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Association of prenatal exposure to PAHs with anti-Müllerian hormone (AMH) levels and birth outcomes of newborns

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HIGHLIGHTS

- Higher maternal PAH exposure is associated with lower cord serum E2 and T levels.
- Maternal PAH exposure is positively correlated with birth weight and negatively correlated with head circumference.
- Maternal PAH exposure may adversely affect the AGD of newborns by affecting AMH.

GRAPHICAL ABSTRACT

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ABSTRACT

Background: Polycyclic aromatic hydrocarbons (PAHs) are chemicals that cause serious concerns because of their carcinogenicity and endocrine disrupting ability.

Objective: In the current study, we studied how urinary PAH metabolites are related with the dose-effects of hormone levels and birth outcomes.

Method: 163 pregnant women without health problems and 163 newborns were enrolled in hospitals in Guiyu (e-waste-exposed area) and Haojiang (reference area) from May 2016 to May 2017. Urine samples were collected to measure hydroxylated PAH (OH-PAH) metabolite levels. Umbilical cord blood was used for measurement of hormone levels. Anthropometric parameters of newborns, such as anogenital distance (AGD), were also measured.

Results: Eight of ten urinary PAH metabolites in the exposed group were significantly higher than in the reference group. Levels of umbilical cord serum estradiol (E2) and testosterone (T) in the exposed group were significantly lower than those in the reference group. Birth weight was positively correlated with 2-OHFlu (2-hydroxyfluorene). Head circumference was negatively correlated with 9-OHFlu, 3-OHPhe (3-hydroxyphenanthrene), 9-OHPhe, and ΣOHFlu (sum of 2-OHFlu and 9-OHFlu). Serum E2 and T levels were negatively correlated with most OH-PAHs. In addition, we found that serum anti-Müllerian hormone (AMH) level was positively correlated with AGD, and serum E2 level was negatively correlated with neonatal head circumference.

Conclusions: PAH exposure in pregnant women may adversely affect the birth outcomes of newborns, especially AGD; and AMH may be involved in the process. Establishing a baseline for the relationship between PAH exposure and health is important to protect the health of mothers and children living in electronic waste (e-waste) recycling areas.

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Abbreviations: PAHs, polycyclic aromatic hydrocarbons; OH-PAH, hydroxylated PAH; AMH, anti-Müllerian hormone; T, testosterone; E2, estradiol; AGD, anogenital distance.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are produced by incomplete combustion of organic materials (Abdel-Shafy and Mansour, 2016). The high volatility of PAHs makes for easy accumulation in soil, plants, particulate matter, sediment and water (Gao et al., 2018; Hoa et al., 2019; Meng et al., 2019; Sushkova et al., 2019). Humans are exposed to PAHs through inhalation, skin contact and oral intake (Beyer et al., 2010; Dong et al., 2012; Kim et al., 2013; Ma and Harrad, 2015; Ruby et al., 2016; Veltman et al., 2011; Wang et al., 2012; Zhu et al., 2019) and become metabolized to estrogenic hydroxyl PAHs (OH-PAHs) (Arcaro et al., 1999; Fertuck et al., 2001; Liu et al., 2017; Machala et al., 2001; Ohta et al., 2012; Tran et al., 1996; Van de Wiele et al., 2005). Short-term exposure to PAHs impairs lung function and brings a risk of thrombosis to patients with coronary heart disease (Kim et al., 2013; Xu et al., 2013). Long-term exposure of PAHs impairs reproduction and immune function by damaging cells through gene mutation (Kuo et al., 2003). OH-PAHs in environmental samples lead to estrogenic activity (Hayakawa et al., 2008), with metabolites of PAHs in some cases being anti-estrogenic in MCF-7 human breast carcinoma cells (Arcaro et al., 1999). In addition, fetal development, involving hormone balance, placental physiology, neonate head circumference, birth length and weight, can be affected by maternal exposure to PAHs (Bolden et al., 2017; Guo et al., 2012; Zhang et al., 2016). For example, placental exposure to PAHs can bring about developmental delays and behavioral problems for children (Perera et al., 2009; Perera et al., 2012). PAHs, as an endocrinological disruptor, can also have adverse effects on an intact organism or its progeny and subpopulations (Scinicariello and Buser, 2014).

