

## PEER REVIEW HISTORY

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### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Air pollution exposure in early pregnancy and adverse pregnancy outcomes - a register based cohort study
<b>AUTHORS</b>	Olsson, David; Mogren, Ingrid; Forsberg, Bertil

### VERSION 1 - REVIEW

<b>REVIEWER</b>	Tim Nawrot, Hasselt University, Belgium
<b>REVIEW RETURNED</b>	14-Nov-2012

<b>GENERAL COMMENTS</b>	<p>Based on the Swedish registers including 120 755 births the authors reported associations between first trimester ozone exposure and preterm birth and preclampsia. The article is well written and scientifically sound.</p> <p>I have the following comments:</p> <ul style="list-style-type: none"><li>• The authors should clarify why only exposure during the first trimester was studied.</li><li>• Adjustment for season should be more specifically addressed and made consistent throughout the paper. In the statistical paragraph adjustment for season is given but in the footnote of the tables this variable is not included. I would also appreciate to see more details on the average temperature adjustments: was this linear</li></ul> <p>Minor comments:</p> <ul style="list-style-type: none"><li>• Line 189 should day be month?</li><li>• The attributable fraction was only given for preeclampsia, why not for preterm birth?</li><li>• Discussion: what about trigger component of air pollution as preceding factor of birth. This acute effects are not the scope of the paper but might be addressed a bit in the discussion.</li></ul>
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<b>REVIEWER</b>	Payam Dadvand MD, PhD Research Fellow Centre for Research in Environmental Epidemiology Barcelona, Spain
	I have no conflict of interest.
<b>REVIEW RETURNED</b>	18-Nov-2012

<b>GENERAL COMMENTS</b>	<b>PREVIEW</b>
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Air pollution exposure in early pregnancy and adverse pregnancy outcomes - a register based cohort study.

### **SUMMARY**

This manuscript reports on a semi-ecologic study (area-level exposure and individual-level outcome and covariate data) of the association between maternal exposure to O<sub>3</sub> and NO<sub>x</sub> during the first trimester of pregnancy and preterm birth, small for gestational age (SGA) and preeclampsia among 120,755 pregnancies occurring in Stockholm, Sweden (1998-2006). It relied on register-based data on outcomes and covariates together with daily citywide averages of O<sub>3</sub> and NO<sub>x</sub> levels obtained from routine monitoring network (two monitors for O<sub>3</sub> and three for NO<sub>x</sub>) to assess exposure to these pollutants. This study found an increase risk of preeclampsia and preterm birth in association to O<sub>3</sub> exposure during the first trimester. The results for NO<sub>x</sub> and SGA were less conclusive.

### **GENERAL COMMENTS**

This is a well-conducted and relatively large prospective study based on high-quality register data. The observed modifying effect of maternal asthma on the association between air pollution and pregnancy outcomes and also the impact of O<sub>3</sub> exposure on preeclampsia are main contributions of this paper to available literature. This study also is the first to show an association between the exposure to air pollution during the first trimester and preeclampsia. The exposure assessment of this study was based on daily citywide averages of pollutant levels, overlooking the possible spatial variation in pollutant levels. Currently, an increasing number of studies on the air pollution-pregnancy outcome associations are using more sophisticated spatiotemporal exposure assessment approaches (e.g. landuse regression models) that account for the small-scale within-city variation of pollutants. In this context, the exposure assessment methodology applied by this study can be considered as a rather simplistic approach. This approach might have had a limited impact on the precision of estimating exposure to O<sub>3</sub> because O<sub>3</sub> is less spatial variable; however, for NO<sub>x</sub> (that is highly spatial variable) it could have resulted in a considerable exposure misclassification. As discussed by the authorss, this might explain why the study could find some effects for O<sub>3</sub> but not NO<sub>x</sub>. Also, I am missing the rational on why the study has focused on early gestation as the exposure window. Other exposure windows

like the second or third trimesters or the entire pregnancy can also be relevant in relation with the studied outcomes.

