PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Maternal Lead Exposure and Premature Rupture of Membranes: A Birth Cohort Study in China
AUTHORS	Huang, Sha Xia, Wei Zhang, Bin Chen, Tian Xu, Shunqing Li, Yuanyuan

VERSION 1 - REVIEW

REVIEWER	Dr Caroline Taylor
REVIEW RETURNED	18-Jan-2018

GENERAL COMMENTS	Major points Premature PROM is a relatively rare event and consequently you have few women in this group – at best this limits the strength of the conclusions that you can draw from this part of your analyses. You mention this briefly on line 232, but I think this is a severe limitation of your study of PPROM and should be stated in the final section on study limitations. Similarly you have small numbers in your multiparous group. I have some concerns about the description and reliability of PROM outcome as follows: I am not completely clear on where these data were from – were they collected from the notes? Could you comment on the reliability of these data. For example, will there be cases that are missed because the woman does not have the test done either because they do not present or diagnosis is difficult (either because of lack of obvious leakage or inaccuracy in the nitrazine test -there can be false positives because of infection, urine, blood, etc.) Please comment on this, including the possible effect on the results of your study. PROM should be defined more precisely – usually defined as rupture more than 1 hour before the onset of labour. This also requires an accurate diagnosis of the onset of labour – was this recorded in the notes? There are quite a lot of details missing from the Methods – some of this is detailed in Yang et al. 2016, which describes a similar study on the same cohort. For example, you exclude cases with intrauterine infection for one analysis, but there is no mention of collection of this information. Similarly, details of the results of some aspects of quality control are missing. Minor points English language: the standard of English is generally reasonable but there are many small slips in grammar and spelling. Please check your manuscript carefully on this point. (For example, in the

	opening sentence of the Introduction 'in delivers' should be 'of deliveries'; in the third sentence 'Rupture begins' should be 'Rupture'; etc.). Also the occasional wrong choice of word, e.g. ln 22 factories should be factors. You describe the study as 'prospective' but I would say it is cross-sectional. Ln 68: You cite three reference here to support your argument for adverse birth outcomes and cognition with lead exposure, but the references are quite general. Please include references which are more specially focussed on the outcomes that you mention. Please check all your references on this point – primary sources should be cited where possible. Ln 74: You should explain why lead is a particular problem for pregnant women and the fetus. Ln 119. Please give some details of the external quality control results. Ln 121: Did you exclude women with urine cr <0.3 or > 3 g/l as done by Yang et al. 2016 https://doi.org/10.1016/j.envint.2016.06.003. Presumably your quality control standards for creatinine were identical to those of Yang – if so please refer to Yang et al. here on this point. Ln 141. There are several other factors that have been associated with PROM including previous bleeding, drug use, hydramnios etc. Did you exclude women with any of these? Ln 156. What is the currency for the income? Ln 196: PROM can't lead to preterm birth since it is by definition >37 weeks – only PPROM can lead to a premature delivery. Ln 221. What are the difference in exposure levels? Were they low in reference 17? Ln 245. Urine Pb is favoured for long-term biomonitoring (i.e. repeated measurements over time), rather than as a long-term biomarker of exposure (i.e. one measurement reflects long-term exposure) as it reflects plasma lead. This therefore weakens the argument for your use of urine rather than blood lead for a single measurement. Please amend and add comment on this limitation. Ln 274. A further limitation is that your outcome occurs at the same time as the exposure, so it is not possible to infer causality. Table 1. Can you give some statistical information for comparisons? The urine Pb was lower in women exposure to passive smoking, which seems counterintuitive. Please comment.

REVIEWER	Salvatore Andrea Mastrolia
REVIEW RETURNED	08-Mar-2018

GENERAL COMMENTS	Comments for the author General comments: this is a very interesting study investigating the pathogenesis of one of the Great Obstetrical Syndromes. I read the manuscript with interest and here is a list of major concerns that the authors may want to address: 1) The manuscript need a thorough language revision. Therefore I suggest the authors to refer to a professional proof reading service since, at the moment, the written English is a severe limitation to the publication of this study 2) Why was the second delivery record excluded in women who gave birth twice?