Anogenital distance (AGD), as a fetal androgen measurement of intrauterine exposure to androgens and antiandrogens, is important for the evaluation of adverse impact of in utero exposure to environmental endocrine disrupting chemicals and the clinical measurement of endocrine-sensitive endpoints in the first year of life (Liu et al., 2014). AGD is a unique tool with which researchers can investigate in utero androgen secretion (Liu et al., 2014). It is able to predict adult testis size by fetal androgen action (Scott et al., 2008). Androgen action within the masculinization programming window also affects reproductive parameters at birth and in adulthood, as shown by their correlation with AGD in human studies (Dean and Sharpe, 2013). AGD in men is reduced if their mothers smoked during pregnancy (Fowler et al., 2011), indicating that androgen exposure might increase in the early programming window (Suzuki et al., 2012). While a previous study showed that mono-2-ethyl-hexyl phthalate concentration is negatively associated with anogenital index [AGI, $AGI = AGD / \text{weight at 18 months (mm/kg)}$], another study involving urine samples from 111 Japanese pregnant women gave the opposite answer, i.e. that maternal smoking status is positively associated with AGI (Suzuki et al., 2012). A former study showed that levels of pyrene (Pyr) and naphthalene (Nap), and anti-Müllerian hormone (AMH) and estradiol (E2), are negatively related to exposure to PAHs, whereas serum follicle-stimulating hormone in umbilical cord blood is positively associated with the levels of PAHs (Yin et al., 2017). AMH is an important glycoprotein hormone related to both gonadal development and abnormal sexual differentiation. In males, AMH is secreted by Sertoli cells of the testis, and causes degeneration of the Müller canal in early embryo development. The concentration of AMH is high before puberty, and then gradually decreases (Josso et al., 2013). The decrease of AMH during puberty is related with the development stage, with the concentration of AMH decreasing to its lowest levels in Tanner II and III, which happens simultaneously with the increase of the testosterone (T) concentration in the testes (Grinspon and Rey, 2010). In the early developmental stages of a female fetus, the lack of AMH allows the Müller canal to develop, leading to the development of female anatomy (La Marca et al., 2009).

So far, few studies examine how PAH exposure correlates with the change in reproductive hormone levels. Due to the lack of proper e-

waste recycling methods, our previous studies have shown that PAH levels in plants and soil in Guiyu, an e-waste recycling area, are significantly higher than those in the reference areas (Alabi et al., 2012). In our previous studies, we observed total PAH levels of 56–567 ng/g in the soil and 181–3034 ng/g in the sediment of Guiyu (Leung et al., 2015). Therefore, we propose that PAH exposure in Guiyu would affect the levels of AMH in neonates.

2. Materials and methods

2.1. Study population and sample collection

A total of 163 pregnant women and 160 newborns were enrolled during May 2016 to May 2017 at maternity wards located in Guiyu and Haojiang, China. Guiyu, a town in southeast China, is one of the largest e-waste destinations and recycling areas in the world, and has a >40-year history of informal e-waste disposal and recycling. E-waste recycling operations are performed predominantly by family-run workshops using informal techniques (Hou et al., 2020; Huo et al., 2019a; Huo et al., 2019b; Zheng et al., 2019). Haojiang (reference area) is located 31.6 km to the east of Guiyu and lacks e-waste recycling. Maternal sociodemographic characteristics (age, weight, height, gestational age, education level and family income), living habits (smoking frequency, alcohol consumption), and health and medical history were obtained from questionnaires collected before or around the time of delivery. Neonatal sex, birth length, birth weight, head circumference and Apgar score were obtained from birth records. This study was approved by the Human Ethics Committee of the Shantou University College of Medicine in China.

Umbilical cord blood samples were collected in vacuum blood collection tubes at birth, and were centrifuged (1000g for 10 min) to separate serum samples, which were stored in a -80°C refrigerator until hormone measurement. Urine samples were collected from all pregnant women on the same days, and were stored at -20°C until PAH metabolite and creatinine measurement.

2.2. Measurement of AGD

Neonatal AGD was measured by trained nurses with a Vernier caliper (Type W41101, USA) at birth. Measurement criteria were as follows: for males, AGD was defined as the distance from the center of the anus to the base of the scrotum where the skin changes from rugate to smooth; for females, AGD was defined as the distance from the center of the anus to the base of the posterior fourchette where the skin folds fuse (Liu et al., 2016).

