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Maternal Lead Exposure and Premature Rupture of Membranes: A Birth Cohort Study in China

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-021565
Article Type:	Research
Date Submitted by the Author:	09-Jan-2018
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Keywords:	lead exposure, premature rupture of membranes, maternal urine, birth cohort

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4 **1 Maternal Lead Exposure and Premature Rupture of Membranes: A**
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6 **2 Birth Cohort Study in China**

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41 18 **Running Title:** Lead Exposure and Premature Rupture of Membranes
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46 20 **Keywords:** lead exposure, premature rupture of membranes, maternal urine, birth
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54 23 **Word count:** 2887
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24 Abstract

25 **Objectives** Maternal exposure to lead (Pb) has been suggested to be related with
26 adverse birth outcomes, but evidence of an association between Pb exposure and
27 premature rupture of membranes (PROM) is limited. The aim of our study was to
28 investigate whether maternal Pb exposure was associated with PROM.

29 **Design** Prospective cohort study.

30 **Study population** This study involved 7290 pregnant women from the Healthy Baby
31 Cohort (HBC) in Wuhan, China, during 2012 to 2014.

32 **Main outcome measures** PROM was defined as spontaneous rupture of amniotic
33 membranes before the onset of labor, and was determined with a pH \geq 6.5 for vaginal
34 fluid. Maternal urinary Pb levels were adjusted by creatinine concentrations and its
35 relationship with PROM was analyzed by logistic regression.

36 **Results** The interquartile range of maternal urinary Pb concentrations of the study
37 population was 2.30-5.64 $\mu\text{g/g}$ creatinine with a median of 3.44 $\mu\text{g/g}$ creatinine.
38 Increased risk of PROM was significantly associated with elevated levels of Pb in
39 maternal urine [adjusted odds ratio (OR) = 1.23; 95% confidence interval (CI) =
40 1.02-1.47 for the medium tertile; adjusted OR = 1.51; 95% CI = 1.27-1.80 for the
41 highest tertile]. A higher risk for preterm PROM associated with Pb levels was
42 observed when compared with the lowest tertile (adjusted OR = 1.24; 95% confidence
43 interval (CI) = 0.80-1.92 for the medium tertile; adjusted OR = 1.73; 95% CI =
44 1.15-2.60). In addition, the relationship between Pb and PROM was more pronounced
45 among primiparous women than multiparous women (p for interaction $<$ 0.01).

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4 46 **Conclusions** Our study found that higher levels of maternal Pb exposure was
5
6 47 associated with increased risk of PROM, indicating that exposure to Pb during
7
8 48 pregnancy may be an important risk factor for PROM.
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14 50 **Strengths and limitations of this study**
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- 18 • This study was conducted with a large sample size, which included 7290
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20 mother-singleton pairs from a birth cohort study in China.
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- 23 • All information of the participants was collected from personal interviews and
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25 medical records, which allowed us to adjust for other potential risk factors for PROM.
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- 28 • Although we included many potential confounders for analysis, the potential for
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30 residual confounding cannot be ruled out.
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52 **Introduction**

53 Premature rupture of membranes (PROM), referred to maternal membranes rupture
54 spontaneously before the onset of labor, accounts for approximately 8%-12% in
55 delivers [1 2]. PROM is related to significant maternal, fetal, and neonatal risk, such
56 as maternal infection, prematurity and neonatal sepsis [3 4]. Rupture begins prior to
57 37 weeks of gestation is considered as preterm PROM, which appears in 1%-3% of all
58 pregnancies and occurs in one-third of preterm delivery, and thus is a leading cause
59 for perinatal morbidity and mortality [4 5]. The etiology of PROM has been shown to
60 be multifactorial, but increasing evidence regarding exposure to environmental
61 pollutants has been shown as risk factors for PROM [6-8].

62 Lead (Pb), a ubiquitous non-biodegradable heavy metal that persists in the
63 environment, is widely used in various industries, such as automobiles, paint, batteries,
64 and plastics [9 10]. Due to these industrial processes, Pb has become the most widely
65 scattered toxic heavy metal worldwide [10]. High levels of Pb exposure have been
66 demonstrated to be associated with preeclampsia, pregnancy-induced hypertension,
67 miscarriage, prematurity, congenital abnormalities, and even impaired cognitive
68 function problems in childhood [11-13]. However, the association between maternal
69 Pb exposure and risk of PROM is limited, and the results were inconsistent. Some
70 studies have found significant relationship between the risk of PROM with maternal
71 Pb levels [8 14-16], while a previous study has failed to observe such an association
72 [17].

73 China is the largest raw and refined Pb producing and consuming country around the

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4 74 world [18]. Pb pollution poses a significant threat for human health, especially for
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6 75 pregnant women and fetus. Given this background, the present study involving 7290
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8 76 participants was designed to explore whether Pb exposure during pregnancy could
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11 77 increase the risk of PROM and preterm PROM in Chinese pregnant women.
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16 79 **Materials and Methods**

17 18 80 **Study population and data collection**

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21 81 The study participants (n = 11311) were enrolled during September 2012 to October
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23 82 2014 from the Healthy Baby Cohort (HBC) study at Wuhan Medical and Health
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25 83 Center for Women and Children in China, and the eligibility criteria has been
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27
28 84 described elsewhere [19]. For this study, we excluded women without urine samples
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30 85 (n = 3947), and who delivered an infant with congenital malformation (n = 62), as
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32 86 well as those who reported smoking (n = 7) and drinking (n = 2) during pregnancy.
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35 87 For women who gave birth twice in HBC (n = 3), we excluded the second delivery
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38 88 record (n = 3). Finally, 7290 pregnant women were included for the present study. All
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40 89 participating mothers signed written informed consent at enrollment. The Ethical
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42 90 Committees of Tongji Medical College, Huazhong University of Science and
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45 91 Technology, and the study hospital approved the study protocol.

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47 92 All participants filled out a structural questionnaire after labor during a face-to-face
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50 93 interview by specially trained nurses. Information on the demographic and
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52 94 socioeconomic background, (e.g., maternal age, educational level, and occupational
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55 95 status), pre-pregnancy body mass index (BMI) (calculated on the basis of
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3 96 self-reported weight and height before pregnancy), and daily-life habits during
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6 97 pregnancy (e.g., alcohol and tobacco consumption) were collected during this process.
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9 98 Medical/reproductive histories and outcomes (e.g., maternal diseases, complications
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11 99 and infant sex) were gathered from medical records. Last menstrual period (LMP) was
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13 100 used to calculate maternal gestational week.

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16 101 PROM was defined as spontaneous rupture of amniotic membranes prior to the onset
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18 102 of labor, and was determined with a $\text{pH} \geq 6.5$ for vaginal fluid. Rupture occurred less
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20 103 than 37 weeks' gestation was considered as preterm PROM.
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25 105 **Urine sample collection and lead exposure measurement**

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28 106 Maternal urine samples were collected when they admitted to the hospital while
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30 107 waiting for delivery and were stored immediately in polypropylene tubes at $-20\text{ }^{\circ}\text{C}$ for
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32 108 further treatment. The detection method for urinary Pb was introduced previously [20].
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35 109 Briefly, prior to analysis, urine specimens were thawed at room temperature until
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38 110 treatment. Then, 1 mL of supernatant urine with 4 mL of 3% HNO_3 were added into
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40 111 15 mL polypropylene tubes for overnight nitrification, and were digested by
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42 112 ultrasound for 1 hour at $40\text{ }^{\circ}\text{C}$. After that, inductively coupled plasma mass
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45 113 spectrometry (ICP-MS; Agilent 7700, Agilent Technologies, Waldbronn) was used to
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47 114 measure maternal urinary Pb concentrations. Assessment of the instrument
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50 115 performance was conducted by the Standard Reference Material Human Urine
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52 116 (SRM2670a Toxic Elements in Urine, National Institute of Standards and Technology,
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55 117 USA) as external quality control sample in each batch. The detection rate of maternal
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3 118 urinary Pb concentrations in this study was 99%, and the samples below the limit of
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6 119 detection (LOD) (0.01 µg/L) were replaced by 1/2 LOD. The intra-day coefficient of
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8 120 variation was below 2.0%, and the inter-day coefficient of variation was under 3.0%.

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10 121 Urine creatinine concentrations measured by an automatic biochemical analyzer
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12 122 (BS-200, Mindray, Shenzhen, China), were used for the adjustment of Pb
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14 123 concentrations to control variable urine dilutions. And the adjusted urinary Pb
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16 124 concentrations were presented as µg/g creatinine.
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126 **Statistical analyses**

127 The distribution of Pb concentration was skewed towards right when tested by
128 Kolmogorov–Smirnov normality test. The Wilcoxon signed rank test was used to
129 compare concentrations of Pb between PROM and non-PROM women. To evaluate
130 the association between Pb exposure and PROM, logistic regression analyses were
131 conducted to calculate crude and adjusted odds ratios (ORs) and 95% confidence
132 intervals (CIs). Maternal urinary Pb levels were categorized into tertiles, of which the
133 lowest level was used as the reference. We detected the linear trends of Pb with
134 PROM via modeling the median values of tertiles of Pb concentration as a continuous
135 variable, and used the Wald test to evaluate the statistical significance. The adjustment
136 for potential confounders was based on known factors associated with PROM, such as
137 household income, passive smoking, parity, and pregnancy-induced hypertension [3 5
138 21-23]. Additionally, covariates that altered parameter estimate of Pb for the effect of
139 PROM by over 10% were also included in the final model. Covariates, including

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3 140 maternal age, family income, pre-pregnancy BMI, parity, passive smoking and
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6 141 pregnancy-induced hypertension, were adjusted for in this analysis. We also
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8 142 performed a sensitivity analysis excluding participants with intrauterine infection
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10 143 during pregnancy in consideration of their potential influence on PROM [24 25].
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13 144 Besides, we analyzed the ORs for PROM stratified by maternal parity (primiparous vs.
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15 145 multiparous), because of the difference in these variables has been previously reported
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17 146 to be related with PROM [23]. An interaction term was added into the model to assess
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19 147 the effect of Pb and maternal parity on the outcome of PROM.
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23 148 All data analyses were performed by SAS (version 9.4; SAS Institute Inc., NC,
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25 149 USA), and two-sided *p* values below 0.05 were considered statistically significant.
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30 151 **Results**

31
32 152 **Table 1** presents the basic characteristics and urinary Pb concentrations of 7290
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34 153 participants. In this study, the prevalence of PROM and preterm PROM was 12.1%
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36 154 and 2%, respectively. Maternal age at labor ranged 18-46 years with 28 years on
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38 155 average. Most of the mothers were primiparous (84.5%), and had a high educational
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40 156 attainment (> 12 years) (67.2%), high annual family income (\geq 50,000 per year)
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42 157 (56.9%), and normal pre-pregnancy BMI (18.5-23.9 kg/m²) (66.2%). The average
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44 158 gestational age at delivery was 39.2 weeks. About 22.9 % were passively exposed to
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46 159 smoking during pregnancy. Approximately 3.9% of the pregnant women had
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48 160 hypertension during pregnancy, and 53.4% of the mothers gave birth to a male infant.
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53 161 The median of maternal urinary Pb concentrations in PROM mothers (3.88 μ g/g
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4 162 creatinine) was higher than that of non-PROM mothers (median = 3.39 $\mu\text{g/g}$
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6 163 creatinine) ($p < 0.05$). Compared with women without preterm PROM (median = 3.43
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8 164 $\mu\text{g/g}$ creatinine), the median of maternal urinary Pb concentrations in preterm PROM
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11 165 mothers was also higher (median = 3.96 $\mu\text{g/g}$ creatinine) ($p < 0.05$).

