Author’s response to reviews

**Title:** Maternal exposure to air pollution before and during pregnancy related to changes in newborn’s cord blood lymphocyte subpopulations. The EDEN Study Cohort.

**Authors:**

- Nour Baïz (baiz@u707.jussieu.fr)
- Rémy Slama (remy.slama@ujf-grenoble.fr)
- Marie-Christine Béné (marie-christine.bene@medecine.uhp-nancy.fr)
- Marie-Aline Charles (marie-aline.charles@inserm.fr)
- Marie-Nathalie Kolopp-Sarda (mkolopps@grip.u-nancy.fr)
- Antoine Magnan (antoine.magnan@univ-nantes.fr)
- Olivier Thiebaugeorges (o.thiebaugeorges@maternite.chu-nancy.fr)
- Gilbert Faure (faure@medecine.uhp-nancy.fr)
- Isabella Annesi-Maesano (annesi-maesano@u707.jussieu.fr)

**Version:** 2 **Date:** 28 September 2011

**Author’s response to reviews:** see over
Title: Maternal exposure to air pollution before and during pregnancy related to changes in newborn’s cord blood lymphocyte subpopulations. The EDEN Study Cohort.

Dear Editor,

I would like to thank you and the reviewers for the careful reviewing of our article. The raised criticisms and comments have greatly contributed to improve our paper. Please find below our reply to them. We hope that you will find the amended version of our article suitable for publication in your journal.

Point-by-point reply

Major Compulsory Revisions

Methods:
1) Lymphocyte immunophenotyping – p. 7
I recommend explaining and justifying why these specific lymphocyte subtypes were chosen to characterise the immune status. What is the advantage of using these indicators of immune status? Why, for example, didn’t authors choose to use general indicators of immune status, such as serum IgA, IgG and IgM levels? It will not be that obvious for readers of BMC Pregnancy and Childbirth

Answer: Our choice to focus on these specific T-cells subtypes as well as on NK cells was based on evidence from previous studies showing that T-cells (1) and NK cells (2) play a critical role in the pathogenesis of allergy and asthma. In addition, percentages of these cells and the ratio (CD4+/CD8+) are considered to be of high prognostic significance for immunocompromised individuals and relevant variables for assessing maturation of the immune system (3). IgA, IgG and IgM levels were not considered as having priority for the study of asthma and allergies because of previous studies. The importance of targeting new cells has been added in the amended version of the paper.

2) Maternal exposure to air pollution – p. 8
The authors should give more details on the exposure assessment to ambient air pollution. They refer to one of the co-author’s paper (line 6) that used land-use regression for exposure estimation to ambient air pollution. Does it mean that in this study the same method was used? It is not clear from the description in the Methods whether the PM10 and NO2 levels measured by air monitoring stations located within less than 2km (results, p.11, line 1) from a maternal residential address were used for calculating the means for the study periods of exposure.

Answer: There was indeed a mistake in the choice of the reference. We did not use land-use regression (LUR) for exposure estimation to ambient air pollution. As indicated in the methods, maternal exposure to NO2 and PM10 was assessed by air monitoring stations located within less than 2 km from the maternal residential address after their geolocalisation through Geographic Information System (GIS). NO2 and PM10 were measured on an hourly basis at permanent background monitoring sites. 7-day average levels were calculated, as well as monthly average and quarterly average levels (3 months before the beginning of pregnancy and each trimester of gestation). The appropriate reference, Hampel R et al. (4), is indicated in the text.
Minor essential revisions

1) Background, p. 4, sentence 3: I am not happy that the authors combined in one sentence the evidence available for environmental tobacco smoke (EST) and urban air pollution, as it does not give the reader a clear idea of the associations found between each of those environmental exposures and birth outcomes. It gives a reference to one study only for urban air pollution, which did not examine all the outcomes listed in that sentence. For example, Slama et al did not examine prematurity and postneonatal mortality in that study. Please change this.

Answer: The evidence available for environmental tobacco smoke (EST) and urban air pollution have been separated and references have been reviewed.

2) Page 6, lines 22-24: Please give more information on how this sample of 370 women was formed. Was it a random collection of cord blood samples?

Answer: Additional information about the sampling of the 370 women has been included in the manuscript.