2.3. Measurement of hormone levels in umbilical cord serum

All serum hormone levels were measured by a UniCel DxI 800 Access (Beckman Coulter, USA) in the clinical laboratory of Shantou Central Hospital. Serum E2 concentration was measured with Access Estradiol Kits (Beckman Coulter, USA) based on an indirect competitive enzyme-linked immunosorbent assay (IC-ELISA); serum testosterone concentration was measured with Access Testosterone Kits (Beckman Coulter, USA) based on IC-ELISA; serum AMH concentration was measured with Access AMH Kits (Beckman Coulter, USA) based on an ELISA double sandwich method. All experiments were carried out according to the manufacturer's instructions.

Regression coefficients (R^2) of the standard curves of the three hormones all exceeded 0.99. The precision of the method was determined by calculating the relative standard deviation percentage (%RSD) of the quality control samples for repeated measurement, and the relative standard deviation percentages of the three hormones were all determined to be <10%.

2.4. Measurement of urinary PAH metabolites and creatinine

Ten urinary PAH metabolites [1-hydroxynaphthalene (1-OHNaP), 2-hydroxynaphthalene (2-OHNaP), 2-hydroxyfluorene (2-OHFlu), 9-hydroxyfluorene (9-OHFlu), 1-hydroxyphenanthrene (1-OHPhe), 2-hydroxyphenanthrene (2-OHPhe), 3-hydroxyphenanthrene (3-OHPhe), 4-hydroxyphenanthrene (4-OHPhe), 9-hydroxyphenanthrene (9-OHPhe), and 1-hydroxypyrene (1-OHPyr)] were measured according to previous studies (Campo et al., 2008; Huo et al., 2019b; Zheng et al., 2019). In brief, 5 mL of urine was transferred to a 15 mL glass vial and added to 20 μ L of internal standard, 2 mL of acetate/acetic acid buffer (0.5 M, pH = 5) and 20 μ L of β -glucuronidase/sulphatase. The mixture was shaken and incubated at 37 °C overnight. After hydrolysis, the solution was cooled at room temperature for 30 min and then 2 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was added. Samples were extracted twice with 2 mL aliquots of n-hexane, centrifuging each time for 10 min at 3000 rpm to facilitate phase separation. The organic extracts were combined in a 15 mL glass vial and then evaporated under a gentle stream of nitrogen at room temperature. The residue was reconstituted with 100 μ L BSTFA [N, O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane] and incubated for 45 min at 90 °C. After derivatization, samples were cooled at room temperature and rapidly transferred into a 2 mL vial containing a 200 μ L conical insert, sealed and analyzed by gas chromatography-mass spectrometry (GC/MS, 7890A/5975C, Agilent, USA).

The regression coefficients (R^2) of the standard curves for all OH-PAHs were above 0.99. Quality control was carried out by performing five repeated analyses of the quality control samples. The recovery rates of the quality control samples corresponding to all OH-PAHs were 85–120%. Urinary creatinine level was measured with a Colorimetric Assay Kit (Item No. 500701, Cayman Chemical, USA). Urinary OH-PAH concentrations were corrected by urinary creatinine levels and expressed as $\mu\text{mol/mol}$ creatinine (Cr).

2.5. Statistical analysis

The independent sample *t*-test was utilized to compare means between the two groups, such as age, birth length, head circumference, Apgar score, and AGD. The Mann-Whitney *U* test was utilized to compare medians across groups, such as serum AMH, T, and E2 levels. Spearman's rank correlation analysis was performed to analyze urinary PAH metabolite concentrations and potential related factors, and the correlation of urinary OH-PAHs and serum hormone levels. Linear regression analysis was used to investigate the effect of PAH exposure on birth outcomes. Statistical analyses were conducted with statistical software SPSS version 23.0 (IBM, America). Two-tailed tests considered $P < 0.05$ as significant.

3. Results

3.1. Characteristics of the study population

There were 163 pregnant women in this study, of which 100 were in the exposed group and 63 were in the reference group. No significant differences were found in maternal age, weight, height, BMI, smoking frequency, alcohol consumption, frequency of sickness, and gestational age between the two groups (Table 1). The percentage of women having a college education or above was 21.2% for the reference group and 3.1% in the exposed group. In addition, family income of women in the exposed group was higher than that in the reference group.