	3) Why where women who delivered neonates with malformations excluded? Same question for women with reported smoking and drinking. 4) A section of clinical definitions is needed. To provide the definition of PROM is not enough. All other clinical variables should be adequately defined. 5) Lines 205-206: the authors state they excluded women with intrauterine infection. A very precise definition of the diagnosis of intrauterine infection should be provided. 6) It would have been of interest to compare the concentration of Pb in women who experienced PROM or pPROM to women who delivered at term. This way the authors would have been able to claim that Pb was the cause for the development of the Obstetrical Syndrome. With this study they can suggest that the highest is the concentration of Pb the higher is the risk of PROM or pPROM

REVIEWER	Prof. Michael Tchirikov
REVIEW RETURNED	10-Apr-2018

GENERAL COMMENTS	The authors present the relation between a PROM and the exposure to lead. Other articles published by the same authors describe the relation between Prom and Cadmium as well as Chromium ("Association between maternal urinary chromium and premature rupture of membranes in the Healthy Baby Cohort study in China.", "Predictors of thallium exposure and its relation with preterm birth.", Maternal urinary cadmium concentrations in relation to preterm birth in the Healthy Baby CohortStudy in China.). Including 7290 women from the "Healthy Baby Cohort" in Wuhan China (2012- 2014), the study shows a significant correlation between maternal high urinary Pb-concentrations and the event of a PROM in primiparous mothers. Questionnaires, medical records and urin samples were used. For the statistical part the Kolmogrov- Smirnov normality test, Wilcoxon, Wald- Test and Odds-Ratio were used. Abstract: Clear, well-chosen information to give a short overview of the article. L 28: The aim of our study was to investigate whether maternal Pb exposure was associated with PROM "and preterm Prom" should be added , because in the result-part the increased risk for preterm PROM is mentioned too. Main paper Introduction: L54: There are sources who show a higher risk than 8-12% but 15%. L56: PROM is related to significant maternal, fetal, and neonatal risk, such as maternal infection, prematurity and neonatal sepsis. Another risk which should me mentioned. The new review PPROM paper (PMID:28710882) could be cited.

	L 57: Uncomplete sentence.” When the” is missing. When the rupture begins (occurs?) prior to 37 weeks of gestation it is considered as preterm PROM Materials and Methods: L86: It seems unreal, that only 7 of 7290 women were smoking and 2 were drinking during pregnancy. L123: Delete “And” L137: Other risk factors for PROM would be infection, cervical insufficiency, and premature contractions. Conclusion: L 282: Significant results are only presented for primiparous mothers as the authors mention earlier (L181-L182). I would recommend going over the manuscript for grammar and spelling errors.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Dr Caroline Taylor
Institution and Country: University of Bristol, UK

Major points
Premature PROM is a relatively rare event and consequently you have few women in this group – at best this limits the strength of the conclusions that you can draw from this part of your analyses. You mention this briefly on line 232, but I think this is a severe limitation of your study of PPROM and should be stated in the final section on study limitations. Similarly you have small numbers in your multiparous group.

Response: Thank you for this comment. We agree that the relatively small numbers of PPROM and multiparous women are limitations of this study. We have now touched on these points in the final section in lines 340-341.

I have some concerns about the description and reliability of PROM outcome as follows: I am not completely clear on where these data were from – were they collected from the notes? Could you comment on the reliability of these data. For example, will there be cases that are missed because the woman does not have the test done either because they do not present or diagnosis is difficult (either because of lack of obvious leakage or inaccuracy in the nitrazine test -there can be false positives because of infection, urine, blood, etc.) Please comment on this, including the possible effect on the results of your study.

Response: Wuhan Medical and Health Center for Women and Children determines PROM by the visualization of amniotic fluid passing from the cervical canal and pooling in the vagina, plus the nitrazine test of a pH higher than 6.5 for vaginal fluid by the attending physician (lines 110-113). The nitrazine test is a simple and rapid bedside method to diagnose PROM and is widely used in many Chinese hospitals with a relatively high reliability (Liang et al., 2014) (lines 113-114). The hospital subsequently registered the PROM outcome information into the medical record, where we directly collected the information about PROM outcome from. We admit that there may be false negative (e.g., minimal leakage) or false positive results (e.g., infection, urine, blood, etc.). However, when the diagnosis remains uncertain after a full evaluation of the positive history, speculum examination and positive nitrazine test by the physician, PROM will be confirmed by ultrasonography.

