Author’s response to reviews

Title: Association between the plasma/whole blood lead ratio and history of spontaneous abortion: a nested cross-sectional study

Authors:

Hector Lamadrid-Figueroa (hlamadrid@insp.mx)
Martha M Tellez-Rojo (mmtellez@insp.mx)
Mauricio Hernandez-Avila (mherman@insp.mx)
Belem Trejo-Valdivia (bvaldivia@insp.mx)
Maritsa Solano-Gonzalez (msolano@insp.mx)
Adriana Mercado-Garcia (adrianam@insp.mx)
Donald Smith (smith@etox.ucsc.edu)
Howard Hu (howardhu@umich.edu)
Robert O Wright (robert.wright@channing.harvard.edu)

Version: 2 Date: 13 July 2007

Author’s response to reviews: see over
Cuernavaca, Mexico July 11th 2007  
Dr Lolu da-Silva  
Assistant Editor, BMC-series journals

Dear Lolu,

Enclosed you will find a revised version of our manuscript entitled “Association between the plasma/whole blood lead ratio and history of spontaneous abortion: a nested cross-sectional study” which incorporates the suggestions and comments of the reviewers of the original version of the manuscript. As requested, we also send a copy of the revised paper with the highlighted changes.

Below is a point by point response to the reviewer comments (which appear in italics).

Reviewer I:

Dear Editor,

Thank you very much for the opportunity to collaborate with the respectful BMC Pregnancy and Childbirth journal through the revision of the paper entitled “Association between the plasma/whole blood lead ratio and history of spontaneous abortion: a nested cross-sectional study” submitted to publication.

The authors present the results of a cross-sectional study nested in two cohorts of pregnant women, conducted in Mexico City, aiming to assess the association between plasma/whole blood Pb ratio and risk of miscarriage. The research question posed by the authors is very interesting and addresses a new step for comprehension of the effect of lead toxicity over the results of human pregnancy. They conducted a careful and appropriate analysis of the data set and attended the relevant standards for reporting of scientific studies. They also addressed at the manuscript the limitations of the study and discussed their implications over the observed results. There is, however, a severe limitation that jeopardizes the biological plausibility of the results. Since the conclusion of the analyses was that high plasma/whole blood Pb ratio was associated with increased risk of miscarriage in past pregnancies and considering that maternal exposure to Pb is cumulative over life, it is hard to understand how the current pregnancy was not affected by plasma lead concentration. It would be more methodologically sound if the study had included all the women who had had lead levels measured when enrolled in the cohorts (as a sub-sample of the main cohort), and if the outcome had been ascertained for the current pregnancy (incident abortions). It would have not only increased sample size from 207 to 312 pregnant women (since primigravidae would not be excluded), but also strengthened plausibility of the study. Trying to understand reasons that conducted the authors to the decision they made, it is possible that the number of cohort women who presented an abortion during the follow-up period was small, what would impair study power to detect
an existing association. Even so, the cohort design would have had methodological advantages. Some of the criteria of causality, like temporality and prevention of information bias, could have been guaranteed. And most importantly, in terms of causality, lack of power would be more scientifically acceptable than lack of plausibility, turning it more reasonable to recommend the study publication in the cohort than in the cross-sectional design.

As a conclusion, in my opinion, although the study subject is highly relevant in terms of public health, especially regarding the health of exposed workers, and despite the appropriate description of the study and well-conducted analyses of the data set, the methodological limitation derived from the sample selection criterion above discussed unfortunately prevents the recommendation for manuscript publication.

**Response:** We appreciate the valuable comments of the reviewer. We fully agree that a cohort design would be much more appropriate to test the hypothesis at hand. In fact, we acknowledge this in the conclusions section of the manuscript. We analyzed data available from previous studies designed for other purposes. Throughout the manuscript, we admit that it is not possible to draw any causal inferences from these data. However, we believe that our observations are consistent with our hypothesis, and suggest that this issue is worth further exploring.

Regarding our methods, although both studies whence our data came from were cohorts, we believe it would not be appropriate to analyze them prospectively for this research question since recruitment occurred at week 10 at the earliest. Since most miscarriages occur during the 1st trimester, the number of miscarriages we observed in the cohorts was quite small (only 12 cases, of which only 4 had plasma and blood Pb measurements), which does not give us enough statistical power (we acknowledge this in page 15). In any case, were we to carry on this proposed analysis, we would be analyzing the effect of plasma lead on 2nd trimester miscarriages, which are only a small fraction of all spontaneous abortions and have characteristics different from 1st trimester miscarriages.

Furthermore, as we explain in the methods section, we did not include women who were undergoing their first pregnancy in this analysis since being currently pregnant with more than 10 weeks of gestational age was an inclusion criterion for all studied women. This means that by definition, spontaneous abortion could not have occurred in this primigravidae women, and the 10 or more weeks of gestation before recruitment can not be considered time at risk of suffering a miscarriage. This restriction on the timing of spontaneous abortions does not apply to past pregnancies and therefore, it is very likely that pooling primigravidae with the rest of the subjects would introduce selection bias. We discuss on this methodological issue and its implications in a detailed fashion in the last section of the Discussion.

**Reviewer II:**
General

This is an original study that evaluates for the first time the plasma/whole blood lead ratio as a marker of susceptibility for the toxic effects of lead exposure. This hypothesis is based on the fact that the plasmatic fraction is the toxicologically active fraction of lead. Therefore, women with higher plasma/whole blood lead ratio would have a higher risk of miscarriage due to higher plasma lead crossing the placental barrier for a given whole blood lead.