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13 166 **Table 2** shows the relationships of tertiles of creatinine-adjusted Pb levels in
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15 167 maternal urine with PROM and preterm PROM. Compared with the lowest tertile, a
16
17 168 significantly positive association of PROM with increasing levels of maternal urinary
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19 169 Pb concentrations was observed (adjusted OR = 1.23; 95% CI = 1.02-1.47 for the
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21 170 medium tertile; adjusted OR = 1.51; 95% CI = 1.27-1.80 for the highest tertile) (p
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23 171 trend < 0.01). A higher risk estimate for preterm PROM in association with Pb levels
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25 172 was found when compared with the lowest tertile (adjusted OR = 1.24; 95% CI =
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27 173 0.80-1.92 for the medium tertile; adjusted OR = 1.73; 95% CI = 1.15-2.60 for the
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29 174 highest tertile) (p trend < 0.01). In addition, the results of the sensitivity analysis
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31 175 (Supplementary material, **Table S1**) excluding subjects with intrauterine infection
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33 176 during pregnancy demonstrated an essentially unchanged associations of Pb with
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35 177 PROM and preterm PROM.

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37 178 **Table 3** presents the results stratified by maternal parity. Among the 6159
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39 179 primiparous women, we observed a significant trend in elevated Pb concentrations
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41 180 and increased risk of PROM (adjusted OR = 1.24; 95% CI = 1.03-1.50 for the
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43 181 medium tertile; adjusted OR = 1.52; 95% CI = 1.27-1.83 for the highest tertile) (p
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45 182 trend < 0.01). However, no statistical significant association was found between
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47 183 PROM and Pb in multiparous mothers (adjusted OR = 1.21; 95% CI = 0.65-2.25 for
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4 184 the medium tertile; adjusted OR = 1.57; 95% CI = 0.87-2.83 for the highest tertile) (*p*
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6 185 trend = 0.13). The risk estimates for PROM women in relation to Pb levels in those
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8 186 who were primiparous and who were multiparous was significantly different (*p* for
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11 187 interaction < 0.01).

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14 15 16 189 **Discussion**

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18 190 Our study examined the association between maternal urinary Pb exposure before
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20 191 delivery and risk of PROM in Chinese pregnant women, and we found that increased
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22 192 concentrations of urinary Pb were significantly positively related to PROM and
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24 193 preterm PROM. Besides, the results suggested that the relationship between Pb and
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26 194 PROM may vary by maternal parity, since it appeared more pronounced in
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28 195 primiparous women than in multiparous women.

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32 196 PROM, especially preterm PROM, is the leading factor contributing to preterm birth
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34 197 and neonatal complications, such as perinatal infections, respiratory distress syndrome,
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36 198 umbilical cord compression, intraventricular hemorrhage, sepsis and even death [4 5].
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38 199 Furthermore, it has been linked with long-term harmful neurodevelopmental
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41 200 consequence for neonatal development [26]. Although there is limited understanding
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43 201 for the cause of PROM currently, there are growing evidences recently suggesting that
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45 202 environmental factors play important roles in inducing PROM by stimulating
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47 203 oxidative stress and inflammation that predispose premature membranes rupture [6 7].
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52 204 In this study, we observed that maternal exposure to higher levels of Pb before
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54 205 delivery was correlated with increased risk of PROM and preterm PROM. After we
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4 206 excluded the subjects with intrauterine infection during pregnancy, which has been
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6 207 implicated as one of the major factors in the pathogenesis of PROM [5], we also
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8 208 observed an unchanged significant association of Pb with PROM and preterm PROM.
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10 209 Consistent with our findings, a study including 332 pregnant women in Iran reported
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12 210 that one unit increase in logarithm of maternal blood Pb concentration was associated
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14 211 with a several-fold risk of PROM [8]. Similarly, a study involving 502 pregnant
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16 212 mothers in Columbia found that higher blood levels was associated with an increase in
17
18 213 the incidence of PROM [14]. Besides, elevated Pb concentrations in the umbilical
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20 214 cord were reported to be associated with increased PROM risk in a cohort study of
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22 215 749 mother-infant pairs in a Pb-smelter community in South Australia [15].
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24 216 Additionally, in a study including 89 mother-infant pairs in the southeast of Spain,
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26 217 Falcon et al. observed a higher placental Pb concentration in the PROM cases than
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28 218 that in the normal deliveries [16]. In contrast, an early study demonstrated that there
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30 219 was no correlation between the risk of PROM delivery with blood Pb concentrations
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32 220 measured in 635 samples from the umbilical cord blood [17]. The discrepancy in
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34 221 results may be due to the differences in Pb exposure levels.

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36 222 PROM has been reported to be associated with multiple factories, including cigarette
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38 223 smoking, low income, parity, infection, and pregnancy-induced hypertension [3 5
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40 224 21-23]. In the present study, inclusion of the potential confounding factors for
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42 225 adjustment did not attenuate the association between increased levels of Pb exposure
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44 226 and risk of PROM. In the stratified analysis by parity, our results suggested that there
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46 227 was a significant difference in parity for the risk of PROM related with maternal Pb
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4 228 exposure. A significantly positive association between PROM and urinary Pb
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6 229 concentrations was observed in primiparous mothers. A similar positive relationship
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8 230 of Pb exposure with PROM delivery was also found in multiparous mothers, though
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10 231 the results were not significant. The possible explanation may attribute to the
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12 232 insufficient sample size of multiparous mothers. Future researches with more
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14 233 multiparous women are warranted to further evaluate this parity difference in the
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16 234 effect of Pb on PROM.

17
18 235 The etiology and mechanism of Pb effect on PROM are not clear. One possible
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20 236 explanation is that Pb can induce toxicity by triggering oxidative stress through the
21
22 237 generation of reactive oxygen species (ROS) [9 27], which is responsible for the
23
24 238 structural weakness of collagen fibrils and causes the membranes losing strength and
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26 239 elasticity [28 29]. As a result, Pb-induced ROS contributing to membrane rupture
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28 240 occurs via the damage of collagen in fetal membranes [8]. Furthermore, findings have
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30 241 shown that Pb can induce an increase in pro-inflammatory cytokines, like TNF α [30],
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32 242 inflammatory responses may be implicated and predisposed the membranes to rupture
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34 243 by promoting alterations of membrane fluidity and impairing membrane barrier
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36 244 function [31].

37
38 245 Urinary Pb has been favored as a long-term biomarker of exposure, and is widely
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40 246 used in the assessment Pb exposure levels [32 33]. In the present study, maternal
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42 247 urinary Pb concentration [arithmetic mean (AM) = 7.40 $\mu\text{g/g}$ creatinine; geometric
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44 248 mean (GM) = 3.69 $\mu\text{g/g}$ creatinine; median = 3.44 $\mu\text{g/g}$ creatinine] was higher than
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46 249 pregnant mothers reported in developed countries, like Australia (AM = 0.87 $\mu\text{g/g}$

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4 250 creatinine; median = 0.7µg/g creatinine) [34], Japan (GM = 0.48µg/g creatinine)
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6 251 [35],and American (GM = 0.63µg/L) [36]. However, there was also an overlap in the
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8 252 concentration of urinary Pb in our study participants with other countries, like Spain
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10 253 (AM = 5.2 µg/g creatinine; median = 3.9 µg/g creatinine) [37]. In addition, Pb
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12 254 concentration in the present subjects was lower in comparison with pregnant women
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14 255 reported in the developing country, like Nigeria (AM = 28.5 µg/g creatinine) [38].
15
16 256 Although Pb petrol has been phased out since 2000 in China, Pb pollution remains a
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18 257 huge challenge for the environment, since large amounts of Pb pollutants from various
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20 258 sources of Pb consumption increase rapidly, due to the unprecedented development of
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22 259 Chinese economy [39 40]. In the past decades, elevated accumulations of Pb have
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24 260 been reported to be a great threat existed in soil and dust in many provinces of China
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26 261 [18 40]. Pb contamination has become a public health concern for human health in
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28 262 China, since Pb can enter into the body of the humans from atmosphere and soil by
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30 263 drinking water, ingesting food, and inhaling air that contains Pb contaminants [10 40].
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32 264 As a consequence, excessive Pb emission in China poses serious adverse health
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34 265 effects for humans, especially for the susceptible pregnant women.
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43 266 The strength of this study is as follows: First, it was conducted with a large sample
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45 267 size, which included 7290 mother-singleton pairs from a birth cohort study in China.
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47 268 We preformed sensitivity analyses and stratified analyses to evaluate the relationship
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49 269 of maternal Pb exposure with PROM and found a significant association between
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51 270 increased levels of Pb exposure and elevated risk of PROM. Moreover, all
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53 271 information about the participants such as demographic characteristics,
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4 272 socioeconomic status and pregnancy outcomes were gathered from personal
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6 273 interviews and medical records, which made it possible for us to adjust for other
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8 274 potential risk factors for PROM. However, there were still some other potential
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10 275 confounders that we may not be able to control. In addition, urinary Pb collected and
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12 276 measured at labor may fail to accurately reflect the whole period of maternal Pb
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14 277 exposure during pregnancy, further studies from multiple time points and different
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16 278 populations are needed to confirm the observed relationship between Pb and PROM.
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279

280 **Conclusion**

281 In this study, we observed a positive relationship between maternal urinary Pb and
282 risk of PROM, indicating that maternal exposure to Pb may be a potential risk factor
283 for PROM. This finding suggests that appropriate public health measures needed to be
284 taken to control maternal Pb exposure during pregnancy.
285

286 **Footnotes**

287 SH and WX contributed equally to this work.

288 **Contributors:** SH carried out the statistical analyses and drafted the manuscript.

289 WX assisted in the statistical analyses, critically reviewed and revised the manuscript.

290 BZ, TC, SX contributed to the study design and developed the initial protocol. YL

291 contributed to the study design, critically reviewed and revised the manuscript. All

292 authors read and approved the final manuscript.

293 **Funding:** This work was supported by the National Natural Science Foundation of

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4 294 China (91743103, 91643207, 21437002, 81372959, and 81402649), the National Key
5
6 295 Research and Development Plan (2016YFC0206700, 2016YFC0206203), and the
7
8 296 Fundamental Research Funds for the Central Universities HUST (2016YXZD043,
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11 297 2015ZDTD047).

12
13 298 **Competing interests:** None declared.

14
15 299 **Ethics approval:** The Ethical Committees of Tongji Medical College, Huazhong
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18 300 University of Science and Technology, and the study hospital approved the study
19
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21 301 protocol.

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23 302 **Provenance and peer review:** Not commissioned; externally peer reviewed.

