all subsequent analyses to approximate normal distribution. Pearson correlation coefficients between 3-PBA concentrations during pregnancy and at ages of 2, 4, 6, and 8 were calculated. Intraclass correlation coefficients (ICCs; two-way mixed models, single rater, absolute agreement option: ICC(3,1)) were computed to examine differences of ARS scores (ages 6 and 8) and 3-PBA concentrations (at ages of 2, 4, 6, and 8 years) of the children over time.

The ARS scores at age 6 and 8 were right skewed (Figure S1), thus ARS scores were analyzed using Poisson regression. Univariate Poisson regression models were used to investigate the association between potential covariates and ARS scores and linear regression models were used to explore the association between those covariates and 3-PBA concentrations at different ages.

We compared the parental characteristics of the participants included in this study and those excluded using independent t-tests (for continuous variables) or chi-square tests (for categorical variables) (Table 1). The characteristics of the children at ages 6 and 8 were also compared (Table 2).

We examined the association between 3-PBA concentrations and ARS scores using multivariable Poisson regression models at multiple

Table 1
Characteristics of the mothers of included and excluded children in the study.

Variables	Included children (n = 524)	Excluded children (n = 202)	P-value
Maternal age at pregnancy, years, mean (SD)	31.2 (3.6)	31.7 (3.6)	0.12
Maternal Education, N (%)			
< College education	92 (17.6)	45 (22.3)	0.17
≥ College graduate	432 (82.4)	157 (77.7)	
Paternal education, N (%)			
< College education	88 (16.8)	32 (15.8)	0.82
≥ College graduate	435 (83.2)	170 (84.2)	
Smoking during pregnancy, yes, N (%)			1.0
Ex-smoker	80 (15.6)	30 (15.4)	
Never smoker	432 (84.4)	165 (84.6)	
DM during pregnancy, yes, N (%)	22 (4.2)	1 (0.5)	**0.01**
Maternal FSIQ, mean (SD)	116.4 (11.6)	117.4 (11.2)	0.62
Season of exposure, prenatal, N (%)			**0.03**
Spring	196 (37.6)	56 (27.9)	
Summer	92 (17.7)	52 (25.9)	
Autumn	115 (22.1)	48 (23.9)	
Winter	118 (22.6)	45 (22.4)	

Abbreviations: SD, standard deviation; DM, diabetes mellitus; FSIQ, full-scale intelligence quotient.
P-value for difference of characteristics between included and excluded children (chi-square test for categorical variables and *t*-test for continuous variables).

Table 2
Sociodemographic characteristics of the children at age 6 and 8.

Variables	Age 6 (n = 449)	Age 8 (n = 414)	p-value
Age, months, mean (SD)	71.2 (1.5)	95.1 (1.5)	**<0.01**
Sex, boys, N (%)	238 (53.0)	219 (52.9)	1.00
BMI, kg/m^2, mean (SD)	15.8 (1.9)	16.9 (2.5)	**<0.01**
Birth order, N (%)			0.63
1st	266 (59.2)	252 (60.9)	
2nd or higher	183 (40.8)	162 (39.1)	
Delivery mode, N (%)			0.83
Vaginal delivery	299 (66.6)	279 (67.4)	
Cesarean section	150 (33.4)	135 (32.6)	
Low birth weight, yes, N (%)	12 (2.7)	11 (2.7)	> 0.99
Prematurity, yes, N (%)	21 (4.7)	18 (4.3)	0.82
Breastfeeding, N (%)			0.94
Exclusive breastfeeding	150 (33.4)	140 (33.8)	
Mixed feeding	290 (64.6)	267 (64.5)	
Formula feeding	9 (2.0)	7 (1.7)	
Monthly family income, N(%)			**<0.01**
< $3500	142 (31.6)	88 (21.3)	
≥$3500	307 (68.4)	326 (78.7)	
Child FSIQ, mean (SD)	109.8 (12.8)	109.7 (12.9)	0.91
ARS scores, mean (SD)			
ARS total score	6.3 (5.5)	6.9 (5.6)	0.11
ARS inattention score	3.5 (3.0)	4.2 (3.4)	**<0.01**
ARS hyperactivity-impulsivity score	2.8 (2.9)	2.7 (2.7)	0.60
Season of 3-PBA exposure, N (%)			0.25
Spring	124 (27.6)	105 (25.4)	
Summer	126 (28.1)	133 (32.1)	
Autumn	155 (34.5)	125 (30.2)	
Winter	44 (9.8)	51 (12.3)	
3-PBA concentrations, μg/L, GM (GSD)	0.8 (2.5)	0.7 (3.9)	0.65