Comparison of birth outcomes between the two groups (100 in the exposed group and 63 in the reference group) is shown in Table 2. No significant differences were found in birth weight and Apgar score (5 min) between the two groups. Birth length of newborns in the exposed group was shorter than those in the reference group (49.50 ± 2.08 cm vs. 50.32 ± 1.25 cm, $P = 0.002$). Head circumference of the

newborns in the exposed group was lower than in the reference group (33.15 ± 2.22 cm vs. 34.57 ± 1.49 cm, $P < 0.001$). Compared with the reference group, the Apgar score (1 min) was decreased in the exposed group (9.56 ± 0.92 vs. 10.00 ± 0.00 , $P < 0.001$). AGD of the newborns was significantly longer in the exposed group than in the reference group (10.34 ± 4.18 mm vs. 8.21 ± 3.41 mm, $P = 0.001$).

3.2. PAH metabolite concentrations and potential related factors

Eight of ten OH-PAHs were significantly higher in the exposed group than in the reference group (except 1-OHPhe and 1-OHPyr, Table 2). These compounds constituted the main homologue pattern; and most of them were highly correlated to each other based on Spearman correlation analysis (Table S1). Spearman correlation analysis was performed to investigate the potential influencing factors of PAH exposure (Table 3). The results showed that ΣOHPAH (sum of ten OH-PAHs), ΣOHFlu (sum of 2-OHFlu and 9-OHFlu) and ΣOHPhe (sum of 1-OHPhe, 2-OHPhe, 3-OHPhe, 4-OHPhe and 9-OHPhe) were negatively correlated with education level ($r_s = -0.274$, $r_s = -0.302$, $r_s = -0.187$, respectively, all $P < 0.05$). ΣOHFlu was negatively associated with duration of maternal residence in Guiyu ($r_s = -0.173$, $P < 0.05$).

3.3. Comparison of hormone concentrations between the two groups

As shown in Table 2, the levels of umbilical cord serum E2 and T in the exposed group were significantly lower than those in the reference group (all $P < 0.05$). There was no significant difference in serum AMH level between the two groups.

3.4. Effects of PAH exposure on birth outcomes

Spearman correlation was performed to explore the correlation between PAH and birth outcomes (Table 4). Birth weight was positively correlated with 2-OHFlu ($r_s = 0.182$, $P < 0.05$). Head circumference was negatively correlated with 9-OHFlu, 3-OHPhe, 9-OHPhe, and ΣOHFlu ($r_s = -0.174$, $r_s = -0.176$, $r_s = -0.233$, $r_s = -0.178$, respectively, all $P < 0.05$). Gestational age was positively correlated with 1-OHNaP, 2-OHFlu, 3-OHPhe, and ΣOHNaP (sum of 1-OHNaP and 1-OHNaP) ($r_s = 0.183$, $r_s = 0.175$, $r_s = 0.167$, $r_s = 0.170$, respectively, all $P < 0.05$). No significant correlations were found between OH-PAHs and birth length, birth BMI, AGD, and Apgar score. Further, linear regression analysis showed no significant associations between urinary ΣOHPAH level and all birth outcomes, before or after adjusting for maternal age, maternal weight, height, maternal education, family income, smoking, and drinking (Table S2).

3.5. Association between urinary OH-PAHs, hormones and birth outcomes

Spearman correlation analysis showed that serum E2 level was negatively correlated with 1-OHNaP, 9-OHPhe, and ΣOHNaP (all $P < 0.05$); serum T level was negatively correlated with 1-OHNaP, 2-OHNaP, 2-OHFlu, 9-OHFlu, 2-OHPhe, 3-OHPhe, 9-OHPhe, ΣOHNaP , and ΣOHFlu (all $P < 0.05$). No significant correlations were found between OH-PAHs and serum AMH level (Table 4). Further, linear regression analysis showed that no significant associations were found between urinary ΣOHPAH and three hormones, before or after adjusting for maternal age, maternal weight, height, maternal education, family income, smoking, and drinking (Table S2). Based on Spearman correlation analysis, we found that the serum AMH level was positively correlated with AGD ($r_s = 0.563$, $P < 0.01$, Table S3), and that serum E2 level was negatively correlated with birth BMI and head circumference ($r_s = -0.214$ and $r_s = -0.164$, all $P < 0.05$, Table S3).