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25 303 **Data sharing statement:** Extra data is available by emailing to the corresponding
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28 304 author at liyuanyuan@hust.edu.cn.

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306 **References**

- 307 1. Ural SH, Nagey DA. Premature Rupture of Membranes. Topics in Obstetrics &
308 Gynecology 1998;**18**(19):1-3
- 309 2. ACOG Practice Bulletin No. 80: premature rupture of membranes. Clinical
310 management guidelines for obstetrician-gynecologists. Obstetrics and
311 gynecology 2007;**109**(4):1007-19 doi:
312 10.1097/01.AOG.0000263888.69178.1f[published Online First: Epub Date]].
- 313 3. Poma PA. Premature rupture of membranes. Journal of the National Medical
314 Association 1996;**88**(1):27
- 315 4. Mercer BM. Preterm premature rupture of the membranes. Obstetrics and
316 gynecology 2003;**101**(1):178-93
- 317 5. Simhan HN, Canavan TP. Preterm premature rupture of membranes: diagnosis,
318 evaluation and management strategies. BJOG: An International Journal of
319 Obstetrics & Gynaecology 2005;**112**:32-37 doi:
320 10.1111/j.1471-0528.2005.00582.x[published Online First: Epub Date]].
- 321 6. Wallace ME, Grantz KL, Liu D, et al. Exposure to Ambient Air Pollution and
322 Premature Rupture of Membranes. American journal of epidemiology
323 2016;**183**(12):1114-21 doi: 10.1093/aje/kwv284[published Online First: Epub
324 Date]].
- 325 7. Dadvand P, Basagana X, Figueras F, et al. Air pollution and preterm premature
326 rupture of membranes: a spatiotemporal analysis. American journal of
327 epidemiology 2014;**179**(2):200-7 doi: 10.1093/aje/kwt240[published Online

- 1
2
3
4 328 First: Epub Date]].
- 5
6 329 8. Vige M, Yokoyama K, Shinohara A, et al. Early pregnancy blood lead levels and
7
8 330 the risk of premature rupture of the membranes. *Reproductive toxicology*
9
10 331 (Elmsford, NY) 2010;**30**(3):477-80 doi:
11
12 332 10.1016/j.reprotox.2010.05.007[published Online First: Epub Date]].
- 13
14
15 333 9. Flora G, Gupta D, Tiwari A. Toxicity of lead: a review with recent updates.
16
17 334 *Interdiscip Toxicol* 2012;**5**(2):47-58
- 18
19
20 335 10. Cheng H, Hu Y. Lead (Pb) isotopic fingerprinting and its applications in lead
21
22 336 pollution studies in China: A review. *Environmental Pollution*
23
24 337 2010;**158**(5):1134-46 doi:
25
26 338 <http://dx.doi.org/10.1016/j.envpol.2009.12.028>[published Online First: Epub
27
28 339 Date]].
- 29
30
31 340 11. Winder C. Lead, reproduction and development. *Neurotoxicology*
32
33 341 1993;**14**(2-3):303-17
- 34
35
36 342 12. O'Halloran K, Spickett JT. The interaction of lead exposure and pregnancy.
37
38 343 *Asia-Pacific journal of public health* 1992;**6**(2):35-9
- 39
40
41 344 13. Needleman H. Lead poisoning. *Annu Rev Med* 2004;**55**:209-22
- 42
43
44 345 14. Fahim MS, Fahim Z, Hall DG. Effects of subtoxic lead levels on pregnant women
45
46 346 in the state of Missouri. *Res Commun Chem Pathol Pharmacol*
47
48 347 1976;**13**(2):309-31
- 49
50
51 348 15. Baghurst PA, Robertson EF, Oldfield RK, et al. Lead in the placenta, membranes,
52
53 349 and umbilical cord in relation to pregnancy outcome in a lead-smelter
54
55
56
57
58
59
60

- 1
2
3
4 350 community. Environmental health perspectives 1991;**90**:315-20
5
6 351 16. Falcon M, Vinas P, Luna A. Placental lead and outcome of pregnancy. Toxicology
7
8 352 2003;**185**(1-2):59-66
9
10
11 353 17. Angell NF, Lavery JP. The relationship of blood lead levels to obstetric outcome.
12
13 354 American journal of obstetrics and gynecology 1982;**142**(1):40-6
14
15
16 355 18. Chen HY, Li AJ, Finlow DE. The lead and lead-acid battery industries during 2002
17
18 356 and 2007 in China. Journal of Power Sources 2009;**191**(1):22-27 doi:
19
20 357 <http://dx.doi.org/10.1016/j.jpowsour.2008.12.140>[published Online First:
21
22 Epub Date]].
23
24
25 359 19. Yang J, Huo W, Zhang B, et al. Maternal urinary cadmium concentrations in
26
27 relation to preterm birth in the Healthy Baby Cohort Study in China. Environ
28
29 Int 2016;**94**:300-6 doi: 10.1016/j.envint.2016.06.003[published Online First:
30
31 361 Epub Date]].
32
33 362
34
35 363 20. Xia W, Du X, Zheng T, et al. A Case-Control Study of Prenatal Thallium Exposure
36
37 and Low Birth Weight in China. Environmental health perspectives
38
39 364 2016;**124**(1):164-9 doi: 10.1289/ehp.1409202[published Online First: Epub
40
41 365 Date]].
42
43 366
44
45 367 21. Lee T, Silver H. Etiology and epidemiology of preterm premature rupture of the
46
47 368 membranes. Clinics in perinatology 2001;**28**(4):721-34
48
49
50 369 22. Zhou Q, Zhang W, Xu H, et al. Risk factors for preterm premature rupture of
51
52 370 membranes in Chinese women from urban cities. International journal of
53
54 371 gynaecology and obstetrics: the official organ of the International Federation

- 1
2
3
4 372 of Gynaecology and Obstetrics 2014;**127**(3):254-9 doi:
5
6 373 10.1016/j.ijgo.2014.06.020[published Online First: Epub Date]].
7
8 374 23. Naeye RL. Factors that predispose to premature rupture of the fetal membranes.
9
10 375 Obstetrics and gynecology 1982;**60**(1):93-8
11
12
13 376 24. Parry S, Strauss JF. Premature Rupture of the Fetal Membranes. New England
14
15 377 Journal of Medicine 1998;**338**(10):663-70 doi:
16 378 doi:10.1056/NEJM199803053381006[published Online First: Epub Date]].
17
18
19
20 379 25. Simmons LE, Rubens CE, Darmstadt GL, et al. Preventing Preterm Birth and
21
22 380 Neonatal Mortality: Exploring the Epidemiology, Causes, and Interventions.
23
24 381 Seminars in Perinatology 2010;**34**(6):408-15 doi:
25
26 382 <http://dx.doi.org/10.1053/j.semperi.2010.09.005>[published Online First: Epub
27
28 383 Date]].
29
30
31
32 384 26. Clark EA, Varner M. Impact of preterm PROM and its complications on long-term
33
34 385 infant outcomes. Clinical obstetrics and gynecology 2011;**54**(2):358-69
35
36
37 386 27. Hsu PC, Liu MY, Hsu CC, et al. Lead exposure causes generation of reactive
38
39 387 oxygen species and functional impairment in rat sperm. Toxicology
40
41 388 1997;**122**(1-2):133-43
42
43
44 389 28. Moore RM, Mansour JM, Redline RW, et al. The physiology of fetal membrane
45
46 390 rupture: insight gained from the determination of physical properties. Placenta
47
48 391 2006;**27**(11-12):1037-51 doi: 10.1016/j.placenta.2006.01.002[published
49
50 392 Online First: Epub Date]].
51
52
53
54 393 29. Wall PD, Pressman EK, Woods JR, Jr. Preterm premature rupture of the
55
56
57
58
59
60

- 1
2
3
4 394 membranes and antioxidants: the free radical connection. Journal of perinatal
5
6 395 medicine 2002;**30**(6):447-57 doi: 10.1515/jpm.2002.071[published Online
7
8 396 First: Epub Date]].
- 10
11 397 30. Ghareeb DA, Hussien HM, Khalil AA, et al. Toxic effects of lead exposure on the
12
13 398 brain of rats: Involvement of oxidative stress, inflammation,
14
15 399 acetylcholinesterase, and the beneficial role of flaxseed extract. Toxicological
16
17 & Environmental Chemistry 2010;**92**(1):187-95
18
19
- 20
21 401 31. Gervasi MT, Chaiworapongsa T, Naccasha N, et al. Maternal intravascular
22
23 402 inflammation in preterm premature rupture of membranes. The Journal of
24
25 403 Maternal-Fetal & Neonatal Medicine 2002;**11**(3):171-75 doi:
26
27 404 10.1080/jmf.11.3.171.175[published Online First: Epub Date]].
- 28
29
30 405 32. Barbosa Jr F, Tanus-Santos JE, Gerlach RF, et al. A critical review of biomarkers
31
32 406 used for monitoring human exposure to lead: advantages, limitations, and
33
34 407 future needs. Environmental health perspectives 2005:1669-74
35
36
37 408 33. Abadin H, Ashizawa A, Stevens YW, et al. Agency for Toxic Substances and
38
39 409 Disease Registry (ATSDR) Toxicological Profiles. Toxicological Profile for
40
41 410 Lead. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US),
42
43 411 2007.
44
45
46
- 47 412 34. Hinwood AL, Callan AC, Ramalingam M, et al. Cadmium, lead and mercury
48
49 413 exposure in non smoking pregnant women. Environmental research
50
51 414 2013;**126**:118-24 doi:
52
53 <http://dx.doi.org/10.1016/j.envres.2013.07.005>[published Online First: Epub
54
55
56
57
58
59
60

- 1
2
3
4 416 Date]].
- 5
6 417 35. Shirai S, Suzuki Y, Yoshinaga J, et al. Maternal exposure to low-level heavy
7
8 418 metals during pregnancy and birth size. *Journal of Environmental Science and*
9
10
11 419 *Health Part A* 2010;**45**(11):1468-74
- 12
13 420 36. Jain RB. Effect of pregnancy on the levels of urinary metals for females aged 17–
14
15 421 39 years old: data from National Health and Nutrition Examination Survey
16
17
18 422 2003–2010. *Journal of Toxicology and Environmental Health, Part A*
19
20 423 2013;**76**(2):86-97
- 21
22
23 424 37. Fort M, Cosín-Tomás M, Grimalt JO, et al. Assessment of exposure to trace metals
24
25 425 in a cohort of pregnant women from an urban center by urine analysis in the
26
27 426 first and third trimesters of pregnancy. *Environmental Science and Pollution*
28
29 427 *Research* 2014;**21**(15):9234-41
- 30
31
32 428 38. Adekunle IM, Ogundele JA, Oguntoke O, et al. Assessment of blood and urine
33
34 429 lead levels of some pregnant women residing in Lagos, Nigeria.
35
36 430 *Environmental Monitoring and Assessment* 2010;**170**(1):467-74 doi:
37
38 431 10.1007/s10661-009-1247-4[published Online First: Epub Date]].
- 39
40
41 432 39. Yan CH, Xu J, Shen XM. Childhood lead poisoning in China: challenges and
42
43 433 opportunities. *Environmental health perspectives* 2013;**121**(10):A294
- 44
45
46 434 40. Duzgoren-Aydin NS. Sources and characteristics of lead pollution in the urban
47
48 435 environment of Guangzhou. *Science of The Total Environment* 2007;**385**(1–
49
50 436 3):182-95 doi: <http://dx.doi.org/10.1016/j.scitotenv.2007.06.047>[published
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53 437 Online First: Epub Date]]

Table 1 Basic characteristics and urinary Pb concentrations ($\mu\text{g/g}$ creatinine) of the 7290 pregnant women.