PROM should be defined more precisely – usually defined as rupture more than 1 hour before the onset of labour. This also requires an accurate diagnosis of the onset of labour – was this recorded in the notes?

Response: Thank you for this good suggestion. We have now added “PROM – refers to maternal membranes rupture more than one hour before the onset of labour” in lines 54-55. The diagnosis of the onset of labour was determined by regular painful contractions and a cervical dilatation of 3 cm or greater, which has been added in lines 115-116. Unfortunately, the onset of labour was not recorded in the medical records.

There are quite a lot of details missing from the Methods – some of this is detailed in Yang et al. 2016, which describes a similar study on the same cohort. For example, you exclude cases with intrauterine infection for one analysis, but there is no mention of collection of this information. Similarly, details of the results of some aspects of quality control are missing.

Response: We agree with the reviewer and have now added the related details missing from the Methods. The information about intrauterine infection was collected from medical records (line 107). We have also provided the details of the results of quality control as “The concentrations of the quality controls were measured within the certified range recommended by the manufacturer (5%). The samples were analyzed with an external calibration method using eight standard concentrations ranging from 0 to 500 mg/L. Field and procedure blanks were also included to assess potential contamination, and Pb was not detected in the containers or storage tubes” (lines 143-148).

Minor points
English language: the standard of English is generally reasonable but there are many small slips in grammar and spelling. Please check your manuscript carefully on this point. (For example, in the opening sentence of the Introduction ‘in delivers’ should be ‘of deliveries’; in the third sentence ‘Rupture begins’ should be ‘Rupture’; etc.). Also the occasional wrong choice of word, e.g. ln 22 factories should be factors.
You describe the study as ‘prospective’ but I would say it is cross-sectional.

Response: Thank you for the correction and suggestion. We have checked our manuscript carefully and revised the errors throughout the manuscript with the help of a native speaker. We have also changed “prospective” to “cross-sectional” in line 29.

Ln 68: You cite three reference here to support your argument for adverse birth outcomes and cognition with lead exposure, but the references are quite general. Please include references which are more specially focussed on the outcomes that you mention. Please check all your references on this point – primary sources should be cited where possible.

Response: The references cited have been adjusted according to the reviewer’s suggestion (line 71).

Ln 74: You should explain why lead is a particular problem for pregnant women and the fetus.

Response: We thank the reviewer for this good comment and have now added this information in lines 77-79, as “Pb pollution poses a significant threat to human health, especially for pregnant women and the vulnerable fetus, who are more susceptible to Pb exposure since Pb can freely cross the placenta”

Ln 119. Please give some details of the external quality control results.

Response: As stated above, we have now provided the related details of the external quality control results in lines 143-148.

Ln 121: Did you exclude women with urine cr <0.3 or > 3 g/l as done by Yang et al. 2016 https://doi.org/10.1016/j.envint.2016.06.003. Presumably your quality control standards for creatinine were identical to those of Yang – if so please refer to Yang et al. here on this point.

Response: The WHO guidelines for assessing urine dilution based on creatinine may be overly restrictive for women. As data from NHANES suggest that women usually have low urinary creatinine, and the cut-off of < 0.3 g/L may be inappropriate for the female population (Barr et al., 2005), so we have performed a sensitivity analysis that only excludes women with creatinine > 3 g/L according to both the guidelines of the WHO and the NHANES based recommendations in Supplementary Table S2. Still, we observed a significantly positive association between maternal urinary Pb and PROM/preterm PROM. Please see the changes with track in the Methods part (lines 177-180), Results (lines 223-225), Discussion (lines 258-260) and Supplementary Table S2.

In addition, our quality control standards for creatinine were identical to those of Yang, and we have referred to Yang et al. on this point (lines 154-155).

Ln 141. There are several other factors that have been associated with PROM including previous bleeding, drug use, hydramnios etc. Did you exclude women with any of these?

Response: Thank you for this excellent point. We agree that other factors, including the ones mentioned by the reviewer, have been associated with PROM. Even though we did not collect detailed information about these risk factors for PROM in this study, we performed a sensitivity analysis (Supplementary Table S1) that excluded all the possible risk factors associated with PROM (intrauterine infection, vaginitis, cervicitis, pelvic inflammatory disease, previous vaginal bleeding, hydramnios, and fetal malposition) that we collected from medical record. The significant association between urinary Pb levels and PROM remained. Based on the reviewer's comment, we will collect these factors and evaluate their possible association with PROM in our future studies (line 338-340). Please see the changes with track in the Methods part (lines 174-177), Results (lines 219-223), Discussion (lines 256-260) and Supplementary Table S1.