3) Methods: Terms ‘preterm birth’ used in Table 1 and ‘prematurity’ used on page 10, line 9 are not the equivalents so they should be defined and should not be used to replace each other. The definition of preterm birth (as well as that of low birth weight, LBW) is important to give; in particular, as they differ from the WHO definitions (see Table 1). According to the WHO definition, LBW is weight at birth of <2500g (not # 2500g) and preterm birth is birth at <37 completed weeks of gestation (not # 37 weeks) (see Results page 11, line 8).

Answer: The variables “preterm birth” and “low birth weight” have been corrected, based on their WHO definition (<37 weeks and <2500 g), and have been defined in the result paragraph. Results did not change when considering such definitions.

4) Methods: Statistical analysis: p. 9, line 10. It is stated that for continuous variables means and standard errors were calculated, while in Tables 1-3 means and standard deviations are given.

Answer: Indeed, it is the standard deviations that were calculated and not the standard errors. This has been corrected in the manuscript.

5) Results: p. 11, line 4: The BETX was spelled out when used first time (p. 8,lines 9-10) so it does not need to be given here again.

Answer: Thank you, this has been corrected.
6) Results: p. 11, line 5: Please add “…the total sample (n=2002), from which our…”.
   Answer: It has been added.

7) Results, p. 12: it would be preferable to number Table 4 as Table 3 as their results are described first, and current Table 3 should be numbered Table 4 as it is presented later in the Results section.
   Answer: The tables numbering has been changed as suggested by the reviewer.

8) Results p. 12, lines 12-14 and Table 3: I cannot see how from table 3 it can be concluded about temporal and intra-individual variations in exposure to air pollution. Also, based on the description of exposure assessment, I would not name these average levels of maternal exposure to ambient air pollution as individual maternal exposure in contrast to individual BETX assessment.
   Answer: Thank you, it is indeed a mistake, since the table does not show any information about temporal and intra-individual variations in exposure to air pollution. So this comment has been erased from the manuscript.

9) Results p. 12, lines 18-19: I suggest specifying during which trimesters of pregnancy CD8+ T-cell and NK cell percentages increased significantly with an increase in PM10 and NO2 concentrations, as this does not seem to be the case for each trimester (e.g. NK cell increased with an increase in PM10 in the first trimester).
   Answer: The trimesters during which cells percentages increased have been added in the text, as suggested by the reviewer.

10) Discussion, p. 14, line 6: PM10 and NO2 abbreviations are given in the methods on page 8, they do not need to be spelled out again.
    Answer: This has been corrected.

Discretionary revisions:

1) Abstract, methods, line 8: I suggest moving ‘in 370 women’ to after “…was assessed…” so it would read: Exposure to background particulate matter less than 10 µm in diameter (PM10) and nitrogen dioxide (NO2) was assessed in 370 women three months before pregnancy and during each trimester using monitoring stations data.
2) Methods: I would recommend moving the Ethics statement subsection to the end of the Methods section as this is the usual practice in the scientific medical journals.
3) Results: p. 11, line 3: Change to “Table 1 presents… living within less that 2km…”
4) Table 3 (a) and (b): I would recommend aligning tables by the beginning of the table.
   Answer: These suggested corrections were made.

Typos:

1) Background, page 4, line 7: an extra comma needs to be removed after ‘impaired fetal growth [5].’; line 8: there is no need for a space before the comma after the word ‘prematurity’, but a space should be added after that comma before ‘postneonatal…’; line 12: a space should be added after the dot, before the new sentences starting with ‘Few studies…’
2) Methods: Statistical analysis: p. 9, line 12: Please change “between each air pollutant and each lymphocyte phenotype...”

3) Methods: Statistical analysis: p. 9, line 15-16: Please amend: “… lymphocyte percentages for a 10 mg/m3 increase in PM10 and NO2 …for a 1 mg/m3 increase in log-transformed benzene exposure.

4) Results: p. 11, line 6: “… study population was sampled”.

5) Table 2 footnote and Results, p.11, line 18: Chi-square test

6) Table 5 footnote: “…for each 10 mg/m3 increase in PM10 or NO2.”

7) Results p. 13, line 3 and title for Figure 2: “…increase in…”

8) Discussion, p.16, line 20: a full stop is missing at the end of the sentence.

9) Discussion, p.17, line 15: misclassification

Answer: Corrected.