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Ambient air pollutants in the first trimester of pregnancy and the risk of birth defects: an observational study

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4 1 **Ambient air pollutants in the first trimester of pregnancy and the risk of birth**
5
6 2 **defects: an observational study**

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4 42 **Abstract**

5 43 **Objectives** As current studies on the association of air pollutants and birth defects
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8 44 were not fully elucidated, this study aimed to examine the effects of maternal air
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10 45 pollutants exposure during the 1st trimester of pregnancy on the risk of birth defects.

11 46 **Design** An observational study.

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13 47 **Participants** We obtained 70,854 singletons with gestational age <20 weeks who
14
15 48 delivered at a large maternal and child health care center in Wuhan, China.

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17 49 **Outcome measures** Birth defects data and daily average concentrates of ambient
18
19 50 particulate matter ≤ 10 μm diameter (PM₁₀), particulate matter ≤ 2.5 μm diameter
20
21 51 (PM_{2.5}), sulfur dioxide (SO₂), and nitrogen dioxide (NO₂) were obtained. Logistic
22
23 52 regression analysis was applied to examine the effects of maternal air pollutants
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25 53 exposure during 1st trimester on the risks of total birth defects, congenital heart
26
27 54 defects (CHDs), limb defects, and orofacial clefts.

28
29 55 **Results** There were a total of 1,352 birth defects cases included in this study, with a
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31 56 prevalence of 19.08%. Maternal exposed to high concentrations of PM₁₀, PM_{2.5}, NO₂,
32
33 57 and SO₂ in the 1st trimester were significantly associated with elevated risks of birth
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35 58 defects (ORs ranged from 1.10 to 1.19). Additionally, for male fetuses, maternal
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37 59 exposed to high PM_{2.5} concentration was associated with an elevated risk of CHDs
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39 60 (OR=1.29, 95% CI: 1.08, 1.54). In the cold season, the risk of birth defects was
40
41 61 significantly increased among women exposed to PM_{2.5} (OR=1.31, 95% CI: 1.15,
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43 62 1.50) and NO₂ (OR=1.14, 95% CI: 1.02, 1.26).

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45 63 **Conclusions** This study showed unfavorable effects of air pollutants exposure on the
46
47 64 risk of birth defects. Especially, maternal PM_{2.5} exposure could have an elevated risk
48
49 65 of CHDs among male fetuses, and stronger effects of PM_{2.5} and NO₂ exposure on
50
51 66 birth defects were observed in the cold season.

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53 67 **Keywords:** air pollutants; birth defects; congenital heart defects; limb defects;
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55 68 orofacial clefts; pregnancy

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4 71 **Strengths and limitations of this study**

- 5 72 ➤ This study provided the first evidence on the positive associations between
6
7 73 maternal exposed to PM_{2.5} during the 1st trimester and the risk of CHDs among
8
9 74 male fetuses but not the female fetuses.
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11 75 ➤ We also novelly found that stronger effects of air pollutants exposure during the
12
13 76 1st trimester on the risk of birth defects were observed in cold season.
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15 77 ➤ The selected participants are located in a large tertiary maternal care center in
16
17 78 Wuhan and the representative of this study was undermined.
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19 79 ➤ Maternal air pollutants exposure indoor or at other living residents including
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21 80 work, dining, and shopping were not included and other covariates including
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23 81 health behaviors and genetic factors were failed to obtain.
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84 INTRODUCTION

85 Birth defects are structural or functional abnormalities occurred during embryonic
86 development, most of them forming in the 1st trimester of pregnancy.¹ Birth defects
87 are the leading cause of fetal death, and associated with the elevated risk of childhood
88 mortality and reduced long-term survival.^{2,3} According to the Institute for Health
89 Metrics and Evaluation, the prevalence of congenital birth defects increased from
90 6.08% in 2005 to 6.29% in 2019.⁴ Although the upward trends were reported in
91 severe congenital heart defects, single ventricle, atrioventricular septal defects, and
92 tetralogy of Fallot in Europe during 1980-2012, the prevalence of birth defects are
93 much lower (1.0-4.1‰).⁵ In China, the overall prevalence of birth defects was
94 increased from 12.83‰ in 1986 to 15.70‰ in 2014.⁶ There is a necessary to explore
95 the potential hazard factors contributed to the high prevalence of birth defects in
96 China based on current knowledge.

97 Due to rapid urbanization, China has experienced severe air pollution in recent
98 years. Report on China's implementation of the Millennium Development Goals
99 (2000-2015) documented that particulate matter ≤ 10 μm diameter (PM₁₀), sulfur
100 dioxide (SO₂), and nitrogen dioxide (NO₂) were major air pollutants in urban areas.⁷
101 Moreover, ambient particulate matter ≤ 2.5 μm diameter (PM_{2.5}) produced by coal
102 combustion, industry sources, and vehicular emissions, is also one of the main air
103 pollutants of industrialization.⁸ The expanding coverage of ambient air pollutants
104 surveillance has contributed to a flow of studies on the adverse pregnancy outcome,
105 including small for gestational age and low birth weight, preterm birth, spontaneous
106 abortion, and stillbirth in recent years.⁹⁻¹¹ Moreover, there is a growing interest on the
107 associations between ambient air pollutants and birth defects, but the results of
108 previous studies are controversial. Most studies reported that ambient air pollutants
109 were associated with increased risks of birth defects.¹²⁻¹⁴ However, Parkes et al. and
110 Dolk et al. pointed out that high concentrates of PM₁₀ and NO₂ exposure was not
111 related to birth defects.^{15,16} A recent meta-analysis conducted by Ma et al. even
112 reported a protective effect of SO₂ on atrial septal defects.¹⁷ These inconsistent results
113 indicate that the effects of ambient air pollutants on the risk of birth defects could be

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4 114 varied by cultures, ethnics, or geographical distribution. More evidence is needed to
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6 115 clarify the risk of birth defects derived by air pollutants during the rapid
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8 116 social-economical development worldwide.

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10 117 As current studies on the associations of air pollutants and birth defects were not
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12 118 fully elucidated, this study aimed to examine the effects of air pollutants on birth
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14 119 defects. Furthermore, the 1st trimester of pregnancy is vital for fetal development
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16 120 because fetal major organs and systems are formed at this stage and the fetus is most
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18 121 susceptible to environmental hazards. As a result, we mainly focused on the
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20 122 associations between maternal exposure to air pollutants including PM₁₀, PM_{2.5}, SO₂,
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22 123 and NO₂ during the 1st trimester and the risk of birth defects in Wuhan, China.

23 124 **METHODS**

24 125 **Study site and population**

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26
27 126 This observational study was conducted in Wuhan city. Wuhan is the capital city of
28
29 127 Hubei Province and a megacity in Central China. Its geographical location is 29° 58' -
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31 128 31° 22' N and 113° 41' - 115° 05' E. The permanent resident population of Wuhan
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33 129 was over 10 million. Wuhan has four distinct seasons of hot summer and cold winter,
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35 130 with an annual average temperature of 15.8 °C - 17.5 °C.¹⁸

36
37 131 There were a total of 130,186 perinatal women with detailed home addresses
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39 132 who delivered at Maternal and Child Health Hospital of Hubei Province, and 98,877
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41 133 of them lived in Wuhan City during the 1st trimester of pregnancy. Then we obtained
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43 134 74,336 participants with distances less than 10 km from home to the nearest air
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45 135 surveillance station. After removing 3,333 mothers of multiple pregnancies and 149
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47 136 with gestational age <20 weeks, 70,854 participants were finally included in this
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49 137 study.

50 138 **Birth defects**

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52 139 Birth defect cases with gestational age ≥ 20 weeks and 0-7 days after birth including
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54 140 elective termination of pregnancy. According to the Implementation of National
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56 141 Hospital Birth Defects Surveillance of China, 23 types of common birth defects were
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58 142 categorized according to the 10th Revision of the International Classification of
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60 143 Diseases (ICD-10, Q00–Q99). The top three birth defects including congenital heart

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4 144 defects (CHDs) (Q20–Q28), limb defects (Q69–Q72), and orofacial clefts (Q35–Q37)
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6 145 were further examined by an obstetrician, pediatrician, or pediatric cardiologist.

7 146 **Exposure assessment**

9 147 Data of the daily concentrations of air pollutants (PM₁₀, PM_{2.5}, NO₂, and SO₂) and the
11 148 geographical locations of the air surveillance stations were obtained from China's
13 149 National Urban Air Quality Real-time Publishing Platform
15 150 (<http://106.37.208.233:20035>). Moreover, the geographical location data was
17 151 converted from the detailed home address of participants by Baidu Map. The
19 152 individual air pollutants were obtained according to the nearest surveillance station
21 153 within 10 km from home, which was confirmed by other studies.^{15,19} The median
23 154 distance from home to the nearest station was 3.50 km in this study. Participants' air
25 155 pollutants exposure during the 1st trimester of pregnancy was estimated by mean
27 156 levels of daily concentrations.

29 157 **Statistical analysis**

31 158 Logistic regression analysis was applied to evaluate the effects of air pollutants
33 159 exposure in the 1st trimester on the risk of total birth defects, CHDs, limb defects, and
35 160 orofacial clefts. Maternal exposures to daily average air pollutants concentration
37 161 during the 1st trimester were divided by interquartile range. These models were
39 162 adjusted for covariates including the year of conception (2013-2018), maternal age
41 163 (<25, 25-29, 30-34, and >34 years), and gravidity (1, 2-3, >3). Stratified analyses by
43 164 neonatal sex (male and female fetus) and season of conception (March to August and
45 165 September to February) were applied to further explore the associations between air
47 166 pollutants exposure in the 1st trimester of pregnancy and the risk of birth defects. The
49 167 adjusted odds ratio (OR) and 95% confidence interval (CI) were provided in each
51 168 model. Statistical analyses were performed by SAS 9.4 (SAS Institute, Inc., Cary,
53 169 NC).

54 170 **RESULTS**

56 171 There were a total of 1,352 birth defect cases among 70,854 singletons, with a
58 172 prevalence of 19.08‰ (Table 1). The prevalence of birth defects increased from
60 173 21.16‰ in 2013 to 24.08‰ in 2018. It ranks first place in subpopulations who

174 conceived in winter (26.98‰), aged <25 years (28.10‰), or with gravidity >3 times
 175 (23.93‰). In addition, the male fetuses had a higher prevalence of birth defects than
 176 female fetuses (20.57‰ vs 16.80‰).

177

178 **Table 1 Prevalence of birth defects among the subgroups of enrolled participants**
 179 **in Wuhan, China**

Variables	Birth cases/Total	defect	Prevalence (‰)
Year of conception			
2013	272/12,856		21.16
2014	203/11,236		18.07
2015	174/11,274		15.43
2016	227/12,261		18.51
2017	226/12,844		17.60
2018	250/10,383		24.08
Season of conception			
Spring	321/18,312		17.53
Summer	295/18,853		15.65
Autumn	287/17,045		16.84
Winter	449/16,644		26.98
Age (years)			
<25	122/4,341		28.10
25–29	632/34,412		18.37
30–34	401/23,783		16.86
>34	197/8,318		23.68
Gravidity			
1	604/35,590		16.97
2-3	372/19,552		19.03
>3	376/15,712		23.93
Neonatal sex			
Male	771/37,488		20.57
Female	560/33,337		16.80
Total	1,352/70,854		19.08

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181

182 Table 2 shows the distributions of daily average ambient air pollutants
 183 concentrations in the 1st trimester among the 5 groups including non-defects, birth
 184 defects, CHDs, limb defects, and orofacial clefts. The median exposures of PM₁₀,
 185 PM_{2.5}, NO₂, and SO₂ during the 1st trimester were 101.78µg/m³, 61.98µg/m³,
 186 53.64µg/m³, and 13.97µg/m³ respectively among the birth defects groups.

187
 188 **Table 2 Quartile concentrations (µg/m³) of exposure for ambient air pollutants**
 189 **among birth defects groups in 1st trimester of pregnancy**

Air pollutants	Non-defects N=69,502	Birth defects N=1,352	CHDs N=265	Limb defects N=210	Orofacial clefts N=119
PM ₁₀					
Min	36.86	39.08	49.82	40.76	48.85
25th	80.84	84.05	84.05	85.86	83.98
Median	102.42	101.78	99.46	105.80	96.77
75th	123.86	124.39	120.48	127.34	116.36
Max	231.49	225.91	225.91	220.82	191.37
PM _{2.5}					
Min	21.27	21.27	22.77	22.16	22.98
25th	39.37	45.02	44.88	42.63	41.26
Median	59.21	61.98	61.33	63.00	60.98
75th	80.20	80.73	78.81	84.66	77.38
Max	178.20	165.92	154.42	154.79	155.35
NO ₂					
Min	9.71	15.22	29.033	25.78	16.8901
25th	43.40	45.70	45.4945	44.31	44.0934
Median	51.49	53.64	53.6923	53.43	53.8407
75th	59.08	59.98	59.4615	62.84	59.989
Max	93.60	93.37	92.9341	93.37	87.5824
SO ₂					
Min	2.93	3.02	3.93	3.77	3.99
25th	9.07	9.36	9.43	10.55	9.46
Median	14.47	13.97	13.77	16.09	12.55
75th	23.54	23.87	23.36	25.23	21.28
Max	71.74	63.36	63.36	62.99	53.75

190 Abbreviations: CHDs, congenital heart defects; NO₂, nitrogen dioxide; PM₁₀, particulate matter
 191 ≤10 µm diameter; PM_{2.5}, particulate matter <2.5 µm diameter; SO₂, sulfur dioxide.

192
 193 Table 3 presents the associations of ambient air pollutants exposure in the 1st
 194 trimester of pregnancy and the risk of birth defects. We also provided the risk of birth

defects in the 2nd and 3rd trimesters, as well as the entire pregnancy in the supplementary Tables S1-S3. High concentrate exposure of air pollutants was significantly associated with an increased risk of the total birth defects, yielding adjusted ORs of 1.10, 1.19, 1.16, and 1.15 for PM₁₀, PM_{2.5}, NO₂, and SO₂, respectively. Moreover, similar elevated risk of CHDs was observed for maternal exposure of PM_{2.5} (OR=1.19, 95%CI: 1.05, 1.35), NO₂ (OR=1.12, 95%CI: 1.00, 1.25), and SO₂ (OR=1.21, 95%CI: 1.02, 1.43), but not PM₁₀ (1.07, 95% CI: 0.94, 1.22).

For male fetuses, there were significantly increased risks of birth defects among mothers exposed to PM₁₀, PM_{2.5}, NO₂, and SO₂ by interquartile increased concentrations during the 1st trimester (OR=1.10-1.21). Moreover, a high concentration of PM_{2.5} exposure was significantly associated with an elevated risk of CHDs (OR=1.29, 95% CI: 1.08, 1.54). For female fetuses, elevated risks of birth defects (OR=1.10-1.18) were observed among maternal exposed to heavy concentrated PM₁₀, PM_{2.5}, NO₂, and SO₂.

After stratified by season, the results show that the risk of birth defects was significantly increased among women who conceived in Autumn/Winter and were exposed to PM_{2.5} (OR=1.31, 95% CI: 1.15, 1.50) and NO₂ (OR=1.14, 95% CI: 1.02, 1.26). PM_{2.5} was also positively related to the increased risk of CHDs (OR=1.44, 95%CI: 1.08, 1.93). Elevated risk of orofacial clefts was observed among women who conceived in cold season exposed to heavy NO₂ concentrations (OR=1.51, 95% CI: 1.02, 2.24). However, in the warm season, the risk of limb defects was elevated among women exposed to SO₂ (OR=1.34, 95% CI: 1.01, 1.77), but decreased risk of orofacial clefts was observed among them (OR=0.64, 95% CI: 0.44, 0.94).