Ln 156. What is the currency for the income?

Response: RMB (yuan). We have added "yuan" in line 200.

Ln 196: PROM can't lead to preterm birth since it is by definition >37 weeks – only PPROM can lead to a premature delivery.

Response: Thank you for the correction. We have corrected it in line 245.

Ln 221. What are the difference in exposure levels? Were they low in reference 17?

Response: Different biosamples have been used to assess the Pb level across these studies, such as blood, umbilical cord, and placenta, whereas our study used urine. Therefore, we do not think these levels can be compared side by side directly. The reason to this discrepancy between reference 17 and our study is currently unknown, but it may be, at least in part, due to the differences in Pb exposure levels (lines 273-274).

Ln 245. Urine Pb is favoured for long-term biomonitoring (i.e. repeated measurements over time), rather than as a long-term biomarker of exposure (i.e. one measurement reflects long-term exposure)

as it reflects plasma lead. This therefore weakens the argument for your use of urine rather than blood lead for a single measurement. Please amend and add comment on this limitation.

Response: Thank you for this comment and we have amended it accordingly (lines 303-304). We admit that one measurement of urinary Pb only reflects plasma lead, and a single measurement may weaken the argument for our use of urine Pb for a single measurement. However, urinary Pb, as a valid biomarker (Barbosa et al., 2005), has been shown to significantly correlate with blood Pb. Another study also reported a good correlation (0.72) between blood and urine Pb levels (Yorita Christensen, 2013). We have added comment on our limitation as “In addition, urinary Pb collected and measured at labour only reflects plasma Pb level at labour, which may not accurately reflect the dynamic maternal Pb exposure throughout the whole pregnancy. Therefore, further studies with urine samples collected at multiple time points and from different populations are needed to confirm the observed relationship between Pb and PROM” (lines 341-348).

Ln 274. A further limitation is that your outcome occurs at the same time as the exposure, so it is not possible to infer causality.

Response: We agree that one limitation of our study is the time of outcome and exposure, and we have now commented on this in lines 344-345.

Table 1. Can you give some statistical information for comparisons? The urine Pb was lower in women exposure to passive smoking, which seems counterintuitive. Please comment.

Response: Thank you for your suggestion. We have used the Wilcoxon signed rank test (line160) to compare concentrations of Pb between these two groups, and added the p value in Table 1. No difference was observed in urinary Pb concentration among pregnant women with or without passive smoking ($p > 0.05$).

Reviewer: 2
Reviewer Name: Salvatore Andrea Mastrolia
Institution and Country: Maternal Fetal Medicine Unit, Fondazione MBBM, San Gerardo Hospital, University of Milano Bicocca, Via Pergolesi 33, Monza, 20900, Monza e Brianza, Italy

Comments for the author
General comments: this is a very interesting study investigating the pathogenesis of one of the Great Obstetrical Syndromes. I read the manuscript with interest and here is a list of major concerns that the authors may want to address:

1) The manuscript need a thorough language revision. Therefore I suggest the authors to refer to a professional proof reading service since, at the moment, the written English is a severe limitation to the publication of this study

Response: Thank you very much for the suggestion. We realize our English may cause confusion sometimes, and therefore have now had a native speaker thoroughly revised the manuscript, which we hope could meet the journal’s requirement now.

2) Why was the second delivery record excluded in women who gave birth twice?

Response: For women who gave birth twice in the study period (n = 3), we only managed to keep their first delivery record. We have added the related information in line 96.

3) Why where women who delivered neonates with malformations excluded? Same question for women with reported smoking and drinking.

Response: We excluded women who delivered neonates with congenital malformations (n = 62) because congenital malformations may be the result of an abnormal pregnancy (line 91).

Evidence has shown smoking or drinking during pregnancy has adverse effects on fetal growth, and the small number of smoking (n = 7) or drinking (n = 2) group did not allow them to be treated as covariates. Therefore, participants who reported smoking and drinking during pregnancy were also excluded (lines 92-95).