Abbreviations: SD, standard deviation; BMI, body mass index; FSIQ, full-scale intelligence quotient; ARS, ADHD Rating Scale IV ; 3-PBA, 3-phenoxybenzoic acid; GM, geometric mean; GSD, geometric standard deviation.
P-value for difference of characteristics between included and excluded children (chi-square test for categorical variables and *t*-test for continuous variables).

time points to identify susceptible exposure windows. We investigated relationships by pairing 3-PBA concentrations at pregnancy, ages 2, 4, 6 or 8 (exposure windows) and ARS scores at age 6 or 8 (ages of outcome). We adjusted for child's age (month), sex, family income, season of exposure and urine creatinine concentration for the prenatal 3-PBA analyses and age, sex, family income, season of exposure, DM during pregnancy, prematurity, breastfeeding and urine creatinine concentration for the postnatal 3-PBA analyses. As eligible participants differed by follow-up ages, we included 449 and 414 children at the age 6 and age 8 for ARS analyses, respectively (n = 169 and n = 158 for the age 2 exposure windows). We repeated the analyses separately for the inattention and hyperactivity-impulsivity ARS subscale scores. We repeated the same analyses after stratification by the children's sex, and also investigated the interaction between sex and 3-PBA concentrations (significance set at $p < 0.05$), as epidemiological research has provided evidence supporting differential effects of pesticide toxicity by sex (Garcia 2003; Lee et al. 2019).

We examined the effects of exposure to 3-PBA on ARS scores at age 6 and 8 using a generalized linear mixed model with a log-link function for repeated analysis (n = 521), adjusting for age, sex, family income, season of exposure, DM during pregnancy, prematurity, breastfeeding and urine creatinine concentration. We also estimated the association in separate sexes. To see if sustained exposures to 3-PBA impact ARS scores, we also constructed models including no covariates, but only the main effects and interactions between 3-PBA concentrations at different exposure periods. We excluded cross-sectional 3-PBA exposures from the models, as cross-section associations may be caused by reverse causation. For the age 6 ARS analysis, we constructed a model including prenatal, age 2, and age 4 3-PBA concentrations, and also another excluding age 2 (only including prenatal and age 4 exposure), as inclusion of age 2 resulted in small sample sizes. For the age 8 ARS analysis, we constructed a model including prenatal, age 2, age 4, and age 6 3-PBA concentrations, and also another excluding age 2 (only including prenatal, age 4 and age 6 exposure), as inclusion of age 2 resulted in small sample sizes.

To test for robustness, we performed sensitivity analyses by adjusting for covariates that were selected when using the forward selection process, which were age, sex, family income, season of exposure, birth order, maternal age at pregnancy and urine creatinine concentration in prenatal models and age, sex, family income, season of exposure, birth order, maternal age at pregnancy, breastfeeding, prematurity and urine creatinine concentration in postnatal models. Another sensitivity analysis was conducted by adjusting for all 3-PBA concentrations measured prior to exposure window of interest (e.g., adjusted for prenatal and age 2 3-PBA concentrations for the age 4 3-PBA analysis). As the sample size was significantly reduced when adding the 3-PBA concentration at age 2 in the postnatal models, we constructed another main model (n = 169 and n = 158 in postnatal analyses) for comparison. Finally, we reran the analyses after restricting to the population that had visited at both ages 6 and 8 (n = 412 for prenatal and ages 4, 6 and 8 exposure windows; n = 157 for age 2 exposure window).

All statistical analyses were performed using SPSS for Windows software (ver. 22.0; Armonk, NY: IBM Corp.) and R version 4.0.2 (The Comprehensive R Archive Network, Vienna, Austria; http://cran-r-project.org). Statistical significance was defined as $p < 0.05$ (two-tailed). As the 3-PBA concentrations were $\log_2$-transformed and the ARS scores were analyzed using a log-link function, the β coefficients were interpreted as $(e^{\beta}-1)*100$ % change for doubling in exposure.