Table 3 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 1st trimester and birth defects

Birth defects ^a	PM ₁₀ OR (95%CI)	PM _{2.5} OR (95%CI)	NO ₂ OR (95%CI)	SO ₂ OR (95%CI)
Total				
Birth defects	1.10(1.03,1.17)	1.19(1.13,1.26)	1.16(1.11,1.22)	1.15(1.07,1.25)
CHDs	1.07(0.94,1.22)	1.19(1.05,1.35)	1.12(1.00,1.25)	1.21(1.02,1.43)

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Limb defects	1.07(0.92,1.23)	1.10(0.95,1.27)	1.12(0.99,1.27)	1.16(0.96,1.40)
Orofacial clefts	0.91(0.75,1.10)	1.04(0.86,1.26)	1.10(0.93,1.30)	0.93(0.73,1.20)
Male fetus				
Birth defects	1.10(1.01,1.19)	1.21(1.12,1.30)	1.15(1.08,1.23)	1.16(1.05,1.29)
CHDs	1.18(0.98,1.42)	1.29(1.08,1.54)	1.11(0.95,1.30)	1.19(0.94,1.50)
Limb defects	1.08(0.90,1.30)	1.07(0.90,1.28)	1.12(0.95,1.30)	1.16(0.91,1.46)
Orofacial clefts	0.88(0.69,1.13)	1.10(0.86,1.41)	1.06(0.85,1.31)	0.99(0.72,1.37)
Female fetus				
Birth defects	1.10(1.00,1.20)	1.18(1.08,1.29)	1.18(1.09,1.28)	1.15(1.03,1.29)
CHDs	0.94(0.78,1.13)	1.05(0.88,1.27)	1.11(0.95,1.31)	1.21(0.95,1.55)
Limb defects	1.06(0.83,1.35)	1.13(0.89,1.45)	1.14(0.92,1.42)	1.14(0.83,1.58)
Orofacial clefts	0.97(0.71,1.32)	0.94(0.70,1.27)	1.21(0.92,1.58)	0.84(0.57,1.23)
Autumn/Winter				
Birth defects	0.90(0.80,1.02)	1.31(1.15,1.50)	1.14(1.02,1.26)	1.04(0.90,1.20)
CHDs	0.90(0.70,1.18)	1.44(1.08,1.93)	1.23(0.97,1.57)	1.17(0.86,1.60)
Limb defects	1.05(0.75,1.46)	1.26(0.87,1.83)	1.26(0.94,1.68)	0.96(0.66,1.41)
Orofacial clefts	0.86(0.57,1.29)	1.36(0.86,2.13)	1.51(1.02,2.24)	1.29(0.79,2.09)
Summer/Spring				
Birth defects	1.08(0.98,1.20)	1.02(0.91,1.16)	1.09(1.00,1.19)	1.05(0.94,1.19)
CHDs	1.00(0.80,1.25)	0.89(0.68,1.17)	0.89(0.73,1.09)	1.07(0.82,1.39)
Limb defects	1.09(0.86,1.38)	1.11(0.83,1.48)	1.12(0.92,1.38)	1.34(1.01,1.77)
Orofacial clefts	0.75(0.54,1.04)	0.69(0.46,1.03)	0.89(0.66,1.19)	0.64(0.44,0.94)

222 Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen dioxide; OR, odds ratio; PM₁₀, particulate matter ≤10 µm diameter; PM_{2.5}, particulate matter <2.5 µm diameter; SO₂, sulfur dioxide.

223 ^a adjusted for year of conception, maternal age, and gravidity.

226

227 DISCUSSION

228 In the current study, the prevalence of birth defects was close to what was reported in
229 Hunan Province (19.18%) and higher than that of Jiangsu Province (7.15%).^{20,21}
230 Moreover, this study examined the associations between the risk of birth defects and
231 air pollutants exposure in the 1st trimester, and we further explored the relationships
232 stratified by fetal sex and season of conception.

233 This study showed that maternal exposure to ambient air pollutants including
234 PM₁₀, PM_{2.5}, NO₂, and SO₂ during the 1st trimester could have higher risks of birth
235 defects, which has been well documented previously. A case-control study conducted
236 by Al Noaimi et al. showed a positive association between PM_{2.5} exposure in the 1st
237 trimester of pregnancy and the risk of birth defects (OR=1.05).²² Wang et al. showed

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4 238 that maternal exposure to PM₁₀ and NO₂ in the early pregnancy significantly
5 239 increased the risk of birth defects by 10.3% per 10 µg/m³ and 3.4% per 10 µg/m³,
6
7 240 respectively.²³

9 241 As was observed among the total participants, the risk of birth defects among
10 242 both male fetuses and female fetuses was significantly related to PM₁₀, PM_{2.5}, NO₂,
11 243 and SO₂. The underlying mechanisms between air pollutants exposure and the
12 244 development of birth defects are still unclear. Maternal air pollutants exposures might
13 245 cause changes in epigenetic signatures and permanent modifications in gene
14 246 expression.²⁴ Studies based on mice models showed that maternal exposure to PM_{2.5}
15 247 during pregnancy leads to spatial memory dysfunction, neurodevelopmental
16 248 impairment, and disturbed cerebral cortex development of mice offspring.^{25,26}
17 249 Another study showed that maternal exposure to a high concentrate of PM_{2.5} during
18 250 the 1st trimester of pregnancy could result in the decreased placental expression of
19 251 *BDNF* and *SYNI*, which may undermine fetal neurodevelopment.²⁷ Moreover, SO₂ is
20 252 a systemic toxic agent which can induce chromosomal aberrations, sister chromatid
21 253 exchanges, and micronuclei in mammalian cells.²⁸

22 254 Except for PM₁₀, this study demonstrated adverse associations between PM_{2.5},
23 255 NO₂, and SO₂ exposure and the risk of CHDs. Previous studies in Taiwan and
24 256 Northeast England also reported insignificant associations between PM₁₀ exposure in
25 257 the 1st trimester and the risk of CHDs,^{29,30} whereas others confirmed the increased risk
26 258 of CHDs caused by PM₁₀.^{12,23,31-34} Furthermore, Huang et al. showed that PM_{2.5}
27 259 exposure per each interquartile increase during gestational weeks 3-8 was related to
28 260 an increased risk of CHDs (OR=1.21).³⁵ Additionally, the positive association
29 261 between the risk of CHDs and maternal exposure to NO₂ and SO₂ was documented by
30 262 studies conducted by Baldacci et al. and Vrijheid et al.^{36,37} Moreover, a case-control
31 263 study conducted by Jiang et al. showed that maternal exposure to SO₂ during the 1st
32 264 trimester was significantly associated with increased risk of CHD (OR=1.78-2.04),
33 265 and Hansen et al. also confirmed that a 0.6 ppb increase in SO₂ was associated with an
34 266 increased risk of aortic artery and valve defects (OR=10.76).^{19,38} The heterogeneity in
35 267 these studies might be explained by the variation in the population, the gestational

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4 268 periods, or the measurement air pollutants exposure. The physiopathological
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6 269 mechanism of the associations between air pollutants and CHDs was not fully
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8 270 elucidated. It is hypothesized that air pollutants exposure during the 1st pregnancy
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10 271 might strengthen the genetically and environmental interaction on the risk of CHDs.³⁷
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12 272 Also, Air pollutants might change the molecule of DNA sequence or alter epigenetics
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14 273 in related to CHDs.³⁹

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16 274 Interestingly, only male fetuses showed an elevated risk of CHDs who were
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18 275 exposed to a higher PM_{2.5} concentration. This result indicates that PM_{2.5} might have a
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20 276 stronger effect on the expression of specific CHDs genes located on the Y
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22 277 chromosome. A study of mice models showed that increased pathological damage in
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24 278 hearts of offspring mice was observed among maternal mice exposure to PM_{2.5}, and
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26 279 these effects in the male group were more obvious than in the female group.
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28 280 Meanwhile, this study suggested that heart damage caused by maternal exposure to
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30 281 PM_{2.5} was worse in male mice in contrast to female mice.⁴⁰

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32 282 After stratified by season, maternal exposure of PM_{2.5} and NO₂ were positively
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34 283 associated with the risk of birth defects in the cold season, but this relationship was
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36 284 not observed in the warm season. Zhao et al. reported that the effects of air pollutants
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38 285 on the risk of birth defects were more obvious in the warm season in Hohhot.⁴¹ This
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40 286 disparity could be partly explained by the uneven levels of dwellings' air pollutants.
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42 287 Compared to cities with lower GDP, cities with a higher GDP and a large population
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44 288 might have lower concentrations of indoor particulate matter.⁴² Additionally, this
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46 289 study reported a negative association between SO₂ exposure and the risk of orofacial
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48 290 clefts in the warm season, which was in contrast with the results reported
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50 291 previously.³⁸ This result might be partly explained by the hypothesis that air
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52 292 pollutants could impact the survival of the fetus with birth defects, rather than causing
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54 293 the malformation development.⁴³ Moreover, Wuhan is a well-known city of hot
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56 294 summer. Most of the residential buildings are equipped with air conditioners, which
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58 295 could help to improve indoor air quality in hot weather. Thus, the interpretation of
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60 296 results should be cautious that significant associations between air pollutants exposure
297 and the risk of birth defects in the warm season in Wuhan.

298 **Strengths**

299 This study firstly provided the evidence on the elevated risk of CHDs among
300 mothers with heavy PM_{2.5} exposure during the 1st trimester. We also novelly found
301 the increased risks of CHDs and orofacial clefts among women who conceived in the
302 cold season exposed to high concentrations of air pollutants.

303 **Limitations**

304 This study has several limitations. Firstly, the selected participants are located in
305 a large tertiary maternal care center in Wuhan and the representative of this study was
306 undermined. Secondly, the birth defects data of this study are manually collected and
307 checked, but mistakes and omissions are inevitable. Thirdly, maternal air pollutants
308 exposure indoor or at other living residents including work, dining, and shopping
309 were not included in this study. Fourthly, covariates including health behaviors and
310 genetic factors which might interfere with the relationship between maternal air
311 pollutants exposure and the risk of birth defects were failed to obtain. More research
312 is needed to explore the pathogenic mechanism of air pollutants exposure during
313 pregnancy and the associated birth defects.

314 **CONCLUSIONS**

315 Our study confirmed the unfavorable effects of maternal exposure to air pollutants
316 (PM₁₀, PM_{2.5}, NO₂, and SO₂) on the risk of birth defects during the 1st trimester of
317 pregnancy. We firstly provided the evidence on the positive associations between
318 PM_{2.5} exposure and the risk of CHDs among male fetuses but not the female fetuses.
319 Moreover, stronger effects of of PM_{2.5} and NO₂ exposure on the risk of birth defects
320 were observed in the cold season in Wuhan. As a result, it should be noted for the risk
321 of birth defects due to air pollutants, and reducing individual air pollutants exposure
322 during the 1st trimester might help to birth defect control in the context of the rapid
323 development all over the world.

324 **Abbreviations**

325 CHDs: congenital heart defects; CI: confidence interval; NO₂: nitrogen dioxide; OR:
326 odds ratio; PM₁₀: particulate matter ≤10 μm diameter; PM_{2.5}: particulate matter ≤2.5
327 μm diameter; SO₂: sulfur dioxide.

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4 328 **Ethics approval and consent to participate**

5 329 This study was approved by the Ethics Committee of Maternal and Child Health
6 Hospital of Hubei Province (2021IECLW025). This study was based on the
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8 330 retrospective clinical data without any individual patient identifiers.
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11 332 **Patient consent for publication**

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14 333 Not applicable.

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17 334 **Patient and public involvement**

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19 335 Patients or the public were not involved in the design, or conduct, or reporting, or
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22 336 dissemination plans of our research.

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24 337 **Availability of data and materials**

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26 338 The data used in this study are available from corresponding author on reasonable
27
28 339 request.

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30 340 **Competing interests**

31
32 341 The authors declare that they have no competing interests.

33
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37
38 344 **Authors' contributions**

39
40 345 Yao Cheng and Jieyun Yin contributed equally to this manuscript. Yao Cheng
41
42 346 collected the data, performed data analysis, and draft the manuscript. Jieyun Yin
43
44 347 collected the data, performed data analysis, and edit the manuscript. Lijun Yang
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46 348 designed the study, collected the data, and performed data analysis. Man Xu, Xinfeng
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48 349 Lu, and Wenting Huang collected the data, interpreted the data, and reviewed the
49
50 350 manuscript. Guoqiang Sun: designed the study, collected the data, and supervised the
51
52 351 project. All authors gave final approval of the version to be submitted and agreed to
53
54 352 be accountable for all aspects of the work.

55
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Table S1 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 2nd trimester and birth defects

Birth defects ^a	PM ₁₀	PM _{2.5}	NO ₂	SO ₂
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Total				
Birth defects	0.98(0.91,1.06)	0.95(0.89,1.02)	0.99(0.93,1.05)	0.90(0.82,0.98)
CHDs	0.99(0.80,1.22)	0.88(0.73,1.05)	0.94(0.79,1.11)	0.79(0.62,1.01)
Limb defects	1.02(0.87,1.20)	0.95(0.82,1.10)	1.04(0.91,1.19)	1.02(0.83,1.24)
Orofacial clefts	1.19(0.82,1.71)	1.40(1.00,1.95)	1.26(0.93,1.71)	1.29(0.82,2.03)
Male fetus				
Birth defects	0.98(0.89,1.09)	0.95(0.87,1.04)	0.94(0.87,1.02)	0.91(0.81,1.03)
CHDs	0.84(0.63,1.13)	0.69(0.53,0.91)	0.69(0.54,0.88)	0.60(0.43,0.85)
Limb defects	1.10(0.89,1.34)	1.00(0.84,1.20)	1.07(0.91,1.26)	1.10(0.86,1.40)
Orofacial clefts	1.12(0.69,1.81)	1.17(0.77,1.77)	1.22(0.83,1.82)	1.10(0.62,1.94)
Female fetus				
Birth defects	0.98(0.87,1.11)	0.96(0.87,1.07)	1.06(0.96,1.17)	0.88(0.77,1.01)
CHDs	1.16(0.86,1.57)	1.09(0.84,1.41)	1.28(1.00,1.64)	1.05(0.74,1.49)
Limb defects	0.90(0.69,1.17)	0.86(0.67,1.10)	1.00(0.80,1.26)	0.88(0.63,1.22)
Orofacial clefts	1.32(0.73,2.38)	1.97(1.07,3.62)	1.30(0.80,2.13)	1.72(0.78,3.76)
Summer/Spring				
Birth defects	0.99(0.89,1.11)	0.92(0.83,1.02)	1.02(0.93,1.12)	0.96(0.83,1.10)
CHDs	0.96(0.72,1.27)	0.82(0.62,1.07)	0.99(0.78,1.26)	0.73(0.51,1.04)
Limb defects	1.08(0.85,1.37)	0.88(0.71,1.11)	1.06(0.86,1.29)	0.92(0.68,1.23)
Orofacial clefts	1.19(0.66,2.13)	1.46(0.82,2.62)	1.06(0.65,1.72)	1.67(0.75,3.74)
Autumn/Winter				
Birth defects	0.96(0.86,1.07)	1.00(0.91,1.10)	0.98(0.89,1.07)	0.89(0.78,1.02)
CHDs	1.01(0.74,1.39)	0.99(0.76,1.29)	0.93(0.73,1.20)	0.97(0.67,1.41)
Limb defects	0.96(0.76,1.20)	1.00(0.82,1.22)	1.03(0.86,1.24)	1.11(0.84,1.46)
Orofacial clefts	1.20(0.75,1.94)	1.31(0.87,1.97)	1.35(0.90,2.02)	1.01(0.58,1.76)

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen dioxide; OR, odds ratio; PM₁₀, particulate matter ≤10 µm diameter; PM_{2.5}, particulate matter <2.5 µm diameter; SO₂, sulfur dioxide.

^a adjusted for year of conception, maternal age, and gravidity.