4) A section of clinical definitions is needed. To provide the definition of PROM is not enough. All other clinical variables should be adequately defined.

Response: Thank you for raising this point. Detailed definitions of all clinical variables in this study have now been provided accordingly, which can be found in lines 121-129.

5) Lines 205-206: the authors state they excluded women with intrauterine infection. A very precise definition of the diagnosis of intrauterine infection should be provided.

Response: Similarly, a precise definition of the diagnosis of intrauterine infection is also provided now in lines 117-121.

6) It would have been of interest to compare the concentration of Pb in women who experienced PROM or pPROM to women who delivered at term. This way the authors would have been able to claim that Pb was the cause for the development of the Obstetrical Syndrome. With this study they can suggest that the highest is the concentration of Pb the higher is the risk of PROM or pPROM

Response: Thank you for this good suggestion. We have compared the concentration of Pb in women who experienced preterm PROM to women who delivered at term in Table 1. Pb levels in women who experienced preterm PROM is slightly higher than in those who delivered at term, with an unreached statistical significance ($p > 0.05$).

Reviewer: 3
Reviewer Name: Prof. Michael Tchirikov
Institution and Country: Martin-Luther-University, Halle, Germany

Abstract:
Clear, well-chosen information to give a short overview of the article.

L28: The aim of our study was to investigate whether maternal Pb exposure was associated with PROM “and preterm Prom” should be added, because in the result-part the increased risk for preterm PROM is mentioned too.

Response: Thank you for the suggestion. We have added the related information accordingly (line 28).

Main paper
Introduction:
L54: There are sources who show a higher risk than 8-12% but 15%.

Response: Thank you for pointing this out. This has now been revised in line 56 with references cited.

L56: PROM is related to significant maternal, fetal, and neonatal risk, such as maternal infection, prematurity and neonatal sepsis.
Another risk which should me mentioned. The new review PPROM paper (PMID:28710882) could be cited.

Response: Per the reviewer's suggestion, the related information is now added in line 58.

L57: Uncomplete sentence." When the" is missing. When the rupture begins (occurs?) prior to 37 weeks of gestation it is considered as preterm PROM

Response: We have revised the sentence as "When the rupture occurs prior to 37 weeks of gestation it is considered as preterm PROM" (lines 58-60).

Materials and Methods:
L86: It seems unreal, that only 7 of 7290 women were smoking and 2 were drinking during pregnancy.

Response: Thank you for raising this concern. First of all, the information on smoking and drinking during pregnancy were obtained by the face-to-face interview, as described in the method. In fact, Chinese women rarely smoke or drink, especially during pregnancy, which is largely different from women in Western countries (West R. Tobacco control: present and future. British medical bulletin 2006;77(1):123-36; Cochrane J, Chen H, Conigrave KM, et al. Alcohol use in China. Alcohol and Alcoholism 2003;38(6):537-42). We have added the related information accordingly (lines 92-94).

L123: Delete "And"

Response: This is now deleted (line 155).

L137: Other risk factors for PROM would be infection, cervical insufficiency, and premature contractions.

Response: Thank you for this comment. As discussed above, we are fully aware of other risk factors for PROM, including infection, cervical insufficiency, premature contractions, hydramnios, etc. However, since we did not collect information on all the risk factors for PROM in this study, we have performed a sensitivity analysis (Supplementary Table S1) that excluded these possible risk factors (intrauterine infection, vaginitis, cervicitis, pelvic inflammatory disease, previous vaginal bleeding, hydramnios, and fetal malposition) associated with PROM. The result still showed a significant association between urinary Pb levels and PROM. Also, we will collect information on these factors in our future studies to evaluate their relationship with PROM (lines 338-340).

Conclusion:
L282: Significant results are only presented for primiparous mothers as the authors mention earlier (L181-L182).

Response: Thank you for this suggestion. We have added the related information in line 353-354.

I would recommend going over the manuscript for grammar and spelling errors.

Response: We apologize for the inconvenience our English may cause. We have now carefully checked and revised the errors of grammar and spelling throughout the manuscript with the help of a native speaker.

VERSION 2 – REVIEW

REVIEWER	Caroline Taylor
REVIEW RETURNED	16-May-2018

GENERAL COMMENTS	The author has addressed all my previous queries adequately.