**SPECIFIC COMMENTS:**

**Abstract**

1. Line 29: It could be more informative to mention NOx instead of vehicle exhaust.

**Background**

- 1- Line 52. The authors have mentioned the findings of another study by their team. Since that other study was also conducted in the same study region (Greater Stockholm area) with similar exposure (O3 and NO2) and outcome (preterm birth), it could be informative to elaborate what this study can add to the results of the pervious study.
- 2- Line 94- I am missing the rational on why the study has focused on early gestation as the exposure window. For preeclampsia, it is sensible to think that periconceptional exposure to be relevant as it can interfere with placental implantation and abnormal placental implantation has been suggested to be involved in the pathogenesis of preeclampsia. But for preterm birth and SGA, there other window periods (e.g. the second and third trimesters or the entire pregnancy period) seem to be relevant as well (as discussed by the authors in lines 323-325). For instance, a recently published systematic review and meta-analysis of the available literature on the impact of maternal exposure to air pollution on pregnancy outcomes (Stieb DM, Chen L, Eshoul M, Judek S. Ambient air pollution, birth weight and preterm birth: A systematic review and meta-analysis. Environ Res. 2012; 117:100-111) has suggested that the third trimester of pregnancy could be a more relevant exposure window for preterm birth (The authors may wish to

cite this reference in the manuscript). More elaboration on the rational behind limiting the analysis to exposure during early gestation is therefore necessary.

## Methods

- 1- As far as I can understand, the authors have adjusted the analyses of all outcomes (preterm birth, SGA, and preeclampsia) for an identical set of covariates which to my opinion is rather simplistic as these outcomes have different pathogenesis/mechanisms. For instance, as mentioned at lines 80-81 of the Background, preeclampsia is responsible for 15% of preterm births and at the same time preeclampsia is reported to be associated with air pollution (as this study and some other studies have found), so preeclampsia can act as a possible confounder in the association between air pollution and preterm birth.
- 2- Line 151. It is not clear whether the maternal smoking was during pregnancy. I am also wondering if there is any data available on maternal alcohol consumption during pregnancy. If not, then this needs to be mentioned as a limitation of study in the Discussion because maternal alcohol consumption has been linked to the outcomes analysed in this study. Also, in step 4 models, both O<sub>3</sub> and NO<sub>x</sub> were included in the models. There is no information presented on the correlation between these two pollutants (both involved in photochemical reactions with each other and therefore possibly correlated) to see if there could be any concern about collinearity when having them both in the same model.
- 3- As mentioned by the authors, the data on BMI, smoking, and family situation were “suffered from non-random missing values” and that was why the authors used an extra step in their analysis to adjust for these covariates. As expected, the 95% confidence intervals for the risk estimates by this step are wider because those participants with missing data should have been dropped from the analysis in this step leading to smaller sample size and larger standard error (e.g. for the associations between O<sub>3</sub> and preterm birth and preeclampsia this interval became wide enough to include null OR of 1 and make the association statistically non-significant). Another way of dealing with this missing data is to carry out a sensitivity analysis using imputation techniques (e.g. multiple imputation) to impute the missing data, enabling authors to run the last step without losing participants with missing data on this covariates and therefore gaining statistical

power. If the authors are not willing to do so, then they may need to present the comparison of the outcome and exposure variables and key covariates for the participants with missing variables with those of participants without any missing data to give the readers an idea of how these two groups were different (this can be shown as a supplementary material).

4- Line 136: *“If there were missing data for more than one monitoring station, no imputation was performed. Daily city-wide levels were then calculated using all monitoring stations. Mean pollution levels and average levels for the meteorological variables were calculated for the first trimester of gestation for each pregnancy if fewer than 20 days of daily averages were missing during the period.”*

It could be informative to mention the number (proportion) of the study participants that were not assigned any exposure (i.e. missing exposure data) due to these constraints.

5- Exposure Data: It could be informative to mention the type of monitors (background, traffic, etc.)

6- The authors have properly conducted the analyses of preterm birth with and without including those with elective caesarean sections. This is not obvious to readers until going through the Results and needs to be mentioned in the Materials and Methods. Also the stratified analysis for Asthmatic/non-asthmatic mothers should be described in the Materials and Methods.

7- The authors may wish to (non-obligatory, just a suggestion) have a look at the impact of maternal exposure to air pollution on gestational age at delivery as a continuous outcome variable. This is important, as the burden of the reduction in gestational age has been shown to increase progressively as gestational age at delivery declines.

## Results

1- It can be informative to mention the temporal trend in outcomes and also O3 levels during the study period.