Table S2 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 3rd trimester and birth defects

Birth defects ^a	PM ₁₀	PM _{2.5}	NO ₂	SO ₂
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Total				
Birth defects	0.87(0.82,0.92)	0.91(0.87,0.96)	0.92(0.87,0.96)	0.93(0.88,0.99)
CHDs	0.90(0.79,1.03)	0.88(0.78,0.99)	0.90(0.81,1.00)	0.88(0.77,1.01)
Limb defects	0.96(0.83,1.11)	0.91(0.80,1.05)	0.96(0.85,1.09)	0.99(0.85,1.15)
Orofacial clefts	0.79(0.65,0.95)	0.98(0.82,1.16)	0.91(0.77,1.08)	0.91(0.74,1.12)
Male fetus				
Birth defects	0.87(0.81,0.94)	0.90(0.83,0.96)	0.92(0.86,0.98)	0.96(0.89,1.05)
CHDs	0.88(0.73,1.05)	0.84(0.71,0.99)	0.87(0.75,1.02)	0.81(0.67,0.97)
Limb defects	0.92(0.77,1.10)	0.90(0.76,1.06)	0.92(0.79,1.07)	1.03(0.85,1.25)
Orofacial clefts	0.77(0.60,0.99)	0.94(0.75,1.18)	0.91(0.74,1.13)	0.95(0.73,1.24)
Female fetus				
Birth defects	0.87(0.79,0.95)	0.94(0.87,1.02)	0.92(0.85,0.99)	0.89(0.81,0.98)
CHDs	0.94(0.78,1.14)	0.96(0.81,1.14)	0.95(0.81,1.11)	0.99(0.81,1.21)
Limb defects	1.01(0.78,1.29)	0.93(0.74,1.18)	1.05(0.85,1.29)	0.91(0.71,1.18)
Orofacial clefts	0.81(0.60,1.10)	1.02(0.77,1.35)	0.89(0.69,1.15)	0.86(0.63,1.19)
Summer/Spring				
Birth defects	1.11(1.01,1.23)	1.29(1.18,1.42)	1.12(1.03,1.22)	1.05(0.96,1.15)
CHDs	1.13(0.90,1.42)	1.27(1.03,1.57)	1.21(1.00,1.45)	0.94(0.77,1.15)
Limb defects	1.10(0.86,1.40)	0.97(0.75,1.26)	0.99(0.80,1.23)	1.01(0.81,1.26)
Orofacial clefts	0.96(0.68,1.37)	1.39(1.02,1.91)	1.24(0.93,1.64)	1.02(0.75,1.39)
Autumn/Winter				
Birth defects	0.68(0.60,0.78)	0.78(0.70,0.87)	0.81(0.73,0.90)	0.87(0.74,1.01)
CHDs	0.84(0.63,1.12)	0.72(0.56,0.93)	0.70(0.56,0.87)	0.86(0.61,1.22)
Limb defects	0.81(0.60,1.10)	0.82(0.63,1.08)	0.94(0.73,1.21)	1.01(0.70,1.48)
Orofacial clefts	0.44(0.30,0.66)	0.78(0.54,1.10)	0.61(0.45,0.83)	0.64(0.40,1.03)

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen dioxide; OR, odds ratio; PM₁₀, particulate matter ≤10 µm diameter; PM_{2.5}, particulate matter <2.5 µm diameter; SO₂, sulfur dioxide.

^a adjusted for year of conception, maternal age, and gravidity.

Table S3 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the entire pregnancy and birth defects

Birth defects ^a	PM ₁₀	PM _{2.5}	NO ₂	SO ₂
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Total				
Birth defects	0.85(0.75,0.97)	0.98(0.87,1.11)	1.05(0.96,1.13)	0.91(0.80,1.05)
CHDs	0.87(0.61,1.24)	0.96(0.70,1.32)	1.01(0.82,1.26)	0.70(0.50,1.00)
Limb defects	0.91(0.69,1.19)	0.86(0.66,1.12)	1.11(0.93,1.31)	1.00(0.75,1.33)
Orofacial clefts	0.68(0.38,1.23)	1.18(0.66,2.12)	1.33(0.90,1.97)	1.35(0.71,2.59)
Male fetus				
Birth defects	0.81(0.68,0.96)	0.91(0.77,1.07)	0.99(0.89,1.10)	0.90(0.76,1.08)
CHDs	0.71(0.43,1.16)	0.78(0.50,1.22)	0.70(0.52,0.94)	0.56(0.34,0.91)
Limb defects	0.94(0.67,1.33)	0.79(0.58,1.09)	1.16(0.93,1.43)	0.97(0.68,1.38)
Orofacial clefts	0.65(0.30,1.41)	1.19(0.57,2.45)	1.37(0.82,2.28)	0.89(0.40,1.99)
Female fetus				
Birth defects	0.92(0.75,1.12)	1.11(0.92,1.34)	1.13(1.00,1.28)	0.93(0.75,1.14)
CHDs	1.08(0.65,1.78)	1.20(0.76,1.92)	1.55(1.11,2.17)	0.91(0.55,1.50)
Limb defects	0.84(0.53,1.34)	1.02(0.64,1.61)	1.02(0.76,1.36)	1.05(0.63,1.75)
Orofacial clefts	0.72(0.29,1.83)	1.15(0.44,3.02)	1.26(0.68,2.31)	2.89(0.96,8.74)
Autumn/Winter				
Birth defects	0.91(0.76,1.10)	1.14(0.97,1.35)	1.06(0.95,1.18)	1.07(0.88,1.30)
CHDs	0.94(0.58,1.53)	1.13(0.75,1.71)	1.21(0.92,1.60)	0.84(0.53,1.35)
Limb defects	0.96(0.64,1.44)	0.89(0.61,1.29)	1.06(0.84,1.33)	0.82(0.54,1.24)
Orofacial clefts	0.89(0.33,2.38)	1.60(0.60,4.25)	1.07(0.61,1.87)	3.37(1.04,10.85)
Summer/Spring				
Birth defects	0.76(0.63,0.92)	0.84(0.70,1.00)	1.03(0.91,1.17)	0.82(0.67,0.99)
CHDs	0.75(0.44,1.26)	0.77(0.47,1.27)	0.78(0.55,1.10)	0.63(0.36,1.07)
Limb defects	0.85(0.58,1.24)	0.83(0.57,1.20)	1.17(0.90,1.52)	1.21(0.81,1.82)
Orofacial clefts	0.59(0.28,1.24)	0.95(0.45,1.98)	1.58(0.90,2.76)	0.79(0.35,1.75)

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen dioxide; OR, odds ratio; PM₁₀, particulate matter ≤10 µm diameter; PM_{2.5}, particulate matter <2.5 µm diameter; SO₂, sulfur dioxide.

^a adjusted for year of conception, maternal age, and gravidity.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(b) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	-
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	6
		(b) Describe any methods used to examine subgroups and interactions	6
		(c) Explain how missing data were addressed	-
		(d) If applicable, explain how loss to follow-up was addressed	-
		(e) Describe any sensitivity analyses	-
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6-7
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6-7
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	Report numbers of outcome events or summary measures over time	6-7
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	-
		(b) Report category boundaries when continuous variables were categorized	8
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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4 1 **Ambient air pollutants in the first trimester of pregnancy and birth defects: an**
5
6 2 **observational study**

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4 42 **Abstract**

5 43 **Objectives** As current studies on the relationships between air pollutants exposure
6 during the 1st trimester and birth defects were not fully elucidated, this study aimed to
7 assess the association between selected air pollutants and birth defects.
8
9 45

10
11 46 **Design** An observational study.

12
13 47 **Participants** We obtained 70,854 singletons with gestational age <20 weeks who
14 were delivered at a large maternal and child health care center in Wuhan, China.
15
16 48

17 49 **Outcome measures** Birth defects data and daily average concentration of ambient
18 particulate matter ≤ 10 μm diameter (PM₁₀), particulate matter ≤ 2.5 μm diameter
19 (PM_{2.5}), sulfur dioxide (SO₂), and nitrogen dioxide (NO₂) were obtained. Logistic
20 regression analysis was applied to assess the association between maternal air
21 pollutants exposure during 1st trimester and total birth defects, congenital heart defects
22 (CHDs), limb defects, and orofacial clefts adjusted to potential covariates.
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27 54
28
29 55 **Results** There were a total of 1,352 birth defect cases included in this study, with a
30 prevalence of 19.08%. Maternal exposed to high concentrations of PM₁₀, PM_{2.5}, NO₂,
31 and SO₂ in the 1st trimester were significantly associated with elevated odds ratios of
32 birth defects (ORs ranged from 1.13 to 1.23). Additionally, for male fetuses, maternal
33 exposed to high PM_{2.5} concentration was associated with an elevated odd of CHDs
34 (OR=1.27, 95% CI: 1.06, 1.52). In the cold season, the odds ratios of birth defects
35 were significantly increased among women exposed to PM_{2.5} (OR=1.64, 95% CI:
36 1.41, 1.91), NO₂ (OR=1.22, 95% CI: 1.08, 1.38), and SO₂ (OR=1.26, 95%CI: 1.07,
37 1.47).
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46 64 **Conclusions** This study showed unfavorable effects of air pollutants exposure during
47 the 1st trimester on birth defects. Especially, the association between maternal PM_{2.5}
48 exposure and CHDs was only observed among male fetuses, and stronger effects of
49 PM_{2.5}, NO₂, and SO₂ exposure on birth defects were observed in the cold season.
50
51 66

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54 68 **Keywords:** air pollutants; birth defects; congenital heart defects; limb defects;
55 orofacial clefts; pregnancy
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4 72 **Strengths and limitations of this study**

- 5 73 ➤ This study explored the associations between maternal air pollutants exposure
6 during 1st trimester and a wide range of outcomes classified by total birth defects,
7 CHDs, limb defects, and orofacial clefts based on a large population.
8
9 75
10
11 76 ➤ The stratification analysis by neonatal sex and season of conception indicated the
12 specific high-risk population, which provided critical evidence of the air quality
13 control policies.
14
15 78
16
17 79 ➤ The selected participants are located in a large tertiary maternal care center in
18 Wuhan and the representativeness of this study was undermined.
19
20 80
21 81 ➤ Maternal air pollutants exposure indoors or other living residents including work,
22 dining, and shopping were not included and other covariates including health
23 behaviors and genetic factors were failed to obtain.
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86 INTRODUCTION

87 Birth defects are structural or functional abnormalities occurred during embryonic
88 development, most of them forming in the 1st trimester of pregnancy.¹ Birth defects
89 are the leading cause of fetal death and are associated with the elevated risk of
90 childhood mortality and reduced long-term survival.^{2,3} According to the Institute for
91 Health Metrics and Evaluation, the prevalence of congenital birth defects increased
92 from 6.08% in 2005 to 6.29% in 2019.⁴ Although the upward trends were reported in
93 severe congenital heart defects, single ventricle, atrioventricular septal defects, and
94 tetralogy of Fallot in Europe during 1980-2012, the prevalence of birth defects are
95 much lower (1.0-4.1‰).⁵ In China, the overall prevalence of birth defects increased
96 from 12.83‰ in 1986 to 15.70‰ in 2014.⁶ There is a necessity to explore the
97 potential hazard factors contributed to the high prevalence of birth defects in China
98 based on current knowledge.

99 Due to rapid urbanization, China has experienced severe air pollution in recent
100 years. The Report on China's implementation of the Millennium Development Goals
101 (2000-2015) documented that particulate matter $\leq 10 \mu\text{m}$ diameter (PM₁₀), sulfur
102 dioxide (SO₂), and nitrogen dioxide (NO₂) were major air pollutants in urban areas.⁷
103 Moreover, ambient particulate matter $\leq 2.5 \mu\text{m}$ diameter (PM_{2.5}) produced by coal
104 combustion, industry sources, and vehicular emissions, is also one of the main air
105 pollutants of industrialization.⁸ The expanding coverage of ambient air pollutants
106 surveillance has contributed to a flow of studies on the adverse pregnancy outcome,
107 including small for gestational age and low birth weight, preterm birth, spontaneous
108 abortion, and stillbirth in recent years.⁹⁻¹¹ Moreover, there is a growing interest in the
109 associations between ambient air pollutants and birth defects, but the results of
110 previous studies are controversial. Most studies reported that ambient air pollutants
111 were associated with increased risks of birth defects.¹²⁻¹⁴ However, Parkes et al. and
112 Dolk et al. pointed out that high concentrations of PM₁₀ and NO₂ exposure were not
113 related to birth defects.^{15,16} A recent meta-analysis conducted by Ma et al. even
114 reported a protective effect of SO₂ on atrial septal defects.¹⁷ These inconsistent results
115 indicate that the effects of ambient air pollutants on birth defects could be varied by

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4 116 culture, ethnicity, or geographical distribution. More evidence is needed to clarify the
5
6 117 risk of birth defects derived from air pollutants during the rapid social-economical
7
8 118 development worldwide.

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10 119 As current studies on the relationships between air pollutants and birth defects
11
12 120 were not fully elucidated, this study aimed to assess the association between selected
13
14 121 air pollutants and birth defects. Furthermore, the 1st trimester of pregnancy is vital for
15
16 122 fetal development because fetal major organs and systems are formed at this stage and
17
18 123 the fetus is most susceptible to environmental hazards. As a result, we mainly focused
19
20 124 on the associations between maternal exposure to air pollutants including PM₁₀,
21
22 125 PM_{2.5}, SO₂, and NO₂ during the 1st trimester and birth defects in Wuhan, China.

23 126 **METHODS**

24 127 **Patient and public involvement**

25
26
27 128 Patients or the public were not involved in any part of the design, conduct, reporting,
28
29 129 or dissemination plans of this study.

30 130 **Study design, site and population**

31
32
33 131 This observational study was conducted in Wuhan city. Wuhan is the capital city of
34
35 132 Hubei Province and a megacity in Central China. Its geographical location is 29° 58' -
36
37 133 31° 22' N and 113° 41' - 115° 05' E. The permanent resident population of Wuhan
38
39 134 was over 10 million. Wuhan has four distinct seasons of hot summer and cold winter,
40
41 135 with an annual average temperature of 15.8 °C - 17.5 °C.¹⁸

42
43 136 There were a total of 130,186 perinatal women with detailed home addresses
44
45 137 who delivered at the Maternal and Child Health Hospital of Hubei Province, and
46
47 138 98,877 of them lived in Wuhan City during the 1st trimester of pregnancy. Then we
48
49 139 obtained 74,336 participants with distances less than 10 km from home to the nearest
50
51 140 air surveillance station. After removing 3,333 mothers of multiple pregnancies and
52
53 141 149 with gestational age <20 weeks, 70,854 participants were finally included in this
54
55 142 study.

56 143 **Birth defects**

57
58 144 Birth defect cases with gestational age ≥ 20 weeks and 0-7 days after birth including
59
60 145 elective termination of pregnancy. Based on the requirements of the Maternal and

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4 146 Child Health Monitoring Manual in China and the Implementation of National
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6 147 Hospital Birth Defects Surveillance of China, 23 types of common birth defects were
7
8 148 categorized according to the 10th Revision of the International Classification of
9
10 149 Diseases (ICD-10, Q00–Q99). The top three birth defects including congenital heart
11
12 150 defects (CHDs) (Q20–Q28), limb defects (Q69–Q72), and orofacial clefts (Q35–Q37)
13
14 151 were further examined by trained obstetricians, pediatricians, or pediatric
15
16 152 cardiologists based on clinical observation, physical examination, biochemical index,
17
18 153 and image examination results. Strict quality control of the reported data was
19
20 154 performed by the assigned county-level inspector every quarter year and further
21
22 155 checked by the city-level inspector semiannually. Detailed descriptions of the 23
23
24 156 types of birth defects were provided in Supplementary Table 1.

157 **Exposure assessment**

158 Data on the daily concentrations of air pollutants (PM₁₀, PM_{2.5}, NO₂, and SO₂) and the
159
160 geographical locations (longitude and latitude) of the air surveillance stations were
161
162 obtained from China's National Urban Air Quality Real-time Publishing Platform
163
164 (<http://106.37.208.233:20035>). Moreover, the geographical location data was
165
166 converted from the detailed home address of participants by Baidu Map. The distance
167
168 from each home address of participants to all of the air surveillance stations were
169
170 calculated. Then the individual daily air pollutants data were obtained according to
171
172 the nearest surveillance station from home, and we only keep participants who lived
173
174 within 10km from the nearest surveillance station, which was confirmed by other
175
176 studies.^{15,19} The median distance from home to the nearest station was 3.50 km in this
177
178 study. Participants' average air pollutants exposure during the 1st trimester of
179
180 pregnancy was estimated by mean levels of daily concentrations.