3. Results

3.1. General characteristics of the participants

Table 1 presents the parental characteristics of our participants (n = 524). The mean maternal age at pregnancy was 31.2 ± 3.6 years. The proportion of mothers who were college graduates were 82.4%. During pregnancy, none of the mothers were current smokers and 15.6% were ex-smokers. We found that the characteristics of those included in this study were not significantly different compared to those who were excluded in our analyses (n = 202), except for DM during pregnancy and season of prenatal 3-PBA exposure. Regarding the child characteristics,

the proportion of boys was similar at ages 6 and 8 (53.0% and 52.9%, respectively), and the majority were from families with a higher income (68.4% and 78.7% at ages 6 and 8: Table 2). The ICC of total ADHD symptoms from age 6 to 8 was 0.55, indicating relatively stable levels of ADHD symptoms in a child. The inattention subscale scores were higher at age 8 compared to age 6 (3.5 ± 3.0 at age 6 vs 4.2 ± 3.4 at age 8). Twelve children at age 6 and 19 children at age 8 surpassed the cut-off value of possible clinical ADHD.

3.2. 3-PBA concentrations

The distribution of 3-PBA concentrations at each age is presented in Table S3. The geometric mean concentrations of 3-PBA exposure were 0.7 ± 3.7, 0.5 ± 3.5, 0.9 ± 3.4, 0.8 ± 2.5, and 0.7 ± 3.9 μg/L, during pregnancy, and at age 2, 4, 6 and 8, respectively. Childhood concentrations of 3-PBA at age 4 to 8 were higher compared to pregnancy, and decreased from age 4 to age 8. Boys were exposed to higher concentrations compared to girls at all ages. The detection frequencies of 3-PBA were > 99% at all time points (99.2% during pregnancy, 100% at ages 2, 4 and 6, 99.8% at age 8). The ICC of 3-PBA from age 2 to 8 was 0.16, and 0.19 from ages 4 to 8. The correlation coefficients of 3-PBA pairs at different ages were small (range −0.03 – 0.29: Figure S2).

3.3. Association between 3-PBA exposure and ADHD symptoms

The association between 3-PBA exposure and ARS scores at multiple time points are shown in Table 3 and Fig. 2. Doubling of 3-PBA concentrations during pregnancy was associated with 2.7% (95% CI: 0.3, 5.2) increase in ARS scores at 6 years of age. Also, doubling of 3-PBA concentrations at age 2 was associated with 5.2% (95% CI: 0.5, 10.2) increase in ARS scores at 6 years of age. Doubling in 3-PBA concentrations at 2 and age 4 years of age was associated with increased ARS scores at 8 years (2.7% change [95% CI: 0.3, 5.3]; 3.3% change [95% CI: 0.2, 6.4], respectively). The effect sizes of associations showed a decreasing trend with age, from age 2 to age 8 (% change [95% CI]: 4.0 [-0.5, 8.7], 2.7 [0.3, 5.3], 3.3 [0.2, 6.4], 0.1 [-2.0, 2.2] at ages 2,4, 6 and 8, respectively).

None of the sex-interaction terms were significant for the significant associations found in the main analysis (Table 3). Significant sex × 3-PBA interaction was found in the association between 3-PBA exposure at age 6 and ARS at age 6, and also in the association between 3-PBA exposure at age 8 and ARS at age 8. However, these were cross-sectional findings and can be caused by reverse causation. According to the sex-stratified analyses, the association between 3-PBA during pregnancy and ARS scores at age 6 was significant in boys, but not girls (4.3% change [95% CI: 1.1, 7.6] for boys vs 0.1% [95% CI: −3.4, 3.7] for girls; Fig. 2). In postnatal exposure windows, the associations were stronger among girls in some exposure windows, as the associations of 3-PBA concentrations at ages 2 with ARS scores at age 6 and 8 were significant in girls, but not boys (4.6% change [95% CI: −0.8, 10.3] for boys versus 11.9% change [95% CI: 0.7, 24.2] for girls at age 2 with age 6 ARS scores; 1.9% change [95% CI: −2.9, 6.9] for boys versus 16.7% change [95% CI: 3.8, 31.1] for girls at age 2 with age 8 ARS scores). Exposure to 3-PBA at age 6 was associated with ARS scores at age 8 in girls only (1.5% change [95% CI: −2.1, 5.2] for boys versus 6.8% change [95% CI: 1.2, 12.7] for girls).