2- Line 189. “day during the year conception occurred”. It seems that “day” needs to be replaced by “month” (according to line 167).

- 3- Line 194. Would be informative to add the correlation coefficient between O3 and temperature/humidity to give readers a better idea about this reported collinearity.
- 4- There is no mention of the results for the O3-asthma interaction term described in line 173. Also, it would be informative to present results (as a supplementary table) for O3 analyses for SGA and preeclampsia stratified asthma condition as is presented for preterm birth in table 2.

### **Discussion**

- 1- There is no discussion of biological plausibility of the findings of this study. A description of possible underlying mechanisms behind these findings needs to be added.
- 2- The findings for the stratified analyses based on asthma status is one of the main contributions of this study to literature and merits more elaboration than just recapping the findings. For example, the authors can add some sentences on the consistency of these findings with those of others, biological plausibility of these findings, and their implications for public health.
- 3- Apart from the possible exposure misclassification (due to overlooking the within-city spatial variation in pollutant levels and merely relying on temporal contrast in pollutant levels), there is no discussion of any other limitation of study and this needs to be added to the end of the Discussion section. For example, the authors can mention the unavailability of data for some relevant covariates (e.g. alcohol consumption during pregnancy, second-hand smoking, etc.), the possible impact of missing data on the findings, and possible exposure misclassification due to relying on ambient air pollution level as a surrogate for personal exposure levels.
- 4- It can be informative to discuss how these findings could be of importance for health professional and policy makers. For example, the authors can use their calculated attributed fraction and add a discussion on the burden of disease associated with preeclampsia and elaborate more on the burden of preterm birth.

## VERSION 1 – AUTHOR RESPONSE

Reviewer: Tim Nawrot,  
Hasselt University, Belgium

Based on the Swedish registers including 120 755 births the authors reported associations between first trimester ozone exposure and preterm birth and preeclampsia. The article is well written and scientifically sound.

I have the following comments:

- The authors should clarify why only exposure during the first trimester was studied.

We have added a sentence on this aspect in the study aim.

- Adjustment for season should be more specifically addressed and made consistent throughout the paper. In the statistical paragraph adjustment for season is given but in the footnote of the tables this variable is not included. I would also appreciate to see more details on the average temperature adjustments: was this linear

This has been clarified in statistical analysis and results sections. With this wide exposure windows temperature was included as a linear predictor.

Minor comments:

- Line 189 should day be month?
- The attributable fraction was only given for preeclampsia, why not for preterm birth?

We have performed the new analysis and added this result in the paper.

- Discussion: what about trigger component of air pollution as preceding factor of birth. This acute effects are not the scope of the paper but might be addressed a bit in the discussion.

We have commented on this in the discussion.

Reviewer: I have no conflict of interest.

Payam Dadvand MD, PhD  
Research Fellow  
Centre for Research in Environmental Epidemiology Barcelona, Spain

There is no mention of ethical approval.

Information on ethical approval has been added in the end of the methods section.

This manuscript reports on a semi-ecologic study (area-level exposure and individual-level outcome and covariate data) of the association between maternal exposure to O<sub>3</sub> and NO<sub>x</sub> during the first trimester of pregnancy and preterm birth, small for gestational age (SGA) and preeclampsia among

120,755 pregnancies occurring in Stockholm, Sweden (1998-2006). It relied on register-based data on outcomes and covariates together with daily citywide averages of O<sub>3</sub> and NO<sub>x</sub> levels obtained from routine monitoring network (two monitors for O<sub>3</sub> and three for NO<sub>x</sub>) to assess exposure to these pollutants. This study found an increase risk of preeclampsia and preterm birth in association to O<sub>3</sub> exposure during the first trimester. The results for NO<sub>x</sub> and SGA were less conclusive.