Characteristics	N (%)	Median (IQR) Pb ($\mu\text{g/g}$ creatinine)
Total	7290	3.44 (2.30-5.64)
Maternal age (years)		
< 25	805 (11.0)	3.37 (2.27-5.52)
25–29	3985 (54.7)	3.49 (2.32-5.77)
30–34	2011 (27.6)	3.40 (2.29-5.47)
≥ 35	489 (6.7)	3.41 (2.15-5.94)
Education background (years)		
≤ 9	1001 (13.7)	3.45 (2.40-5.63)
9–12	1389 (19.1)	3.47 (2.35-5.56)
> 12	4898 (67.2)	3.42 (2.26-5.66)
Missing	2 (0.03)	5.69(3.50-7.90)
Family income(yuan/year)		
< 50,000	3021 (41.4)	3.52 (2.31-5.75)
$\geq 50,000$	4148 (56.9)	3.39 (2.28-5.60)
Missing	121 (1.7)	3.50 (2.38-5.83)
Parity		
primiparous	6159 (84.5)	3.45 (2.30-5.66)
multiparous	1131 (15.5)	3.38 (2.26-5.59)
Pre-pregnancy BMI (kg/m^2)		
< 18.5	1527 (20.95)	3.47 (2.27-5.77)
18.5–23.9	4832 (66.28)	3.41 (2.29-5.56)
≥ 24	910 (12.48)	3.60 (2.39-6.04)
Missing	21 (0.29)	3.87 (2.07-8.12)
Passive smoking during pregnancy		

Yes	1670 (22.9)	3.30 (2.29-5.32)
No	5620 (77.1)	3.47 (2.30-5.71)
Pregnancy-induced hypertension		
Yes	286 (3.9)	3.26 (2.18-5.21)
No	7004 (96.1)	3.44 (2.30-5.66)
Intrauterine infection		
Yes	987 (13.5)	3.59 (2.41-6.04)
No	6303 (86.5)	3.42 (2.28-5.60)
Gestational age (weeks)		
< 37	291 (4.0)	3.99 (2.62-7.54)
≥ 37	6999 (96.0)	3.42 (2.29-5.60)
Infant gender		
Male	3890 (53.4)	3.48 (2.34-5.78)
Female	3400 (46.6)	3.40 (2.25-5.52)
PROM		
Yes	881 (12.1)	3.88 (2.55-6.59)
No	6409 (87.9)	3.39 (2.27-5.55)
Preterm PROM		
Yes	147 (2.0)	3.96 (2.63-7.67)
No	7143 (98.0)	3.43 (2.29-5.61)

Abbreviation: BMI, body mass index; IQR, interquartile range; PROM, Premature

Rupture of Membranes.

Table 2 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine.

Pb ($\mu\text{g/g}$ creatinine)	case	OR ^a (95% CI)	OR ^b (95% CI)
PROM			
Tertile1 (< 2.65)	241	1.00	1.00
Tertile2 (2.65–4.70)	291	1.24 (1.03, 1.48)	1.23 (1.02, 1.47)
Tertile3 (\geq 4.70)	349	1.52 (1.27, 1.81)	1.51 (1.27, 1.80)
<i>p</i> for trend		< 0.01	< 0.01
Preterm PROM			
Tertile1 (< 2.65)	37	1.00	1.00
Tertile2 (2.65–4.70)	46	1.25 (0.81, 1.93)	1.24 (0.80, 1.92)
Tertile3 (\geq 4.70)	64	1.74 (1.16, 2.62)	1.73 (1.15, 2.60)
<i>p</i> for trend		< 0.01	< 0.01

Abbreviations: OR, odds ratio; CI, confidence interval.

^a Unadjusted odds ratio.

^b Adjusted for maternal age, family income, pre-BMI, parity, passive smoking and pregnancy-induced hypertension.

Table 3 Risk of PROM associated with the levels of Pb in maternal urine, stratified by parity.

Pb levels ^a (µg/g creatinine)	Primiparous (n = 6159)			Multiparous (n = 1131)			<i>p</i> for interaction
	N	OR ^b (95% CI)	OR ^c (95% CI)	N	OR ^b (95% CI)	OR ^c (95% CI)	
T1	220	1.00	1.00	20	1.00	1.00	< 0.01
T2	268	1.25 (1.04, 1.51)	1.24 (1.03, 1.50)	24	1.21 (0.66, 2.23)	1.21 (0.65, 2.25)	
T3	318	1.52 (1.27, 1.83)	1.52 (1.27, 1.83)	31	1.59 (0.89, 2.84)	1.57 (0.87, 2.83)	
<i>P</i> for trend		< 0.01	< 0.01		0.11	0.13	

Abbreviations: OR, odds ratio; CI, confidence interval; T: tertile;

^a Pb levels: primiparous, T1 (< 2.66), T2 (2.66-4.71), T3 (≥ 4.71); multiparous, T1 (< 2.61), T2 (2.61-4.61), T3 (≥ 4.61).

^b Unadjusted odds ratio.

^c Adjusted for maternal age, family income, pre-BMI, passive smoking and pregnancy-induced hypertension.

Table S1 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine, after excluding intrauterine infection during pregnancy (n = 6303)

Pb ($\mu\text{g/gcreatinine}$)	case	OR ^a (95% CI)	OR ^b (95% CI)
PROM			
Tertile1 (< 2.63)	208	1.00	1.00
Tertile2 (2.63–4.65)	257	1.27 (1.04, 1.54)	1.26 (1.04, 1.53)
Tertile3 (\geq 4.65)	302	1.52 (1.26, 1.84)	1.51 (1.25, 1.83)
<i>p</i> for trend		< 0.01	< 0.01
preterm PROM			
Tertile1 (< 2.63)	34	1.00	1.00
Tertile2 (2.63–4.65)	37	1.09 (0.68, 1.74)	1.07 (0.67, 1.71)
Tertile3 (\geq 4.65)	56	1.66 (1.08, 2.55)	1.64 (1.07, 2.53)
<i>p</i> for trend		0.011	0.015

Abbreviations: OR, odds ratio; CI, confidence interval.

^a Unadjusted odds ratio.

^b Adjusted for maternal age, family income, pre-BMI, parity, passive smoking and pregnancy-induced hypertension.

BMJ Open

Maternal Lead Exposure and Premature Rupture of Membranes: A Birth Cohort Study in China

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-021565.R1
Article Type:	Research
Date Submitted by the Author:	09-May-2018
Complete List of Authors:	Huang, Sha; Huazhong University of Science and Technology Tongji Medical College Xia, Wei ; Huazhong University of Science and Technology Tongji Medical College Sheng, Xia; Huazhong University of Science and Technology Tongji Medical College, School of Public Health Qiu, Lin; Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science and Technology Zhang, Bin; Wuhan Medical and Healthcare Center for Women and Children Chen, Tian ; Huazhong University of Science and Technology Tongji Medical College Xu, Shunqing; Tongji Medical College, Huazhong University of Science and Technology, Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, and State Key Laboratory of Environmental Health, School of Public Health Li, Yuanyuan
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Epidemiology, Occupational and environmental medicine, Public health
Keywords:	lead exposure, premature rupture of membranes, maternal urine, birth cohort

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Manuscripts

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4 **1 Maternal Lead Exposure and Premature Rupture of Membranes: A**
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6 **2 Birth Cohort Study in China**

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8 3 Sha Huang¹, Wei Xia¹, Xia Sheng¹, Lin Qiu¹, Bin Zhang², Tian Chen¹, Shunqing Xu¹,
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44 **19 Running Title:** Lead Exposure and Premature Rupture of Membranes

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47 **20 Keywords:** lead exposure, premature rupture of membranes, maternal urine, birth
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51 **22 Word count:** 3500

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23 **Abstract**

24 **Objectives** Maternal exposure to lead (Pb) has been suggested to correlate with
25 adverse birth outcomes, but evidence supporting an association between Pb exposure
26 and premature rupture of membranes (PROM) is limited. The aim of our study was to
27 investigate whether maternal Pb exposure was associated with PROM and preterm
28 PROM.

29 **Design** Cross-sectional cohort study.

30 **Study population** The present study involved 7290 pregnant women from the
31 Healthy Baby Cohort (HBC) in Wuhan, China, during 2012 to 2014.

32 **Main outcome measures** PROM was defined as spontaneous rupture of amniotic
33 membranes before the onset of labour and was determined with a pH \geq 6.5 for vaginal
34 fluid. Maternal urinary Pb level was adjusted by creatinine concentration, and its
35 relationship with PROM was analyzed by logistic regression.

36 **Results** The interquartile range of maternal urinary Pb concentrations of the study
37 population was 2.30-5.64 $\mu\text{g/g}$ creatinine with a median of 3.44 $\mu\text{g/g}$ creatinine.
38 Increased risk of PROM was significantly associated with elevated levels of Pb in
39 maternal urine [adjusted odds ratio (OR) = 1.23, 95% confidence interval (CI) =
40 1.02-1.47 for the medium tertile; adjusted OR = 1.51, 95% CI = 1.27-1.80 for the
41 highest tertile]. The risk of preterm PROM associated with Pb levels was significantly
42 higher when compared to the lowest tertile (adjusted OR = 1.24, 95% CI = 0.80-1.92
43 for the medium tertile; adjusted OR = 1.73, 95% CI = 1.15-2.60 for the highest tertile).
44 In addition, the relationship between Pb and PROM was more pronounced among

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4 45 primiparous women than multiparous women (p for interaction < 0.01).

5
6 46 **Conclusions** Our study found that higher levels of maternal Pb exposure was
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8 47 associated with increased risk of PROM, indicating that exposure to Pb during
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10 48 pregnancy may be an important risk factor for PROM.
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50 **Strengths and limitations of this study**

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- 20 • This study was conducted with a large sample size, which included 7290
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22 mother-singleton pairs from a birth cohort study in China.
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25 • All information about the participants was collected from personal interviews and
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27 medical records, which allowed us to adjust for other potential risk factors for PROM.
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- 31 • Although many potential confounders were taken into account for analysis, other
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33 confounding factors may remain.
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52 **Introduction**

53 Premature rupture of membranes (PROM), refers to maternal membranes rupture
54 more than one hour before the onset of labour, occurs in approximately 5%-15% of
55 deliveries [1-3]. PROM is related to significant maternal, fetal, and neonatal risks,
56 such as maternal infection, prematurity, neonatal sepsis, as well as adverse
57 neurological outcomes [4-6]. When the rupture occurs prior to 37 weeks of gestation,
58 it is considered as preterm PROM. Preterm PROM appears in 1%-3% of all
59 pregnancies and one-third of preterm deliveries, and thus is a leading cause of
60 perinatal morbidity and mortality [5 7]. The etiology of PROM has been shown to be
61 multifactorial, and increasing evidence has regarded exposure to environmental
62 pollutants as risk factors for PROM [8-10].