When examining the subscale scores separately, 3-PBA was not found to be associated with a specific subscale score prominently. Doubling in 3-PBA concentrations at age 4 were associated with a 3.8% decrease (95% CI: −7.3, −0.3) in the hyperactivity-impulsivity subscale scores at age 6 (Table S4). The inattention subscale scores at age 2 were related to 3-PBA concentrations at age 8 (5.8% change, [95% CI: 1.9, 9.8]; Table S4).

The mixed model results were not significant (Table S5), indicating that increase in $\log_2$-transformed 3-PBA metabolites were not associated with increase in ARS scores from age 6 to 8 (-0.4% change, 95% CI: −3.3, 2.6). The 2-way interaction between prenatal and age 4 3-PBA concentrations was found to be associated with an increase in age 6 ARS scores (1.5 % change, 95% CI 0.3, 2.8). The 3-way interaction between prenatal, age 4 and age 6 3-PBA concentrations was also linked to an increase in age 8 ARS scores (2.0% change, 95% CI: 1.0, 3.1; Table S6).

3.4. Sensitivity analyses

In the sensitivity analyses, adjustment for covariates selected by forward selection (Model 1) did not change the significant associations between exposure window-specific 3-PBA concentrations and ARS scores (Table S7). When we reduced the sample size to match the sample size of age 2, only prenatal and age 2 3-PBA exposure was associated with age 6 ARS scores (Main Model 2). However, adjusting for all prior 3-PBA concentrations of early exposure period did not change the results in comparison to the main model (Model 2). When restricting to the population that visited at both ages 6 and 8, the results remained similar

Table 3
Associations between 1-unit increase in exposure to 3-PBA and changes in ARS scores by exposure windows.

Age of ARS assessment	Exposure windows	Total[a]		Boys[b]		Girls[b]		Sex × 3-PBA
		(β [95% CI])	p-value	(β [95% CI])	p-value	(β [95% CI])	p-value	p-value
Age 6	Pregnancy	**0.03 (3.40*10^{-3}, 0.05)**	**0.03**	**0.04 (0.01, 0.07)**	**0.01**	9.32*10^{-4} (-0.04, 0.04)	0.96	0.36
(n = 449	Age 2*	**0.05 (5.27*10^{-3}, 0.10)**	**0.03**	0.05 (-8.05*10^{-3}, 0.10)	0.10	**0.11 (7.36*10^{-3}, 0.22)**	**0.04**	0.44
Boys: n = 238	Age 4	−0.02 (-0.04, 0.10)	0.20	−7.46*10^{-3} (-0.04, 0.02)	0.63	−0.03 (-0.07, 0.02)	0.24	0.74
Girls:n = 211)	Age 6	−0.01 (-0.04, 0.02)	0.40	0.01 (-0.03, 0.05)	0.54	**−0.06 (-0.11, −1.56*10^{-3})**	**0.04**	**<0.01**
Age 8	Pregnancy	2.24*10^{-3} (-0.02, 0.03)	0.86	0.02 (-0.01, 0.05)	0.29	−0.02 (-0.06, 0.02)	0.31	0.25
(n = 414	Age 2*	0.04 (-5.72*10^{-3}, 0.08)	0.09	0.02 (-0.03, 0.07)	0.45	**0.15 (0.04, 0.27)**	**0.01**	0.40
Boys:n = 219	Age 4	**0.03 (2.85*10^{-3}, 0.05)**	**0.03**	0.03 (-1.51*10^{-3}, 0.06)	0.06	0.02 (-0.02, 0.06)	0.30	0.31
Girls:n = 195)	Age 6	**0.03 (2.57*10^{-3}, 0.06)**	**0.03**	0.02 (-0.02, 0.05)	0.42	**0.07 (0.01, 0.12)**	**0.02**	0.72
	Age 8	1.01*10^{-3} (-0.02, 0.02)	0.93	0.02 (-6.28*10^{-3}, 0.05)	0.13	−0.03 (-0.06, 4.91*10^{-3})	0.10	**0.03**