181 **Statistical analysis**

182 Observed outcomes were classified as total birth defects, CHDs, limb defects, and
183
184 orofacial clefts. Maternal exposure variables including PM₁₀, PM_{2.5}, NO₂, and SO₂
185
186 were divided by interquartile range based on the daily average concentration during
187
188 the 1st trimester. Covariates including the year of conception (2013-2018), maternal
189
190 age (<25, 25-29, 30-34, and >34 years), gravidity (1, 2-3, and >3), and urban/rural

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4 176 were obtained. Moreover, other covariates including per capita of cars,
5
6 177 unemployment, per capita area of roads, per capita of medication beds were retrieved
7
8 178 from the official website of Wuhan Bureau of Statistics (<http://tjj.wuhan.gov.cn/>).
9
10 179 Logistic regression analysis was applied to assess the association between maternal
11
12 180 air pollutants exposure during 1st trimester and birth defects adjusted for potential
13
14 181 covariates. Stratified analyses by neonatal sex (male and female fetus) and season of
15
16 182 conception by last menstrual period (March to August and September to February)
17
18 183 were applied to further explore the associations between air pollutants exposure in the
19
20 184 1st trimester of pregnancy and birth defects. The adjusted odds ratio (OR) and 95%
21
22 185 confidence interval (CI) were provided in each model. Statistical analyses were
23
24 186 performed by SAS 9.4 (SAS Institute, Inc., Cary, NC).

187 RESULTS

188 There were a total of 1,352 birth defect cases among 70,854 singletons, with a
189
190 prevalence of 19.08‰ (Table 1). The prevalence of birth defects increased from
191
192 21.16‰ in 2013 to 24.08‰ in 2018. It ranks first place in subpopulations who
193
194 conceived in winter (26.98‰), aged <25 years (28.10‰), or with gravidity >3 times
195
196 (23.93‰). In addition, the male fetuses had a higher prevalence of birth defects than
197
198 female fetuses (20.57‰ vs 16.80‰).

195 **Table 1 Prevalence of birth defects among the subgroups of enrolled participants**
196 **in Wuhan, China**

Variables	Birth defect cases	Total cases	Prevalence (‰)
Year of conception			
2013	272	12,856	21.16
2014	203	11,236	18.07
2015	174	11,274	15.43
2016	227	12,261	18.51
2017	226	12,844	17.60
2018	250	10,383	24.08
Season of conception			
Spring	321	18,312	17.53
Summer	295	18,853	15.65
Autumn	287	17,045	16.84
Winter	449	16,644	26.98
Age (years)			

<25	122	4,341	28.10
25–29	632	34,412	18.37
30–34	401	23,783	16.86
>34	197	8,318	23.68
Gravidity			
1	604	35,590	16.97
2-3	372	19,552	19.03
>3	376	15,712	23.93
Neonatal sex			
Male	771	37,488	20.57
Female	560	33,337	16.80
Total	1,352	70,854	19.08

197

198 Table 2 shows the distributions of daily average ambient air pollutants
 199 concentrations in the 1st trimester among the 5 groups including non-defects, birth
 200 defects, CHDs, limb defects, and orofacial clefts. The median exposures of PM₁₀,
 201 PM_{2.5}, NO₂, and SO₂ during the 1st trimester were 101.78µg/m³, 61.98µg/m³,
 202 53.64µg/m³, and 13.97µg/m³ respectively among the birth defects groups.

203

204 **Table 2 Quartile concentrations (µg/m³) of exposure for ambient air pollutants**
 205 **among birth defects groups in 1st trimester of pregnancy in Wuhan, China**

Air pollutants	Non-defects N=69,502	Birth defects N=1,352	CHDs N=265	Limb defects N=210	Orofacial clefts N=119
PM ₁₀					
Min	36.86	39.08	49.82	40.76	48.85
25th	80.84	84.05	84.05	85.86	83.98
Median	102.42	101.78	99.46	105.80	96.77
75th	123.86	124.39	120.48	127.34	116.36
Max	231.49	225.91	225.91	220.82	191.37
PM _{2.5}					
Min	21.27	21.27	22.77	22.16	22.98
25th	39.37	45.02	44.88	42.63	41.26
Median	59.21	61.98	61.33	63.00	60.98
75th	80.20	80.73	78.81	84.66	77.38
Max	178.20	165.92	154.42	154.79	155.35
NO ₂					
Min	9.71	15.22	29.03	25.78	16.89
25th	43.40	45.70	45.49	44.31	44.09
Median	51.49	53.64	53.69	53.43	53.84
75th	59.08	59.98	59.46	62.84	59.99
Max	93.60	93.37	92.93	93.37	87.58

SO ₂						
Min	2.93	3.02	3.93	3.77	3.99	
25th	9.07	9.36	9.43	10.55	9.46	
Median	14.47	13.97	13.77	16.09	12.55	
75th	23.54	23.87	23.36	25.23	21.28	
Max	71.74	63.36	63.36	62.99	53.75	

206 Abbreviations: CHDs, congenital heart defects; NO₂, nitrogen dioxide; PM₁₀, particulate
207 matter ≤10 µm diameter; PM_{2.5}, particulate matter <2.5 µm diameter; SO₂, sulfur dioxide.

208

209 Table 3 presents the associations between ambient air pollutants exposure in the
210 1st trimester of pregnancy and birth defects. We also provided the odds ratios of birth
211 defects in the 2nd and 3rd trimesters, as well as the entire pregnancy in the
212 supplementary Tables S2-S4. High concentrate exposure to air pollutants was
213 significantly associated with increased odds of total birth defects, yielding adjusted
214 ORs of 1.13, 1.23, 1.18, and 1.19 for PM₁₀, PM_{2.5}, NO₂, and SO₂, respectively.
215 Moreover, similar elevated odds of CHDs was observed for maternal exposure of
216 PM_{2.5} (OR=1.21, 95%CI: 1.06, 1.38), NO₂ (OR=1.13, 95%CI: 1.01, 1.27), and SO₂
217 (OR=1.24, 95%CI: 1.03, 1.48), but not PM₁₀ (1.08, 95% CI: 0.95, 1.24).

218 For male fetuses, there were significantly increased odds of total birth defects
219 among mothers exposed to PM₁₀, PM_{2.5}, NO₂, and SO₂ by interquartile increased
220 concentrations during the 1st trimester (OR=1.13-1.23). Moreover, a positive
221 association between PM_{2.5} exposure during the 1st trimester and CHDs was detected
222 (OR=1.27, 95% CI: 1.06, 1.52). For female fetuses, elevated odds of total birth
223 defects (OR=1.14-1.22) were also observed among maternal exposed to heavy
224 concentrated PM₁₀, PM_{2.5}, NO₂, and SO₂.

225 After stratified by season, the results show that the odds of total birth defects
226 were significantly increased among women who conceived in Autumn/Winter and
227 were exposed to PM_{2.5} (OR=1.64, 95% CI: 1.41, 1.91), NO₂ (OR=1.22, 95% CI: 1.08,
228 1.38), or SO₂ (OR=1.26, 95% CI: 1.07, 1.47). PM_{2.5}, NO₂, and SO₂ were also
229 positively related to CHDs (ORs=1.36-1.84). Elevated hazard of orofacial clefts was
230 observed among women who conceived in the cold season exposed to heavy NO₂
231 concentrations (OR=1.85, 95% CI: 1.16, 2.93). However, in the warm season, the odd

232 of total birth defects was elevated among women exposed to PM₁₀ (OR=1.12, 95%
233 CI: 1.00, 1.24).

234

235 **Table 3 Adjusted odds ratios and 95% confidence interval of ambient air**
236 **pollutants for each interquartile increase during the 1st trimester and birth defects**
237 **in Wuhan, China**

Birth defects ^a	PM ₁₀ OR (95%CI)	PM _{2.5} OR (95%CI)	NO ₂ OR (95%CI)	SO ₂ OR (95%CI)
Total				
Birth defects	1.13(1.07,1.21)	1.23(1.15,1.30)	1.18(1.12,1.24)	1.19(1.10,1.30)
CHDs	1.08(0.95,1.24)	1.21(1.06,1.38)	1.13(1.01,1.27)	1.24(1.03,1.48)
Limb defects	1.11(0.96,1.29)	1.09(0.93,1.26)	1.15(1.01,1.31)	1.16(0.95,1.43)
Orofacial clefts	0.90(0.74,1.10)	1.04(0.85,1.26)	1.10(0.93,1.31)	0.95(0.73,1.22)
Male fetus				
Birth defects	1.13(1.04,1.22)	1.23(1.14,1.34)	1.18(1.10,1.26)	1.19(1.07,1.33)
CHDs	1.16(0.96,1.40)	1.27(1.06,1.52)	1.11(0.95,1.30)	1.18(0.93,1.50)
Limb defects	1.10(0.91,1.33)	1.08(0.90,1.30)	1.15(0.98,1.35)	1.14(0.89,1.47)
Orofacial clefts	0.86(0.66,1.12)	1.09(0.85,1.41)	1.07(0.86,1.34)	0.99(0.71,1.37)
Female fetus				
Birth defects	1.14(1.04,1.25)	1.22(1.12,1.34)	1.19(1.10,1.29)	1.21(1.07,1.37)
CHDs	0.99(0.82,1.20)	1.11(0.91,1.34)	1.14(0.97,1.34)	1.30(1.00,1.71)
Limb defects	1.08(0.84,1.38)	1.15(0.90,1.47)	1.15(0.93,1.43)	1.18(0.84,1.66)
Orofacial clefts	0.98(0.71,1.34)	0.95(0.69,1.30)	1.16(0.88,1.52)	0.88(0.59,1.30)
Autumn/Winter				
Birth defects	1.05(0.92,1.20)	1.64(1.41,1.91)	1.22(1.08,1.38)	1.26(1.07,1.47)
CHDs	1.05(0.79,1.39)	1.84(1.32,2.57)	1.36(1.03,1.80)	1.43(1.00,2.03)
Limb defects	1.19(0.84,1.70)	1.48(0.99,2.22)	1.35(0.97,1.87)	1.06(0.70,1.61)
Orofacial clefts	0.95(0.61,1.47)	1.63(0.98,2.70)	1.85(1.16,2.93)	1.40(0.82,2.40)
Summer/Spring				
Birth defects	1.12(1.00,1.24)	1.06(0.93,1.21)	1.09(1.00,1.19)	1.12(0.98,1.28)
CHDs	0.99(0.77,1.26)	0.89(0.65,1.21)	0.88(0.71,1.08)	1.24(0.92,1.66)
Limb defects	1.18(0.92,1.51)	1.21(0.89,1.63)	1.17(0.95,1.43)	1.23(0.89,1.71)
Orofacial clefts	0.72(0.50,1.03)	0.66(0.41,1.04)	0.88(0.65,1.19)	0.72(0.47,1.10)

238 Bold values represent statistical significance (two-sided $P < 0.05$).

239 Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen
240 dioxide; OR, odds ratio; PM₁₀, particulate matter $\leq 10 \mu\text{m}$ diameter; PM_{2.5}, particulate matter
241 $< 2.5 \mu\text{m}$ diameter; SO₂, sulfur dioxide.

242 ^a Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of
243 roads, per capita of medication beds, unemployment, and per capita of cars.

244

245 DISCUSSION

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4 246 In the current study, the prevalence of birth defects was close to what was reported in
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6 247 Hunan Province (19.18%) and higher than that of Jiangsu Province (7.15%).^{20,21}
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8 248 Moreover, this study examined the associations between birth defects and air
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10 249 pollutants exposure in the 1st trimester, and we further explored the relationships
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12 250 stratified by fetal sex and season of conception.

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14 251 This study showed that maternal exposure to ambient air pollutants including
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16 252 PM₁₀, PM_{2.5}, NO₂, and SO₂ during the 1st trimester could have higher odds of birth
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18 253 defects, which has been well documented previously. Moreover, we have adopted
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20 254 peak exposure on the thresholds at the 95th percentile to derive the accumulated days
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22 255 of high dose exposure, which double confirmed the positive association between high
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24 256 dose of NO₂ exposure during the 1st trimester and birth defects (Supplementary Table
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26 257 5). A case-control study conducted by Al Noaimi et al. showed a positive association
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28 258 between PM_{2.5} exposure in the 1st trimester of pregnancy and birth defects
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30 259 (OR=1.05).²² Wang et al. applied Poisson generalized additive models on the
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32 260 time-series data adjusted for temperature, relative humidity, season, and time trend,
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34 261 which showed that maternal exposure to PM₁₀ and NO₂ in early pregnancy
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36 262 significantly increased the risk of birth defects by 10.3% per 10 µg/m³ and 3.4% per
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38 263 10 µg/m³, respectively.²³

39 264 As was observed among the total participants, the risk of birth defects among
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41 265 both male fetuses and female fetuses was significantly related to PM₁₀, PM_{2.5}, NO₂,
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43 266 and SO₂. The underlying mechanisms between air pollutants exposure and the
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45 267 development of birth defects are still unclear. Maternal air pollutants exposures might
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47 268 cause changes in epigenetic signatures and permanent modifications in gene
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49 269 expression.²⁴ Studies based on mice models showed that maternal exposure to PM_{2.5}
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51 270 during pregnancy leads to spatial memory dysfunction, neurodevelopmental
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53 271 impairment, and disturbed cerebral cortex development of mice offspring.^{25,26}
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55 272 Another study showed that maternal exposure to a high concentrate of PM_{2.5} during
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57 273 the 1st trimester of pregnancy could result in the decreased placental expression of
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59 274 *BDNF* and *SYNI*, which may undermine fetal neurodevelopment.²⁷ Moreover, SO₂ is
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275 a systemic toxic agent which can induce chromosomal aberrations, sister chromatid

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4 276 exchanges, and micronuclei in mammalian cells.²⁸

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6 277 Except for PM₁₀, this study demonstrated adverse associations of CHDs with
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8 278 PM_{2.5}, NO₂, and SO₂ exposure. Previous studies in Taiwan and Northeast England
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10 279 also reported insignificant associations between PM₁₀ exposure in the 1st trimester and
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12 280 CHDs,^{29,30} whereas others confirmed the increased risk of CHDs caused by
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14 281 PM₁₀.^{12,23,31-34} Furthermore, Huang et al. showed that PM_{2.5} exposure per each
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16 282 interquartile increase during gestational weeks 3-8 was related to an increased risk of
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18 283 CHDs (OR=1.21).³⁵ Additionally, the positive association between CHDs and
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20 284 maternal exposure to NO₂ and SO₂ was documented by studies conducted by Baldacci
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22 285 et al. and Vrijheid et al.^{36,37} Moreover, a case-control study conducted by Jiang et al.
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24 286 showed that maternal exposure to SO₂ during the 1st trimester was significantly
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26 287 associated with increased risk of CHD (OR=1.78-2.04), and Hansen et al. also
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28 288 confirmed that a 0.6 ppb increase in SO₂ was associated with an increased risk of
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30 289 aortic artery and valve defects (OR=10.76).^{19,38} The heterogeneity in these studies
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32 290 might be explained by the variation in the population, the gestational periods, or the
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34 291 measurement of air pollutants exposure. The pathophysiological mechanism of the
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36 292 associations between air pollutants and CHDs was not fully elucidated. It is
37
38 293 hypothesized that air pollutants exposure during the 1st pregnancy might strengthen
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40 294 the genetic and environmental interaction on CHDs.³⁷ Also, Air pollutants might
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42 295 change the molecule of DNA sequence or alter epigenetics in related to CHDs.³⁹

43 296 The epidemiological difference in CHDs between male and female fetuses has
44
45 297 been widely reported.^{40,41} This disparity could be explained by the sex
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47 298 chromosome-linked genes expression and their interactions with hormonal effects
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49 299 during early development.⁴² Interestingly, only male fetuses showed an elevated risk
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51 300 of CHDs who were exposed to a higher PM_{2.5} concentration. This result indicates that
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53 301 PM_{2.5} might emphasize the disparity in the embryonic origins of sexual dimorphism.
54
55 302 Moreover, PM_{2.5} might have a stronger effect on the expression of specific CHDs
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57 303 genes located on the Y chromosome. A study of mice models showed that increased
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59 304 pathological damage in the hearts of offspring mice was observed among maternal
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305 mice exposed to PM_{2.5}, and these effects in the male group were more obvious than in

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4 306 the female group. PM_{2.5} exposure in utero inhibited the expression of the *GATA4* gene
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6 307 in male mice, which related to the formation of CHDs.⁴³

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8 308 After stratified by season, maternal exposure of PM_{2.5}, NO₂, and SO₂ were
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10 309 positively associated with birth defects in the cold season, but this relationship was
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12 310 not observed in the warm season. Zhao et al. reported that the effects of air pollutants
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14 311 on birth defects were more obvious in the warm season in Hohhot.⁴⁴ This disparity
15
16 312 could be partly explained by the uneven levels of dwellings' air pollutants. Compared
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18 313 to cities with lower GDP, cities with a higher GDP and a large population might have
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20 314 lower concentrations of indoor particulate matter.⁴⁵ Moreover, Wuhan is a
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22 315 well-known city with hot summer. Most of the residential buildings are equipped with
23
24 316 air conditioners, which could help to improve indoor air quality in hot weather. Thus,
25
26 317 the interpretation of results should be cautious that significant associations between
27
28 318 air pollutants exposure and birth defects in the warm season in Wuhan.