Table 1

Descriptive statistics of pregnant women in the exposed and reference groups.

	N	Reference group	N	Exposed group	Statistics	P-value
Maternal age [median (IQR), years]	63	27.00 (24.00, 30.50)	93	28.00 (25.00, 31.00)	$Z = -1.293$	0.196
Maternal weight [median (IQR), kg]	51	49.00 (45.00, 55.00)	87	50.00 (45.00, 25.00)	$Z = -0.374$	0.708
Maternal height [median (IQR), m]	54	1.58 (1.55, 1.60)	95	1.58 (1.55, 1.60)	$Z = -0.382$	0.702
BMI [median (IQR), kg/m ²]	49	20.13 (18.34, 21.56)	86	19.78 (18.33, 21.26)	$Z = -0.723$	0.470
Gestational age (mean $\pm$ SD, weeks)	62	39.34 $\pm$ 1.16	91	39.59 $\pm$ 1.11		0.180
Maternal education level [n (%)]	57		97		$\chi^2 = 22.713$	0.000
Elementary school		6 (10.5%)		28 (28.9%)		
Junior school		28 (49.1%)		59 (60.8%)		
High school		11 (19.3%)		7 (7.2%)		
College or above		12 (21.1%)		3 (3.1%)		
Family income (¥) [n (%)]	48		95		$\chi^2 = 9.941$	0.019
<30,000		14 (29.2%)		34 (35.8%)		
30,000–80,000		16 (33.3%)		47 (49.5%)		
80,000–120,000		13 (27.1%)		9 (9.5%)		
120,000–300,000		5 (10.4%)		5 (5.3%)		
Smoking during pregnancy [n (%)]	61	1 (1.6%)	96	2 (2.1%)	$\chi^2 = 0.039$	0.843
Alcohol consumption [n (%)]	63	0 (0%)	95	2 (2.0%)	$\chi^2 = 1.301$	0.254
Frequency of floor cleaning [n (%)]	50		95		$\chi^2 = 10.572$	0.005
More than twice a week		20 (40.0%)		54 (56.8%)		
Once to twice a week		21 (42.0%)		38 (40.0%)		
Less than once a week		9 (18.0%)		3 (3.2%)		
Sick during pregnancy [n (%)]	57	23 (40.4%)	94	42 (44.7%)	$\chi^2 = 0.271$	0.602
Parity	53		95		$\chi^2 = 24.561$	0.000
1		16 (30.2%)		13 (13.7%)		
2		28 (52.8%)		29 (30.5%)		
>2		9 (17.0%)		53 (55.7%)		
Puerpera is a permanent resident of Guiyu [n (%)]	56	9 (16.1%)	93	55 (59.1%)	$\chi^2 = 24.459$	0.000
Number of years puerpera lived in Guiyu [n (%)]	15	0.0 (0.0, 1.0)	85	22.0 (3.5, 28.5)	$\chi^2 = 40.067$	0.128

4. Discussion

In this study, we found higher levels of eight urinary PAH metabolites in the e-waste-exposed group. Results of hormones showed that cord serum E2 and T levels are lower in the exposed group.

Moreover, serum T and E2 levels correlated with more than one urinary PAH metabolite, and serum AMH concentration is positively correlated with AGD. These results suggest that PAHs from irregular e-waste disassembly may adversely affect maternal hormones levels and neonatal outcomes.

Table 2

Urinary PAH metabolite concentrations, birth outcomes and hormones in e-waste-exposed and reference groups.