[a] Adjusted for child's age, sex, family income, season of exposure and urine creatinine for prenatal exposure analyses.
Adjusted for child's age, sex, family income, season of exposure, breastfeeding, prematurity, diabetes mellitus during pregnancy and urine creatinine for postnatal exposure analyses.
[b] Adjusted for child's age, sex, family income, season of exposure and urine creatinine for prenatal exposure analyses.
Adjusted for child's age, sex, family income, season of exposure, breastfeeding, prematurity, diabetes mellitus during pregnancy and urine creatinine for postnatal exposure analyses.
Abbreviations, 3-PBA, 3-phenoxybenzoic acid, ARS, ADHD Rating Scale IV, CI, confidence interval.
Significant results shown in bold.
*Sample size: n = 169 and n = 158 for the age 6 and age 8 analyses, respectively for the total population.
n = 93 and n = 76 for the age 6 and age 8 analyses, respectively for the boys.
n = 88 and n = 70 for the age 6 and age 8 analyses, respectively for the girls.

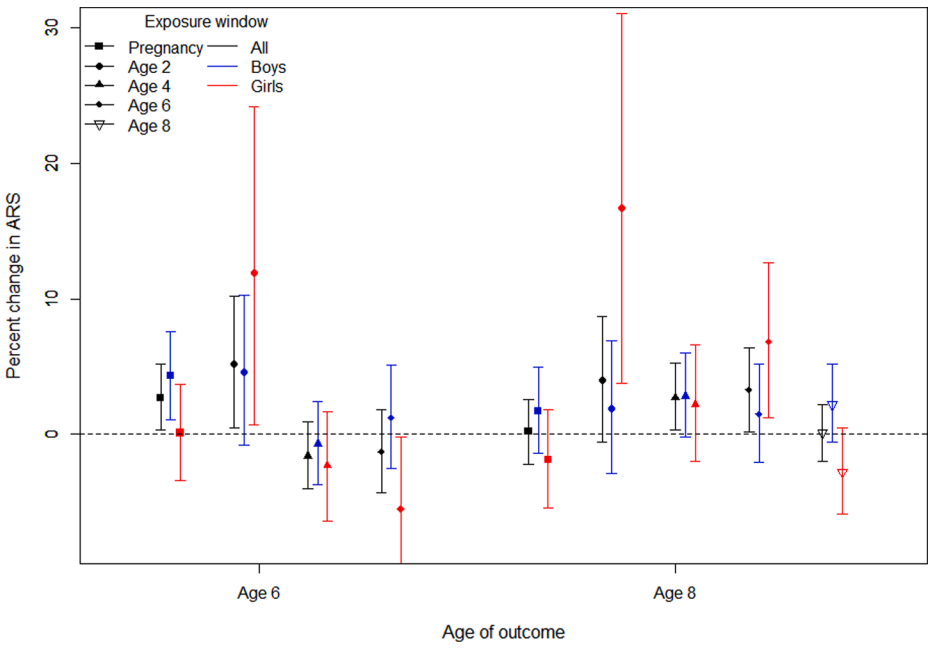

Fig. 2. CI plot of the associations between 3-PBA and ARS scores Adjusted for child's age, sex (for the total sample), family income, season of exposure and urine creatinine for prenatal exposure analyses Adjusted for child's age, sex (for the total sample), family income, season of exposure, breastfeeding, prematurity, diabetes mellitus during pregnancy and urine creatinine for postnatal exposure analyses Abbreviations: CI, confidence interval; 3-PBA, 3-phenoxybenzoic acid; ARS , ADHD Rating Scale.

(Model 3).

4. Discussion

To our knowledge, this is the first study that investigated 1) the effect of both prenatal and early childhood exposure to 3-PBA on ADHD symptoms in school-age children and 2) the susceptible exposure window to 3-PBA neurotoxicity. In utero, ages 2 to 6 were found to be susceptible periods, but each window of exposure affected ADHD symptoms at different ages. The associations did not show a specific pattern by sex. Sustained exposure to 3-PBA during pregnancy and at age 4 was found to impact age 6 ADHD symptoms, while sustained exposure to 3-PBA during pregnancy, at age 4 and age 6 was associated with increase in ARS score at age 8.