29 **Strengths**

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31 320 This study firstly provided evidence on the elevated risk of CHDs among mothers
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33 321 with heavy PM_{2.5} exposure during the 1st trimester. We also found the increased risks
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35 322 of CHDs and orofacial clefts among women who conceived in the cold season
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37 323 exposed to high concentrations of air pollutants.

38 **Limitations**

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41 325 This study has several limitations. Firstly, the selected participants are located in a
42
43 326 large tertiary maternal care center in Wuhan and the representative of this study was
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45 327 undermined. Secondly, the birth defects data of this study are manually collected and
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47 328 checked, but mistakes and omissions are inevitable. Thirdly, maternal air pollutants
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49 329 exposure indoor or other living residents including work, dining, and shopping were
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51 330 not included in this study. Fourthly, other risk factors (including ethnicity, smoking,
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53 331 drinking, medications, drug use, and family history et al.) were failed to obtain, which
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55 332 might undermine the interpretation of the relationship between maternal air pollutants
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57 333 exposure and birth defects. More research is needed to explore the pathogenic
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59 334 mechanism of air pollutants exposure during pregnancy and the associated birth
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335 defects.

336 CONCLUSIONS

337 Our study confirmed the unfavorable effects of maternal exposure to air pollutants
338 (PM₁₀, PM_{2.5}, NO₂, and SO₂) on birth defects during the 1st trimester of pregnancy.
339 We firstly provided the evidence on the positive associations between PM_{2.5} exposure
340 and CHDs among male fetuses but not female fetuses. Moreover, stronger effects of
341 PM_{2.5}, NO₂, and SO₂ exposure on birth defects were observed in the cold season in
342 Wuhan. As a result, it should be noted for birth defects due to air pollutants, and
343 reducing individual air pollutants exposure during the 1st trimester might help to birth
344 defect control in the context of the rapid development all over the world. Moreover,
345 the implementation of air quality protection policies on birth defect control should
346 consider seasonal factor, especially for the cold season in Wuhan, China. Future
347 studies of birth defects and air quality data collected by individual air pollutants
348 monitors are promoted.

349 Abbreviations

350 CHDs: congenital heart defects; CI: confidence interval; NO₂: nitrogen dioxide; OR:
351 odds ratio; PM₁₀: particulate matter ≤10 µm diameter; PM_{2.5}: particulate matter ≤2.5
352 µm diameter; SO₂: sulfur dioxide.

353 Ethics approval and consent to participate

354 This study was approved by the Ethics Committee of Maternal and Child Health
355 Hospital of Hubei Province (2021IECLW025). This study was based on the
356 retrospective clinical data without any individual patient identifiers.

357 Patient consent for publication

358 Not applicable.

359 Patient and public involvement

360 Patients or the public were not involved in the design, or conduct, or reporting, or
361 dissemination plans of our research.

362 Availability of data and materials

363 The data used in this study are available from corresponding author on reasonable

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4 364 request.

5 365 **Competing interests**

7 366 The authors declare that they have no competing interests.

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13 369 **Authors' contributions**

15 370 Yao Cheng and Jieyun Yin contributed equally to this manuscript. Yao Cheng
17 371 collected the data, performed data analysis, draft and revised the manuscript. Jieyun
19 372 Yin collected the data, performed data analysis, and edit the manuscript. Lijun Yang
21 373 designed the study, collected the data, and performed data analysis. Man Xu, Xinfeng
23 374 Lu, and Wenting Huang collected the data, interpreted the data, and reviewed the
25 375 manuscript. Guohong Dai: collected the data, interpreted the data, and revised the
27 376 manuscript. Guoqiang Sun: designed the study, collected the data, and supervised the
29 377 project. All authors gave final approval of the version to be submitted and agreed to
31 378 be accountable for all aspects of the work.

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Table S1 The rank result of the 23 types of common birth defects

Number	Birth defect	N
21	Conjoined twins	0
13	Bladder valgus	1
1	Anencephaly	2
3	Encephalocele	3
20	Gastroschisis	3
8	Small ears (including no ears)	6
10	Esophageal atresia or stenosis	8
2	Spina bifida	12
18	Congenital diaphragmatic hernia	13
19	Omphalocele	18
12	Hypospadias	27
11	Rectoanal atresia or stricture (including anorectal atresia)	30
14	Equinovarus	41
4	Congenital hydrocephalus	45
22	Down's syndrome	79
9	Other malformations of the external ear (except for small ears and no ears)	86
5, 6, 7	Orofacial clefts	119
15, 16, 17	Limb defects	210
23	Congenital heart defects	265

Table S2 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 2^{ed} trimester and birth defects in Wuhan, China

Birth defects ^a	PM ₁₀	PM _{2.5}	NO ₂	SO ₂
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Total				
Birth defects	1.01(0.93,1.09)	0.95(0.89,1.02)	0.99(0.93,1.06)	0.88(0.80,0.97)
CHDs	0.98(0.79,1.22)	0.86(0.72,1.04)	0.94(0.79,1.11)	0.75(0.58,0.97)
Limb defects	1.03(0.87,1.22)	0.95(0.82,1.11)	1.05(0.92,1.21)	1.01(0.83,1.25)
Orofacial clefts	1.27(0.86,1.87)	1.42(1.01,1.99)	1.27(0.93,1.74)	1.38(0.85,2.25)
Male fetus				
Birth defects	1.01(0.91,1.12)	0.95(0.87,1.04)	0.95(0.87,1.03)	0.90(0.79,1.02)
CHDs	0.82(0.61,1.10)	0.69(0.52,0.91)	0.69(0.53,0.88)	0.58(0.41,0.83)
Limb defects	1.12(0.90,1.38)	1.00(0.84,1.20)	1.09(0.92,1.29)	1.09(0.84,1.41)
Orofacial clefts	1.11(0.69,1.81)	1.20(0.79,1.82)	1.25(0.83,1.87)	1.19(0.67,2.12)
Female fetus				
Birth defects	1.01(0.89,1.14)	0.96(0.86,1.07)	1.06(0.96,1.17)	0.87(0.75,1.00)
CHDs	1.21(0.88,1.66)	1.07(0.82,1.39)	1.28(0.99,1.65)	1.00(0.69,1.44)
Limb defects	0.90(0.69,1.18)	0.87(0.68,1.12)	0.99(0.79,1.25)	0.89(0.63,1.25)
Orofacial clefts	1.64(0.80,3.36)	2.02(1.06,3.86)	1.31(0.79,2.18)	2.01(0.75,5.39)
Summer/Spring				
Birth defects	1.04(0.93,1.17)	0.91(0.82,1.01)	1.03(0.94,1.13)	0.92(0.79,1.06)
CHDs	0.99(0.73,1.33)	0.79(0.60,1.04)	1.01(0.78,1.29)	0.64(0.44,0.94)
Limb defects	1.10(0.86,1.41)	0.88(0.69,1.11)	1.06(0.87,1.29)	0.92(0.67,1.25)
Orofacial clefts	1.20(0.64,2.25)	1.47(0.81,2.67)	1.07(0.66,1.76)	1.71(0.74,3.97)
Autumn/Winter				
Birth defects	0.96(0.86,1.08)	1.00(0.91,1.10)	0.97(0.89,1.07)	0.87(0.75,1.00)
CHDs	1.02(0.74,1.40)	1.00(0.76,1.30)	0.93(0.72,1.19)	1.00(0.68,1.47)
Limb defects	0.94(0.74,1.18)	0.99(0.82,1.21)	1.05(0.87,1.28)	1.05(0.77,1.43)
Orofacial clefts	1.30(0.78,2.17)	1.34(0.88,2.03)	1.38(0.90,2.12)	1.08(0.58,2.03)

Bold values represent statistical significance (two-sided P<0.05).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen dioxide; OR, odds ratio; PM₁₀, particulate matter ≤10 μm diameter; PM_{2.5}, particulate matter <2.5 μm diameter; SO₂, sulfur dioxide.

^a Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S3 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 3rd trimester and birth defects in Wuhan, China

Birth defects ^a	PM ₁₀	PM _{2.5}	NO ₂	SO ₂
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Total				
Birth defects	0.86(0.81,0.92)	0.89(0.84,0.94)	0.90(0.85,0.95)	0.92(0.86,0.98)
CHDs	0.90(0.79,1.03)	0.87(0.77,0.98)	0.89(0.79,1.00)	0.88(0.76,1.01)
Limb defects	0.96(0.83,1.12)	0.90(0.79,1.04)	0.97(0.85,1.10)	1.00(0.85,1.17)
Orofacial clefts	0.78(0.64,0.95)	0.97(0.81,1.16)	0.90(0.76,1.07)	0.89(0.72,1.10)
Male fetus				
Birth defects	0.87(0.80,0.94)	0.88(0.81,0.94)	0.91(0.85,0.97)	0.96(0.88,1.05)
CHDs	0.85(0.72,1.01)	0.88(0.74,1.05)	0.88(0.76,1.03)	0.81(0.67,0.98)
Limb defects	0.93(0.77,1.11)	0.89(0.75,1.05)	0.92(0.79,1.08)	1.03(0.85,1.25)
Orofacial clefts	0.78(0.60,1.00)	0.94(0.75,1.19)	0.93(0.75,1.16)	0.95(0.72,1.25)
Female fetus				
Birth defects	0.86(0.78,0.94)	0.91(0.84,0.99)	0.89(0.83,0.96)	0.86(0.78,0.95)
CHDs	0.93(0.77,1.14)	0.92(0.76,1.10)	0.91(0.77,1.07)	0.98(0.79,1.21)
Limb defects	1.01(0.79,1.30)	0.94(0.74,1.19)	1.05(0.85,1.31)	0.93(0.72,1.21)
Orofacial clefts	0.78(0.57,1.08)	0.99(0.74,1.32)	0.85(0.66,1.10)	0.79(0.56,1.11)
Summer/Spring				
Birth defects	1.04(0.94,1.16)	1.18(1.07,1.31)	1.06(0.98,1.16)	0.96(0.87,1.06)
CHDs	1.07(0.84,1.36)	1.16(0.91,1.46)	1.13(0.94,1.37)	0.84(0.68,1.05)
Limb defects	1.06(0.82,1.37)	0.89(0.66,1.19)	0.97(0.78,1.21)	0.94(0.74,1.19)
Orofacial clefts	0.96(0.66,1.39)	1.37(0.98,1.91)	1.22(0.91,1.64)	0.93(0.67,1.30)
Autumn/Winter				
Birth defects	0.64(0.56,0.73)	0.78(0.70,0.87)	0.74(0.66,0.82)	0.86(0.73,1.00)
CHDs	0.74(0.55,0.99)	0.73(0.57,0.94)	0.65(0.52,0.82)	0.82(0.58,1.15)
Limb defects	0.78(0.57,1.08)	0.82(0.62,1.08)	1.05(0.78,1.41)	1.07(0.73,1.58)
Orofacial clefts	0.78(0.55,1.10)	0.40(0.27,0.60)	0.52(0.38,0.70)	0.62(0.39,0.99)

Bold values represent statistical significance (two-sided P<0.05).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen dioxide; OR, odds ratio; PM₁₀, particulate matter ≤10 μm diameter; PM_{2.5}, particulate matter <2.5 μm diameter; SO₂, sulfur dioxide.

^a Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S4 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the entire pregnancy and birth defects in Wuhan, China

Birth defects ^a	PM ₁₀	PM _{2.5}	NO ₂	SO ₂
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Total				
Birth defects	0.85(0.74,0.97)	0.95(0.83,1.09)	1.04(0.95,1.13)	0.84(0.72,0.98)
CHDs	0.91(0.64,1.29)	0.86(0.60,1.23)	1.01(0.80,1.27)	0.60(0.41,0.89)
Limb defects	0.91(0.69,1.21)	0.85(0.64,1.13)	1.14(0.95,1.37)	0.97(0.71,1.34)
Orofacial clefts	0.68(0.36,1.26)	1.16(0.62,2.17)	1.34(0.89,2.04)	1.45(0.70,2.98)
Male fetus				
Birth defects	0.81(0.68,0.97)	0.89(0.75,1.06)	0.99(0.89,1.11)	0.83(0.68,1.02)
CHDs	0.81(0.50,1.31)	0.72(0.44,1.17)	0.69(0.50,0.94)	0.52(0.31,0.89)
Limb defects	0.94(0.66,1.33)	0.75(0.53,1.07)	1.22(0.97,1.53)	0.90(0.61,1.33)
Orofacial clefts	0.69(0.32,1.48)	1.36(0.64,2.87)	1.48(0.86,2.53)	1.08(0.46,2.53)
Female fetus				
Birth defects	0.92(0.75,1.13)	1.06(0.86,1.30)	1.11(0.97,1.27)	0.86(0.68,1.09)
CHDs	1.06(0.62,1.82)	1.05(0.63,1.76)	1.59(1.11,2.28)	0.71(0.40,1.28)
Limb defects	0.86(0.54,1.38)	1.09(0.67,1.79)	1.01(0.75,1.37)	1.12(0.64,1.97)
Orofacial clefts	0.64(0.22,1.84)	0.84(0.28,2.54)	1.16(0.61,2.21)	2.99(0.77,11.70)
Autumn/Winter				
Birth defects	0.92(0.76,1.12)	1.07(0.89,1.28)	1.04(0.93,1.17)	0.98(0.79,1.22)
CHDs	0.94(0.57,1.55)	1.02(0.65,1.60)	1.22(0.91,1.63)	0.65(0.38,1.10)
Limb defects	0.97(0.65,1.45)	0.85(0.57,1.27)	1.06(0.84,1.35)	0.82(0.52,1.29)
Orofacial clefts	0.91(0.34,2.43)	1.54(0.55,4.28)	1.10(0.61,1.98)	3.91(1.17,13.04)
Summer/Spring				
Birth defects	0.74(0.60,0.90)	0.80(0.65,0.97)	1.02(0.89,1.17)	0.73(0.58,0.92)
CHDs	0.76(0.44,1.30)	0.76(0.44,1.32)	0.75(0.52,1.08)	0.61(0.33,1.13)
Limb defects	0.82(0.55,1.23)	0.83(0.55,1.26)	1.26(0.95,1.67)	1.12(0.71,1.77)
Orofacial clefts	0.56(0.24,1.30)	0.99(0.43,2.24)	1.62(0.89,2.95)	0.74(0.29,1.91)

Bold values represent statistical significance (two-sided P<0.05).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen dioxide; OR, odds ratio; PM₁₀, particulate matter ≤10 μm diameter; PM_{2.5}, particulate matter <2.5 μm diameter; SO₂, sulfur dioxide.