63 Lead (Pb), a ubiquitous non-biodegradable heavy metal that persists in the
64 environment, is widely used in various industries, such as automobiles, paint, batteries,
65 and plastics [11 12]. Due to these industrial processes, Pb has become the most widely
66 distributed toxic heavy metal worldwide [12]. High levels of Pb exposure have been
67 demonstrated to be associated with preeclampsia, pregnancy-induced hypertension,
68 miscarriage, prematurity, congenital abnormalities, and even impaired cognitive
69 function problems in childhood [13-18]. However, the association between maternal
70 Pb exposure and risk of PROM is limited, and the results were inconsistent. Some
71 studies have found a significant correlation between the risk of PROM and maternal
72 Pb levels [10 19-21], while another study has failed to observe such an association
73 [22].

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4 74 China is the largest Pb (raw and refined) producing and consuming country in the
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6 75 world [23]. Pb pollution poses a significant threat to human health, especially for
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8 76 pregnant women and the vulnerable fetuses, who are more susceptible to Pb exposure
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11 77 since Pb can freely cross the placenta [24]. Given this background, the present study
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13 78 involving 7290 participants was designed to explore whether Pb exposure during
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15 79 pregnancy could increase the risk of PROM and preterm PROM in Chinese pregnant
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17 80 women.
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23 **Materials and methods**

24 **Study population and data collection**

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28 84 The study participants (n = 11311) were enrolled during September 2012 to October
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30 85 2014 from the Healthy Baby Cohort (HBC) study at Wuhan Medical and Health
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32 86 Center for Women and Children in China, and the eligibility criteria has been
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34 87 described previously [25]. For this study, we excluded women without urine samples
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36 88 (n = 3947), as well as those who delivered infants with congenital malformations (n =
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38 89 62), which may be caused by an abnormal pregnancy. The number of cases with
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40 90 smoking (n = 7) or drinking (n = 2) during pregnancy were rather small, in line with
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42 91 previous reports [26 27], and were also excluded, as these lifestyles have been shown
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44 92 to have adverse effects on fetal growth. For women who gave birth twice in HBC (n =
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46 93 3), we excluded the second delivery record and only kept the first one (n = 3). Finally,
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48 94 7290 pregnant women were included in the present study. All participating mothers
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50 95 signed written informed consent at enrollment. The ethical committees of Tongji
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4 96 Medical College, Huazhong University of Science and Technology, and the study
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6 97 hospital approved the study protocol.
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8 98 All participants filled out a structural questionnaire after labour during a face-to-face
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10 99 interview by specially trained nurses. Information on the women's demographic and
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13 100 socioeconomic backgrounds, (e.g., maternal age, educational level, and occupational
14
15 101 status), pre-pregnancy body mass index (BMI) (calculated on the basis of
16
17 102 self-reported weight and height before pregnancy), and daily-life habits during
18
19 20 103 pregnancy (e.g., alcohol and tobacco consumption) were collected during this process.
21
22
23 104 Medical/reproductive histories and outcomes (e.g., intrauterine infection, maternal
24
25 105 diseases, and infant sex) were gathered from medical records. Last menstrual period
26
27 106 (LMP) was used to calculate maternal gestational week.
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30 107 PROM was defined as spontaneous rupture of amniotic membranes prior to the onset
31
32 108 of labour and was determined by the visualization of amniotic fluid passing from the
33
34 109 cervical canal and pooling in the vagina, plus the nitrazine test of a $\text{pH} \geq 6.5$ for
35
36 110 vaginal fluid. The nitrazine test is a simple and rapid bedside method to diagnose
37
38 111 PROM and is widely used in Chinese hospitals with a relatively high reliability [28].
39
40 112 The diagnosis of the onset of labour was determined by regular painful contractions
41
42 113 and a cervical dilatation of 3 cm or greater. Rupture occurred less than 37 weeks of
43
44 114 gestation was considered as preterm PROM. The definition of the clinical diagnosis of
45
46 115 intrauterine infection was considered in the presence of maternal fever ($> 38\text{ }^{\circ}\text{C}$)
47
48 116 accompanied by signs or symptoms of maternal and fetal tachycardia, uterine
49
50 117 tenderness, foul smelling discharge, maternal leucocytosis or positive amniotic fluid
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3 118 cultures from an amniocentesis. Clinical vaginitis was defined by the presence of
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6 119 erythema and an exudative discharge that was associated with symptoms of pruritus
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9 120 or pain. Cervicitis was diagnosed based on cervical erosion with purulent discharge
10
11 121 from the cervix. Pelvic inflammatory disease was clinically defined by the presence of
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13 122 adnexal tenderness and/or the presence of tender adnexal mass on bimanual pelvic
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16 123 examination. Vaginal bleeding was defined as the presence of bleeding in pregnant
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18 124 women prior to 28 weeks of gestation. Polyhydramnios was defined by having an
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20 125 amniotic fluid index of 24 cm or more. Fetal malposition was defined as
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23 126 occipito-transverse or occipito-posterior position.
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128 **Urine sample collection and lead exposure measurement**

129 Maternal urine samples were collected upon their admission to the hospital while
130 waiting for delivery and were stored immediately in polypropylene tubes at -20 °C for
131 further treatment. The detection method for urinary Pb was introduced previously [29].
132 Briefly, prior to analysis, urine specimens were thawed at room temperature. Then, 1
133 mL of supernatant urine with 4 mL of 3% HNO₃ were added into 15 mL
134 polypropylene tubes for overnight nitrification and were digested by ultrasound for 1
135 hour at 40 °C. Next, inductively coupled plasma mass spectrometry (ICP-MS; Agilent
136 7700, Agilent Technologies, Waldbronn) was used to measure maternal urinary Pb
137 concentrations. Assessment of the instrument performance was conducted using the
138 Standard Reference Material Human Urine (SRM2670a Toxic Elements in Urine,
139 National Institute of Standards and Technology, USA) as external quality control

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4 140 sample in each batch. The concentrations of the quality controls were measured within
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6 141 the certified range recommended by the manufacturer (5%). The samples were
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8 142 analyzed with an external calibration method using eight standard concentrations
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10 143 ranging from 0 to 500 mg/L. Field and procedure blanks were also included to assess
11
12 144 potential contamination, and Pb was not detected in the containers or storage tubes.
13
14 145 The detection rate of maternal urinary Pb concentrations in this study was 99%, and
15
16 146 the samples below the limit of detection (LOD) (0.01 µg/L) were replaced by 1/2
17
18 147 LOD. The intra-day coefficient of variation was below 2.0%, and the inter-day
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20 148 coefficient of variation was under 3.0%.

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22
23 149 Urine creatinine concentrations measured by an automatic biochemical analyzer
24
25 150 (BS-200, Mindray, Shenzhen, China), were used for the adjustment of Pb
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27 151 concentrations to control variable urine dilutions. The quality control standards for
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29 152 creatinine were identical to those described previously [19]. The adjusted urinary Pb
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31 153 concentrations were presented as µg/g creatinine.
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40 155 **Statistical analyses**

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42 156 The distribution of Pb concentration was skewed towards the right when tested by the
43
44 157 Kolmogorov–Smirnov normality test. The Wilcoxon signed rank test was used to
45
46 158 compare concentrations of Pb between PROM and non-PROM women. To evaluate
47
48 159 the association between Pb exposure and PROM, logistic regression analyses were
49
50 160 conducted to calculate crude and adjusted odds ratios (ORs) and 95% confidence
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52 161 intervals (CIs). Maternal urinary Pb levels were categorized into tertiles, with lowest
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4 162 one used as the reference. We detected the linear trends of Pb with PROM by
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6 163 modeling the median values of tertiles of Pb concentration as a continuous variable
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8 164 and used the Wald test to evaluate the statistical significance. The adjustment for
9
10 165 potential confounders was based on known factors associated with PROM, such as
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13 166 household income, passive smoking, parity, and pregnancy-induced hypertension [4 7
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16 167 30-32]. Additionally, covariates that altered the parameter estimate of Pb effect on
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18 168 PROM by over 10% were also included in the final model. Covariates, including
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21 169 maternal age, family income, pre-pregnancy BMI, parity, passive smoking and
22
23 170 pregnancy-induced hypertension, were adjusted for in this analysis. We also
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26 171 performed a sensitivity analysis excluding participants with intrauterine infection
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28 172 (chorioamnionitis), vaginitis, cervicitis, pelvic inflammatory disease, previous vaginal
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31 173 bleeding, polyhydramnios, and fetal malposition in consideration of their potential
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33 174 influence on PROM. As data from NHANES suggested that women usually have low
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35 175 urinary creatinine, and the upper cut-off (3 g/L) remained appropriate for the female
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38 176 population [33], a sensitivity analysis that excluded women with creatinine > 3 g/L
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41 177 was also conducted. In addition, we analyzed the ORs for PROM stratified by
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43 178 maternal parity (primiparous vs. multiparous), because the difference in these
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45 179 variables has been previously reported to associate with PROM [32]. An interaction
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48 180 term was added into the model to assess the effect of Pb and maternal parity on the
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50 181 outcome of PROM.

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52 182 All data analyses were performed by SAS (version 9.4; SAS Institute Inc., NC,
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55 183 USA), and two-sided *p* values below 0.05 were considered statistically significant.

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6 185 **Patient involvement**

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8 186 No patients were involved in setting the research question or the outcome measures,
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11 187 nor were they involved in the design or implementation of the study. No patients were
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13 188 involved in the interpretation of study results or writing up of the manuscript. There
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15 189 are no plans to disseminate the results of the research to study participants.
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21 191 **Results**

22
23 192 The basic characteristics and urinary Pb concentrations of 7290 participants is shown
24
25 193 in Table 1. In this study, the prevalence of PROM and preterm PROM was 12.1% and
26
27 194 2%, respectively. Maternal age at labour ranged from 18 to 46 years, with 28 years as
28
29 195 the average age. Most of the mothers were primiparous (84.5%) and had a high
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31 196 educational attainment (> 12 years) (67.2%), high annual family income (\geq 50,000
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33 197 yuan per year) (56.9%), and normal pre-pregnancy BMI (18.5-23.9 kg/m²) (66.3%).
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37 198 The average gestational age at delivery was 39.2 weeks. About 22.9% were passively
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39 199 exposed to smoking during pregnancy. Approximately 3.9% of the pregnant women
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41 200 had hypertension during pregnancy, and 53.4% of the mothers gave birth to a male
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43 201 infant.
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47 202 The median of maternal urinary Pb concentrations in PROM mothers (3.88 μ g/g
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49 203 creatinine) was higher than that of non-PROM mothers (median = 3.39 μ g/g
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51 204 creatinine) ($p < 0.05$). Compared with women without preterm PROM (median = 3.43
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53 205 μ g/g creatinine), the median of maternal urinary Pb concentrations in preterm PROM
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206 mothers was also higher (median = 3.96 µg/g creatinine) ($p < 0.05$).