Since the risk of spontaneous abortion was evaluated retrospectively and plasma lead has a large inter-individual variability the authors suggest that this variability makes it difficult to find associations with current plasma lead measurements. In contrast plasma/whole blood lead ratio could be influenced by polymorphic alleles of genes coding for proteins involved in the partitioning of circulating lead and would be stable over time.

The authors analyzed data from two cohorts with up to date methods for blood and plasma lead measurements. There are no major concerns of selection or information bias.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The authors based their conclusions on the direct evaluation of the effect of the mentioned ratio; however, there is no mention of any assessment of the effect of whole plasma or bone lead on spontaneous abortion stratified by the ratio of plasma/whole blood lead. The statistical advantage of the simultaneous consideration of the susceptibility marker and the current lead exposure as an interaction term in the model would add to the evaluation of the postulated hypothesis that the partitioning modulates the effect of lead exposure. If there is a problem with the statistical power to identify the ratio-current exposure interaction, this could be mentioned in the discussion.

Response: We thank the reviewer for his comments and valuable suggestion. We conducted an additional analysis on the interaction between bone Pb and the PPb/BPb ratio. As the reviewer suggested, we tested the hypothesis that the association between bone Pb and the history of abortions should be stronger in subjects with high PPb/BPb ratios. Although we found the interaction coefficients to be in the expected direction, particularly in the case of the patella model, we believe the sample size was too small to detect a significant effect modification (the subset of subjects with valid bone Pb measurements was only 95 in the case of tibia and 153 in the case of patella). We have added this information to the text of the manuscript (pages 12 and 14).

We chose not to perform this analysis with either plasma or whole blood Pb concentrations. This decision was taken on the basis that these variables reflect current exposure, and we wouldn’t expect them to have any strong association with spontaneous abortion history regardless of the magnitude of the PPb/BPb ratio. We
believe this would be an excellent analysis to carry on in a future prospective study of this issue.

---

**Minor Essential Revisions** (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

---

**Discretionary Revisions** (which the author can choose to ignore)

The description of the Poisson model used could be shortened.

**Response:** We appreciate the reviewer’s suggestion. However, we have decided to keep the section on the Poisson assumption as it is, since we believe it is important to stress that we followed a methodologically rigorous statistical approach to the subject. Furthermore, we believe it is important to stimulate a more explicit description of the statistical methods followed in reports of biomedical research. This could lead to interesting discussions about the appropriateness of the statistics used in this kind of applications, as is done in other quantitative fields.

**Reviewer III**:

**General**

This well written paper represents an interesting study on the effect of lead on spontaneous abortion.

---

**Minor Essential Revisions** (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct).

1. The questions posed by the authors are new. However, the idea of studying the ratio might be more adequately introduced. The essence is presented in Discussion; viz. “Under this hypothesis current blood or plasma Pb would not necessarily be strongly associated to history of miscarriage, since blood or plasma Pb concentrations are more dependent on intra-individual temporal variation than plasma/blood Pb and would therefore be less correlated between pregnancies”. The genetic implications of the hypothesis are interesting.

**Response:** We appreciate the reviewer’s suggestion. We have moved the sentence that the reviewer mentions to the Background section.

2. The methods are appropriate and well described. However, to categorize the study as a nested cross sectional study seems inappropriate. I would rather suggest: a nested retrospective cohort study. The data seem to be sound and limitations are adequately discussed.

**Response.** We appreciate the reviewer’s suggestion. However, we have decided to keep our original title since in order to classify this as a retrospective cohort study we would need to have records of plasma and blood Pb concentrations measured.
before the events (abortions) occurred. The fact that we are lacking this kind of measurements is the main limitation of our study, since it prevents us from reaching any conclusion about potential causal relationships.

3. The discussion is comprehensive. The main finding is the association between subsequent plasma/whole blood ratio and previous abortion. However, I miss a discussion of social class as a confounder. One would expect a higher lead exposure in lower social classes and the question should be addressed whether lead or other factors associated with low social class (e.g. smoking) are the causal ones. Adjustment for schooling as performed might to some extent solve the problem. The effect of this adjustment should be accounted for.

Response: We appreciate the reviewer’s comments and suggestions. We fully agree that social class is a potential a priori confounder, unfortunately there was no recording of a Socio-Economic Index in these cohorts and thus we used schooling as its proxy. We did not find it to exert an important confounding effect on the models we fitted, the estimate of the PPb/BPb effect was practically unchanged (IRR without adjustment for schooling was 1.121, p=0.018). This may be due to the fact that schooling was not found to be correlated with the plasma/blood Pb ratio (Spearman’s $\rho=-0.03$, p=0.67), once again because the partitioning of circulating Pb depends on biological rather than social determinants. We have added this information to the manuscript in pages 12 and 16.

We appreciate the comments of the reviewers, who have provided us with important ideas to strengthen this manuscript. Please let us know if there are any further questions or comments on this revised version.

Sincerely

Hector Lamadrid-Figueroa, MD

Centro de Investigación en Evaluación y Encuestas
Instituto Nacional de Salud Pública,
Ave. Universidad 655, Cuernavaca 62508,
Morelos, Mexico.
Phone (52) (777) 3 29 30 18
Fax (52) (777) 311 11 48
holmamadrid@insp.mx