Previous studies on the effect of pyrethroid exposure on neurodevelopment have been limited in number and have produced inconsistent results. In regard to prenatal exposure, the study by Dalsager et al. found that prenatal exposure to 3-PBA was associated with ADHD related traits at 2–4 years of age (Dalsager et al. 2019). Maternal pyrethroid exposure was reported to be associated with lower cognitive scores at three months of age (Fluegge et al. 2016) and at 12 months of age (Xue et al. 2013), and with poor social-emotional development at age 1 (Eskenazi et al. 2018). One study found that prenatal 3-PBA was associated with generalized behavioral deficits measured at 4–9 years of age (Furlong et al. 2017). Comparatively, prenatal 3-PBA concentrations were only marginally associated with Bayley Scales of infant development at 24 months of age (Watkins et al. 2016). Another study found that greater *in utero* 3-PBA concentrations were associated with a moderate but imprecise increase in risk for autism spectrum disorder compared to typically developing children at age 3-years (Barkoski et al. 2021). A French cohort reported that prenatal 3-PBA was not associated with IQ scores at six years of age (Viel et al. 2015). These studies differed in exposure levels, used different measurement tools of behavior, and assessed behavioral outcome at different ages; thus, results may not be directly comparable across studies. Moreover, exposure misclassification, sex-specific effects, and the effects of pesticide mixtures may have contributed to the discrepancy (Radke et al. 2020). Due to the paucity of literature, further studies are warranted to determine the neurotoxic effect of pyrethroid pesticides.

Only one previous study has investigated the association of prenatal pyrethroid exposure and ADHD symptoms to our knowledge. This study on a mother–child cohort in Denmark (Odense Child Cohort) found that doubling in maternal 3-PBA concentration was associated with a 3% higher expected ADHD score assessed by the Child Behavior Checklist for Ages $1_{1/2}$–5 (95% CI: 1.00, 1.07) (Dalsager et al. 2019), while our study found a 2.7% increase (95% CI: 1.2, 6.0) in ARS scores associated with doubling of maternal 3-PBA concentrations. A cross-sectional study of 8–15-year-olds in the US reported that hyperactive-impulsive symptoms increased by 50% for every 10-fold increase in 3-PBA concentrations (Wagner-Schuman et al. 2015), equivalent to 5% increase in symptoms for every 2-fold increase in 3-PBA. The estimates of effect were similar in these studies and the present study, but the exposure concentrations differed, as the median of prenatal exposure was 0.24 ug/L in the Denmark study, 0.29 ug/L in the US study (2001–2002 National Health and Nutrition Examination Survey), and 0.77 μg/L in our study. Even at lower concentrations in the Denmark and US studies, 3-PBA showed neurotoxic effects. According to the Korean National Environmental Health Survey, 3-PBA concentrations in the general population were>3 times higher in Korea than in the US, probably due to the wider exposure of Koreans to various forms of pyrethroids such as sprays and fumigants (Hu et al. 2021; Yoo et al. 2016). The population burden is determined not only by the effect size but also the distribution of a factor (Bellinger 2012). Although the effect sizes were small, the population burden can be a concern due to the wide-spread usage of pyrethroid pesticides.

We found multiple susceptible periods, spanning from the prenatal phase to age 6. Previous animal studies have suggested that both prenatal and postnatal periods may represent susceptible phases for pyrethroid neurotoxicity (Ahlbom et al. 1994; Burns et al. 2013; Eriksson and Nordberg 1990; Richardson et al. 2015; Talts et al. 1998). Multiple susceptible periods are also plausible considering the continuous brain development from *in utero* to adolescence (Kim et al. 2021). Axon elaboration, synapse formation, as well as axon and synapse elimination are mechanisms that have shown to alter circuit architecture during susceptible periods (Knudsen 2004), therefore, the periods of rapid neurogenesis and synaptic pruning may be periods of heightened sensitivity to external insults (Ismail et al. 2017; Meredith 2015). Neurogenesis is most prominent during early fetal development, whereas synaptogenesis starts from 27 weeks of gestation and intensifies over the first 2 years of life (Johnston et al. 2009). Synaptic pruning occurs

rapidly from the age of 2 years to 10 years (Huttenlocher and Dabholkar 1997). The susceptible period to 3-PBA coincides with periods of neurodevelopment. Identification of susceptible periods could facilitate interventions to reduce exposure on the population level and individual level during these time periods (Stacy et al. 2017).