207 Table 2 shows the relationship between creatinine-adjusted maternal urinary Pb
208 levels and PROM/preterm PROM. Compared with the lowest tertile, a significantly
209 positive correlation between PROM and Pb concentrations was observed (adjusted
210 OR = 1.23, 95% CI = 1.02-1.47 for the medium tertile; adjusted OR = 1.51, 95% CI =
211 1.27-1.80 for the highest tertile) (p trend < 0.01). The risk estimate for preterm PROM
212 in association with Pb levels was significantly higher compared to the lowest tertile
213 (adjusted OR = 1.24, 95% CI = 0.80-1.92 for the medium tertile; adjusted OR = 1.73,
214 95% CI = 1.15-2.60 for the highest tertile) (p trend < 0.01). In addition, the sensitivity
215 analysis (Supplementary material, Table S1) excluding subjects with intrauterine
216 infection, vaginitis, cervicitis, pelvic inflammatory disease, previous vaginal bleeding,
217 polyhydramnios, and fetal malposition demonstrated a similar association between Pb
218 and PROM/preterm PROM. Consistent observation was also made in the sensitivity
219 analysis excluding women with creatinine > 3 g/L in the statistical models
220 (Supplementary material, Table S2).

221 Results stratified by maternal parity are summarized in Table 3. Among the 6159
222 primiparous women, we observed a significantly positive correlation between Pb
223 concentrations and PROM risk (adjusted OR = 1.24, 95% CI = 1.03-1.50 for the
224 medium tertile; adjusted OR = 1.52, 95% CI = 1.27-1.83 for the highest tertile) (p
225 trend < 0.01). However, no statistically significant association was found between
226 PROM and Pb in multiparous mothers (adjusted OR = 1.21, 95% CI = 0.65-2.25 for
227 the medium tertile; adjusted OR = 1.57, 95% CI = 0.87-2.83 for the highest tertile) (p

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4 228 trend = 0.13). The risk estimates for PROM in relation to Pb levels in primiparous and
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6 229 multiparous women were significantly different (p for interaction < 0.01).
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10 231 **Discussion**

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13 232 Our study examined the association between maternal urinary Pb exposure before
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15 233 delivery and risk of PROM in Chinese pregnant women, and we found that urinary Pb
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17 234 concentration was significantly and positively correlated with PROM and preterm
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19 235 PROM incidence. Meanwhile, our study suggested that the effect of Pb on PROM
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21 236 may depend on maternal parity, as the correlation is more pronounced in the
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23 237 primiparous women than in the multiparous ones.
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28 238 Preterm PROM is the leading cause of preterm birth and neonatal complications,
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30 239 such as perinatal infections, respiratory distress syndrome, umbilical cord
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32 240 compression, intraventricular hemorrhage, sepsis and even death [5 7]. Furthermore, it
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34 241 has been linked with long-term adverse neurodevelopmental outcomes [34]. Although
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36 242 our current understanding about the cause of PROM is limited, accumulating evidence
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38 243 has suggested that environmental factors play important roles in inducing PROM by
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40 244 stimulating oxidative stress and inflammation [8 9].
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45 245 In this study, we observed that maternal Pb exposure prior to delivery correlated
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47 246 with increased risk of PROM and preterm PROM. After excluding the subjects with
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49 247 complications that are known to cause PROM, or those with creatinine > 3 g/L,
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51 248 significant associations between Pb and PROM/preterm PROM were still observed.
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54 249 Consistent with our findings, a study including 332 pregnant women in Iran reported
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4 250 that one unit increase in the logarithm of maternal blood Pb concentration was
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6 251 associated with a several-fold risk of PROM [10]. Similarly, a study involving 502
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8 252 pregnant mothers in Columbia found that higher blood levels of Pb was associated
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10 253 with an increase in the incidence of PROM [19]. Furthermore, elevated Pb
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12 254 concentration in the umbilical cord was reported to be associated with increased
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14 255 PROM risk in a cohort study of 749 mother-infant pairs in a Pb-smelter community in
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16 256 South Australia [20]. Additionally, a study of 89 mother-infant pairs in the southeast
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18 257 of Spain observed a higher placental Pb concentration in the PROM cases than in the
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20 258 normal deliveries [21]. However, despite these findings, an early study demonstrated
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22 259 no significant correlation between PROM risk and blood Pb concentration measured
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24 260 in 635 samples from the umbilical cord blood [22]. The reason to this discrepancy is
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26 261 currently unknown, but it may be, at least in part, due to the differences in Pb
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28 262 exposure levels.

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30 263 PROM has been reported to be associated with multiple factors, including cigarette
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32 264 smoking, low income, parity, infection, and pregnancy-induced hypertension [4 7
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34 265 30-32]. In the present study, inclusion of the potential confounding factors for
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36 266 adjustment did not undermine the association between increased levels of Pb exposure
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38 267 and PROM risk. In the stratified analysis by parity, our results suggested that parity
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40 268 status may influence the effect of maternal Pb exposure on the risk of PROM. A
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42 269 significantly positive association between PROM and urinary Pb concentrations was
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44 270 observed in primiparous mothers, whereas a similar positive correlation was also
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46 271 observed in multiparous mothers, despite the unreached statistical significance. One
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4 272 possible explanation may be the unbalanced sample sizes between primiparous and
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6 273 multiparous mothers (6159 vs. 1131). Future studies enrolling more multiparous
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8 274 women will help to further evaluate this difference caused by parity status.

9
10 275 The etiology and mechanism of the effect of Pb on PROM are not clear. One
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13 276 prevailing mechanistic explanation is that Pb can induce toxicity by triggering
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16 277 oxidative stress through the generation of reactive oxygen species (ROS) [11 35],
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18 278 which is responsible for the structural weakness of collagen fibrils and causes the
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21 279 membranes to lose strength and elasticity, and consequently damage the collagen in
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23 280 fetal membrane [10 36 37]. Furthermore, Pb is shown to induce inflammatory
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26 281 responses via upregulating the expression of pro-inflammatory cytokines, such as
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28 282 TNF- α [38], thus predispose the membrane to rupture by promoting alterations of
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31 283 membrane fluidity and impairment in membrane barrier function [39].

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33 284 Urinary Pb is favoured for long-term biomonitoring and is widely used in the
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36 285 assessment of Pb exposure level [40 41]. In the present study, maternal urinary Pb
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39 286 concentration [arithmetic mean (AM) = 7.40 $\mu\text{g/g}$ creatinine, geometric mean (GM) =
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41 287 3.69 $\mu\text{g/g}$ creatinine, median = 3.44 $\mu\text{g/g}$ creatinine] was higher than pregnant
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43 288 mothers reported in several developed countries, such as Australia (AM = 0.87 $\mu\text{g/g}$
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45 289 creatinine, median = 0.7 $\mu\text{g/g}$ creatinine) [42], Japan (GM = 0.48 $\mu\text{g/g}$ creatinine) [43],
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48 290 and the United States (GM = 0.63 $\mu\text{g/L}$) [44]. Yet, the urinary Pb concentration in our
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51 291 study also overlapped with other countries, including Spain (AM = 5.2 $\mu\text{g/g}$ creatinine,
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53 292 median = 3.9 $\mu\text{g/g}$ creatinine) [45]. In addition, Pb concentration in the present
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55 293 subjects was generally lower in comparison with pregnant women reported in
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4 294 developing countries, such as Nigeria (AM = 28.5 µg/g creatinine) [46]. Although
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6 295 Pb-containing petrol has been phased out since 2000 in China, Pb pollution remains a
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8 296 huge environmental challenge, as large amounts of Pb pollutants from various sources
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10 297 of Pb consumption have been increasing rapidly due to the unprecedented economic
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12 298 development [47 48]. In the past decades, elevated accumulations of Pb have been
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14 299 widely spreaded in soil and dust in many Chinese provinces [23 48], raising a major
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16 300 public health concern in China, since Pb can enter human body via intaking
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18 301 Pb-containing food, water, and even air [12 48]. As a consequence, excessive Pb
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20 302 emissions pose serious adverse health effects on humans, especially on the susceptible
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22 303 pregnant women and their fetuses.

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28 304 The strength of this study is as follows: first, it was conducted with a large sample
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30 305 size, which included 7290 mother-singleton pairs from a birth cohort study in China.
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32 306 We preformed sensitivity analyses and stratified analyses to evaluate the relationship
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34 307 between maternal Pb exposure and PROM, where a significant correlation was
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36 308 observed. Moreover, all information about the participants including demographic
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38 309 characteristics, socioeconomic status and pregnancy outcomes were gathered from
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40 310 personal interviews and medical records, which made it possible to adjust for other
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42 311 potential risk factors for PROM.

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47 312 Admittedly, there were other potential confounders that we were not able to control.
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49 313 Unfortunately, several important risk factors for PROM, such as drug use, cervical
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51 314 insufficiency, premature contractions, were not collected in the present study but will
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53 315 be included in the future studies. The small numbers of preterm PROM and
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4 316 multiparous women also limited the power of our study. In addition, urinary Pb
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6 317 collected and measured at labour only reflects plasma Pb level at labour, which may
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8 318 not accurately reflect the dynamic maternal Pb exposure throughout the whole
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11 319 pregnancy and limit the strength to determine the causal effect between maternal
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13 320 urinary Pb level and PROM. Therefore, further studies with urine samples collected at
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15 321 multiple time points and from different populations are needed to confirm the
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17 322 observed relationship between Pb and PROM.
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22 23 324 **Conclusion**

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25 325 In this study, we observed a positive relationship between maternal urinary Pb and the
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27 326 risk of PROM, supporting that maternal exposure to Pb may be a potential risk factor
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29 327 for PROM. Additionally, the significant association was only present in primiparous
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31 328 women and not in multiparous women. This finding suggested that appropriate public
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33 329 health measures need to be implemented to control maternal Pb exposure during
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35 330 pregnancy.
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41 42 332 **Footnotes**

43 333 SH and WX contributed equally to this work.