^a Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S5 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for the duration of peak level exposure during the 1st trimester and birth defects in Wuhan, China

Birth defects ^a	PM10 OR (95%CI)	PM2.5 OR (95%CI)	NO2 OR (95%CI)	SO2 OR (95%CI)
Total				
Birth defects	1.03(0.90,1.17)	1.10(0.94,1.28)	1.40(1.26,1.57)	1.04(0.86,1.27)
CHDs	0.91(0.67,1.23)	1.05(0.74,1.49)	1.39(1.08,1.77)	1.18(0.75,1.85)
Limb defects	1.02(0.74,1.41)	1.24(0.87,1.77)	1.37(1.04,1.81)	1.00(0.64,1.58)
Orofacial clefts	0.67(0.42,1.06)	0.92(0.54,1.54)	1.31(0.91,1.89)	0.90(0.47,1.73)
Male fetus				
Birth defects	1.08(0.90,1.29)	1.04(0.85,1.28)	1.33(1.15,1.54)	0.98(0.75,1.27)
CHDs	1.01(0.67,1.52)	1.03(0.64,1.65)	1.19(0.85,1.67)	1.11(0.59,2.11)
Limb defects	1.07(0.71,1.61)	1.18(0.75,1.85)	1.48(1.05,2.08)	0.85(0.47,1.52)
Orofacial clefts	0.67(0.37,1.22)	0.83(0.41,1.65)	1.17(0.73,1.87)	0.99(0.96,1.02)
Female fetus				
Birth defects	0.99(0.80,1.21)	1.17(0.93,1.47)	1.52(1.29,1.80)	1.08(0.81,1.44)
CHDs	0.78(0.50,1.23)	1.00(0.60,1.65)	1.57(1.10,2.25)	1.09(0.59,2.03)
Limb defects	0.96(0.56,1.63)	1.31(0.74,2.34)	1.21(0.76,1.95)	1.24(0.60,2.56)
Orofacial clefts	0.71(0.35,1.45)	1.01(0.46,2.23)	1.62(0.90,2.91)	1.01(0.39,2.61)
Autumn/Winter				
Birth defects	1.00(0.81,1.23)	1.24(0.99,1.55)	1.18(0.99,1.40)	1.12(0.78,1.61)
CHDs	1.25(0.75,2.08)	1.02(0.63,1.64)	1.46(0.97,2.19)	1.29(0.54,3.08)
Limb defects	1.01(0.59,1.70)	1.46(0.85,2.53)	1.22(0.77,1.94)	1.00(0.46,2.18)
Orofacial clefts	0.77(0.39,1.55)	0.99(0.48,2.03)	2.31(1.15,4.66)	1.48(0.50,4.41)
Summer/Spring				
Birth defects	1.03(0.78,1.36)	1.00(0.61,1.65)	1.23(1.00,1.52)	1.07(0.76,1.51)
CHDs	0.71(0.37,1.39)	0.66(0.16,2.83)	0.77(0.45,1.32)	1.52(0.69,3.37)
Limb defects	1.58(0.81,3.08)	2.17(0.92,5.08)	1.61(1.01,2.57)	0.92(0.42,2.02)
Orofacial clefts	0.35(0.12,1.04)	0.64(0.08,4.94)	0.59(0.25,1.39)	0.68(0.19,2.48)

Bold values represent statistical significance (two-sided P<0.05).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO2, nitrogen dioxide; OR, odds ratio; PM10, particulate matter $\leq 10 \mu\text{m}$ diameter; PM2.5, particulate matter $< 2.5 \mu\text{m}$ diameter; SO2, sulfur dioxide.

^a Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(b) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	-
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	6
		(b) Describe any methods used to examine subgroups and interactions	6
		(c) Explain how missing data were addressed	-
		(d) If applicable, explain how loss to follow-up was addressed	-
		(e) Describe any sensitivity analyses	-
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	6-7 - -
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	6-7 - -
Outcome data	15*	Report numbers of outcome events or summary measures over time	6-7
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	- 8 -
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Ambient air pollutants in the first trimester of pregnancy and birth defects: an observational study

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4 1 **Ambient air pollutants in the first trimester of pregnancy and birth defects: an**
5
6 2 **observational study**

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4 42 **Abstract**

5 43 **Objectives** As current studies on the relationships between air pollutants exposure
6 during the 1st trimester and birth defects were not fully elucidated, this study aimed to
7 assess the association between selected air pollutants and birth defects.
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11 46 **Design** An observational study.

12 47 **Participants** We obtained 70,854 singletons with gestational age <20 weeks who
13 were delivered at a large maternal and child health care center in Wuhan, China.
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16 49 **Outcome measures** Birth defects data and daily average concentration of ambient
17 particulate matter ≤ 10 μm diameter (PM₁₀), particulate matter ≤ 2.5 μm diameter
18 (PM_{2.5}), sulfur dioxide (SO₂), and nitrogen dioxide (NO₂) were obtained. Logistic
19 regression analysis was applied to assess the association between maternal air
20 pollutants exposure during 1st trimester and total birth defects, congenital heart defects
21 (CHDs), limb defects, and orofacial clefts with adjustments of potential covariates.
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29 55 **Results** There were a total of 1,352 birth defect cases included in this study, with a
30 prevalence of 19.08%. Maternal exposed to high concentrations of PM₁₀, PM_{2.5}, NO₂,
31 and SO₂ in the 1st trimester were significantly associated with elevated odds ratios of
32 birth defects (ORs ranged from 1.13 to 1.23). Additionally, for male fetuses, maternal
33 exposed to high PM_{2.5} concentration was associated with an elevated odd of CHDs
34 (OR=1.27, 95% CI: 1.06, 1.52). In the cold season, the odds ratios of birth defects
35 were significantly increased among women exposed to PM_{2.5} (OR=1.64, 95% CI:
36 1.41, 1.91), NO₂ (OR=1.22, 95% CI: 1.08, 1.38), and SO₂ (OR=1.26, 95%CI: 1.07,
37 1.47).
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46 64 **Conclusions** This study showed unfavorable effects of air pollutants exposure during
47 the 1st trimester on birth defects. Especially, the association between maternal PM_{2.5}
48 exposure and CHDs was only observed among male fetuses, and stronger effects of
49 PM_{2.5}, NO₂, and SO₂ exposure on birth defects were observed in the cold season.
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54 68 **Keywords:** air pollutants; birth defects; congenital heart defects; limb defects;
55 orofacial clefts; pregnancy
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4 71 **Strengths and limitations of this study**

- 5 72 ➤ This study explored the associations between maternal air pollutants exposure
6 during 1st trimester and fetal outcomes classified by total birth defects, CHDs,
7 limb defects, and orofacial clefts based on a large population.
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9 74
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11 75 ➤ The stratification analysis by neonatal sex and season of conception indicated the
12 specific high-risk population, which provided critical evidence of the air quality
13 control policies.
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15 77
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17 78 ➤ The selected participants are located in a large tertiary maternal care center in
18 Wuhan and the representative of this study was undermined.
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20 79
21 80 ➤ Maternal air pollutants exposure indoor or in other living residents including
22 work, dining, and shopping were not included, and other covariates including
23 health behaviors and genetic factors were failed to obtain.
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85 INTRODUCTION

86 Birth defects are structural or functional abnormalities occurred during embryonic
87 development, most of them forming in the 1st trimester of pregnancy.¹ Birth defects
88 are the leading cause of fetal death and are associated with the elevated risk of
89 childhood mortality and reduced long-term survival.^{2,3} According to the Institute for
90 Health Metrics and Evaluation, the prevalence of congenital birth defects increased
91 from 6.08% in 2005 to 6.29% in 2019.⁴ Although the upward trends were reported in
92 severe congenital heart defects, single ventricle, atrioventricular septal defects, and
93 tetralogy of Fallot in Europe during 1980-2012, the prevalence of birth defects are
94 much lower (1.0-4.1‰).⁵ In China, the overall prevalence of birth defects increased
95 from 12.83‰ in 1986 to 15.70‰ in 2014.⁶ There is a necessity to explore the
96 potential hazard factors contributed to the high prevalence of birth defects in China
97 based on current knowledge.

98 Due to rapid urbanization, China has experienced severe air pollution in recent
99 years. The Report on China's implementation of the Millennium Development Goals
100 (2000-2015) documented that particulate matter ≤ 10 μm diameter (PM₁₀), sulfur
101 dioxide (SO₂), and nitrogen dioxide (NO₂) were major air pollutants in urban areas.⁷
102 Moreover, ambient particulate matter ≤ 2.5 μm diameter (PM_{2.5}) produced by coal
103 combustion, industry sources, and vehicular emissions, is also one of the main air
104 pollutants of industrialization.⁸ The expanding coverage of ambient air pollutants
105 surveillance has contributed to adverse pregnancy outcomes including small for
106 gestational age, low birth weight, preterm birth, spontaneous abortion, and stillbirth in
107 recent years.⁹⁻¹¹ Moreover, there is a growing interest in the associations between
108 ambient air pollutants and birth defects, but the results of previous studies are
109 controversial. Most studies reported that ambient air pollutants were associated with
110 increased risks of birth defects.¹²⁻¹⁴ However, Parkes et al. and Dolk et al. pointed out
111 that high concentrations of PM₁₀ and NO₂ exposure were not related to birth
112 defects.^{15,16} A recent meta-analysis conducted by Ma et al. even reported a protective
113 effect of SO₂ on atrial septal defects.¹⁷ These inconsistent results indicate that the
114 effects of ambient air pollutants on birth defects could be varied by culture, ethnicity,

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4 115 or geographical distribution. More evidence is needed to clarify the risk of birth
5 116 defects derived from air pollutants during the rapid social-economical development
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7 117 worldwide.
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10 118 As current studies on the relationships between air pollutants and birth defects
11 119 were not fully elucidated, this study aimed to assess the association between selected
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13 120 air pollutants and birth defects. Furthermore, the 1st trimester of pregnancy is vital for
14
15 121 fetal development because fetal major organs and systems are formed at this stage and
16
17 122 the fetus is most susceptible to environmental hazards. As a result, we mainly focused
18
19 123 on the associations between maternal exposure to air pollutants including PM₁₀,
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21 124 PM_{2.5}, SO₂, and NO₂ during the 1st trimester and birth defects in Wuhan, China.
22

23 125 **METHODS**

24 126 **Patient and public involvement**

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26
27 127 Patients or the public were not involved in any part of the design, conduct, reporting,
28
29 128 or dissemination plans of this study.
30

31 129 **Study design, site and population**

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33 130 This observational study was conducted in Wuhan city. Wuhan is the capital city of
34
35 131 Hubei Province and a megacity in Central China. Its geographical location is 29° 58' -
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37 132 31° 22' N and 113° 41' - 115° 05' E. The permanent resident population of Wuhan
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39 133 was over 10 million. Wuhan has four distinct seasons of hot summer and cold winter,
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41 134 with an annual average temperature of 15.8 °C - 17.5 °C.¹⁸

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43 135 There were a total of 130,186 perinatal women with detailed home addresses
44
45 136 who delivered at the Maternal and Child Health Hospital of Hubei Province, and
46
47 137 98,877 of them lived in Wuhan City during the 1st trimester of pregnancy. Then we
48
49 138 obtained 74,336 participants with distances less than 10 km from home to the nearest
50
51 139 air surveillance station. After removing 3,333 mothers of multiple pregnancies and
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53 140 149 with gestational age <20 weeks, 70,854 participants were finally included in this
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55 141 study.

56 142 **Birth defects**

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58 143 Birth defect cases with gestational age \geq 20 weeks and 0-7 days after birth including
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60 144 elective termination of pregnancy. Based on the requirements of the Maternal and

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4 145 Child Health Monitoring Manual in China and the Implementation of National
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6 146 Hospital Birth Defects Surveillance of China, 23 types of common birth defects were
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8 147 categorized according to the 10th Revision of the International Classification of
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10 148 Diseases (ICD-10, Q00–Q99). The top three birth defects including congenital heart
11
12 149 defects (CHDs) (Q20–Q28), limb defects (Q69–Q72), and orofacial clefts (Q35–Q37)
13
14 150 were further examined by trained obstetricians, pediatricians, or pediatric
15
16 151 cardiologists based on clinical observation, physical examination, biochemical index,
17
18 152 and image examination results. Strict quality control of the reported data was
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20 153 performed by the assigned county-level inspector every quarter year and further
21
22 154 checked by the city-level inspector semiannually. Detailed descriptions of the 23
23
24 155 types of birth defects were provided in Supplementary Table 1.

25 156 **Exposure assessment**

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27 157 Data on the daily concentrations of air pollutants (PM₁₀, PM_{2.5}, NO₂, and SO₂) and the
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29 158 geographical locations (longitude and latitude) of the air surveillance stations were
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31 159 obtained from China's National Urban Air Quality Real-time Publishing Platform
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33 160 (<http://106.37.208.233:20035>). Moreover, the geographical location data was
34
35 161 converted from the detailed home address of participants by Baidu Map. The distance
36
37 162 from each home address of participants to all of the air surveillance stations were
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39 163 calculated. Then the individual daily air pollutants data were obtained according to
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41 164 the nearest surveillance station from home, and we only keep participants who lived
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43 165 within 10km from the nearest surveillance station, which was confirmed by other
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45 166 studies.^{15,19} The median distance from home to the nearest station was 3.50 km in this
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47 167 study. Participants' average air pollutants exposure during the 1st trimester of
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49 168 pregnancy was estimated by mean levels of daily concentrations.

50 169 **Statistical analysis**

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52 170 Observed outcomes were classified as total birth defects, CHDs, limb defects, and
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54 171 orofacial clefts. Maternal exposure variables including PM₁₀, PM_{2.5}, NO₂, and SO₂
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56 172 were divided by interquartile range based on the daily average concentration during
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58 173 the 1st trimester. Covariates including the year of conception (2013-2018), maternal
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60 174 age (<25, 25-29, 30-34, and >34 years), gravidity (1, 2-3, and >3), and urban/rural

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4 175 were obtained. Moreover, other covariates including per capita of cars,
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6 176 unemployment, per capita area of roads, per capita of medication beds were retrieved
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8 177 from the official website of Wuhan Bureau of Statistics (<http://tjj.wuhan.gov.cn/>).
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10 178 Logistic regression analysis was applied to assess the association between maternal
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12 179 air pollutants exposure during 1st trimester and birth defects adjusted for potential
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14 180 covariates. Stratified analyses by neonatal sex (male and female fetus) and season of
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16 181 conception by last menstrual period (March to August and September to February)
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18 182 were applied to further explore the associations between air pollutants exposure in the
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20 183 1st trimester of pregnancy and birth defects. The adjusted odds ratio (OR) and 95%
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22 184 confidence interval (CI) were provided in each model. Statistical analyses were
23
24 185 performed by SAS 9.4 (SAS Institute, Inc., Cary, NC).

186 RESULTS

187 There were a total of 1,352 birth defect cases among 70,854 singletons, with a
188 prevalence of 19.08‰ (Table 1). The prevalence of birth defects increased from
189 21.16‰ in 2013 to 24.08‰ in 2018. Subpopulations who conceived in winter
190 (26.98‰), aged <25 years (28.10‰), or with gravidity >3 times (23.93‰) ranks the
191 first place among corresponding categories. In addition, the male fetuses had a higher
192 prevalence of birth defects than female fetuses (20.57‰ vs 16.80‰).

193
194 **Table 1 Prevalence of birth defects among the subgroups of enrolled participants**
195 **in Wuhan, China**

Variables	Birth defect cases	Total cases	Prevalence (‰)
Year of conception			
2013	272	12,856	21.16
2014	203	11,236	18.07
2015	174	11,274	15.43
2016	227	12,261	18.51
2017	226	12,844	17.60
2018	250	10,383	24.08
Season of conception			
Spring	321	18,312	17.53
Summer	295	18,853	15.65
Autumn	287	17,045	16.84
Winter	449	16,644	26.98
Age (years)			

<25	122	4,341	28.10
25–29	632	34,412	18.37
30–34	401	23,783	16.86
>34	197	8,318	23.68
Gravidity			
1	604	35,590	16.97
2-3	372	19,552	19.03
>3	376	15,712	23.93
Neonatal sex			
Male	771	37,488	20.57
Female	560	33,337	16.80
Total	1,352	70,854	19.08

196

197 Table 2 shows the distributions of daily average ambient air pollutants
 198 concentrations in the 1st trimester among the 5 groups including non-defects, birth
 199 defects, CHDs, limb defects, and orofacial clefts. The median exposures of PM₁₀,
 200 PM_{2.5}, NO₂, and SO₂ during the 1st trimester were 101.78µg/m³, 61.98µg/m³,
 201 53.64µg/m³, and 13.97µg/m³ respectively among the birth defects groups.