There were no clear sex-specific association patterns found in this study. The associations between prenatal 3-PBA exposure and ADHD symptoms in the Odense Child Cohort were not modified by sex, either (Dalsager et al., 2019). However, other studies on the association between pyrethroid exposure and neurodevelopment have reported mixed findings regarding sex-modification. Watkins et al. found that the negative association between prenatal 3-PBA concentration and neurodevelopment scores at 24 months of age was stronger in girls compared to boys (Watkins et al. 2016), whereas another study observed increased odds of an ADHD diagnosis associated with pyrethroid biomarkers in boys, but not girls (Wagner-Schuman et al. 2015). A rodent study by Meng et al. found that pyrethroid exposure during puberty was more prominently associated with spatial learning and memory impairments in females (Meng et al. 2011), but male mice have also been reported to be more vulnerable to pyrethroid-induced hyperactivity than female mice (Lazarini et al. 2001). Pyrethroid exposure led to decreased testosterone and estradiol synthesis and androgen receptor expression in the cerebral cortex of pubertal mice (Liu et al. 2011), and this may underlie the sex-dependent action of the pyrethroid on neurodevelopment. The mechanism underlying the sex-specific susceptible periods are undetermined, and further studies investigating sex hormones *in vivo* may bring further insight to the mechanism underlying 3-PBA neurotoxicity.

Among ADHD symptoms, pyrethroid exposure was not found to be specifically related to hyperactivity-impulsivity symptoms or inattentive symptoms in children. However, although not significant, prenatal 3-PBA exposure was marginally significantly associated with hyperactivity-impulsivity scores at age 6 (3.7% change, 95% CI: −0.03, 7.5). This is in line with a previous cross-sectional study in 8–15-year-old participants, which reported that hyperactive-impulsive symptoms increased by 50% for every 10-fold increase in 3-PBA concentrations (Wagner-Schuman et al. 2015). This effect size was comparable to our study, which showed a 3.7% increase in hyperactivity-impulsivity symptoms for a 2-fold increase in prenatal 3-PBA exposure. The relation of pyrethroids and hyperactivity-impulsivity have also been reported in previous animal studies, demonstrating that pyrethroid exposure during gestation and early childhood results in hyperactivity and impulsive-like behavior (Eriksson and Fredriksson 1991; Richardson et al. 2015). Another study demonstrated up-regulation of striatal dopamine transporters (DAT) in mice given intraperitoneal doses of pyrethroid permethrin, which has been linked to the pathophysiology underlying ADHD hyperactive symptoms (Gillette and Bloomquist 2003). ADHD is a heterogeneous disorder with various symptom profiles, and clinical distinction of subtype may be important as comorbidities, functional impairment, and underlying pathophysiology may differ among subtypes (Levy et al. 2005; Saad et al. 2017). The association of 3-PBA with a specific subtype of ADHD may also be a factor contributing to the inconsistent results on 3-PBA and ADHD association. Further studies exploring the relationship of 3-PBA with ADHD subtypes are warranted.

The differential effects of 3-PBA according to timing of exposure can be partially explained by epigenetic processes such as DNA methylation. First, *in utero*, ages 2 to 6 were prone to 3-PBA neurotoxicity. These findings are in line with previous studies investigating age-related methylation changes. Acevedo et al reported that DNA methylation changes during the first five years of life and weakens around the third year (Acevedo et al. 2015). From these findings, we can speculate that the methylome would be more prone to environmentally toxic materials in the early stages of life, when changes are more dynamic. Second, the effect of prenatal 3-PBA exposure affected ADHD symptoms at six years of age, suggesting long-term effects. Transient environmental exposure during critical windows are known to affect epigenetic marks, which are evident at birth (Hogg et al. 2012) and may persist until later life (Reynolds et al. 2013; Waterland and Michels 2007). Third, the adverse effects of prenatal 3-PBA exposure were attenuated at age 8. Prenatal 3-PBA may have affected DNA methylation regions related to ADHD symptom manifestation, but this affect may have been compensated by positive factors that also affect DNA methylation (Choi et al. 2020).