44
45 334 **Contributors:** SH carried out the statistical analyses and drafted the manuscript.
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47 335 WX, XS assisted in the statistical analyses, critically reviewed and revised the
48
49 336 manuscript. LQ, BZ, TC, SX contributed to the study design and developed the initial
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51 337 protocol. YL contributed to the study design, critically reviewed and revised the
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4 338 manuscript. All authors read and approved the final manuscript.
5

6 339 **Funding:** This work was supported by the National Natural Science Foundation of
7
8 340 China (91743103, 91643207, 21437002, 81372959, and 81402649), the National Key
9
10 341 Research and Development Plan (2016YFC0206700, 2016YFC0206203), and the
11
12 342 Fundamental Research Funds for the Central Universities HUST (2016YXZD043,
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14 343 2015ZDTD047).
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18 344 **Competing interests:** None declared.
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20 345 **Ethics approval:** The ethical committees of Tongji Medical College, Huazhong
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22 346 University of Science and Technology, and the study hospital approved the study
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24 347 protocol.
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28 348 **Provenance and peer review:** Not commissioned; externally peer reviewed.
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30 349 **Data sharing statement:** Extra data is available by emailing to the corresponding
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32 350 author at liyuanyuan@hust.edu.cn.
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352 **References**

- 353 1. Ural SH, Nagey DA. Premature Rupture of Membranes. Topics in Obstetrics &
354 Gynecology 1998;**18**(19):1-3
- 355 2. ACOG Practice Bulletin No. 80: premature rupture of membranes. Clinical
356 management guidelines for obstetrician-gynecologists. Obstetrics and
357 gynecology 2007;**109**(4):1007-19 doi:
358 10.1097/01.AOG.0000263888.69178.1f[published Online First: Epub Date]].
- 359 3. Perinatal complications associated with maternal tobacco use. Seminars in
360 Neonatology; 2000. Elsevier.
- 361 4. Poma PA. Premature rupture of membranes. Journal of the National Medical
362 Association 1996;**88**(1):27
- 363 5. Mercer BM. Preterm premature rupture of the membranes. Obstetrics and
364 gynecology 2003;**101**(1):178-93
- 365 6. Tchirikov M, Schlabritz-Loutsevitch N, Maher J, et al. Mid-trimester preterm
366 premature rupture of membranes (PPROM): etiology, diagnosis, classification,
367 international recommendations of treatment options and outcome. Journal of
368 perinatal medicine 2017
- 369 7. Simhan HN, Canavan TP. Preterm premature rupture of membranes: diagnosis,
370 evaluation and management strategies. BJOG: An International Journal of
371 Obstetrics & Gynaecology 2005;**112**:32-37 doi:
372 10.1111/j.1471-0528.2005.00582.x[published Online First: Epub Date]].
- 373 8. Wallace ME, Grantz KL, Liu D, et al. Exposure to Ambient Air Pollution and

- 1
2
3
4 374 Premature Rupture of Membranes. American journal of epidemiology
5
6 375 2016;**183**(12):1114-21 doi: 10.1093/aje/kwv284[published Online First: Epub
7
8 376 Date]].
- 9
10
11 377 9. Dadvand P, Basagana X, Figueras F, et al. Air pollution and preterm premature
12
13 378 rupture of membranes: a spatiotemporal analysis. American journal of
14
15 379 epidemiology 2014;**179**(2):200-7 doi: 10.1093/aje/kwt240[published Online
16
17 380 First: Epub Date]].
- 18
19
20 381 10. Vigeh M, Yokoyama K, Shinohara A, et al. Early pregnancy blood lead levels and
21
22 382 the risk of premature rupture of the membranes. Reproductive toxicology
23
24 383 (Elmsford, NY) 2010;**30**(3):477-80 doi:
25
26 384 10.1016/j.reprotox.2010.05.007[published Online First: Epub Date]].
- 27
28
29 385 11. Flora G, Gupta D, Tiwari A. Toxicity of lead: a review with recent updates.
30
31 386 Interdiscip Toxicol 2012;**5**(2):47-58
- 32
33
34 387 12. Cheng H, Hu Y. Lead (Pb) isotopic fingerprinting and its applications in lead
35
36 388 pollution studies in China: A review. Environmental Pollution
37
38 389 2010;**158**(5):1134-46 doi:
39
40 390 <http://dx.doi.org/10.1016/j.envpol.2009.12.028>[published Online First: Epub
41
42 391 Date]].
- 43
44
45 392 13. Vigeh M, Yokoyama K, Ramezanzadeh F, et al. Lead and other trace metals in
46
47 393 preeclampsia: a case–control study in Tehran, Iran. Environmental research
48
49 394 2006;**100**(2):268-75
- 50
51
52 395 14. Chen X-K, Yang Q, Smith G, et al. Environmental lead level and
53
54
55
56
57
58
59
60

- 1
2
3 396 pregnancy-induced hypertension. *Environmental research* 2006;**100**(3):424-30
4
5
6 397 15. Linn S, Schoenbaum S. The relationship between prenatal exposure to lead and
7
8 398 congenital anomalies. *Jama* 1984;**251**:2956-59
9
10
11 399 16. Dietrich KN, Succop PA, Berger OG, et al. Lead exposure and the cognitive
12
13 400 development of urban preschool children: the Cincinnati Lead Study cohort at
14
15 401 age 4 years. *Neurotoxicology and teratology* 1991;**13**(2):203-11
16
17
18 402 17. Hertz-Picciotto I. The evidence that lead increases the risk for spontaneous
19
20 403 abortion. *Am J Ind Med* 2000;**38**(3):300-9
21
22
23 404 18. Vigeh M, Yokoyama K, Seyedaghamiri Z, et al. Blood lead at currently acceptable
24
25 405 levels may cause preterm labour. *Occupational and environmental medicine*
26
27 406 2010:oem. 2009.050419
28
29
30 407 19. Fahim MS, Fahim Z, Hall DG. Effects of subtoxic lead levels on pregnant women
31
32 408 in the state of Missouri. *Res Commun Chem Pathol Pharmacol*
33
34 409 1976;**13**(2):309-31
35
36
37 410 20. Baghurst PA, Robertson EF, Oldfield RK, et al. Lead in the placenta, membranes,
38
39 411 and umbilical cord in relation to pregnancy outcome in a lead-smelter
40
41 412 community. *Environmental health perspectives* 1991;**90**:315-20
42
43
44 413 21. Falcon M, Vinas P, Luna A. Placental lead and outcome of pregnancy. *Toxicology*
45
46 414 2003;**185**(1-2):59-66
47
48
49 415 22. Angell NF, Lavery JP. The relationship of blood lead levels to obstetric outcome.
50
51 416 *American journal of obstetrics and gynecology* 1982;**142**(1):40-6
52
53
54 417 23. Chen HY, Li AJ, Finlow DE. The lead and lead-acid battery industries during 2002
55
56
57
58
59
60

- 1
2
3
4 418 and 2007 in China. Journal of Power Sources 2009;**191**(1):22-27 doi:
5
6 419 <http://dx.doi.org/10.1016/j.jpowsour.2008.12.140>[published Online First:
7
8 420 Epub Date]].
- 9
10
11 421 24. Shannon M. Severe lead poisoning in pregnancy. Ambulatory Pediatrics
12
13 422 2003;**3**(1):37-39
- 14
15
16 423 25. Yang J, Huo W, Zhang B, et al. Maternal urinary cadmium concentrations in
17
18 424 relation to preterm birth in the Healthy Baby Cohort Study in China. Environ
19
20 425 Int 2016;**94**:300-6 doi: 10.1016/j.envint.2016.06.003[published Online First:
21
22 426 Epub Date]].
- 23
24
25 427 26. West R. Tobacco control: present and future. British medical bulletin
26
27
28 428 2006;**77**(1):123-36
- 29
30 429 27. Cochrane J, Chen H, Conigrave KM, et al. Alcohol use in China. Alcohol and
31
32 430 Alcoholism 2003;**38**(6):537-42
- 33
34
35 431 28. Liang Dk, Qi Hb, Luo X, et al. Comparative study of placental α -
36
37 432 microglobulin - 1, insulin - like growth factor binding protein - 1 and
38
39 433 nitrazine test to diagnose premature rupture of membranes: A randomized
40
41 434 controlled trial. Journal of Obstetrics and Gynaecology Research
42
43 435 2014;**40**(6):1555-60
- 44
45
46
47 436 29. Xia W, Du X, Zheng T, et al. A Case-Control Study of Prenatal Thallium Exposure
48
49 437 and Low Birth Weight in China. Environmental health perspectives
50
51 438 2016;**124**(1):164-9 doi: 10.1289/ehp.1409202[published Online First: Epub
52
53 439 Date]].
- 54
55
56
57
58
59
60

- 1
2
3
4 440 30. Lee T, Silver H. Etiology and epidemiology of preterm premature rupture of the
5
6 441 membranes. *Clinics in perinatology* 2001;**28**(4):721-34
7
8
9 442 31. Zhou Q, Zhang W, Xu H, et al. Risk factors for preterm premature rupture of
10
11 443 membranes in Chinese women from urban cities. *International journal of*
12
13 444 *gynaecology and obstetrics: the official organ of the International Federation*
14
15 445 *of Gynaecology and Obstetrics* 2014;**127**(3):254-9 doi:
16
17 446 10.1016/j.ijgo.2014.06.020[published Online First: Epub Date]].
18
19
20 447 32. Naeye RL. Factors that predispose to premature rupture of the fetal membranes.
21
22 448 *Obstetrics and gynecology* 1982;**60**(1):93-8
23
24
25 449 33. Barr DB, Wilder LC, Caudill SP, et al. Urinary creatinine concentrations in the
26
27 450 U.S. population: implications for urinary biologic monitoring measurements.
28
29 451 *Environmental health perspectives* 2005;**113**(2):192-200
30
31
32 452 34. Clark EA, Varner M. Impact of preterm PROM and its complications on long-term
33
34 453 infant outcomes. *Clinical obstetrics and gynecology* 2011;**54**(2):358-69
35
36
37 454 35. Hsu PC, Liu MY, Hsu CC, et al. Lead exposure causes generation of reactive
38
39 455 oxygen species and functional impairment in rat sperm. *Toxicology*
40
41 456 1997;**122**(1-2):133-43
42
43
44 457 36. Moore RM, Mansour JM, Redline RW, et al. The physiology of fetal membrane
45
46 458 rupture: insight gained from the determination of physical properties. *Placenta*
47
48 459 2006;**27**(11-12):1037-51 doi: 10.1016/j.placenta.2006.01.002[published
49
50 51 Online First: Epub Date]].
52
53 460
54
55 461 37. Wall PD, Pressman EK, Woods JR, Jr. Preterm premature rupture of the
56
57
58
59
60

- 1
2
3
4 462 membranes and antioxidants: the free radical connection. Journal of perinatal
5
6 463 medicine 2002;**30**(6):447-57 doi: 10.1515/jpm.2002.071[published Online
7
8 464 First: Epub Date]].
- 10
11 465 38. Ghareeb DA, Hussien HM, Khalil AA, et al. Toxic effects of lead exposure on the
12
13 466 brain of rats: Involvement of oxidative stress, inflammation,
14
15 467 acetylcholinesterase, and the beneficial role of flaxseed extract. Toxicological
16
17 & Environmental Chemistry 2010;**92**(1):187-95
18
19
- 20
21 469 39. Gervasi MT, Chaiworapongsa T, Naccasha N, et al. Maternal intravascular
22
23 470 inflammation in preterm premature rupture of membranes. The Journal of
24
25 471 Maternal-Fetal & Neonatal Medicine 2002;**11**(3):171-75 doi:
26
27 10.1080/jmf.11.3.171.175[published Online First: Epub Date]].
28
29
- 30
31 473 40. Barbosa Jr F, Tanus-Santos JE, Gerlach RF, et al. A critical review of biomarkers
32
33 474 used for monitoring human exposure to lead: advantages, limitations, and
34
35 475 future needs. Environmental health perspectives 2005:1669-74
36
37
- 38 476 41. Abadin H, Ashizawa A, Stevens YW, et al. Agency for Toxic Substances and
39
40 477 Disease Registry (ATSDR) Toxicological Profiles. Toxicological Profile for
41
42 478 Lead. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US),
43
44 2007.
45
46
- 47
48 480 42. Hinwood AL, Callan AC, Ramalingam M, et al. Cadmium, lead and mercury
49
50 481 exposure in non smoking pregnant women. Environmental research
51
52 482 2013;**126**:118-24 doi:
53
54 <http://dx.doi.org/10.1016/j.envres.2013.07.005>[published Online First: Epub
55
56