	N	Reference group	N	Exposed group	P-value
OH-PAH ($\mu\text{mol/mol Cr}$)/median (25th, 75th)					
1-OHNap	60	1.359 (0.612, 3.001)	86	3.263 (1.312, 12.181)	0.000 ^a
2-OHNap	60	1.822 (0.770, 2.890)	86	4.177 (1.312, 10.139)	0.000 ^a
2-OHFlu	60	2.812 (1.438, 5.083)	86	6.973 (3.421, 15.147)	0.000 ^a
9-OHFlu	60	11.097 (7.314, 20.120)	86	43.790 (17.778, 84.337)	0.000 ^a
1-OHPhe	60	22.298 (10.220, 42.526)	86	23.762 (9.807, 53.910)	0.588 ^a
2-OHPhe	60	1.640 (0.707, 2.601)	86	4.880 (1.783, 9.331)	0.000 ^a
3-OHPhe	60	3.171 (1.348, 4.834)	86	10.615 (3.996, 19.953)	0.000 ^a
4-OHPhe	60	6.592 (2.450, 11.838)	86	9.643 (4.645, 19.922)	0.005 ^a
9-OHPhe	60	1.490 (1.055, 3.292)	86	5.887 (2.504, 11.867)	0.000 ^a
1-OHPyr	60	18.966 (7.563, 33.561)	86	24.682 (13.477, 68.513)	0.054 ^a
ΣOHNap^b	60	3.679 (1.544, 5.960)	86	7.645 (3.435, 23.988)	0.000 ^a
ΣOHFlu^c	60	14.421 (8.653, 24.334)	86	52.778 (22.111, 107.896)	0.000 ^a
ΣOHPhe^d	60	36.402 (15.180, 69.112)	86	57.542 (27.898, 128.148)	0.003 ^a
ΣOHPAH^e	60	81.042 (42.382, 130.022)	86	160.195 (85.726, 396.929)	0.000 ^a
Outcomes/mean $\pm$ SD					
Birth weight (g)	62	3121.77 $\pm$ 414.16	98	3187.24 $\pm$ 360.51	0.293 ^f
Birth length (cm)	62	50.32 $\pm$ 1.25	98	49.50 $\pm$ 2.08	0.002 ^f
Head circumference (cm)	62	34.57 $\pm$ 1.49	98	33.15 $\pm$ 2.22	0.000 ^f
Apgar score (1 min)	61	10.00 $\pm$ 0.00	98	9.56 $\pm$ 0.92	0.000 ^f
Apgar score (5 min)	61	10.00 $\pm$ 0.00	98	9.97 $\pm$ 0.30	0.432 ^f
Anogenital distance (mm)	62	8.21 $\pm$ 3.41	100	10.34 $\pm$ 4.18	0.001 ^f
Hormones/median (25th, 75th)					
AMH (ng/mL)	57	0.85 (0.08, 21.40)	95	0.31 (0.07, 15.41)	0.156 ^a
E2 (pg/mL)	51	8440.00 (6253.00, 11,451.00)	85	5808.00 (3649.50, 8677.50)	0.000 ^a
T (ng/mL)	62	1.30 (1.01, 1.54)	97	1.08 (0.83, 1.34)	0.025 ^a

AMH: anti-Müllerian hormone; E2: estradiol; T: testosterone.

^a Analysis by Mann-Whitney *U* test for differences between the exposed and reference groups.^b Combined sum of the two examined naphthalene metabolites.^c Combined sum of the fluorene metabolites.^d Combined sum of the five examined phenanthrene metabolites.^e Combined sum of the ten examined PAH metabolites.^f Analysis by independent-sample *t*-test for differences between the exposed and reference groups.

Table 3

Spearman correlation analysis between urinary metabolites of PAHs and related factors.

Related factors	1-OHPyr	ΣOHPAH ^a	ΣOHNap ^b	ΣOHFlu ^c	ΣOHPh ^d
	r _s	r _s	r _s	r _s	r _s
Education level	−0.153	−0.274**	−0.134	−0.302**	−0.187*
Income	0.064	−0.112	−0.135	−0.137	−0.120
Smoking	0.032	−0.018	−0.012	−0.057	−0.038
Alcohol consumption	0.015	−0.022	−0.010	−0.085	−0.015
Duration of maternal residence in Guiyu	−0.092	−0.155	−0.161	−0.173*	−0.124
Number of maternal visits to e-waste recycling workshops	−0.072	−0.095	−0.028	−0.078	−0.131

* $P < 0.05$.** $P < 0.01$.^a Combined sum of the ten examined PAH metabolites.^b Combined sum of the two examined naphthalene metabolites.^c Combined sum of the two examined fluorene metabolites.^d Combined sum of the five examined phenanthrene metabolites.