## GENERAL COMMENTS

This is a well-conducted and relatively large prospective study based on high-quality register data. The observed modifying effect of maternal asthma on the association between air pollution and pregnancy outcomes and also the impact of O<sub>3</sub> exposure on preeclampsia are main contributions of this paper to available literature. This study also is the first to show an association between the exposure to air pollution during the first trimester and preeclampsia. The exposure assessment of this study was based on daily citywide averages of pollutant levels, overlooking the possible spatial variation in pollutant levels. Currently, an increasing number of studies on the air pollution-pregnancy outcome associations are using more sophisticated spatiotemporal exposure assessment approaches (e.g. landuse regression models) that account for the small-scale within-city variation of pollutants. In this context, the exposure assessment methodology applied by this study can be considered as a rather simplistic approach. This approach might have had a limited impact on the precision of estimating exposure to O<sub>3</sub> because O<sub>3</sub> is less spatial variable; however, for NO<sub>x</sub> (that is highly spatial variable) it could have resulted in a considerable exposure misclassification. As discussed by the authors, this might explain why the study could find some effects for O<sub>3</sub> but not NO<sub>x</sub>. Also, I am missing the rationale on why the study has focused on early gestation as the exposure window. Other exposure windows like the second or third trimesters or the entire pregnancy can also be relevant in relation with the studied outcomes.

## SPECIFIC COMMENTS:

### Abstract

1. Line 29: It could be more informative to mention NO<sub>x</sub> instead of vehicle exhaust.

This has been altered.

### Background

1- Line 52. The authors have mentioned the findings of another study by their team. Since that other study was also conducted in the same study region (Greater Stockholm area) with similar exposure (O<sub>3</sub> and NO<sub>2</sub>) and outcome (preterm birth), it could be informative to elaborate what this study can add to the results of the previous study.

We have added this in the study aim.

2- Line 94- I am missing the rationale on why the study has focused on early gestation as the exposure window. For preeclampsia, it is sensible to think that periconceptional exposure to be relevant as it can interfere with placental implantation and abnormal placental implantation has been suggested to

be involved in the pathogenesis of preeclampsia. But for preterm birth and SGA, there other window periods (e.g. the second and third trimesters or the entire pregnancy period) seem to be relevant as well (as discussed by the authors in lines 323-325). For instance, a recently published systematic review and meta-analysis of the available literature on the impact of maternal exposure to air pollution on pregnancy outcomes (Stieb DM, Chen L, Eshoul M, Judek S. Ambient air pollution, birth weight and preterm birth: A systematic review and meta-analysis. *Environ Res.* 2012; 117:100-111) has suggested that the third trimester of pregnancy could be a more relevant exposure window for preterm birth (The authors may wish to cite this reference in the manuscript). More elaboration on the rationale behind limiting the analysis to exposure during early gestation is therefore necessary.

We have clarified this in the study aim. One problem with the third trimester as an exposure window is that the averaging time is shorter for preterm deliveries supporting more extreme mean values.

## Methods

1- As far as I can understand, the authors have adjusted the analyses of all outcomes (preterm birth, SGA, and preeclampsia) for an identical set of covariates which to my opinion is rather simplistic as these outcomes have different pathogenesis/mechanisms. For instance, as mentioned at lines 80-81 of the Background, preeclampsia is responsible for 15% of preterm births and at the same time preeclampsia is reported to be associated with air pollution (as this study and some other studies have found), so preeclampsia can act as a possible confounder in the association between air pollution and preterm birth.

We assess that preeclampsia is a step along the pathway for preterm birth and as such should not be considered as a confounder. However, we have tested to adjust for it. Adding preeclampsia as a covariate did not alter any of the reported associations with preterm birth.

2- Line 151. It is not clear whether the maternal smoking was during pregnancy. I am also wondering if there is any data available on maternal alcohol consumption during pregnancy. If not, then this needs to be mentioned as a limitation of study in the Discussion because maternal alcohol consumption has been linked to the outcomes analysed in this study. Also, in step 4 models, both O<sub>3</sub> and NO<sub>x</sub> were included in the models. There is no information presented on the correlation between these two pollutants (both involved in photochemical reactions with each other and therefore possibly correlated) to see if there could be any concern about collinearity when having them both in the same model.

As stated in the discussion, restricting the study to temporal variation in air pollution restricts the possible sources of confounding factors to risk factors that might vary over time. We have clarified that it was smoking status at first antenatal care visit. We have added information on correlation between the air pollutants.