202

203 **Table 2 Quartile concentrations (µg/m³) of exposure for ambient air pollutants**
 204 **among birth defects groups in 1st trimester of pregnancy in Wuhan, China**

Air pollutants	Non-defects N=69,502	Birth defects N=1,352	CHDs N=265	Limb defects N=210	Orofacial clefts N=119
PM ₁₀					
Min	36.86	39.08	49.82	40.76	48.85
25th	80.84	84.05	84.05	85.86	83.98
Median	102.42	101.78	99.46	105.80	96.77
75th	123.86	124.39	120.48	127.34	116.36
Max	231.49	225.91	225.91	220.82	191.37
PM _{2.5}					
Min	21.27	21.27	22.77	22.16	22.98
25th	39.37	45.02	44.88	42.63	41.26
Median	59.21	61.98	61.33	63.00	60.98
75th	80.20	80.73	78.81	84.66	77.38
Max	178.20	165.92	154.42	154.79	155.35
NO ₂					
Min	9.71	15.22	29.03	25.78	16.89
25th	43.40	45.70	45.49	44.31	44.09
Median	51.49	53.64	53.69	53.43	53.84
75th	59.08	59.98	59.46	62.84	59.99
Max	93.60	93.37	92.93	93.37	87.58

SO ₂						
Min	2.93	3.02	3.93	3.77	3.99	
25th	9.07	9.36	9.43	10.55	9.46	
Median	14.47	13.97	13.77	16.09	12.55	
75th	23.54	23.87	23.36	25.23	21.28	
Max	71.74	63.36	63.36	62.99	53.75	

205 Abbreviations: CHDs, congenital heart defects; NO₂, nitrogen dioxide; PM₁₀, particulate
206 matter ≤10 µm diameter; PM_{2.5}, particulate matter <2.5 µm diameter; SO₂, sulfur dioxide.

207
208 Table 3 presents the associations between ambient air pollutants exposure in the
209 1st trimester of pregnancy and birth defects. We also provided the odds ratios of birth
210 defects in the 2nd and 3rd trimesters, as well as the entire pregnancy in the
211 supplementary Tables S2-S4. High concentrate exposure to air pollutants was
212 significantly associated with increased odds of total birth defects, yielding adjusted
213 ORs of 1.13, 1.23, 1.18, and 1.19 for PM₁₀, PM_{2.5}, NO₂, and SO₂, respectively.
214 Moreover, similar elevated odds of CHDs was observed for maternal exposure of
215 PM_{2.5} (OR=1.21, 95%CI: 1.06, 1.38), NO₂ (OR=1.13, 95%CI: 1.01, 1.27), and SO₂
216 (OR=1.24, 95%CI: 1.03, 1.48), but not PM₁₀ (1.08, 95% CI: 0.95, 1.24).

217 For male fetuses, there were significantly increased odds of total birth defects
218 among mothers exposed to PM₁₀, PM_{2.5}, NO₂, and SO₂ by interquartile increased
219 concentrations during the 1st trimester (OR=1.13-1.23). Moreover, a positive
220 association between PM_{2.5} exposure during the 1st trimester and CHDs was detected
221 (OR=1.27, 95% CI: 1.06, 1.52). For female fetuses, elevated odds of total birth
222 defects (OR=1.14-1.22) were also observed among maternal exposed to heavy
223 concentrated PM₁₀, PM_{2.5}, NO₂, and SO₂.

224 After stratified by season, the results show that the odds of total birth defects
225 were significantly increased among women who conceived in Autumn/Winter and
226 were exposed to PM_{2.5} (OR=1.64, 95% CI: 1.41, 1.91), NO₂ (OR=1.22, 95% CI: 1.08,
227 1.38), or SO₂ (OR=1.26, 95% CI: 1.07, 1.47). PM_{2.5}, NO₂, and SO₂ were also
228 positively related to CHDs (ORs=1.36-1.84). Elevated hazard of orofacial clefts was
229 observed among women who conceived in the cold season exposed to heavy NO₂
230 concentrations (OR=1.85, 95% CI: 1.16, 2.93). However, in the warm season, the odd

231 of total birth defects was elevated among women exposed to PM₁₀ (OR=1.12, 95%
232 CI: 1.00, 1.24).

233

234 **Table 3 Adjusted odds ratios and 95% confidence interval of ambient air**
235 **pollutants for each interquartile increase during the 1st trimester and birth defects**
236 **in Wuhan, China**

Birth defects ^a	PM ₁₀ OR (95%CI)	PM _{2.5} OR (95%CI)	NO ₂ OR (95%CI)	SO ₂ OR (95%CI)
Total				
Birth defects	1.13(1.07,1.21)	1.23(1.15,1.30)	1.18(1.12,1.24)	1.19(1.10,1.30)
CHDs	1.08(0.95,1.24)	1.21(1.06,1.38)	1.13(1.01,1.27)	1.24(1.03,1.48)
Limb defects	1.11(0.96,1.29)	1.09(0.93,1.26)	1.15(1.01,1.31)	1.16(0.95,1.43)
Orofacial clefts	0.90(0.74,1.10)	1.04(0.85,1.26)	1.10(0.93,1.31)	0.95(0.73,1.22)
Male fetus				
Birth defects	1.13(1.04,1.22)	1.23(1.14,1.34)	1.18(1.10,1.26)	1.19(1.07,1.33)
CHDs	1.16(0.96,1.40)	1.27(1.06,1.52)	1.11(0.95,1.30)	1.18(0.93,1.50)
Limb defects	1.10(0.91,1.33)	1.08(0.90,1.30)	1.15(0.98,1.35)	1.14(0.89,1.47)
Orofacial clefts	0.86(0.66,1.12)	1.09(0.85,1.41)	1.07(0.86,1.34)	0.99(0.71,1.37)
Female fetus				
Birth defects	1.14(1.04,1.25)	1.22(1.12,1.34)	1.19(1.10,1.29)	1.21(1.07,1.37)
CHDs	0.99(0.82,1.20)	1.11(0.91,1.34)	1.14(0.97,1.34)	1.30(1.00,1.71)
Limb defects	1.08(0.84,1.38)	1.15(0.90,1.47)	1.15(0.93,1.43)	1.18(0.84,1.66)
Orofacial clefts	0.98(0.71,1.34)	0.95(0.69,1.30)	1.16(0.88,1.52)	0.88(0.59,1.30)
Autumn/Winter				
Birth defects	1.05(0.92,1.20)	1.64(1.41,1.91)	1.22(1.08,1.38)	1.26(1.07,1.47)
CHDs	1.05(0.79,1.39)	1.84(1.32,2.57)	1.36(1.03,1.80)	1.43(1.00,2.03)
Limb defects	1.19(0.84,1.70)	1.48(0.99,2.22)	1.35(0.97,1.87)	1.06(0.70,1.61)
Orofacial clefts	0.95(0.61,1.47)	1.63(0.98,2.70)	1.85(1.16,2.93)	1.40(0.82,2.40)
Summer/Spring				
Birth defects	1.12(1.00,1.24)	1.06(0.93,1.21)	1.09(1.00,1.19)	1.12(0.98,1.28)
CHDs	0.99(0.77,1.26)	0.89(0.65,1.21)	0.88(0.71,1.08)	1.24(0.92,1.66)
Limb defects	1.18(0.92,1.51)	1.21(0.89,1.63)	1.17(0.95,1.43)	1.23(0.89,1.71)
Orofacial clefts	0.72(0.50,1.03)	0.66(0.41,1.04)	0.88(0.65,1.19)	0.72(0.47,1.10)

237 Bold values represent statistical significance (two-sided $P < 0.05$).

238 Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen
239 dioxide; OR, odds ratio; PM₁₀, particulate matter $\leq 10 \mu\text{m}$ diameter; PM_{2.5}, particulate matter
240 $< 2.5 \mu\text{m}$ diameter; SO₂, sulfur dioxide.

241 ^a Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of
242 roads, per capita of medication beds, unemployment, and per capita of cars.

243

244 DISCUSSION

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4 245 In the current study, the prevalence of birth defects was close to what was reported in
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6 246 Hunan Province (19.18%) and higher than that of Jiangsu Province (7.15%).^{20,21}
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8 247 Moreover, this study examined the associations between birth defects and air
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10 248 pollutants exposure in the 1st trimester, and we further explored the relationships
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12 249 stratified by fetal sex and season of conception.

13
14 250 This study showed that maternal exposure to ambient air pollutants including
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16 251 PM₁₀, PM_{2.5}, NO₂, and SO₂ during the 1st trimester could have higher odds of birth
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18 252 defects, which has been well documented previously. Moreover, we have adopted
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20 253 peak exposure on the thresholds at the 95th percentile to derive the accumulated days
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22 254 of high dose exposure, which further confirmed the positive association between high
23
24 255 dose of NO₂ exposure during the 1st trimester and birth defects (Supplementary Table
25
26 256 5). A case-control study conducted by Al Noaimi et al. showed a positive association
27
28 257 between PM_{2.5} exposure in the 1st trimester of pregnancy and birth defects
29
30 258 (OR=1.05).²² Wang et al. applied Poisson generalized additive models on the
31
32 259 time-series data adjusted for temperature, relative humidity, season, and time trend,
33
34 260 which showed that maternal exposure to PM₁₀ and NO₂ in early pregnancy
35
36 261 significantly increased the risk of birth defects by 10.3% per 10 µg/m³ and 3.4% per
37
38 262 10 µg/m³, respectively.²³

39 263 As was observed among the total participants, the risk of birth defects among
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41 264 both male fetuses and female fetuses was significantly related to PM₁₀, PM_{2.5}, NO₂,
42
43 265 and SO₂. The underlying mechanisms between air pollutants exposure and the
44
45 266 development of birth defects are still unclear. Maternal air pollutants exposures might
46
47 267 cause changes in epigenetic signatures and permanent modifications in gene
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49 268 expression.²⁴ Animal studies showed that maternal exposure to PM_{2.5} during
50
51 269 pregnancy leads to spatial memory dysfunction, neurodevelopmental impairment, and
52
53 270 disturbed cerebral cortex development of mice offspring.^{25,26} Another study showed
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55 271 that maternal exposure to a high concentrate of PM_{2.5} during the 1st trimester of
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57 272 pregnancy could result in the decreased placental expression of *BDNF* and *SYN1*,
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59 273 which may undermine fetal neurodevelopment.²⁷ Moreover, SO₂ is a systemic toxic
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274 agent which can induce chromosomal aberrations, sister chromatid exchanges, and

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4 275 micronuclei in mammalian cells.²⁸

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6 276 Except for PM₁₀, this study demonstrated adverse associations of CHDs with
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8 277 PM_{2.5}, NO₂, and SO₂ exposure. Previous studies in Taiwan and Northeast England
9
10 278 also reported insignificant associations between PM₁₀ exposure in the 1st trimester and
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12 279 CHDs,^{29,30} whereas others confirmed the increased risk of CHDs related with
13
14 280 PM₁₀.^{12,23,31-34} Furthermore, Huang et al. showed that PM_{2.5} exposure per each
15
16 281 interquartile increase during gestational weeks 3-8 was related to an increased risk of
17
18 282 CHDs (OR=1.21).³⁵ Additionally, the positive association between CHDs and
19
20 283 maternal exposure to NO₂ and SO₂ was documented by studies conducted by Baldacci
21
22 284 et al. and Vrijheid et al.^{36,37} Moreover, a case-control study conducted by Jiang et al.
23
24 285 showed that maternal exposure to SO₂ during the 1st trimester was significantly
25
26 286 associated with increased risk of CHD (OR=1.78-2.04), and Hansen et al. also
27
28 287 confirmed that a 0.6 ppb increase in SO₂ was associated with an increased risk of
29
30 288 aortic artery and valve defects (OR=10.76).^{19,38} The heterogeneity in these studies
31
32 289 might be explained by the variation in the population, the gestational periods, or the
33
34 290 measurement of air pollutants exposure. The physiopathological mechanism of the
35
36 291 associations between air pollutants and CHDs was not fully elucidated. It is
37
38 292 hypothesized that air pollutants exposure during the 1st pregnancy might strengthen
39
40 293 the genetic and environmental interaction on CHDs.³⁷ Also, Air pollutants might
41
42 294 change the molecule of DNA sequence or alter epigenetics related to CHDs.³⁹

43 295 In line with this study, the epidemiological difference in CHDs between male
44
45 296 and female fetuses has been reported previously.^{40,41} This disparity could be explained
46
47 297 by the sex chromosome-linked genes expression and their interactions with hormonal
48
49 298 effects during early development.⁴² Interestingly, only male fetuses showed an
50
51 299 elevated risk of CHDs who were exposed to a higher PM_{2.5} concentration. This result
52
53 300 indicates that PM_{2.5} might emphasize the disparity in the embryonic origins of sexual
54
55 301 dimorphism. Moreover, PM_{2.5} might have a stronger effect on the expression of
56
57 302 specific CHDs genes located on the Y chromosome. A study of mice models showed
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59 303 that increased pathological damage in the hearts of offspring mice was observed
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304 among maternal mice exposed to PM_{2.5}, and these effects in the male group were

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4 305 more obvious than in the female group. PM_{2.5} exposure in utero inhibited the
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6 306 expression of the *GATA4* gene in male mice, which was related to the formation of
7
8 307 CHDs.⁴³

9
10 308 After stratified by season, maternal exposure of PM_{2.5}, NO₂, and SO₂ were
11
12 309 positively associated with birth defects in the cold season instead of the warm season.
13
14 310 Zhao et al. reported that the effects of air pollutants on birth defects were more
15
16 311 obvious in the warm season in Hohhot.⁴⁴ This disparity could be partly explained by
17
18 312 the uneven levels of dwellings' air pollutants. Compared to cities with lower GDP,
19
20 313 cities with a higher GDP and a large population might have lower concentrations of
21
22 314 indoor particulate matter.⁴⁵ Moreover, Wuhan is a well-known city with hot summer.
23
24 315 Most of the residential buildings are equipped with air conditioners, which could help
25
26 316 to improve indoor air quality in hot weather. Thus, the interpretation of results should
27
28 317 be cautious that significant associations between air pollutants exposure and birth
29
30 318 defects in the warm season in Wuhan.

31 319 **Strengths**

32
33 320 This study firstly provided evidence on the elevated risk of CHDs among mothers
34
35 321 with heavy PM_{2.5} exposure during the 1st trimester. We also found the increased risks
36
37 322 of CHDs and orofacial clefts among women who conceived in the cold season and
38
39 323 exposed to high concentrations of air pollutants.

40 324 **Limitations**

41
42 325 This study has several limitations. Firstly, the selected participants are located in a
43
44 326 large tertiary maternal care center in Wuhan and the representative of this study was
45
46 327 undermined. Secondly, the birth defects data of this study are manually collected and
47
48 328 checked, but mistakes and omissions are inevitable. Thirdly, maternal air pollutants
49
50 329 exposure indoor or in other living residents including work, dining, and shopping
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52 330 were not included in this study. Fourthly, other risk factors (including ethnicity,
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54 331 smoking, drinking, medications, drug use, and family history et al.) were failed to
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56 332 obtain, which might influence the interpretation of the relationship between maternal
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58 333 air pollutants exposure and birth defects. More research is needed to explore the
59
60 334 pathogenic mechanism of air pollutants exposure during pregnancy and the associated

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4 335 birth defects.

5 336 **CONCLUSIONS**

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7 337 Our study confirmed the unfavorable effects of maternal exposure to air pollutants
8
9 338 (PM₁₀, PM_{2.5}, NO₂, and SO₂) on birth defects during the 1st trimester of pregnancy.
10
11 339 We firstly provided the evidence on the positive associations between PM_{2.5} exposure
12
13 340 and CHDs among male fetuses but not female fetuses. Moreover, stronger effects of
14
15 341 PM_{2.5}, NO₂, and SO₂ exposure on birth defects were observed in the cold season in
16
17 342 Wuhan. As a result, it should be noted for birth defects due to air pollutants, and
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19 343 reducing individual air pollutants exposure during the 1st trimester might help to birth
20
21 344 defect control in the context of the rapid development all over the world. Moreover,
22
23 345 the implementation of air quality protection policies on birth defect control should
24
25 346 consider seasonal factor, especially for the cold season in Wuhan, China. Future
26
27 347 studies of birth defects and air quality data collected by individual air pollutants
28
29 348 monitors are promoted.

30
31 349 **Abbreviations**

32
33 350 CHDs: congenital heart defects; CI: confidence interval; NO₂: nitrogen dioxide; OR:
34
35 351 odds ratio; PM₁₀: particulate matter ≤10 µm diameter; PM_{2.5}: particulate matter ≤2.5
36
37 352 µm diameter; SO₂: sulfur dioxide.

38
39 353 **Ethics approval and consent to participate**

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41 354 This study was approved by the Ethics Committee of Maternal and Child Health
42
43 355 Hospital of Hubei Province (2021IECLW025). This study was based on the
44
45 356 retrospective clinical data without any individual patient identifiers.

46
47 357 **Patient consent for publication**

48
49 358 Not applicable.