The mean ARS scores in this study (6.3 at age 6, 6.9 at age 8) were similar to those of the Korean study of 29,914 community-based children aged 5–7 years (9.5 in boys and 6.8 in girls; Choi et al. 2019). The proportion of those surpassing the ARS cut-off was low (2.7% at age 6, 4.6% at age 8), indicating a generally healthy population. However, ADHD traits are dimensional and are continuously distributed in the general population (Riglin et al. 2021; Thapar and Cooper 2016). Even subclinical ADHD symptoms have been found to be associated with higher rates of comorbid psychopathologies and cognitive, interpersonal, and school functioning deficits (Biederman et al. 2018). Furthermore, ADHD traits share common environmental and genetic risks as clinical disorders (Sullivan et al. 2012). Further studies are warranted to determine if the effect of 3-PBA on ADHD traits can be expandable to a clinical population.

ADHD symptoms were relatively stable at age 6 and 8 within our study population. Studies have suggested that during childhood and adolescence, the majority showed onset of some symptoms of impairment before age 7, but a significant proportion of cases report the onset between 7 and 16 years of age (Asherson and Agnew-Blais 2019). Data from an adult population survey showed that only 50% of individuals with clinical features recalled an onset before age 7; by contrast, 95% recalled onset before age 12 and 99% before age 16 (Kessler et al. 2005). 3-PBA exposure at age 4 and age 6 affected ADHD symptoms at age 8 only, and this could have affected new onset symptom manifestation at age 8, or contributed to symptom stability from early ages. Further studies investigating ADHD trajectories are warranted to determine this speculation.

There are some limitations to this study. This study was conducted in a community-based cohort and did not target a clinical population with formal ADHD diagnoses. Therefore, further studies using a diagnostic interview that can confirm ADHD diagnosis is needed. The associations at different exposure periods night have been influenced by difference in sample size (statistical power) across age groups. The sample size at age 2 years was less than half of the sample sizes at other ages, so the results of this exposure period are less reliable. There was a large confidence interval at age 2 in girls. Further, concentration of 3-PBA exposure was measured at a single time point using spot urine; considering the fast clearance (7 h) and high intra-individual variability of 3-PBA exposure, calculation of mean 3-PBA concentrations across multiple time points or average 24-hour urine 3-PBA concentrations could more accurately reflect 3-PBA exposure level (Morgan et al. 2016; Ratelle et al. 2015). The 3-PBA concentrations in urine may not represent entirely the exposure to parent pyrethroid compounds, but also reflect exposures in the environment (Trunnelle et al. 2014); this may have caused the high variation and low ICC of 3-PBA between different age groups. We only measured 3-PBA, and other pyrethroid metabolites levels, like *cis*-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-DBCA), *cis*-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-DCCA), and *trans*-DCCA, were not available. Although we accounted for various covariates, factors like diet and other endocrine-disrupting chemicals could have confounded the results. Lastly, we studied a Korean cohort living in urban regions, thus limiting the generalizability of our findings to other ethnicities or populations in rural areas.

Despite these limitations, the major strengths of this study include the longitudinal design and repetitive assessment of 3-PBA exposure and ADHD symptoms. We were able to identify multiple susceptible periods in early life, and also examine the change in neurotoxicity effects across time. Where some studies have used agricultural pesticide applications as a proxy for pesticide exposure, we used a biomarker, which captures

all exposure routes.

5. Conclusions

Exposure to 3-PBA during pregnancy and at age 2 was associated with increased ADHD symptoms at age 6, while exposure at ages 4 and 6 was associated with ADHD symptoms at age 8. These findings indicate there are multiple susceptible periods in early life that can contribute to the manifestation of ADHD symptoms during the school-age period. Regulatory efforts to decrease pyrethroid exposure in this age range could result in large benefits to public mental health. Further replication studies are necessary to confirm these results.

CRediT authorship contribution statement

Kyung-Shin Lee: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Youn-Hee Lim:** Conceptualization, Methodology. **Young Ah Lee:** Conceptualization, Data curation. **Choong Ho Shin:** Conceptualization, Data curation. **Bung-Nyun Kim:** Supervision, Writing – review & editing. **Yun-Chul Hong:** Funding acquisition, Writing – review & editing, Supervision. **Johanna Inhyang Kim:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107124.

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