3- As mentioned by the authors, the data on BMI, smoking, and family situation were “suffered from non-random missing values” and that was why the authors used an extra step in their analysis to adjust for these covariates. As expected, the 95% confidence intervals for the risk estimates by this step are wider because those participants with missing data should have been dropped from the analysis in this step leading to smaller sample size and larger standard error (e.g. for the associations between O<sub>3</sub> and preterm birth and preeclampsia this interval became wide enough to include null OR of 1 and make the association statistically non-significant). Another way of dealing with this missing data is to carry out a sensitivity analysis using imputation techniques (e.g. multiple imputation) to impute the missing data, enabling authors to run the last step without losing participants with missing

data on this covariates and therefore gaining statistical power. If the authors are not willing to do so, then they may need to present the comparison of the outcome and exposure variables and key covariates for the participants with missing variables with those of participants without any missing data to give the readers an idea of how these two groups were different (this can be shown as a supplementary material).

For all years except one the excluded subject had a O3 mean deviating 1.5 % or less from the included subjects. This has been added to results.

4- Line 136: "If there were missing data for more than one monitoring station, no imputation was performed. Daily city-wide levels were than calculated using all monitoring stations. Mean pollution levels and average levels for the meteorological variables were calculated for the first trimester of gestation for each pregnancy if fewer than 20 days of daily averages were missing during the period." It could be informative to mention the number (proportion) of the study participants that were not assigned any exposure (i.e. missing exposure data) due to these constraints.

We have added this information.

5- Exposure Data: It could be informative to mention the type of monitors (background, traffic, etc.)

This information has been added (background sites).

6- The authors have properly conducted the analyses of preterm birth with and without including those with elective caesarean sections. This is not obvious to readers until going through the Results and needs to be mentioned in the Materials and Methods. Also the stratified analysis for Asthmatic/non-asthmatic mothers should be described in the Materials and Methods.

This has been clarified. It is not a stratified analysis, it is an interaction term.

7- The authors may wish to (non-obligatory, just a suggestion) have a look at the impact of maternal exposure to air pollution on gestational age at delivery as a continuous outcome variable. This is important, as the burden of the reduction in gestational age has been shown to increase progressively as gestational age at delivery declines.

We agree that this is an important outcome to study, and we have investigated this relation in our previous paper. However, it is beyond the scope of this manuscript to cover all aspects.

## Results

1- It can be informative to mention the temporal trend in outcomes and also O3 levels during the study period.

We have added this.

2- Line 189. "day during the year conception occurred". It seems that "day" needs to be replaced by "month" (according to line 167).

This mistake has been corrected.

3- Line 194. Would be informative to add the correlation coefficient between O3 and

temperature/humidity to give readers a better idea about this reported collinearity.

We have added information on the correlation coefficient.

4- There is no mention of the results for the O<sub>3</sub>-asthma interaction term described in line 173. Also, it would be informative to present results (as a supplementary table) for O<sub>3</sub> analyses for SGA and preeclampsia stratified asthma condition as is presented for preterm birth in table 2.

The table is not from a stratified analysis; it is from the interaction term.

## Discussion

1- There is no discussion of biological plausibility of the findings of this study. A description of possible underlying mechanisms behind these findings needs to be added.

The suggested mechanisms and related references are presented in the introduction.

2- The findings for the stratified analyses based on asthma status is one of the main contributions of this study to literature and merits more elaboration than just recapping the findings. For example, the authors can add some sentences on the consistency of these findings with those of others, biological plausibility of these findings, and their implications for public health.

We have added a proposed pathway through which ozone exposure among asthmatic women may lead to preterm birth.

3- Apart from the possible exposure misclassification (due to overlooking the within-city spatial variation in pollutant levels and merely relying on temporal contrast in pollutant levels), there is no discussion of any other limitation of study and this needs to be added to the end of the Discussion section. For example, the authors can mention the unavailability of data for some relevant covariates (e.g. alcohol consumption during pregnancy, second-hand smoking, etc.), the possible impact of missing data on the findings, and possible exposure misclassification due to relying on ambient air pollution level as a surrogate for personal exposure levels.

See about temporal contrasts in Methods point 3.

4- It can be informative to discuss how these findings could be of importance for health professional and policy makers. For example, the authors can use their calculated attributed fraction and add a discussion on the burden of disease associated with preeclampsia and elaborate more on the burden of preterm birth.

We added policy implications in the conclusions.

## VERSION 2 – REVIEW

<b>REVIEWER</b>	Tim Nawrot, associate professor of epidemiology Hasselt University, Belgium  no competing interests
<b>REVIEW RETURNED</b>	22-Dec-2012
<b>GENERAL COMMENTS</b>	Authors addressed the comments well