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52 359 **Patient and public involvement**

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55 360 Patients or the public were not involved in the design, or conduct, or reporting, or
56
57 361 dissemination plans of our research.

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59 362 **Availability of data and materials**

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4 363 The data used in this study are available from corresponding author on reasonable
5
6 364 request.

7 365 **Competing interests**

8
9 366 The authors declare that they have no competing interests.

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13 369 **Authors' contributions**

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16
17 370 Yao Cheng and Jieyun Yin contributed equally to this manuscript. Yao Cheng
18
19 371 collected the data, performed data analysis, draft and revised the manuscript. Jieyun
20
21 372 Yin collected the data, performed data analysis, and edit the manuscript. Lijun Yang
22
23 373 designed the study, collected the data, and performed data analysis. Man Xu, Xinfeng
24
25 374 Lu, and Wenting Huang collected the data, interpreted the data, and reviewed the
26
27 375 manuscript. Guohong Dai: collected the data, interpreted the data, and revised the
28
29 376 manuscript. Guoqiang Sun: designed the study, collected the data, and supervised the
30
31 377 project. All authors gave final approval of the version to be submitted and agreed to
32
33 378 be accountable for all aspects of the work.

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Table S1 The rank result of the 23 types of common birth defects

Number	Birth defect	N
21	Conjoined twins	0
13	Bladder valgus	1
1	Anencephaly	2
3	Encephalocele	3
20	Gastroschisis	3
8	Small ears (including no ears)	6
10	Esophageal atresia or stenosis	8
2	Spina bifida	12
18	Congenital diaphragmatic hernia	13
19	Omphalocele	18
12	Hypospadias	27
11	Rectoanal atresia or stricture (including anorectal atresia)	30
14	Equinovarus	41
4	Congenital hydrocephalus	45
22	Down's syndrome	79
9	Other malformations of the external ear (except for small ears and no ears)	86
5, 6, 7	Orofacial clefts	119
15, 16, 17	Limb defects	210
23	Congenital heart defects	265

Table S2 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 2^{ed} trimester and birth defects in Wuhan, China

Birth defects ^a	PM ₁₀	PM _{2.5}	NO ₂	SO ₂
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Total				
Birth defects	1.01(0.93,1.09)	0.95(0.89,1.02)	0.99(0.93,1.06)	0.88(0.80,0.97)
CHDs	0.98(0.79,1.22)	0.86(0.72,1.04)	0.94(0.79,1.11)	0.75(0.58,0.97)
Limb defects	1.03(0.87,1.22)	0.95(0.82,1.11)	1.05(0.92,1.21)	1.01(0.83,1.25)
Orofacial clefts	1.27(0.86,1.87)	1.42(1.01,1.99)	1.27(0.93,1.74)	1.38(0.85,2.25)
Male fetus				
Birth defects	1.01(0.91,1.12)	0.95(0.87,1.04)	0.95(0.87,1.03)	0.90(0.79,1.02)
CHDs	0.82(0.61,1.10)	0.69(0.52,0.91)	0.69(0.53,0.88)	0.58(0.41,0.83)
Limb defects	1.12(0.90,1.38)	1.00(0.84,1.20)	1.09(0.92,1.29)	1.09(0.84,1.41)
Orofacial clefts	1.11(0.69,1.81)	1.20(0.79,1.82)	1.25(0.83,1.87)	1.19(0.67,2.12)
Female fetus				
Birth defects	1.01(0.89,1.14)	0.96(0.86,1.07)	1.06(0.96,1.17)	0.87(0.75,1.00)
CHDs	1.21(0.88,1.66)	1.07(0.82,1.39)	1.28(0.99,1.65)	1.00(0.69,1.44)
Limb defects	0.90(0.69,1.18)	0.87(0.68,1.12)	0.99(0.79,1.25)	0.89(0.63,1.25)
Orofacial clefts	1.64(0.80,3.36)	2.02(1.06,3.86)	1.31(0.79,2.18)	2.01(0.75,5.39)
Summer/Spring				
Birth defects	1.04(0.93,1.17)	0.91(0.82,1.01)	1.03(0.94,1.13)	0.92(0.79,1.06)
CHDs	0.99(0.73,1.33)	0.79(0.60,1.04)	1.01(0.78,1.29)	0.64(0.44,0.94)
Limb defects	1.10(0.86,1.41)	0.88(0.69,1.11)	1.06(0.87,1.29)	0.92(0.67,1.25)
Orofacial clefts	1.20(0.64,2.25)	1.47(0.81,2.67)	1.07(0.66,1.76)	1.71(0.74,3.97)
Autumn/Winter				
Birth defects	0.96(0.86,1.08)	1.00(0.91,1.10)	0.97(0.89,1.07)	0.87(0.75,1.00)
CHDs	1.02(0.74,1.40)	1.00(0.76,1.30)	0.93(0.72,1.19)	1.00(0.68,1.47)
Limb defects	0.94(0.74,1.18)	0.99(0.82,1.21)	1.05(0.87,1.28)	1.05(0.77,1.43)
Orofacial clefts	1.30(0.78,2.17)	1.34(0.88,2.03)	1.38(0.90,2.12)	1.08(0.58,2.03)

Bold values represent statistical significance (two-sided P<0.05).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen dioxide; OR, odds ratio; PM₁₀, particulate matter ≤10 μm diameter; PM_{2.5}, particulate matter <2.5 μm diameter; SO₂, sulfur dioxide.

^a Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S3 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the 3rd trimester and birth defects in Wuhan, China

Birth defects ^a	PM ₁₀	PM _{2.5}	NO ₂	SO ₂
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Total				
Birth defects	0.86(0.81,0.92)	0.89(0.84,0.94)	0.90(0.85,0.95)	0.92(0.86,0.98)
CHDs	0.90(0.79,1.03)	0.87(0.77,0.98)	0.89(0.79,1.00)	0.88(0.76,1.01)
Limb defects	0.96(0.83,1.12)	0.90(0.79,1.04)	0.97(0.85,1.10)	1.00(0.85,1.17)
Orofacial clefts	0.78(0.64,0.95)	0.97(0.81,1.16)	0.90(0.76,1.07)	0.89(0.72,1.10)
Male fetus				
Birth defects	0.87(0.80,0.94)	0.88(0.81,0.94)	0.91(0.85,0.97)	0.96(0.88,1.05)
CHDs	0.85(0.72,1.01)	0.88(0.74,1.05)	0.88(0.76,1.03)	0.81(0.67,0.98)
Limb defects	0.93(0.77,1.11)	0.89(0.75,1.05)	0.92(0.79,1.08)	1.03(0.85,1.25)
Orofacial clefts	0.78(0.60,1.00)	0.94(0.75,1.19)	0.93(0.75,1.16)	0.95(0.72,1.25)
Female fetus				
Birth defects	0.86(0.78,0.94)	0.91(0.84,0.99)	0.89(0.83,0.96)	0.86(0.78,0.95)
CHDs	0.93(0.77,1.14)	0.92(0.76,1.10)	0.91(0.77,1.07)	0.98(0.79,1.21)
Limb defects	1.01(0.79,1.30)	0.94(0.74,1.19)	1.05(0.85,1.31)	0.93(0.72,1.21)
Orofacial clefts	0.78(0.57,1.08)	0.99(0.74,1.32)	0.85(0.66,1.10)	0.79(0.56,1.11)
Summer/Spring				
Birth defects	1.04(0.94,1.16)	1.18(1.07,1.31)	1.06(0.98,1.16)	0.96(0.87,1.06)
CHDs	1.07(0.84,1.36)	1.16(0.91,1.46)	1.13(0.94,1.37)	0.84(0.68,1.05)
Limb defects	1.06(0.82,1.37)	0.89(0.66,1.19)	0.97(0.78,1.21)	0.94(0.74,1.19)
Orofacial clefts	0.96(0.66,1.39)	1.37(0.98,1.91)	1.22(0.91,1.64)	0.93(0.67,1.30)
Autumn/Winter				
Birth defects	0.64(0.56,0.73)	0.78(0.70,0.87)	0.74(0.66,0.82)	0.86(0.73,1.00)
CHDs	0.74(0.55,0.99)	0.73(0.57,0.94)	0.65(0.52,0.82)	0.82(0.58,1.15)
Limb defects	0.78(0.57,1.08)	0.82(0.62,1.08)	1.05(0.78,1.41)	1.07(0.73,1.58)
Orofacial clefts	0.78(0.55,1.10)	0.40(0.27,0.60)	0.52(0.38,0.70)	0.62(0.39,0.99)

Bold values represent statistical significance (two-sided P<0.05).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen dioxide; OR, odds ratio; PM₁₀, particulate matter ≤10 μm diameter; PM_{2.5}, particulate matter <2.5 μm diameter; SO₂, sulfur dioxide.

^a Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S4 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for each interquartile increase during the entire pregnancy and birth defects in Wuhan, China

Birth defects ^a	PM ₁₀	PM _{2.5}	NO ₂	SO ₂
	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
Total				
Birth defects	0.85(0.74,0.97)	0.95(0.83,1.09)	1.04(0.95,1.13)	0.84(0.72,0.98)
CHDs	0.91(0.64,1.29)	0.86(0.60,1.23)	1.01(0.80,1.27)	0.60(0.41,0.89)
Limb defects	0.91(0.69,1.21)	0.85(0.64,1.13)	1.14(0.95,1.37)	0.97(0.71,1.34)
Orofacial clefts	0.68(0.36,1.26)	1.16(0.62,2.17)	1.34(0.89,2.04)	1.45(0.70,2.98)
Male fetus				
Birth defects	0.81(0.68,0.97)	0.89(0.75,1.06)	0.99(0.89,1.11)	0.83(0.68,1.02)
CHDs	0.81(0.50,1.31)	0.72(0.44,1.17)	0.69(0.50,0.94)	0.52(0.31,0.89)
Limb defects	0.94(0.66,1.33)	0.75(0.53,1.07)	1.22(0.97,1.53)	0.90(0.61,1.33)
Orofacial clefts	0.69(0.32,1.48)	1.36(0.64,2.87)	1.48(0.86,2.53)	1.08(0.46,2.53)
Female fetus				
Birth defects	0.92(0.75,1.13)	1.06(0.86,1.30)	1.11(0.97,1.27)	0.86(0.68,1.09)
CHDs	1.06(0.62,1.82)	1.05(0.63,1.76)	1.59(1.11,2.28)	0.71(0.40,1.28)
Limb defects	0.86(0.54,1.38)	1.09(0.67,1.79)	1.01(0.75,1.37)	1.12(0.64,1.97)
Orofacial clefts	0.64(0.22,1.84)	0.84(0.28,2.54)	1.16(0.61,2.21)	2.99(0.77,11.70)
Autumn/Winter				
Birth defects	0.92(0.76,1.12)	1.07(0.89,1.28)	1.04(0.93,1.17)	0.98(0.79,1.22)
CHDs	0.94(0.57,1.55)	1.02(0.65,1.60)	1.22(0.91,1.63)	0.65(0.38,1.10)
Limb defects	0.97(0.65,1.45)	0.85(0.57,1.27)	1.06(0.84,1.35)	0.82(0.52,1.29)
Orofacial clefts	0.91(0.34,2.43)	1.54(0.55,4.28)	1.10(0.61,1.98)	3.91(1.17,13.04)
Summer/Spring				
Birth defects	0.74(0.60,0.90)	0.80(0.65,0.97)	1.02(0.89,1.17)	0.73(0.58,0.92)
CHDs	0.76(0.44,1.30)	0.76(0.44,1.32)	0.75(0.52,1.08)	0.61(0.33,1.13)
Limb defects	0.82(0.55,1.23)	0.83(0.55,1.26)	1.26(0.95,1.67)	1.12(0.71,1.77)
Orofacial clefts	0.56(0.24,1.30)	0.99(0.43,2.24)	1.62(0.89,2.95)	0.74(0.29,1.91)

Bold values represent statistical significance (two-sided P<0.05).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO₂, nitrogen dioxide; OR, odds ratio; PM₁₀, particulate matter ≤10 μm diameter; PM_{2.5}, particulate matter <2.5 μm diameter; SO₂, sulfur dioxide.

^a Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

Table S5 Adjusted odds ratios and 95% confidence interval of ambient air pollutants for the duration of peak level exposure during the 1st trimester and birth defects in Wuhan, China

Birth defects ^a	PM10 OR (95%CI)	PM2.5 OR (95%CI)	NO2 OR (95%CI)	SO2 OR (95%CI)
Total				
Birth defects	1.03(0.90,1.17)	1.10(0.94,1.28)	1.40(1.26,1.57)	1.04(0.86,1.27)
CHDs	0.91(0.67,1.23)	1.05(0.74,1.49)	1.39(1.08,1.77)	1.18(0.75,1.85)
Limb defects	1.02(0.74,1.41)	1.24(0.87,1.77)	1.37(1.04,1.81)	1.00(0.64,1.58)
Orofacial clefts	0.67(0.42,1.06)	0.92(0.54,1.54)	1.31(0.91,1.89)	0.90(0.47,1.73)
Male fetus				
Birth defects	1.08(0.90,1.29)	1.04(0.85,1.28)	1.33(1.15,1.54)	0.98(0.75,1.27)
CHDs	1.01(0.67,1.52)	1.03(0.64,1.65)	1.19(0.85,1.67)	1.11(0.59,2.11)
Limb defects	1.07(0.71,1.61)	1.18(0.75,1.85)	1.48(1.05,2.08)	0.85(0.47,1.52)
Orofacial clefts	0.67(0.37,1.22)	0.83(0.41,1.65)	1.17(0.73,1.87)	0.99(0.96,1.02)
Female fetus				
Birth defects	0.99(0.80,1.21)	1.17(0.93,1.47)	1.52(1.29,1.80)	1.08(0.81,1.44)
CHDs	0.78(0.50,1.23)	1.00(0.60,1.65)	1.57(1.10,2.25)	1.09(0.59,2.03)
Limb defects	0.96(0.56,1.63)	1.31(0.74,2.34)	1.21(0.76,1.95)	1.24(0.60,2.56)
Orofacial clefts	0.71(0.35,1.45)	1.01(0.46,2.23)	1.62(0.90,2.91)	1.01(0.39,2.61)
Autumn/Winter				
Birth defects	1.00(0.81,1.23)	1.24(0.99,1.55)	1.18(0.99,1.40)	1.12(0.78,1.61)
CHDs	1.25(0.75,2.08)	1.02(0.63,1.64)	1.46(0.97,2.19)	1.29(0.54,3.08)
Limb defects	1.01(0.59,1.70)	1.46(0.85,2.53)	1.22(0.77,1.94)	1.00(0.46,2.18)
Orofacial clefts	0.77(0.39,1.55)	0.99(0.48,2.03)	2.31(1.15,4.66)	1.48(0.50,4.41)
Summer/Spring				
Birth defects	1.03(0.78,1.36)	1.00(0.61,1.65)	1.23(1.00,1.52)	1.07(0.76,1.51)
CHDs	0.71(0.37,1.39)	0.66(0.16,2.83)	0.77(0.45,1.32)	1.52(0.69,3.37)
Limb defects	1.58(0.81,3.08)	2.17(0.92,5.08)	1.61(1.01,2.57)	0.92(0.42,2.02)
Orofacial clefts	0.35(0.12,1.04)	0.64(0.08,4.94)	0.59(0.25,1.39)	0.68(0.19,2.48)

Bold values represent statistical significance (two-sided P<0.05).

Abbreviations: CHDs, congenital heart defects; CI, confidence interval; NO2, nitrogen dioxide; OR, odds ratio; PM10, particulate matter $\leq 10 \mu\text{m}$ diameter; PM2.5, particulate matter $< 2.5 \mu\text{m}$ diameter; SO2, sulfur dioxide.

^a Adjusted for year of conception, maternal age, gravidity, urban/rural, per capita area of roads, per capita of medication beds, unemployment, and per capita of cars.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(b) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	-
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	6
		(b) Describe any methods used to examine subgroups and interactions	6
		(c) Explain how missing data were addressed	-
		(d) If applicable, explain how loss to follow-up was addressed	-
		(e) Describe any sensitivity analyses	-
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	6-7 - -
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	6-7 - -
Outcome data	15*	Report numbers of outcome events or summary measures over time	6-7
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	- 8 -
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10-11
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.