- 1
2
3
4 484 Date]].
- 5
6 485 43. Shirai S, Suzuki Y, Yoshinaga J, et al. Maternal exposure to low-level heavy
7
8 486 metals during pregnancy and birth size. *Journal of Environmental Science and*
9
10
11 487 *Health Part A* 2010;**45**(11):1468-74
- 12
13 488 44. Jain RB. Effect of pregnancy on the levels of urinary metals for females aged 17–
14
15 489 39 years old: data from National Health and Nutrition Examination Survey
16
17
18 490 2003–2010. *Journal of Toxicology and Environmental Health, Part A*
19
20 491 2013;**76**(2):86-97
- 22
23 492 45. Fort M, Cosín-Tomás M, Grimalt JO, et al. Assessment of exposure to trace metals
24
25 493 in a cohort of pregnant women from an urban center by urine analysis in the
26
27 494 first and third trimesters of pregnancy. *Environmental Science and Pollution*
28
29 495 *Research* 2014;**21**(15):9234-41
- 31
32
33 496 46. Adekunle IM, Ogundele JA, Oguntoke O, et al. Assessment of blood and urine
34
35 497 lead levels of some pregnant women residing in Lagos, Nigeria.
36
37 498 *Environmental Monitoring and Assessment* 2010;**170**(1):467-74 doi:
38
39 499 10.1007/s10661-009-1247-4[published Online First: Epub Date]].
- 41
42 500 47. Yan CH, Xu J, Shen XM. Childhood lead poisoning in China: challenges and
43
44 501 opportunities. *Environmental health perspectives* 2013;**121**(10):A294
- 45
46
47 502 48. Duzgoren-Aydin NS. Sources and characteristics of lead pollution in the urban
48
49 503 environment of Guangzhou. *Science of The Total Environment* 2007;**385**(1–
50
51 504 3):182-95 doi: <http://dx.doi.org/10.1016/j.scitotenv.2007.06.047>[published
52
53 505 Online First: Epub Date]].
- 54
55
56
57 506

Table 1 Basic characteristics and urinary Pb concentrations ($\mu\text{g/g}$ creatinine) of the 7290 pregnant women.

Characteristics	N (%)	Median (IQR) Pb ($\mu\text{g/g}$ creatinine)	<i>P</i> value
Total	7290	3.44 (2.30-5.64)	
Maternal age (years)			0.43
< 25	805 (11.0)	3.37 (2.27-5.52)	
25–29	3985 (54.7)	3.49 (2.32-5.77)	
30–34	2011 (27.6)	3.40 (2.29-5.47)	
≥ 35	489 (6.7)	3.41 (2.15-5.94)	
Education background (years)			0.63
≤ 9	1001 (13.7)	3.45 (2.40-5.63)	
9–12	1389 (19.1)	3.47 (2.35-5.56)	
> 12	4898 (67.2)	3.42 (2.26-5.66)	
Missing	2 (0.03)	5.69(3.50-7.90)	
Family income(yuan/year)			0.37
< 50,000	3021 (41.4)	3.52 (2.31-5.75)	
$\geq 50,000$	4148 (56.9)	3.39 (2.28-5.60)	
Missing	121 (1.7)	3.50 (2.38-5.83)	
Parity			0.43
primiparous	6159 (84.5)	3.45 (2.30-5.66)	
multiparous	1131 (15.5)	3.38 (2.26-5.59)	
Pre-pregnancy BMI (kg/m^2)			0.10
< 18.5	1527 (20.9)	3.47 (2.27-5.77)	
18.5–23.9	4832 (66.3)	3.41 (2.29-5.56)	
≥ 24	910 (12.5)	3.60 (2.39-6.04)	
Missing	21 (0.3)	3.87 (2.07-8.12)	
Passive smoking during pregnancy			0.07

Yes	1670 (22.9)	3.30 (2.29-5.32)	
No	5620 (77.1)	3.47 (2.30-5.71)	
Pregnancy-induced hypertension			0.08
Yes	286 (3.9)	3.26 (2.18-5.21)	
No	7004 (96.1)	3.44 (2.30-5.66)	
Gestational age (weeks)			< 0.01
< 37	291 (4.0)	3.99 (2.62-7.54)	
≥ 37	6999 (96.0)	3.42 (2.29-5.60)	
Infant gender			0.02
Male	3890 (53.4)	3.48 (2.34-5.78)	
Female	3400 (46.6)	3.40 (2.25-5.52)	
PROM	881 (12.1)	3.88 (2.55-6.59)	0.47
Preterm PROM	147 (2.0)	3.96 (2.63-7.67)	
Term PROM	734 (10.1)	3.87 (2.54, 6.34)	

Abbreviation: BMI, body mass index; IQR, interquartile range; PROM, Premature

Rupture of Membranes.

Table 2 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine.

Pb ($\mu\text{g/g}$ creatinine)	case	OR ^a (95% CI)	OR ^b (95% CI)
PROM			
Tertile1 (< 2.65)	241	1.00	1.00
Tertile2 (2.65–4.70)	291	1.24 (1.03, 1.48)	1.23 (1.02, 1.47)
Tertile3 (\geq 4.70)	349	1.52 (1.27, 1.81)	1.51 (1.27, 1.80)
<i>p</i> for trend		< 0.01	< 0.01
Preterm PROM			
Tertile1 (< 2.65)	37	1.00	1.00
Tertile2 (2.65–4.70)	46	1.25 (0.81, 1.93)	1.24 (0.80, 1.92)
Tertile3 (\geq 4.70)	64	1.74 (1.16, 2.62)	1.73 (1.15, 2.60)
<i>p</i> for trend		< 0.01	< 0.01

Abbreviations: OR, odds ratio; CI, confidence interval.

^a Unadjusted odds ratio.

^b Adjusted for maternal age, family income, pre-BMI, parity, passive smoking and pregnancy-induced hypertension.

Table 3 Risk of PROM associated with the levels of Pb in maternal urine, stratified by parity.

Pb levels ^a (µg/g creatinine)	Primiparous (n = 6159)			Multiparous (n = 1131)			<i>p</i> for interaction
	N	OR ^b (95% CI)	OR ^c (95% CI)	N	OR ^b (95% CI)	OR ^c (95% CI)	
T1	220	1.00	1.00	20	1.00	1.00	< 0.01
T2	268	1.25 (1.04, 1.51)	1.24 (1.03, 1.50)	24	1.21 (0.66, 2.23)	1.21 (0.65, 2.25)	
T3	318	1.52 (1.27, 1.83)	1.52 (1.27, 1.83)	31	1.59 (0.89, 2.84)	1.57 (0.87, 2.83)	
<i>P</i> for trend		< 0.01	< 0.01		0.11	0.13	

Abbreviations: OR, odds ratio; CI, confidence interval; T: tertile;

^a Pb levels: primiparous, T1 (< 2.66), T2 (2.66-4.71), T3 (≥ 4.71); multiparous, T1 (< 2.61), T2 (2.61-4.61), T3 (≥ 4.61).

^b Unadjusted odds ratio.

^c Adjusted for maternal age, family income, pre-BMI, passive smoking and pregnancy-induced hypertension.

Supplementary Materials

Maternal Lead Exposure and Premature Rupture of Membranes: A

Birth Cohort Study in China

Sha Huang¹, Wei Xia¹, Xia Sheng¹, Lin Qiu², Bin Zhang², Tian Chen¹, Shunqing Xu¹,
and Yuanyuan Li¹

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Table S1 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine, after excluding intrauterine infection, vaginitis, cervicitis, pelvic inflammatory disease, vaginal bleeding, polyhydramnios, and fetal malposition (n = 6553).

Table S2 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine, after excluding creatinine > 3 g/L (n = 7281).

Table S1 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine, after excluding intrauterine infection, vaginitis, cervicitis, pelvic inflammatory disease, vaginal bleeding, polyhydramnios, and fetal malposition (n = 6553).

Pb ($\mu\text{g/gcreatinine}$)	case	OR ^a (95% CI)	OR ^b (95% CI)
PROM			
Tertile1 (< 2.65)	208	1.00	1.00
Tertile2 (2.65–4.71)	245	1.20 (0.99, 1.46)	1.20 (0.98, 1.46)
Tertile3 (\geq 4.71)	308	1.56 (1.29, 1.87)	1.55 (1.28, 1.87)
<i>p</i> for trend		< 0.01	< 0.01
preterm PROM			
Tertile1 (< 2.65)	33	1.00	1.00
Tertile2 (2.65–4.71)	38	1.15 (0.72, 1.85)	1.15 (0.72, 1.84)
Tertile3 (\geq 4.71)	53	1.62 (1.04, 2.51)	1.61 (1.04, 2.51)
<i>p</i> for trend		0.02	0.01

Abbreviations: OR, odds ratio; CI, confidence interval.

^a Unadjusted odds ratio.

^b Adjusted for maternal age, family income, pre-BMI, parity, passive smoking and pregnancy-induced hypertension.

Table S2 Risk of PROM and preterm PROM associated with the levels of Pb in maternal urine, after excluding creatinine > 3 g/L (n = 7281).

Pb ($\mu\text{g/gcreatinine}$)	case	OR ^a (95% CI)	OR ^b (95% CI)
PROM			
Tertile1 (< 2.66)	241	1.00	1.00
Tertile2 (2.66–4.70)	290	1.23 (1.03, 1.47)	1.22 (1.02, 1.46)
Tertile3 (\geq 4.70)	349	1.52 (1.27, 1.81)	1.51 (1.26, 1.80)
<i>p</i> for trend		< 0.01	< 0.01
preterm PROM			
Tertile1 (< 2.66)	37	1.00	1.00
Tertile2 (2.66–4.70)	46	1.25 (0.81, 1.93)	1.24 (0.80, 1.92)
Tertile3 (\geq 4.70)	64	1.74 (1.16, 2.62)	1.73 (1.15, 2.60)
<i>p</i> for trend		< 0.01	0.01

Abbreviations: OR, odds ratio; CI, confidence interval.

^a Unadjusted odds ratio.

^b Adjusted for maternal age, family income, pre-BMI, parity, passive smoking and pregnancy-induced hypertension.

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
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
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	5-7
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(b) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
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	7-8
Bias	9	Describe any efforts to address potential sources of bias	5-6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8-9
		(b) Describe any methods used to examine subgroups and interactions	8-9
		(c) Explain how missing data were addressed	No missing data
		(d) If applicable, explain how loss to follow-up was addressed	Not applicable
		(e) Describe any sensitivity analyses	9
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	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	No missing data
		(c) Summarise follow-up time (eg, average and total amount)	Not applicable
Outcome data	15*	Report numbers of outcome events or summary measures over time	10
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	11-12
		(b) Report category boundaries when continuous variables were categorized	10
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-12
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-16
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	17

*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.