PAHs as a class of persistent organic pollutants, their toxicity to the immune system, nervous system, cardiovascular system has been widely studied (Huo et al., 2019a; Santiago-Colón et al., 2019; Zheng et al., 2019). We found that eight of ten OH-PAH concentrations in the exposed group are significantly higher than in the reference group, which is consistent with our previous findings (Dai et al., 2019; Huo et al., 2019a, 2019b) that irregular e-waste recycling has resulted in excessive release of PAHs into the air and soil. Therefore, it is an urgent problem to innovate the traditional e-waste recycling technology. Moreover, we found that PAH exposure is negatively correlated with education level and duration of maternal residence in Guiyu. Therefore, strengthening the health awareness of the parents in the e-waste recycling area and providing a clean and hygienic environment for the newborns are policies that need to be promoted.

PAHs could activate aryl hydrocarbon-responsive genes to bring widespread antiestrogenic effects, and their hydroxylated metabolites could lead to estrogenic activity in vitro. In addition, PAHs affect the dynamic balance of organisms by simulating endogenous hormones and therefore are considered endocrine disruptors. The possible endocrine disrupting pathways of PAHs are as follows: binding to ER influences E2 metabolism and ultimately leads to metabolic degradation of E2. Mechanisms such as the association or crosstalk between AhR and various cellular signaling pathways (such as mitogen-activated protein

kinases, nuclear factor-kappa B and cell cycle/apoptosis-related pathways) have been extensively studied to reveal how PAHs stimulate AhR-mediated cell signaling (Hankinson, 1995; Henklova et al., 2008; Puga et al., 2009; Puga et al., 2002; Schmidt and Bradfield, 1996; Tian, 2009). In addition, the finding that testosterone level is negatively associated with PAH exposure level is similar to the findings (Yin et al., 2017), who investigated the effect of maternal PAH exposure on neonates and found a negative relationship between PAH exposure and AMH and E2. However, no significant relationship between PAH exposure and T was shown in their study, whereas we found that the levels of E2 and T in the exposure group are significantly lower, but there is no difference in AMH. These results suggest that E2 and T are more likely to be disturbed by PAHs through the aforementioned pathways.

Many studies on the effects of AMH on gonadal development have been published. The differentiation of reproductive structures is greatly affected by the dimorphic AMH expression in ovary and testis (Guibourdenche et al., 2003; Josso et al., 1993; Lee and Donahoe, 1993). For males, the secretion of AMH declines with Sertoli cell maturation and T production. In the male fetus, AMH mainly exerts Müllerian duct regression and is involved in the development and function of the testicles. For females, the absence of AMH in the embryo before birth allows the Müllerian canal to distinguish and form the uterus, fallopian tube and upper vagina (Josso, 2001). After that, it has been suggested that the AMH level is correlated with the ovarian cycle and follicular development for the whole reproductive life span, and is important for monitoring physiological or pathological follicular exhaustion (Gustafson et al., 1992; Long et al., 2000; Seifer et al., 2002). Previous studies show that E2 and AMH are negatively affected by PAH exposure while follicle-stimulating hormone in umbilical cord serum is positively affected. We found that PAH exposure is negatively correlated with cord serum levels of E2 and T, but not with level of AMH. This suggests that PAHs may more directly affect T and E2 levels, but potentially affect AMH level, leading to changes in neonatal colonization and endocrine disorders.

AGD is a marker of genital development. Thus, the AGD of newborns is indicative of the androgen levels during the fetal period. The higher the androgen exposure in fetal life, the longer the AGD is in newborns (Mira-Escolano et al., 2014). In males, the AGD could reflect androgen exposure and possible endocrine disruption during the fetal period (Fowler et al., 2011). In females, one symptom of congenital adrenal hyperplasia (CAH) is an increased AGD (Dean and Sharpe, 2013). We found that the AGD of newborns is significantly longer in the exposed

Table 4

Spearman analysis of the association between urinary PAH metabolites and birth outcomes and hormones.

	Birth weight	Birth length	Birth BMI	Head circumference	Apgar score (1 min)	Apgar score (5 min)	Gestational age	AGD	AMH	E2	T
1-OHNap	0.142	0.064	0.105	−0.061	−0.060	0.123	0.183*	0.079	−0.046	−0.215*	−0.253**
2-OHNap	0.135	0.004	0.112	−0.014	−0.022	0.109	0.160	0.028	−0.076	−0.133	−0.232**
2-OHFlu	0.182*	−0.010	0.156	−0.090	−0.055	0.135	0.175*	0.114	−0.035	−0.131	−0.194*
9-OHFlu	0.082	−0.048	0.087	−0.174*	−0.079	0.102	0.135	0.073	−0.112	−0.114	−0.216**
1-OHPhe	0.074	0.050	0.026	0.067	0.100	0.082	0.139	−0.040	−0.050	−0.014	−0.073
2-OHPhe	0.149	−0.030	0.139	−0.139	0.054	0.106	0.126	0.130	−0.055	−0.156	−0.215**
3-OHPhe	0.108	−0.044	0.096	−0.176*	−0.080	0.102	0.167*	0.082	−0.110	−0.155	−0.198*
4-OHPhe	0.138	0.076	0.092	−0.007	−0.106	0.074	0.148	0.051	−0.085	−0.036	−0.162
9-OHPhe	0.100	−0.040	0.076	−0.233**	−0.060	0.127	0.132	0.038	−0.121	−0.168*	−0.170*
1-OHPyr	0.124	0.009	0.123	−0.053	−0.029	0.113	0.162	−0.049	−0.008	−0.064	−0.052
ΣOHNap ^a	0.152	0.035	0.123	−0.038	−0.038	0.127	0.170*	0.057	−0.062	−0.192*	−0.266**
ΣOHFlu ^b	0.100	−0.038	0.100	−0.178*	−0.059	0.106	0.160	0.101	−0.096	−0.112	−0.212*
ΣOHPh ^c	0.099	0.014	0.064	−0.056	−0.114	0.096	0.133	0.029	−0.057	−0.071	−0.137
ΣOHPAH ^d	0.128	−0.005	0.114	−0.126	−0.002	0.117	0.160	0.059	−0.044	−0.111	−0.151

AGD: anogenital distance; AMH: anti Müllerian hormone; E2: estradiol; T: testosterone.

^a Combined sum of the two examined naphthalene metabolites.^b Combined sum of the fluorene metabolites.^c Combined sum of the five examined phenanthrene metabolites.^d Combined sum of the ten examined PAH metabolites.* $P < 0.05$.** $P < 0.01$.

group than in the reference group, and serum AMH level is positively correlated with AGD. Although our results show no significant correlation between PAH exposure and AMH levels or AGD, we speculate that small changes in AMH levels caused by PAH exposure may have adverse cascading effects on AGD. In addition, we found that the neonatal weight and head circumference are negatively correlated with PAH exposure, and the levels of E2 and T are negatively correlated with PAH exposure, so we believe that PAH exposure may have adverse effects on neonatal birth outcomes by affecting hormone levels.

Our study has several limitations. First, the relative sample size of maternal and infant pairings cannot be determined by statistical correlation. Second, because of the combined exposure in an e-waste recycling area, the effects of other pollutants on hormones were neglected in our study. Third, Measurement of PAH metabolites in urine samples collected from a single time point does not reflect the overall picture of individual PAH exposure (Gao et al., 2018). Finally, we tested the hormone levels of newborns at birth, but further investigation needs to be performed on how maternal exposure to PAHs affects newborns in the long term or the exact health problems indicated by the longer AGD in the exposed group. A longitudinal cohort study is required to follow up the newborns to answer these questions.

5. Conclusion

Our study indicates that puerperae living in e-waste recycling areas suffer a high risk for PAH exposure. Maternal exposure to PAHs is negatively associated with E2 and T levels in cord serum, which further affects birth outcomes of newborns, including longer AGD. Follow-up of the participants is required to investigate the effects of maternal exposure to PAHs on postnatal development and health in the long term.

CRedit authorship contribution statement

Xiaofan Huang: Investigation, Writing - original draft. **Xijin Xu:** Conceptualization, Project administration. **Yifeng Dai:** Methodology, Data curation, Formal analysis. **Zhiheng Cheng:** Formal analysis, Writing - review & editing. **Xiangbin Zheng:** Investigation. **Xia Huo:** Funding acquisition, Supervision.

Declaration of competing interest

The authors declare they have no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.138009>.

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